Journal of Inherited Metabolic Disease (2018) 41 (Suppl 1):S37–S219
https://doi.org/10.1007/s10545-018-0233-9

ABSTRACTS

Oral Presentations

PARALLEL SESSION 1A: Clycosylation and cardohydrate disorders

O-001

Fertility in classical galactosaemia, *N*-glycan, hormonal and inflammatory gene expression interactions

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Background: Classical Galactosaemia (CG) is caused by deficiency of galactose-1-phosphate uridylyltransferase (GALT). Long-term complications persist in treated patients despite dietary galactose restriction with significant variations in outcomes suggesting epigenetic glycosylation influences. Primary Ovarian Insufficiency (POI) is a very significant complication affecting females with follicular depletion noted in early life.
Methods: We studied specific glycan synthesis, leptin system and inflammatory gene expression in white blood cells as potential biomarkers of infertility in 54 CG adults (27 females and 27 males) (age range 17–51 y) on a galactose-restricted diet in a multi-site Irish and Dutch study. Gene expression profiles were tested for correlation with a serum Ultra Performance Liquid Chromatography (UPLC)-Immunoglobulin (IgG)-*N*-glycan galactose incorporation assay and endocrine measurements.
Results: 25 females (93% of subjects) had clinical and biochemical evidence of POI. As expected, the female patients, the majority of whom were on hormone replacement therapy, and the controls of both genders showed a positive correlation between log leptin and BMI but this correlation was not apparent in CG male subjects. The key glycan synthesis modifier genes *MGAT3* and *FUT8* which influence glycan chain bisecting and fucosylation and subsequently cell signalling and adhesion were significantly upregulated ($p< 0.01$ and $p< 0.05$) as was the glycan synthesis gene *ALG9* ($p< 0.01$). Both the *LEP* and *LEPR* genes were upregulated ($p< 0.01$). The inflammatory genes *ANXA1* and *ICAM1* and the apoptosis gene *SEPT4* were also upregulated, ($p< 0.01$).
Discussion: These findings further elaborate on the systemic glycosylation and cell signalling abnormalities evident in CG which likely influence the pathophysiology of POI.

O-002

Link between glycemia and hyperlipidemia in Glycogen Storage Disease type Ia

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Background: Glycogen Storage Disease type Ia (GSD Ia) is an inborn error of glucose metabolism characterized by fasting hypoglycemia, hyperlipidemia and fatty liver disease. We have previously reported considerable heterogeneity in circulating triglyceride levels between individual GSD Ia patients, a phenomenon that is poorly understood. Interestingly, hypertriglyceridemia in GSD Ia patients appears to be related to glycemic control, but the mechanisms that link glycemia to hyperlipidemia remain unresolved.
Methods: We performed a systematic analysis of whole-body triglyceride metabolism in fed (normoglycemic) and fasted (hypoglycemic) hepatocyte-specific glucose-6-phosphatase deficient (L-$G6pc^{-/-}$) mice, a liver-specific model for GSD Ia.
Results: L-$G6pc^{-/-}$ mice exhibited fatty liver disease as compared to wildtype controls under both conditions, but the increase in hepatic triglyceride content was highest in fasted L-$G6pc^{-/-}$ mice. Hepatic *de novo* fatty acid synthesis substantially contributed to hepatic triglyceride accumulation in fed L-$G6pc^{-/-}$ mice. In contrast, in fasted L-$G6pc^{-/-}$ mice, *de novo* lipogenesis was of less importance and increased liver fat was paralleled by enhanced adipose tissue lipolysis. Plasma lipoprotein analysis showed that VLDL-triglyceride and cholesterol levels were increased in L-$G6pc^{-/-}$ mice under both conditions, but that VLDL levels were significantly higher in fasted versus fed L-$G6pc^{-/-}$ mice. VLDL production rates were doubled under both conditions in L-$G6pc^{-/-}$ mice, while VLDL catabolism was inhibited only in hypoglycemic L-$G6pc^{-/-}$ mice.
Discussion: Our data show that hypoglycemia in GSD Ia promotes adipose tissue lipolysis and arrests VLDL catabolism. These mechanisms likely also contribute to aggravation of fatty liver and hyperlipidemia in patients with poorly controlled glycemia and may contribute to clinical heterogeneity in GSD Ia.v

O-003 Tracing the fate of galactose in PGM1-CDG

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Background: Phosphoglucomutase-1 (***PGM1***) mutations have recently been reclassified as a congenital disorder of glycosylation (PGM1-CDG; MIM 612941). PGM1 interconverts glucose-1-P to glucose-6-P, and is a key enzyme sitting at the crossroads of glycogenesis, glycogenolysis, glycolysis, nucleotide sugar biosynthesis, the hexosamine biosynthesis pathway, and the pentose phosphate pathway (PPP). PGM1-CDG is also one of the few multisystem CDGs with a largely effective treatment – galactose, which has been shown in open label trials to be both safe and reduce symptoms in patients. How galactose benefits these patients though is not understood**.**
Methods: To probe the biochemical basis to galactose treatment, we employed tracer based metabolomics, a technique capable of tracking the activity of multiple pathways simultaneously. Control and PGM1-CDG fibroblasts were cultured with the tagged sugars (^{13}C) glucose, with and without galactose. The ^{13}C tracer is then incorporated into connected pathways after which, the cells were harvested to enable detection of metabolites by the mass-spectrometry.
Results: Our results suggest that the mechanism by which galactose benefits these patients is by restoring depleted levels of the activated sugars UDP-glucose and -galactose, thus restarting stalled glycosylation in these patients. Further, our data indicates that glucose is utilised differently in the presence of galactose.
Discussion: Our results suggest there might be other possible treatment options other than galactose supplementation, which could potentially prove more efficient in correcting the PGM1-CDG phenotype. In addition, this study could shed light on how galactose would benefit other CDGs such as PMM2-CDG.

O-004

Defining a new immune deficiency syndrome; MAN2B2-CDG

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Background: Many types of congenital disorders of glycosylation affect the lysosomal and secretory pathways, however no lysosomal enzyme deficiency has been described so far causing CDG. Alpha-mannosidosis is a clinically heterogeneous lysosomal storage disorder due to mutations in the lysosomal mannosidase gene *MAN2B1*. We present a patient with severe combined immune deficiency harboring a biallelic mutation in the mannosidase gene *MAN2B2,* affecting lysosomal integrity and the glycosylation pathway.
Methods: Exome sequencing was employed to identify disease-causing mutations in a patient born to consanguineous parents. The patient presented with profound immune deficiency, strabismus, dysmorphisms, abnormal chest configuration, and a history of stroke. Pathogenic effects of the mutation were investigated *in vitro* by mass spectrometry, MALDI-TOF free glycan profile analysis and Western blotting in immortalized lymphocytes and fibroblasts.
Results: Exome sequencing identified a predicted pathogenic biallelic mutation p.D38N in *MAN2B2*. Pedigree analysis confirmed segregation of the homozygous mutation with disease. Free glycan profiling showed accumulation of GlcNAc2Man2 with terminal 1,6 Mannose motif in patient cells, indicative of a deficiency of 1,6 mannosidase activity.Glycosylation analysis showed increased expression of hypoglycosylated ICAM-1, and decreased levels of glycosylated ICAM-1 protein in patient immune cells compared to control. Glycosylated LAMP2 protein was found decreased in patient cells compared to control.
Discussion: Our data suggest the *MAN2B2* gene as a novel autosomal recessive disease gene leading to congenital disorder of glycosylation and affecting lysosomal trafficking. Disruption of *MAN2B2* enzymatic activity and subsequent impairment of α1,6-mannosidosis leads to abnormal mannosylation of glycans. Bone-marrow transplantation restores the metabolic defect.

O-005

Ngly1 pathogenic variant causing deglycosylation defect, masquerading as mitochondrial hepatocerebral cytopathy

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Background: Mitochondrial disease and congenital disorders of glycosylation participate in seemingly distinct metabolic pathways but share clinical manifestations and constitute multi-systemic disorders.
Case report and methods: We report on a patient who presented since birth with progressive hepatic failure, hypotonia, oscillating eye movements, psychomotor delay, fluctuating hyperlactatemia and elevated serum α-fetoprotein. She died at age one year. Initially, we attributed her phenotype to hepatocerebral mitochondrial DNA depletion syndrome (HC-MDS), further grounded by electron microscopical finding of hepatocytes filled with abnormal mitochondria with changes in shape, size, cristae and matrix. During three successive pregnancies the growing list of genes linked to HC-MDS was sequenced, including *DGUOK, TK2, SUCLA2 RRM2B, TYMP* and *POLG*. However, no mutation was found, and three healthy boys were born. During recent pregnancy, parents refused genetic tests. A full-term female was born who presented from birth hypotonia, abnormal eye contact, purposeless hand movements, developmental delay, neuropathy, hypertransaminasemia, elevated α-fetoprotein and mild hyperlactatemia. Her biochemical abnormalities improved with time. At age six months whole exome sequencing (WES) was performed.
Results: WES revealed a homozygous novel nonsense mutation in *Ngly1*, found also in her deceased sister.
Discussion: *Ngly1* is a cytoplasmic de-N-glycosylating enzyme, involved in the process of endoplasmic reticulum-associated degradation (ERAD),

a quality control mechanism for newly synthesized proteins. We report for the first time a patient with Ngyl1 deficiency, mimicking a fatal HC-MDS, broadening the Ngly1-CDDG phenotype. This study further supports essential physiological interactions between cellular N-linked deglycosylation capacity, and mitochondrial function. It also underscores the role of WES in avoiding a "diagnostic odyssey", allowing early genetic diagnosis and counseling.

O-006

Longitudinal follow-up of 75 PMM2-CDG patients

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Background: PMM2-CDG is the first described CDG that presents with either a neurological or multivisceral subtype. Little is known about the longitudinal evolution.
Methods: We performed data analysis on PMM2-CDG patients' clinical data (Nijmegen CDG severity score) and laboratory data.
Results: 75 patients (28 male) were followed up from 11.0± 6.91 years for an average of 7.5 years.
On a group level, there was no significant evolution in overall clinical severity. There was some improvement in mobility and communication, liver function, endocrine function, strabismus and eye movements. However, the current clinical function, the system specific involvement and the current clinical assessment remained unchanged.
On follow-up there was improvement of biochemical variables with (near) normalization of aPTT, factor IX, protein C, antithrombin III, thyroid stimulating hormone and AST/ALT.
Coagulation abnormalities (factor IX, Antithrombin III and protein C) correlated significantly with clinical scores.
Discussion: PMM2-CDG patients show a spontaneous biochemical improvement and stable clinical course based on the Nijmegen CDG severity score, which should be used for end points in upcoming clinical trials.

PARALLEL SESSION 1B: New treatments

O-007

Safety and efficacy of KH176 in adult patients with mitochondrial disease due to the m.3243A>G mutation (KHENERGY)

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Background: Mitochondrial diseases are progressive debilitating multi-system disorders with limited therapeutic options. KH176 is a potent intracellular redox-modulating compound being developed to treat mitochondrial disease. Tolerability, safety, pharmacokinetics, pharmacodynamics and efficacy of KH176 in patients with the m.3242A>G (MELAS) mutation was assessed.
Methods: A double blind, randomized, placebo-controlled, single-center, two-way cross-over phase II study with 100 mg twice daily oral KH176 in adult m.3243A>G patients without cardiovascular involvement. Patients were randomly assigned to receive KH176 or placebo for the first 28 days. Efficacy parameters included clinical and functional outcome measures. The trial was registered with ClinicalTrials.gov (NCT02909400).
Results: Twenty patients were recruited. Nineteen entered the first treatment period and were randomly assigned to treatment (n=10 KH176 and n=9 placebo first). Twice daily oral 100 mg KH176 was well tolerated. No serious treatment emergent adverse events were reported. No significant improvements in gait parameters, the primary endpoint, or other functional outcome measures were obtained. KH176 had a positive effect on attentional performance (effect size with alarm −2.2 (95%CI −4.1 to −0.3; p=0.025). The Beck Depression Inventory and the Hospital Anxiety and Depression Scale were statistically in favour of the KH176 treatment (difference of the changes from baseline: −2.9 (95%CI −5.7 to −0.13) p=0.04 and −1.9 (95%CI −3.6 to −0.2) p=0.03 respectively).
Discussion: KH176 was well tolerated and appeared safe at the 100 mg twice a day dose regimen. Although this exploratory 28 day study in this chronic condition did not meet the primary endpoint (gait), secondary functional and clinical endpoints provide the first evidence of a treatment effect on alertness and mood. Proper assesment of motor function need longer duration studies in more severely affected patients.
Conflict of Interest declared.

O-008

Fragment-based drug discovery for inborn errors of metabolism: Primary hyperoxaluria I as exemplar target.

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Background: Small molecule therapy has created limited impact for inborn errors of metabolism (IEM), as most defects result in loss-of-function in enzymes that are not well-studied as drug targets. To overcome these hurdles, we have developed a structural biology pipeline that identifies small molecule binders as starting point for development of pharmacological chaperones and substrate reduction inhibitors. Our approach combines the emerging technologies of high-throughput crystallography and fragment screening, taking advantage of the protein 3D shape to look for pockets that bind fragment molecules (< 300 Da).
Methods: We chose primary hyperoxaluria I (PH1) as a pilot for our pipeline because genetic knockdown of hydroxyacid oxidase 1 (HAO1), upstream of the defective enzyme (AGXT), ameliorates disease phenotype and represents a good inhibitor target. We streamlined and automated the process for generating milligram quantities of recombinant HAO1 protein in the crystalline state, soaking hundreds of crystals with a custom library of chemical fragments, and resolving their crystal structures to identify bound fragments.
Results: From solving 409 structures of human HAO1 (in a matter of days), we identified 12 fragments bound to the active site, tetramer interface, and a novel allosteric pocket. These fragments were validated by biophysical binding assays, and optimized inhibitors were found by structure analysis and biophysics. Promising fragments are being developed into lead inhibitors by cycles of medicinal chemistry and structure based design.
Discussion: To our knowledge, this study is the first example of fragment screening applied to the IEM field representing a step change for its drug discovery. Thanks to its independence from enzyme function and use of fragments to simplify follow-up chemistry, our approach is now being systematically applied to diverse IEM targets for chaperone (e.g. AGXT, ALDH7A1) and inhibitor (e.g. GALK1, NDST1) development in our lab.

O-009

Organoids to evaluate novel treatment strategies for intrahepatic cholestatic disease

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Background: ATP8B1 and ABCB11 deficiency (PFIC1 and PFIC2) are rare pediatric liver diseases characterized by intrahepatic cholestasis. Treatment options are limited to invasive therapies, including liver transplantation. We recently tested promising novel therapies *in vitro* to rescue specific *ATP8B1* genotypes, that might also be applicable to specific *ABCB11* genotypes. However, there is no good *in vitro* model to predict clinical effect of these mutation-specific therapies.
Methods / case report: We used liver organoids to generate a functional preclinical treatment assay for the bile salt export pump (BSEP). BSEP is encoded by *ABCB11,* and functionally dependent on ATP8B1 because of its putative role in plasma membrane composition and localization of apical transmembrane proteins, such as BSEP. To allow preclinical testing in a personalized manner without the need for a liver biopsy, we also evaluated functional assessment of ATP8B1 in rectum organoids.
Results: We generated liver and rectum organoids with ATP8B1 and ABCB11 deficiency from patient tissue and through CRISPR-Cas9 gene editing. We show that BSEP function can be evaluated with fluorescent taurocholate in differentiated liver organoids, which might be used as a functional assay for both ATP8B1 and ABCB11 deficiency. We additionally show we can use the Forskolin induced swell test in rectum organoids to assess activity of the cystic fibrosis transmembrane conductance regulator (CFTR) as a functional assay for ATP8B1.
Discussion: Organoids provide a unique *in vitro* model to evaluate the clinical effect of novel therapeutic strategies for ATP8B1 and ABCB11 deficiency in patients' native cellular and genetic background. After optimization, these assays will be used to study the patient-specific effect of our novel therapeutic strategies.

O-010

An observational study of patients with cerebral adrenoleukodystrophy (CALD) treated with allogeneic hematopoietic stem cell transplant

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Background: Cerebral adrenoleukodystrophy (CALD) is caused by deficiency of the ALD protein and accumulation of very-long chain fatty acids, in the adrenal cortex and white matter leading to progressive loss of neurologic function and death. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) can halt disease progression, if performed early. Here, we aim to better understand outcomes of allo-HSCT in CALD.
Methods: Sixty patients (≤17 years) will be enrolled in the ongoing ALD-103 study. Retrospective data are collected, or patients are followed prospectively, for 4 years after their last allo-HSCT. Data are analyzed for the entire cohort, and for early disease patients (evidence of cerebral disease as established by GdE+ or Loes score ≥0.5 but ≤9, and NFS ≤1).
Results: As of August 2017, 38 patients (22 with early disease) were enrolled; median follow up time was 17.5 months (min-max, 1.0-53.0). Median age at allo-HSCT was 9.0 years (min-max, 2.0-15.0). Median baseline NFS and Loes scores were 0.0 (min-max, 0.0-4.0) and 3.0 (min-max, 0.0-16.0), respectively. Five of the patients with early diseases (22.7%), had engraftment failure and received a second transplant. Cells from an unrelated and related donor were used for 28 and 10 transplants, respectively. Cord blood (19) and bone marrow (17) were the most common sources of HSCTs. Busulfan (Bu)/fludarabine (Flu), Bu/cyclophosphamide (Cy), and Bu/Flu/Cy were used for conditioning in 20, 14, and 4 transplants, respectively. Six patients with advanced (37.5%) and 1 patient with early disease (4.5%) died post-HSCT. Among the 38 transplants, there were 8 cases (21.1%) each of ≥Grade 2 acute GVHD and chronic GVHD; 6 cases of GVHD were in early disease patients.
Discussion: These preliminary data suggest that while safety risks following allo-HSCT are common in advanced disease patients, risks also exist for patients who received treatment early. There is an unmet need for improved safety outcomes in CALD.
Conflict of Interest declared.

O-011

Lenti-D hematopoetic stem cell gene therapy for cerebral adrenoleukodystrophy: safety and efficacy outcomes from an ongoing Ph 2/3 trial

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Background: Adrenoleukodystrophy (ALD) is an X-linked disease caused by dysfunction of the ALD protein which results in the accumulation of very-long chain fatty acids in adrenal and nervous system tissues. Cerebral ALD (CALD), affecting 35-40% of boys with ALD, is characterized by inflammatory demyelination leading to progressive loss of neurologic function and death. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) has a positive impact on indices of cerebral disease progression, if performed early, but can be associated with significant risk.
Methods: Lenti-D Drug Product (DP) is an investigational gene therapy for the treatment of CALD. Boys with CALD (≤17 years) enrolled in an open-label phase 2/3 study of the safety and efficacy of Lenti-D DP underwent full myeloablation followed by infusion of autologous CD34+ cells transduced with elivaldogene tavalentivec (Lenti-D) lentiviral vector. The primary efficacy endpoint is the proportion of patients who are alive and free of major functional disabilities (MFD) at Month 24. Safety assessments included the proportion of patients who experience engraftment failure, acute (≥Grade II) graft-versus-host disease (GVHD), or chronic GVHD.
Results: As of August 2017, 21 patients were treated (median follow-up 30.2 months, min-max, 1–46). Of the 17 patients with evaluable data at Month 24, 15 (88%) remain alive and MFD-free with evidence of disease stabilization. One patient succumbed to disease progression; another was withdrawn from the trial due to radiographic evidence of disease progression. There was no evidence of replication competent lentivirus or insertional oncogenesis. No graft failure, GVHD, or transplant-related mortality were reported. Most adverse events were consistent with myeloablative conditioning.
Discussion: These data suggest that Lenti-D DP may offer an alternative to allo-HSCT in patients with CALD. Additional follow-

up is ongoing to assess durability of efficacy and long-term safety.
Conflict of Interest declared.

O-012

Long-term safety and efficacy of combined therapy of high-dose ambroxol and imiglucerase in neuronopathic Gaucher disease

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Background: High-dose ambroxol (ABX) therapy has been suggested to improve the clinical outcome of patients with neuronopathic Gaucher disease (nGD) and carrying the selective genotypes (N188S and F213I).
Methods: 4 nGD patients with N188S or F213I genotype were enrolled. The duration of enzyme replacement therapy (ERT, Imiglucerase 60 IU/kg q 2wks) before enrollment was 4–5 yrs. ABX dose was gradually increased until 27 mg/kg/day in order to target its blood level of 10 umol/L and maintained for 6–18 months. The biochemical and neuropsychiatric features were assessed during 4.5 years of treatment.
Results: All patients tolerated the high dose of ABX+ERT, although mild proteinuria, hypouricemia and significant respiratory mucus production were noted. At 27 mg/kg/day of ABX, its trough blood level was 4.9±2.9 mmol/L (range, 2.8-8.8 mmol/L). The ABX level in cerebrospinal fluid was 22.8 ±2.6% of its plasma level. The residual glucocerebrosidase (GBA) activity in leukocytes increased from 5.1±0.6% to 14.0±4.6% of mean normal activity. Significant decrease of myoclonic and generalized seizures was noted. However, number of antiepileptic drug was also increased from 4.8±1.7 to 6.3±2.4 per person. Modified severity scoring tool of nGD, intelligence and modified Barthel index scores progressed worse during the first 2 years of the study and then stabilized. NAA/Cr and Chol/Cr levels in brain magnetic resonance spectroscopy were also stationary during the study.
Discussion: High dose ABX+ERT treatment for nGD were safe in the long-term despite the risk of renal and respiratory adverse reaction. The pharmacokinetic property of ABX was different among the patients. This treatment might help halt the progression of nGD in terms of neuropsychiatric functions, but its neuroprotective effect needs to be validated in the longer term. Further study is required to find the optimal therapeutic dosage of ABX with the consideration of individual difference of pharmacokinetics.

PARALLEL SESSION 1C: Phenylketonuria and neurotransmitter disorders

O-013

Clinical characterization of tremor in patients with Phenylketonuria

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Background: In phenylketonuria(PKU) high Phe levels, by reducing availability of dopamine(DA) and 5HT precursors, Tyr and tryptophan respectively, can alter intracerebral neurotransmitter synthesis. The aim of the study was to assess the occurrence, age at onset, distribution, and associated neurological signs of tremor in PKU patients and its relation with metabolic variables affected in the disease.
Methods: 74 PKU [59 early-(ET) and 15 late-treated(LT)] and 43 controls subjects(age range 7–54) underwent individual and familiar tremor history, clinical assessment of tremor(Fahn-Tolosa-Marin Tremor Rating Scale, FTMTRS) and IQ evaluation. Concomitant blood Phe,Tyr and prolactin(PRL) were collected.
Results: 29 out of 74 PKU patients showed tremor. An action hand tremor was detected in 10 patients; an action and postural hand tremor in 18; a resting, action and postural hand tremor in 3; tongue postural tremor in 2; voice tremor in 1; lower limb tremor in 3 patients. Familiar history of tremor was positive in 7/29 PKU patients with tremor. FTMTRS score was 0–27 for ETPKU, 0–19 for LTPKU and 0–5 for controls. Most patients showed brisk tendon reflexes. A significant correlation between FTMTRS part A and PRL($r=.257; p< .05$)was found in PKU. In ETPKU we found a significant correlation between PRL and: FTMTRS total score($r=.352; p< .001$),part A($r=.393; p< .001$),part C($r=.524; p< .001$).
Discussion: PKU tremor clinical features resemble those of essential tremor, but its occurrence is much higher as compared to general population, especially taking into account the young age of the patients. Although PRL levels can be affected from several environmental factors it can be easily used as a parameter of brain DA availability. Interestingly, a positive correlation between severity score at FTMTRS and PRL levels was found, especially in the ETPKU subgroup. It can be hypothesized that PKU patients are more vulnerable to experience tremor and that high Phe levels can anticipate tremor onset.

O-014

Does early treatment of PKU patients with sapropterin dihydrochloride affect brain development?

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Background: Phenylketonuria (PKU) is an inborn error of metabolism caused by phenylalanine hydroxylase (PAH) deficiency. Most patients with mild or moderate PKU can be treated with sapropterin dihydrochloride (SD), which since 2015 is registered for PKU patients from the age of 4 months. SD is a pharmaceutical version of tetrahydrobiopterin (BH_4), a cofactor of PAH. Hyperactivity has recently been reported as a post-marketing observation in PKU patients treated with SD.
Methods: 60 or 120 ng/ml sepiapterin, a stable precursor of BH_4, were added every 24h over three daysto culture media of 3D organotypic rat brain cell cultures at two developmental stages. BH_4 and BH_2 measurements, immunohistochemistry, western blotting, metabolomics and RNA sequencing were performed at different time points.
Results: We confirmed successful conversion of sepiapterin to BH_4. In the earlier developmental stage, all observed effects were already present at 60 ng/ml. Interestingly, none of these effects was observed in the later developmental stage. We found swollen astrocytes, diminished astrocytic fibres, delayed differentiation of oligodendrocytes and perturbation of axonal elongation. We also discovered signs of altered GABAergic neurotransmission. RNA sequencing analyses revealed a number of significantly dysregulated genes. GO enrichment allowed identification of affected key biological processes.

Discussion: We showed deleterious effects of BH_4 on immature developing brain cells in a rat *in vitro* model. This observation raises the question whether the use of SD can be recommended in very young PKU patients as currently licensed. Further *in vivo* studies are needed to confirm our findings.

O-015

Cerebral creatine deficiency and lower weight gain in a new KI rat model of creatine transporter deficiency

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Background: Creatine (Cr) is a nitrogenous organic acid essential for recycling ATP. Cr is synthesized by a 2-step pathway (AGAT and GAMT), and transported by SLC6A8. Cerebral Cr deficiency syndromes (CCDS), due to AGAT, GAMT or SLC6A8 deficiencies, are inborn errors of metabolism causing severe neurodevelopmental delays and intellectual disability, characterized by absence of brain Cr measured by magnetic resonance spectroscopy (MRS). While AGAT and GAMT deficiencies can be improved with Cr treatment, the X-linked SLC6A8 deficiency cannot. Pathological mechanisms are still largely unknown. We present the first characterization of a new rat model of SLC6A8 deficiency.
Methods: Generation of knock-in rats:Codon Tyr389 of *Slc6a8* rat gene was changed to Cys (c.1166A>G) using the CRISPR/Cas9 engineering technology (Sprague–Dawley rats). Progeny were genotyped by PCR. Measurement of brain Cr by MRS: Rats were anesthetized with 1.5-4% isoflurane and ^{1}H-MRS scans were performed on a horizontal 9.4T MRI system in different brain regions. CNS was analyzed on cryosections by immunohistochemistry for the astrocytic marker GFAP and for aquaporin 4.
Results: We have established a $Slc6a8^{Y389C/y}$ rat strain based on one same missense point mutation described in human abolishing completely the Cr transporter activity. Mutant males rats showed absence of Cr peaks in CNS ^{1}H-MRS, and a 40% decrease in body weight gain at 14–18 weeks (as compared to age-matched WT). The homozygous females had the same pattern as mutant males, while heterozygotes were indistinguishable from WT. Astrocytic fibers (GFAP) and microcapillaries (aquaporin 4) appeared disorganized, in particular in the cerebellar cortex.
Discussion: Our first results validate this rat model as a promising tool to better understand SLC6A8 deficiency. In particular, morphological alterations of brain structures in our $Slc6a8^{Y389C/y}$ rats, and their loss of weight gain, may help to comprehend and treat human pathology.

O-016

AGIL-AADC gene therapy in children with AADC deficiency increases dopamine production and sustains motor milestones

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Background: Aromatic L-amino acid decarboxylase (AADC) deficiency is a rare, genetic disorder of neurotransmitter synthesis in children. We evaluated biologic and clinical outcomes through 5 years following administration of AGIL-AADC, a recombinant adeno-associated virus vector containing human complementary DNA encoding the AADC enzyme, in children with severe AADC deficiency.
Methods: In 2 single-arm, open-label clinical studies, children with severe AADC deficiency received an AGIL-AADC total dose of $1.8x10^{11}$ vg as bilateral, intraputaminal, stereotactic infusions during a single operative session. De novo dopamine production was evaluated using F-DOPA PET imaging. Clinical assessments included the achievement of motor milestones using the Peabody Developmental Motor Scale, Second Edition (PDMS-2) and adverse events (AEs). Data from AGIL-AADC patients were compared with a natural history cohort of severe AADC patients using Fisher exact test (α=0.05).
Results: Eighteen patients aged 21 months to 8.5 years were administered AGIL-AADC. At baseline, no patient had developed full head control or the ability to sit unassisted or to stand; these observations were consistent with the natural history cohort (N=82). Following AGIL-AADC administration, all patients had sustained de novo dopamine production. Of 15 patients evaluated 2 years post-treatment, 5 gained full head control (P< 0.0001); 4 could sit unassisted (P=0.0004); and 1 could stand with support. Of 7 patients evaluated 5 years post-treatment, 4 gained full head control and the ability to sit unassisted (P< 0.0001 each); 2 could stand with support (P=0.0054). All patients had clinical improvement in PDMS-2 items. Generally, AEs were associated with the disease.
Discussion: AGIL-AADC administration increased dopamine production and improved motor milestone acquisition in children with severe AADC deficiency. Gene therapy with AGIL-AADC is a potential therapeutic for these patients to maintain motor milestones.
Conflict of Interest declared.

O-017

Neurotransmitter trafficking defect in a patient with the clathrin (CLTC) alteration presenting with hyperphenylalaninemia and Parkinsonism

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Background: Clathrins play a key role in intracellular trafficking and endocytosis of macromolecules, including neurotransmitters (Nts). Defects of Clarhrin Heavy Chain (*CLTC*; GenBank: NM_004859.3), have been so far associated with neurodevelopmental disorders and epileptic encephalopathy.
Case report: This 30-year-old woman presented during the first years of life with psychomotor delay and impairment of social skills. At the age of 4 years, ataxia and proximal limb rigidity became evident. During the following years, a cognitive decline was observed and, at the age of 11, she showed mild intellectual disability, drooling, and slight gait ataxia. An extensive metabolic work-up detected mild increase of blood phenylalanine (Phe). On CSF examination homovanillic, 5-hydroxy-indolacetic, and 5-methylthetrahydrofolic acids were low. *PAH* sequencing disclosed compound heterozygosity for two missense variants, p.[Asp151Glu]:[Thr380Met]. Enzymatic and molecular analysis of BH4 pathway and biogenic amine metabolism was normal. L-DOPA/carbidopa trial was ineffective. Although unusual, the condition was regarded as an atypical presentation of a very mild deficit of PAH (blood Phe < 240 μM). In the following years the girl developed a relapsing-remitting Parkinsonism with severe achalasia and weight loss while her younger brother carrying the same *PAH* molecular and biochemical alteration was normal. The family underwent WES analysis.
Results: WES identified in the proband a *de novo* missense change (c.2669C>T:p.Pro890Leu) in the *CLTC* gene (NM_004859.3).

Discussion: Parkinsonism Plus is never been reported in a *CLTC* defect. We detected a possible metabolic biomarker of the disease such as Nts' depletion in CSF. We suggest this defect as a new disorder of biogenic amine trafficking resulting in movement disorders and/or neurodevelopmental derangement. The interplay in our case between the two independent metabolic alterations deserves further investigations.

O-018

Proteomic study on neurotransmitter defects find several biomarkers pointing towards neurodevelopment dysregulation

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Background: Inborn errors of monoamines (MA) are monogenic diseases of synthesis, catabolism and transport of catecolamines and serotonin. Symptoms include movement disorders, developmental delay and complex encephalopathies. Low levels of CSF MA metabolites are related to severe phenotypes, but no other biomarkers of neuronal dysfunction have been described.
Methods: We have recruited 94 CSF samples from 9 centres (Spain, Netherlands, Germany, Greece, Switzerland, Canada, Turkey, USA, Argentina) including 28 TH, 15 AADC, 10 GTPCH, 8 DHPR, 22 PTPS, 4 SR, 2 DBH, 1 DAT and 4 with no diagnosis. CSF proteomic studies were performed through MS analysis (LCMSMS with LTQ-Orbitrap Velos Pro equipment) after albumin and IgG depletion. For data analysis the bioinformatic tools Panther and gene ontology were used.
Results: A total of 1188 proteins have been detected, 385 proteins being common in 80/92. Regardless of the specific disorder, we found that 10% of the 385 protein pool are related with nervous system development (glutamate receptors, axon guidance and neurexins). After performing the GO enrichement analysis for biological process, we found the next categories enriched: *NMDA glutamate receptor clustering* (7.43E-03), *PSD95 clustering* (1.04E-02), *spermine biosynthetic process* (3.61E-02) and *positive regulation of synapse maturation* (2.31E-02). Panther analysis showed the pathway "*Nervous system development*" (1.57E-03) enriched.
Discussion: This is the first study that uses a novel –omic technique in a large cohort of patients with MA defects. Interestingly, the main category of common proteins belong to crucial neurodevelopmental functions (plasticity, synapse maturation, branching). MA behave as trophic factors in the immature brain and low levels of these molecules from early stages could regulate important developmental functions, explaining the clinical manifestations. Future studies will focus on variables related to specific group of disorders and their phenotype.

PARALLEL SESSION 2A: Nutrition and dietetics

O-019

Prospective study :Glycomacropeptide and conventional amino acid protein substitutes in children effect on blood phenylalanine and growth

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Background: In PKU, the biologically active properties of casein glycomacropeptide (CGMP) when given as a protein substitute is reported to improve gut and bone health and enhance protein utilization. However, the effect of any residual phenylalanine (Phe) in CGMP is usually disregard when used as a protein substitute (PS) in children. A longitudinal, study over 12 m evaluating CGMP (CGMP-AA) (36mg per 20g protein equivalent) compared with a Phe-free L-amino acid supplement (L-AA) examining impact on blood Phe, tyrosine (Tyr), Phe:Tyr ratio, weight, height and BMI z scores.
Methods: 48 children with PKU, median age 9.2y (5-16y), 28 boys, were divided into 2 groups: CGMP-AA, n=29; L-AA, n=19. In both groups total median protein equivalent from PS was 60g /day (20-80g). CGMP-AA replaced L-AA if blood Phe remained within target range. Median blood Phe, Tyr and Phe:Tyr ratio and weight, height and BMI z scores were compared within and between the groups at baseline, 6m and 12m.
Results: At the end of 12m only 48% of subjects were able to completely use CGMP-AA as their single source of PS. At 12m CGMP-AA provided a median of 75% (30–100) of the total PS with the remainder being given as L-AA. Within the CGMP group, blood Phe increased significantly between baseline and12m : [baseline to 6m; baseline Phe 270 μmol/L (170–430); 6m, Phe 300 μmol/L (125–485) p=0.06; baseline to 12m: baseline, Phe 270 μmol/L (170–430), 12m Phe 300 μmol/L (200–490), p< 0.001)]. However, there were no differences between the CGMP and L-AA group for Phe, Tyr, Phe:Tyr ratio or anthropometry at any of the three measured time points. Within the CGMP gp only weight (p=0.0001) and BMI z scores (p=0.0001) increased significantly between baseline to 12m
Discussion: CGMP-AA increases blood Phe concentrations and so it can only be used partly to contribute to PS in some children with PKU. CGMP-AA should be carefully introduced in children with PKU and close monitoring of blood Phe control is essential.
Conflict of Interest declared.

O-020

Effect of phytosterols on serum lipids of children with hypercholesterolemia

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Background: Daily supplementation of 2g of plant sterols/stanols has been included as an adjunct to hypolipidemic diet. The aim of this retrospective study was to assess the effectiveness of plant sterols in reducing LDL-C levels in children and adolescents with hypercholesterolemia.

Methods: The sample of the study consisted of 426 children and adolescents, aged >5 years old, with persistently increased LDL-C levels (≥130 mg/dl) in whom a recommendation for a daily supplemental consumption of 1.5 - 2.5g of plant sterols was given. In all participants lipid profile was investigated before and at least 12 weeks after the recommendation.
Results: Two hundred and twenty (51.6%) out of 426 children enrolled in the study consumed 1.5-2.5g of plant sterols daily for at least 12 weeks prior to the re-examination (group A), while 206 (48.4%) did not consume any plant sterols despite the recommendation (group B). A reduction in LDL-C ≥10% was considered as a response to plant sterols supplementation.In group A, 68.2% of participants reduced LDL-C levels ≥10%, while 66.4% reduced non-HDL-C levels ≥10%. In group B, only 28.2% of participants reduced LDL-C levels ≥10%, while only 28.6% reduced non-HDL-C levels ≥10%. Significant changes were found in the percent and absolute changes in LDL-C and non-HDL-C levels, between the two groups.The reduction in LDL-C and non-HDL-C levels was independent of sex, age, BMI z-score, diet, exercise, baseline levels or the type (yoghurt drink or margarine) and the amount of plant sterol-enriched food. The concentrations of HDL-C, triglycerides and ApoA1 did not show any significant changes between the two groups. No adverse effects were reported.
Discussion: Daily consumption of 1.5 to 2.5 g of plant sterols reduced LDL-C and non-HDL-C levels significantly in children and adolescents, without any effect on HDL-C concentration. Therefore, the use of plant sterols is safe and effective in the management of hypercholesterolemia in childhood.

O-021

Quality of dietary carbohydrates affects gut microbial community of phenylketonuric subjects

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Background: Low-phenylalanine (Phe) diet, the main-stay of treatment for phenylketonuria (PKU), has been shown to increase glycemic index (GI) and glycemic load (GL), affecting the availability of substrates for microbial fermentation. Indeed, changes in the PKU gut microbiota and in microbial metabolites have been previously reported. In this study gut microbial communities of children with PKU and with mild hyperphenylalaninemia (MHP, unrestricted diet) have been compared.
Methods/case report: Forty-twochildren(21males/21females,9–13years)were enrolled. Dietary intakes and gut microbiota analysis, by next-generation sequencing using V3–V4 hypervariable 16S rRNA genomic region, have been performed.
Results: While alpha-diversity analysis revealed no significant differences between PKU and MHP groups, phylogenetic analysis highlighted a significant separation of gut microbiota according to both unweighted (p=0.008) and weighted Unifrac distances (p=0.03). Major differences were seen within the *Firmicutes* phylum. Indeed, PKU children were depleted in *Faecalibacterium* spp. and enriched in *Blautia* spp. and *Clostriudium* spp. We found a divergent response of members of the *Firmicutes* phylum with respect of daily glycemic index, higher in PKU children. *F. prausnitzii,* unclassified *Ruminococcaceae* and, to a lesser extent *Roseburia* spp. negatively correlated with GI, whereas other *Lachnospiraceae* (unclassified) were positively associated. Indicator species analysis suggested *Faecalibacterium prausnitzii* to be related to MHP status, whereas *Ruminococcus bromii* to be associated to PKU.
Discussion: Despite PKU children having a higher vegetable and fiber intake, the quality of carbohydrates ingested seems to particularly affect *F. prausnitzii* abundance, considered a biomarker for a healthy status. It still remains to evaluate whether an improvement of current Phe-free protein substitutes and special low protein products could rebalance the microbial community.

O-022

Body composition in hepatic glycogen storage disease: relationship with uncooked cornstarch

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Background: Hepatic Glycogen Storage Disease (GSD) is a group of hereditary metabolic diseases associated with fasting hypoglycemia whose treatment is essentially dietary and aims to maintain normoglycemia through administration of uncooked cornstarch (UCCS). For reasons not fully understood, but associated with its dietary therapy, hepatic GSD appears to be linked with obesity. However, little is described in the literature about the skeletal muscle mass of these patients. Therefore, this study aims to describe and evaluate the body composition of patients with hepatic GSD through dual-energy x-ray absorptiometry (DEXA) and its associations with treatment aspects.
Methods: Weight and height were assessed and the body mass index (BMI) was calculated. Data on body composition was obtained through bone densitometry and classified. Children under 8 years of age were excluded from body composition evaluation. Clinical and treatment data were assessed through medical records review.
Results: 24 hepatic GSD patients were included. From BMI classification, 79% were overweight/obese and 21% were eutrophic. On fat mass index evaluation, 16/21 participants had excessive adiposity. Regarding relative skeletal muscle index, 2 patients (28%) had sarcopenia (GSDIb/Ixα), one of which presented sarcopenic obesity (Ib). The intake of UCCS per kilogram of weight showed a negative correlation with lean mass and, differently from the expected, fat mass was also negatively associated with UCCS intake per kilogram of weight. DISCUSSION: The results found in this study suggest a high frequency of obesity in Hepatic GSD; however, it could not prove that such condition is a reflex of the adopted diet therapy. This study also shows an unprecedented result on sarcopenia and its negative association with the consumption of UCCS and lean mass on Hepatic GSD patients, suggesting the need of a thorough evaluation if the protein and total calorie intake are appropriate considering the dietary treatment.

PARALLEL SESSION 2B: Lysosomal storage disorders

O-023

Efficient and effective newborn screening for lysosomal disorders

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Background: Since February 2016, more than 116,000 infants born in the Commonwealth of Kentucky have been screened for Krabbe disease,

Mucopolysaccharidosis type I, and Pompe disease. Here we present our screening processes and results.
Methods: The primary screening test is a 6 plex MS/MS method followed by biochemical 2nd tier tests. To determine which cases require the 2nd tier tests, result review of the primary screenig test makes use of covariate-adjusted reference intervals and employs post-analytical interpretive tools created using Collaborative Laboratory Integrated Reports (CLIR; https://clir.mayo.edu), a multivariate pattern recognition software (Minter Baerg MM et al. Genet Med. in press).
Results: Specimens collected before 24 hours and after 1 week of age were 2,833 (2.4%) and 5,961 (5.1%), respectively. Age at collection and birth weight were provided for 99.5% of the specimens. With only a single repeat sample requested since screening began, the false positive rate currently is 0.0009% and the positive predictive value is 87%. Of eight cases referred as abnormal, one was affected with infantile Krabbe disease, five were affected with (late onset) Pompe disease, and one was affected with Mucopolysaccharidosis type I. The remaining case was a heterozygote for Mucopolysaccharidosis type I, a false positive outcome.
Discussion: Our experience shows that newborn screening for lysosomal disorders can be precise when (free) bioinformatics tools are employed in combination with biochemical genetic 2nd tier tests. This approach should allow any newborn screening program to efficiently and effectively expand their screening menu for treatable conditions to the benefit of the newborns they serve.

O-024

Novel treatment of MPS II (Hunter Syndrome) with SB-913 ZFN-mediated *in vivo* human genome editing: Update from a Phase 1/2 clinical trial

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Background: Mucopolysaccharidosis type II (MPS II, Hunter syndrome), is a rare, X-linked metabolic disease caused by mutations in the *IDS* gene, which leads to a deficiency of the lysosomal enzyme iduronate-2-sulfatase (IDS). MPS II is characterized by progressive respiratory and cardiac disease, skeletal abnormalities, and premature death with neurodegeneration in the severe form. SB-913 is a new type of gene therapy being developed for the treatment of MPS II. SB-913 uses zinc finger nuclease (ZFN)-mediated *in vivo* genome editing to insert a normal copy of the *IDS* transgene into liver cells, delivered via AAV2/6 vectors. The precision and specificity of the ZFNs allow for integration at a specified genomic location and result in high, continuous production of IDS, as demonstrated in an MPS II mouse model. SB-913 aims to provide lifelong production of IDS, with the goal of therapeutic benefit and eliminating the need for weekly enzyme replacement therapy.
Methods: A Phase 1/2 clinical trial (CHAMPIONS) is ongoing in the U.S. to determine if SB-913 is safe and tolerable in patients with MPS II. The study is a multicenter, open-label, dose ranging trial with one-time peripheral intravenous infusion of SB-913, followed by three years of observation and testing. IDS activity and clinical endpoints will also be assessed. Key eligibility criteria include age ≥18, diagnosis of attenuated MPS II, and lack of pre-existing antibodies to AAV6.
Results: Four subjects have received a single infusion of SB-913, with two subjects in each of two dose cohorts (5e12 vg/kg and 1e13 vg/kg). The infusions were generally well-tolerated and no serious adverse events related to the study drug were reported, with maximum exposure of up to 5 months post-study drug administration. Additional analysis of the trial data will be presented as available.
Discussion: Initial safety data from this clinical trial support further development of SB-913 for the treatment of MPS II.
Conflict of Interest declared.

O-025

Antisense oligonucleotides promote exon inclusion in iPSC-derived skeletal muscle cells from Pompe patients

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Background: Pompe disease is a recessive autosomal inherited disorder caused by a deficiency of acid α-glucosidase (GAA). This lysosomal storage disease causes accumulation of glycogen in lysosomes, and results in severe skeletal muscle wasting. Enzyme replacement therapy is available, but has limitations, including variable efficacy, antibody formation, and high costs. This warrants the search for alternative treatment options. We aim to develop an RNA-based therapy for the correction of the predominant *GAA* variant in Pompe disease, the c.-32-13T>G (IVS1) variant.
Methods: Antisense oligonucleotides (AONs) were identified in a screen for correction of aberrant splicing caused by the common IVS1 variant. Morpholino-based AONs were designed based on this screen, and tested in patient-derived fibroblasts and skeletal muscle cells obtained via induced pluripotent stem cells.
Results: The IVS1 *GAA* variant caused exon skipping in patient-derived cells. Hits from the screen resulted in the identification of AONs that could promote exon inclusion and restore normal *GAA* splicing in patient derived cells. These AONs induced near-complete restoration of GAA enzyme activity from the IVS1 allele in patient-derived skeletal muscle cells.
Discussion: AON-mediated splicing correction provides a potential novel approach to treat the majority of childhood/adult Pompe patients. Further optimization for in vivo testing is warranted.

O-026

Benefit of MGTA-456 cord blood expansion in enhancing donor derived microglial engraftment as treatment for inherited metabolic disorders

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Background: Hematopoietic Stem Cell Transplant (HSCT) is utilized as therapy for inherited disorders affecting the central nervous system (CNS), such as Hurler syndrome, globoid cell and metachromatic leukodystrophy (GLD, MLD) and cerebral adrenoleukodystrophy (cALD).

The benefit of HSCT is thought to be due to the delivery of the gene product into the CNS through donor derived microglia. However, the ability for HSCT to affect disease progression is limited to patients with early disease and those with attenuated phenotypes. Presumably, enhanced delivery of donor microglia could increase the success of HSCT. The expansion of cord blood stem cells (CD34+ cells) can be achieved using an aryl hydrocarbon receptor (AHR) antagonist and cytokines (MGTA-456). The use of MGTA-456 has been shown to provide more rapid engraftment following HSCT in adults with hematologic malignancies (Wagner et al., Cell Stem Cell, 2016), but its effects on microglial engraftment have not previously been tested.
Methods: We compared the ability of MGTA-456 to engraft the blood and brain microglia in NSG mice.
Results: Mice transplanted with MGTA-456 showed 2.8-fold higher human CD45 engraftment in the blood at week 13 compared to mice transplanted with non-expanded fresh cord blood or mock-treated CD34+ cells. We observed an approximately 10-fold increase in human CD45+ CD11b+ myeloid cells in the brains of transplanted mice with MGTA-456 ($n=15$, $p< 0.0001$). To confirm this, we assessed the presence of Ku80+ Iba1+ microglia in brain by morphology and immunohistochemistry.
Discussion: The limitations of allogeneic HSCT as therapy for inherited lysosomal/peroxisomal and disorders has long been recognized. As the neurologic benefits seem related to the effect of donor derived microglia, the use of MGTA-456 as a means of enhancing microglia engraftment may prove beneficial. A trial is now open at the University of Minnesota exploring the use of MGTA-456 in patients with Hurler, MLD, GLD and cALD.
Conflict of Interest declared.

PARALLEL SESSION 2C: Novel diagnostic approaches

O-027

Contribution of functional studies to validate disease-causing variants identified by NGS in patients with inborn errors of metabolism

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Background: The implementation of Next Generation Sequencing(NGS) has rapidly increased the yield of diagnoses allowing the identification of an important number of new diseases and new disease-causing variants. However, the demonstration of the pathogenic significance of the identified variants is still a challenge to reach a definitive diagnosis.
Methods / Case report: We present 31 families with a broad-spectrum of clinical and biochemical phenotypes. Genetic studies were performed by whole-exome sequencing and gene prioritization was based on a detailed analysis of the clinical and biochemical features, with focus on particular metabolic pathways. Pathogenicity of the identified variants was demonstrated by specific functional validation studies depending on mutation type, gene function and available material. These studies included mRNA/protein expression, aberrant splicing demonstration, enzyme activities, metabolite studies in body fluids, UPR stress-signaling, morphology/dynamics analysis of particular organelles (Golgi-ER-mitochondria), and a comprehensive characterization of the mitochondrial function(respirometry,OXPHOS assembly,lipid composition).
Results: Using this strategy we identified candidate genes in 19 out of the 31 cases while 12 of them remain unsolved and are under further genetic analysis. Only 2 cases showed already reported mutations (*PEX1,SLC39A8*). Functional studies demonstrated the pathogenicity of the identified variants in 10 cases (*SERAC1, TIMM50, TRAPPC11,ITPA,NADK2,ECHS1,NDUFAF4,PKLR,HACE1,MTO1*). In addition, 7 patients are still under functional studies, 5 of which are new pathological entities.
Discussion: We highlight the importance of precise functional studies that demonstrate the impact of genetic variants identified by NGS. The integration of these studies with a detailed clinical and biochemical characterization of the patients is an effective strategy to reach reliable diagnoses and to identify new potential disease-causing genes.

O-028

Large-scale, untargeted metabolomic profiling identifies novel biomarkers and clarifies DNA variants of uncertain significance

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Background: Untargeted genetic testing has increased the challenge of dealing with variants of uncertain significance (VUS) in clinical practice. Because phenotypes are often non-specific, interpretation of VUS is challenging and individualized functional testing through RNA or protein expression studies is often not clinically validated and is prohibitively costly and time-consuming.
Methods: We have previously described the use of untargeted metabolomic profiling in screening for a variety of inborn errors of metabolism (IEMs) using UHPLC MS/MS. We evaluated 180 individuals from our clinical laboratory who had both clinical exome and clinical metabolomic analyses performed for diagnostic purposes. The indication in 90% was neurological and the average age was 8 years.
Results: In 37.8% of cases, metabolomic analysis contributed to interpretation of the variant(s). Of the 62.2% where metabolomic analysis was not helpful, about half of candidate genes did not have a known role in metabolism and the other half were judged to be "solved" by DNA testing alone. Of the 37.8% where metabolomics was helpful, in 75% of cases it excluded a diagnosis, in 19.1% it confirmed pathogenicity of a VUS and in the remaining 14.7% it changed the variant classification. Of the 15 cases where the variant classification was changed, 6 VUS were reclassified as pathogenic; 5 VUS were reclassified as likely pathogenic; 2 variants classified as likely pathogenic were reclassified as pathogenic; one likely benign variant was reclassified as likely pathogenic and one VUS was reclassified as likely benign.
Discussion: We have demonstrated that large-scale untargeted metabolomic profiling can be useful in identifying novel biomarkers and interpreting VUS in a wide variety of IEMs, and we will specifically present the utility in cases of citrate transporter deficiency, GABA transaminase and 17-hydroxysteroid dehydrogenase deficiencies and spondyloepimetaphyseal dysplasia, Genevieve type.
Conflict of Interest declared.

O-029

Signature oligosaccharides change the way we diagnose and monitor the mucopolysaccharidoses

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Background: The diagnosis of mucopolysaccharidoses traditionally relies on stepwise investigations, starting with the non-specific GAG assay, followed by electrophoresis, enzyme assay and mutation analysis. We have developed a new tandem mass spectrometric assay to detect signature oligosaccharides for each of the mucopolysaccharidoses. The assay is rapid, specific, sensitive and can be used for diagnosis and monitoring the effectiveness of therapies.
Methods: The equivalent of 0.5 μmole urinary creatinine was evaporated to dryness and resuspended in derivatising reagent containing internal standard. Following incubation at 70 oC, and partial removal of the derivatising agent with chloroform, oligosaccharides were separated by liquid chromatography and analysed on an AB SCIEX QTRAP 6500 triple quadrupole mass spectrometer, using multiple reaction monitoring for 10 targeted fragments plus the internal standard.
Results: De-identified urines from 723 subjects, comprising 630 unaffected individuals and 93 MPS patients, were analyzed blinded to diagnosis. All 93 MPS samples were correctly identified by the presence of specific "signature" oligosaccharides. Reference intervals were calculated from unaffected urines, with 99% confidence intervals, to yield an assay with 100% specificity and sensitivity. Time to diagnosis is faster. As confirmation of diagnosis requires measurement of a single MPS enzyme, it is also less costly.
Discussion: this test offers superior performance to 1- and 2-dimensional electrophoresis for diagnosis as well as monitoring the effectiveness of enzyme replacement, gene and stem cell therapies for the mucopolysaccharidoses. It has replaced standard testing in our, and 2 other Australian laboratories.

O-030

Cross-omics: merging whole exome sequencing with untargeted metabolomics

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Background: Whole exome sequencing (WES) often results in many variants. Many mutations in metabolic disorders are of the missense type, difficult to interpret and often classified as variants of uncertain significance (VUS) and, depending on filtering, often not even reported. The unfiltered list of genes with variants can exceed 100. To improve diagnostic yield, we developed a cross-omics pipeline that integrates WES with untargeted metabolomics.
Methods: WES was performed using a relatively lenient variant filtering. Genes were selected based on high quality homozygous, compound heterozygous and hemizygous variants with an allele frequency < 1% in the general population. No additional filtering was performed. Based on databases including PathwayCommons, the HGNC and Recon2, an in silico metabolic environment was generated. Direct infusion MS-based untargeted metabolomics was performed on blood spots. An in-house developed bioinformatics pipeline resulted in lists of altered metabolites. Finally, Metabolite Set Enrichment Analysis using an adjusted Fisher's exact test was performed to assess for which genes evidence for functional metabolic consequences exists.
Results: The best overall cross-omics performance is reached with metabolite cut-off Z-scores of < -1.5 & >2.0 in combination with three extensions (metabolic steps away from primary genetic defect). In a training set of 33 DBS, cross-omics successfully resulted in the disease-causing gene in the top 3 (of 100) in 82% of patients. In addition to the training set, cross-omics lead to proper diagnostic classification of HEXA VUSs in a patient with variants in 37 genes.
Discussion: We developed a fully integrated automated genetic-metabolic diagnostic workflow. We propose that untargeted metabolomics should be performed in patients undergoing WES and anticipate that a combined genetic and metabolic approach will provide complementary information, speed up the diagnostics process and improve the diagnostic yield.

O-031

Integrated UPLC-HR-MS and NMR detection of inborn errors of metabolism in urine

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Background: Nuclear magnetic resonance spectroscopy (NMR) shows a high potential of detecting diseases caused by inborn errors of metabolism. With this technique, increased concentrations of endogenous metabolites in bodyfluids, e.g. urine, are detected and quantified precisely and accurately. High resolution mass spectrometry (HR-MS) coupled to UPLC (ultra performance liquid chromatography) performed in the full scan MS mode can add valuable semi quantitative information on the presence and abundance of marker found in a large set of samples and therefore strengthen the results found by the NMR method.
Methods: Urine samples were prepared for NMR by adding 10% of deuterated buffer solution to the sample. For the MS analysis 200uL of urine was diluted with the same volume of 2mM NaN_3 solution for submission to a UPLC-TOF-MS (time of flight mass spectrometry) system. The raw data obtained from the UPLC-MS system were subjected to statistical analysis software and the bucket table was then exported to an Excel spread sheet for further analysis.
Results: More than 2000 urine sample data sets measured in positive and negative ionization mode and subjected to statistical analysis were compared to the results obtained from NMR targeted analysis of metabolites indicating inborn errors of metabolism. The results show a high potential of complementarity especially for metabolites which are not easily detectable by NMR. Examples for diseases are shown.
Discussion: Advantages using NMR detection for the investigation of inborn errors of metabolism are the ease of sample preparation, short measurement time, absence of wearing parts, repeatability, accuracy and precision. However, due to the matrix effect, in some cases the NMR detection limit is increased and the selectivity is decreased. In such cases, semi quantitative mass detection of the marker metabolites leads to a significant improvement of the diagnosis. NMR and UPLC-MS analysis can be easily paralleled.

O-032

A technological upgrade for newborn mass urine screening in the Province of Quebec: from TLC to MS/MS

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Background: The Neonatal Mass Urine Screening Program began in 1971 in Sherbrooke, Quebec, focusing on early detection of newborns with late-onset forms of urea cycle disorders and organic acidurias to prevent morbidity and mortality before the onset of clinical symptoms, some of these diseases being detected only by urine analysis. With the advancement of more refined quantitative tandem mass spectrometry (MS/MS) methodologies, we performed an evaluative research project to assess the feasibility to transfer the existing multiplex thin layer chromatography technique with a sequential-four reagent staining methodology to a quantitative MS/MS approach.
Methods: Twenty-one day-old newborn urine samples are collected voluntarily at home by parents on a filter paper and sent by regular mail to our central laboratory. A 5-cm filter paper disk is punched out after UV light inspection, and extraction performed using 3 mL of 0.01M NH_4OH/H_2O. Samples are vortexed, centrifuged, and the extracts injected into a UPLC-Xevo TQ-S micro tandem MS system (Waters Corp.) with a sample organizer (Waters). An Acquity UPLC CSH C18 (Waters) is used with methanol/water/0.1% formic acid mobile phases.
Results: A rapid 2-minute multiplex MS/MS methodology was devised using positive and negative electrospray ionization modes. Preliminary reference values were established. Urine specimens analyzed from affected newborns with urea cycle disorders and organic acidurias, and ERNDIM specimens revealed abnormal results. By focusing on 20 different biomarkers and their related internal standards, 17 inborn errors of metabolism were targeted, including Triple H syndrome (a founder-effect in Quebec) by targeting orotic acid and uracil. This latter disease cannot be detected in dried blood spots at 2 days of age.
Discussion: It is feasible to perform MS/MS newborn mass urine screening for early and pre-symptomatic detection of 17 inborn errors of metabolism. High-risk screening is also possible.

PARALLEL SESSION 3A: Mitochondrial disorders

O-033

Elucidating the complexity of mitochondrial membrane lipids in Barth Syndrome

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Background: Barth Syndrome (BTHS) is a rare X-linked disease characterized by mitochondrial dysfunction, cardiomyopathy and muscle weakness. The affected *TAZ* gene encodes for tafazzin, a CoA-independent transacylase that remodels the cardiolipin (CL) side chain composition. Pathogenic mutations cause a loss of total CL content combined with elevated monolyso-CL (MLCL) levels, altered mitochondrial ultrastructure and inhibition of apoptosis. Structurally, CLs have a unique dimeric structure carrying four fatty acyl side chains making them the most diverse phospholipids. This side chain composition is tailored for each tissue and regulated by a post-biosynthesis remodeling process, in which tafazzin plays a major role.
Methods: We recently developed a lipidomics method to detect and quantify individual molecular CL species. Furthermore, we use fragmentation data combined with mathematical modeling to extract the acyl side chain composition of each single lipid.
Results:This novel approach grants us detailed insights in membrane lipid composition. In addition to the known loss of CL content and MLCL accumulation, we observed a preferential depletion of polyunsaturated in favor of saturated side chains in BTHS patient fibroblasts. Using the CRISPR/Cas9 system, we generated *TAZ* knock-out strains in HEK and U937 cells and confirmed this phenotype. Furthermore, we supplemented cells with linoleic acid rich heart lipid extracts, which strongly incorporated into CLs and affected mitochondrial activity: cellular respiration was found to be more efficient in intact cells, which could be linked to simultaneous increase of complex I activity.
Discussion: Our novel lipidomics approach allows us to detect even little changes in cardiolipin constitution letting us study its influence on mitochondria. In summary, we could show the depletion of polyunsaturated side chains of CL in *TAZ* deficient cells as well as how the CL lipid composition can impact on mitochondrial functioning.

O-034

Mutations in *QRSL1*, *GATB*, and *GATC* encoding glutamyl-tRNA^{Gln} amidotransferase subunits cause a lethal mitochondrial cardiomyopathy

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Background: Mitochondrial glutaminyl mt-tRNA (mt-tRNA^{Gln}) is first charged with glutamic acid by EARS2 to Glu-tRNA^{Gln} followed by amidotransferase reaction using glutamine to Gln-tRNA^{Gln} by Gat^{CAB} aminoacyl-tRNA amidotransferase consisting of subunits GatA (*QSRL1*), GatB (*GATB*), and GatC (*GATC*). We describe 9 patients with mutations in these novel genes.
Methods: Mutations were identified by exome sequencing. Functional studies evaluated enzyme activities, protein amounts, assembly of protein complexes, respirometry, mitochondrial protein translation, and mt-tRNA charging. The impact of glutamine in tissue culture and of temporarily halting translation was assessed. Lentiviral transfection rescue experiments were used for *GATB*. *QRSL1* and GAT*B* variants were modeled in homologues *S. cerevisiae* genes.
Results: Patients presented with severe cardiomyopathy and lactic acidosis with prenatal to early infancy onset. Variable features were anemia, prenatal edema, hearing loss, and elevated CK. Mutations were present in *QRSL1* (2 families), in novel genes *GATB* (1 family) and *GATC* (2 families). Protein amounts were reduced in GatC and GatB but normal in GatA. All patients had combined enzyme deficiencies affecting heart and muscle more than fibroblasts. Complexes I and V showed incomplete assembly. Mitochondrial protein translation was deficient, but transiently improved after a short halt. Upon fibroblast culture in absence of glutamine, the mt-

$tRNA^{Gln}$ aminoacylation was deficient and oxygen consumption was reduced. Lentiviral transduction experiments using wildtype *GATB* rescued enzyme function. Yeast studies modeling homologous mutations for genes *QRSL1* and *GATB* showed reduced oxygen consumption.
Discussion: All patients with mutations in the GatCAB complex subunits present with fatal cardiomyopathy and lactic acidosis. Cells cultured in high glutamine greatly improved tRNA aminoacylation and functionality. Deficient GatCAB resulted in inefficient mitochondrial translation.

O-035

Genetic defects causing complex movement disorders and basal ganglia degeneration in childhood

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Background: Basal ganglia degeneration (BGD) in childhood causes movement disorders, neurodevelopmental dysfunction, and long-lasting severe disability. Clinical, radiological and biochemical analysis allow classification of BGD in clinical categories, and massive parallel sequencing (MPS) enables genetic diagnosis.
Methods: Observational clinical study, nuclear, and mtDNA gene sequencing combined with biomarkers in a prospective cohort of 40 patients with BGD.
Results: Patients presented at mean age 26.6(0–132) months, the majority (25/40) with acute onset encephalopathy triggered by infection/fever, spasticity(33), dystonia(31), abnormal ocular movements (10), parkinsonism(9), ataxia(5), chorea(3), and severe disability (IV-V GMFCS, 34/40). We achieved genetic diagnosis in 28 (23 probands, 5 affected siblings) using MPS (target multigene panel: 10/32, 31.2%; WES: 7/13, 53.8%;complete mtDNA sequencing: 6/25, 24%). We identified 12 recessive variants, 4 de novo mutations, and one X linked. 8/21 were novel variants in previously known genes. Using complete mtDNA sequencing we identified mutations in the mtDNA. According to phenotypes: 1.Striatal necrosis group(13/31): *NDUFS4* and *NDUFAF6*, *PDHA1*, *SLC25A19* and *MECR*, *ECHS1* and *HIBCH*, and non-mitochondrial genes *TUBB4A, GNAO1*. 2.Calcification(6/8): *IFIH1, ADAR, RNASEH2B* and *RNF213*. 3.Metal deposition(1): *SLC39A14*. Biomarker analysis (hypermanganesemia in *SLC39A14*, interferon signature in *ADAR*) and muscle functional assays (*NDUFAF6* and *NDUFS4* genes) allowed us to validate novel variants.
Discussion: BGD leads to severe disability in early childhood. WES and mtDNA sequencing allows genetic confirmation in 70% of the patients. Patients with striatal necrosis were affected by primary mitochondrial defects, inborn errors of metabolism causing toxicity and secondary mitochondrial dysfunction, and non-mitochondrial genes. In some patients, cofactor supplementation improved clinical outcome after genetic confirmation.

O-036

A novel complex neurological phenotype due to homozygous mutation in *FDX2*

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Background: Defects in [Fe-S] cluster biogenesis are increasingly recognized as causing neurologic disease. Mutations in a number of genes that encode proteins involved in [Fe-S] cluster assembly lead to complex neurological phenotypes. One class of proteins essential in the early cluster assembly are ferredoxins, FDX1 and FDX2. FDX2 is ubiquitously expressed and is essential in the formation of [Fe-S] protein clusters in humans.
Methods: We describe a novel complex neurological syndrome characterized by childhood onset optic atrophy, sensory-motor axonal neuropathy and myopathy with reversible leukoencephalopathy identified in 6 individuals from two unrelated Brazilian families, with a novel homozygous mutation in FDX2. Patients were clinically evaluated, underwent MRI imaging, nerve conduction studies, electromyography and muscle biopsy. To define the genetic etiology, a combination of homozygosity mapping and whole exome sequencing was performed.
Results: MRI imaging disclosed a reversible or partially reversible leukoencephalopathy. Muscle biopsy demonstrated an unusual pattern of regional SDH and COX deficiency with iron accumulation. The phenotype was mapped in both families to the same homozygous missense mutation in *FDX2* (c.431C>T, p.P144L). The deleterious effect of the mutation was validated by Real Time PCR and Western Blot analysis, which demonstrated normal expression of FDX2 mRNA but severely reduced or absent expression of FDX2 protein in muscle tissue.
Discussion: This study describes a novel complex neurological phenotype with unusual MRI and muscle biopsy features, conclusively mapped to a mutation in *FDX2*, which encodes a ubiquitously expressed mitochondrial ferredoxin essential for early Iron-Sulfur cluster biogenesis.

PARALLEL SESSION 3B: Pathogenic mechanisms in inborn errors of metabolism

O-037

MiRNA analysis provides new insights into propionic acidemia related cardiomyopathy.

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Background: Propionic acidemia (PA) patients surviving the neonatal period frequently develop cardiac hypertrophy (CH) or arrhythmias, one of the major causes of mortality. The hypomorphic PA mouse model exhibits CH with an increase in ANP, BNP and β-MHC markers. We have previously identified dysregulated miRNAs in PA mice tissues, some of them also present in altered levels in PA patients' plasma samples, potentially contributing to the pathophysiology. Here, we studied cardiac enriched miRNAs, which could contribute to the establishment of CH through the alteration of signaling pathways.
Methods: qRT-PCR and western blot
Results: We detected 10 miRNAs upregulated in heart from PA mice, correlating in some cases with decreased expression of their direct targets. Furthermore, in PA patients' plasma samples we detected several of these miRNAs in decreased levels compared to matched control samples. To investigate the mechanism of miRNA dysregulation we analyzed the effect of propionate treatment in the cardiac murine cell line HL-1; this resulted in increased levels of several miRNAs and of cardiac hypertrophy marker BNP. In PA mice heart samples, we performed gene expression analysis of the PI3K-Akt pathway, finding altered expression of 33 genes, including *Pten*, *Mtor* or *Fos*. We have also detected increased expression of *Nfatc3* and *Nfatc4* genes, a decrease/absence in the phosphorylation status of the stress kinases JNK and p38 and activation of mTOR signaling pathway correlating with a decrease in autophagy.
Discussion: Our results highlight the possible involvement of miRNAs, their targets and signaling pathways in the development of CH in PA, and their potential role as biomarkers for disease prognosis. The triggering mechanism appears to be the accumulation of propionate, as shown in vitro in HL-1 cells. Of note, miRNAs can be therapeutically modulated, which could provide novel approaches to treat or prevent cardiac complications in PA patients.

O-038

Propionate anions produce acute and sustained remodelling of calcium transients in rat ventricular myocytes

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Background: Propionic acidaemia (PA) is an inborn error of metabolism caused by a deficiency of mitochondrial propionyl-CoA carboxylase (PCC). Arrhythmias and cardiomyopathies are cited as major causes of death. Defective catabolism of propiogenic substrates by PCC leads to metabolic acidosis and an accumulation of propionate derivatives in fluid compartments. Here, we investigated the acute and chronic effects of propionate on cardiac myocyte Ca^{2+} handling, which undergoes remodelling in various cardiac disorders.
Methods: Experiments were conducted on adult or neonatal rat ventricular myocytes under electrical pacing, and excitation-contraction coupling was imaged using fluorescent Ca^{2+} reporters (FuraRed or Fluo3). Test solutions contained 6mM propionate either with acidosis to mimic uncompensated PA ("propionic acidosis"; 0.2mM NH_4Cl, 16mM HCO_3^-, pH ~7.3) or without ("propionate"; 22mM HCO_3^-, pH ~7.4) to mimic compensated PA.
Results: Acute exposure to propionate anions (10–30 minutes) reduced cellular Ca^{2+} extrusion by Na^+/Ca^{2+} exchange (NCX), probed by the recovery from a caffeine-evoked Ca^{2+} release from the sarcoplasmic reticulum (SR) store. Acute propionate exposure also curtailed diastolic Ca^{2+} leak through the SR-embedded ryanodine receptors (RyR), probed by releasing residual SR Ca^{2+} that remains after a 3-minute period of no pacing. Long-term (48-hour) culture in propionate, with or without acidosis, produced a persistent lengthening of the Ca^{2+} transient due to a slowing of the rise-time (i.e. SR Ca^{2+} release flux) and its recovery (i.e. suppressed re-uptake by the SR Ca^{2+} ATPase – SERCA – activity).
Discussion: We demonstrate acute and sustained (gene expression-related) effects of propionate on cardiac myocyte Ca^{2+} handling, which may lead to abnormal heart function.

O-039

α-galactosidase A activity modulates DNA methylation of androgen receptor promoter in Fabry disease vascular endothelial cells

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Background: Fabry disease is caused by deficient activity of α-galactosidase A (α-gal A) and subsequent accumulation of glycosphingolipids, mainly globotriaosylceramide (Gb3). Previous studies showed that a number of genes are abnormally expressed in Fabry patients and a mouse model of the disease. The aberrant gene expression may play important roles in the pathogenesis of Fabry disease; however, the molecular basis by which α-gal A deficiency causes the altered gene expression remains to be elucidated.
METHODS: We tested whether α-gal A deficiency can affect DNA methylation, the most important epigenetic mechanisms for gene regulation. We examined the effect of α-gal A activity on the methylation status of two specific CpG regions in androgen receptor (AR) gene, whose expression is upregulated in Fabry disease. α-Gal A activity in a Fabry patient-derived endothelial cell line was manipulated by stable gene-transduction or short-term treatment with exogenous enzyme or α-gal A-specific inhibitor. Methylation level of each CpG site was quantitatively analyzed by bisulfite pyrosequencing.
Results: We found that decreased α-gal A activity or increased Gb3 accumulation in Fabry disease endothelial cells are associated with decreased methylation level of one of the CpG regions in AR promoter. This CpG region is located within the core promoter of AR gene and its methylation has been shown to be associated with AR gene silencing; thus, hypomethylation of this region might contribute to upregulated AR in Fabry disease. Methylation level of LINE-1, a marker for global methylation, was not affected by α-gal A deficiency.
Discussion: This study provides evidence that α-gal A deficiency and glycosphingolipids storage modulate DNA methylation, and this effect is highly gene- and target site-specific. These findings may have important implication in understanding the role of dysregulated DNA methylation in the pathogenesis of Fabry disease and other lysosomal storage disorders.

O-040

The satellite cell paradox in Pompe disease: breaking the barriers for regeneration

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Background: Skeletal muscle regeneration after damage is completely dependent on muscle stem cells (MuSC). Defects in muscle regeneration contribute to muscle wasting in muscle-degenerative disorders. We recently reported that MuSC activation in Pompe disease (PD) is suppressed, even though the muscle damage due to glycogen accumulation is extensive. PD is a recessive metabolic myopathy caused by deficiency in acid alpha glucosidase (GAA). In this study we investigated the mechanisms behind the suppressed MuSC response and determined the efficacy of a muscle-regenerative therapy.
Methods: Two independent mouse models of Pompe disease were used to study the muscle regeneration potential. Markers of muscle regeneration and muscle wasting were determined by IHC and flow cytometry. Injury experiments were performed to induce regeneration and determine MuSC regenerative and self-renewal potential. Effects on muscle function was determined by Rotarod assay.
Results: Limb muscle of young GAAKO mice showed a progressive increase in levels of glycogen and size of Lamp1-positive lysosomal compartment in the first 15 weeks after birth. In parallel, an endogenous muscle regeneration response was observed. After 15 weeks muscle regeneration activity was lost, and muscle wet weight and Rotarod performance were reduced. Across all ages we detected a strong increase in Pax7-positive MuSCs that were, however, inactive in animals of 15 weeks and older. Surprisingly, GAAKO animals efficiently repaired $BaCl_2$-induced muscle damage, even in aged animals, In serial injury experiments GAAKO MuSC showed efficient selfrenewing capacity.
Discussion: In young GAAKO animals muscle damage and repair are in balance. At 15 weeks the loss of muscle regeneration tips the balance towards muscle wasting. The retained functional competence of GAAKO MuSCs and improvement of muscle morphology and function after forcing regeneration opens opportunities for muscle regenerative therapy for Pompe disease.
Conflict of Interest declared.

PARALLEL SESSION 3C: New disorders and concepts

O-041

GOT2 deficiency: a novel disorder of the malate aspartate shuttle resulting in serine deficiency

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Background: Whole exome sequencing in a patient with serine- and vitamin B6-responsive epileptic encephalopathy and profound psychomotor delay with spastic paresis revealed compound heterozygous variants in *GOT2*, the gene encoding the mitochondrial glutamate oxaloacetate transaminase. GOT2 is an important member of the malate-aspartate shuttle, playing an essential role in the intracellular NAD(H) redox balance by allowing the re-oxidation of cytosolic NADH. Biochemically, the patient presented with low plasma serine, hypercitrullinemia, hypercalcemia, hyperlactatemia and hyperammonemia.
Methods: We measured the GOT activity and performed stable isotope studies on serine *de novo* biosynthesis in the patient's fibroblasts. In addition, to investigate the causal relationship between GOT2 deficiency and serine synthesis, we generated *GOT2*-knockout HEK293 cells by CRISPR/Cas9, and measured the GOT activity and serine *de novo* biosynthesis also in these cells.
Results: *In vitro* analysis of GOT activity in the patient's fibroblasts revealed a markedly decrease in total GOT activity (30% of controls) and a severe decrease in GOT2 activity (< 9% of controls), while our stable isotope studies revealed a decreased serine biosynthesis (37% of controls). GOT2 activity in the GOT2-knockout cells was virtually inexistent (< 2% of controls), while serine synthesis was severely impaired (90-93% less than controls). Hypothesizing that the defect in serine biosynthesis is secondary to an increased cytosolic NADH/NAD^+ ratio, we tested the effect of NAD^+, glycerol and pyruvate on serine synthesis and found that pyruvate supplementation completely restores serine biosynthesis in GOT2-deficient cells.
Discussion: We report GOT2 deficiency as a novel inborn error of metabolism presenting with neurologic impairment and seizures, clinically responsive to serine and vitamin B6, showing *in vitro* amenability to pyruvate supplementation.

O-042

Pharmacologic inhibition of hepatic O-GlcNAcase enhances ureagenesis and ammonia detoxification.

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Background: Hyperammonemia results from inherited defects of the urea-cycle and acquired liver diseases. O-GlcNAcylation is a dynamic post-translational modification of intracellular proteins catalyzed by the enzyme O-GlcNAc transferase whereas O-GlcNAcase (OGA) removes the sugar moiety. Among several functions, O-GlcNAcylation regulates liver metabolism of glucose, lipids, and bile acids.
Methods: We investigated whether pharmacological inhibition of OGA by two different drugs PUGNAc and Thiamet-G was effective at reducing blood ammonia levels in C57BL/6 wild-type mice with acute hyperammonemia induced by intraperitoneal injection of 15N-labeled ammonium chloride. Ammonia removal was monitored by determinations of serum ammonia levels whereas ureagenesis was evaluated by 15N-NMR spectroscopy. O-GlcNAc protein levels were determined by Western blot and hepatic gene expression by qPCR. Whole-liver metabolome analysis was performed by 1H-NMR.

Results: As expected, treatment with OGA inhibitors increased liver O-GlcNAc protein levels. Both PUGNAc and Thiamet-G resulted in reduced concentrations of serum ammonia and increased serum levels of 15N-labeled urea. Hepatic 15N-urea was increased whereas hepatic glutamine was unaffected. Neither the expression of urea cycle enzymes nor other genes involved in ammonia removal were affected by OGA inhibition. Interestingly, whole-liver metabolome was well separated between PUGNAc and control animals, and metabolite set enrichment analysis rated urea cycle as the main pathway implicated. Inhibition of OGA was associated with higher hepatic levels of essential substrates for the functioning of the urea cycle, such as ATP, aspartate, and glutamate.
Discussion: Hepatic O-GlcNAcylation is important for ammonia detoxification and OGA is a promising therapeutic target to treat hyperammonemia.

O-043

Identification of energy balance dysregulation in a mouse model of mut-type methylmalonic aciduria

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Background: Isolated methylmalonic aciduria (MMAuria) is primarily caused by deficiency of methylmalonyl-CoA mutase (MUT). Biochemically, MUT deficiency results in the accumulation of methylmalonic acid (MMA), propionyl-carnitine (C3) and other metabolites. Patients often exhibit lethargy, failure to thrive and metabolic decompensation leading to coma or even death. To investigate metabolic disturbances in MMAuria, we used a mouse model of *Mut* deficiency which combines a knock-in (ki) missense allele with a knock-out (ko) allele to increase severity (*Mut*-ko/ki).
Methods: *Mut*-ko/ki and *Mut*-ki/wt littermate control mice were fed a 51%-protein diet from day 12 of life. Blood MMA and C3 levels were monitored. At the German Mouse Clinic we used indirect calorimetry to measure metabolic rate and substrate utilization, and Time Domain Nuclear Magnetic Resonance to determine body composition. *i.p.* Glucose Tolerance Test was performed and plasma insulin, FGF21 and leptin measured in *ad libitum* fed mice.
Results: Compared to their littermates, *Mut*-ko/ki mice were underweight and showed drastically increased levels of MMA and C3. When normalized to body mass, fat and free fluids were increased in *Mut*-ko/ki mice and lean mass was lower, indicating a switch in body composition. Indirect calorimetry revealed decreased metabolic rate, and females in particular showed a drastic reduction in minimum energy expenditure indicative of metabolic suppression. *Mut*-ko/ki mice exhibited a shift towards lipid oxidation, decreasing their reliance on carbohydrate oxidation, which was accompanied by impaired glucose tolerance, and lower plasma insulin and leptin as well as increased FGF21.
Discussion: Metabolic disturbances found in *Mut*-ko/ki mice indicate growth delay and energy shortage. Specifically, the striking metabolic suppression in *Mut*-ko/ki females reveals a strong effect of MUT deficiency on energy balance regulation. These data suggest energy dysregulation as a common disease mechanism.

O-044

Biallelic *SLC13A3* mutations cause reversible leukoencephalopathy with increased urine excretion of alpha-ketoglutarate.

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Background: *SLC13A3* encodes the plasma membrane Na^+/Dicarboxylate Cotransporter 3 (NaDC3), which imports four to six carbon dicarboxylates (DCA) as well as N-acetylaspartate (NAA). NaDC3 is mainly expressed in kidney and in astrocytes where it might feed neurons with dicarboxylates and participate in NAA metabolism. We describe two unrelated patients presenting with acute, reversible (and recurrent in one) neurological deterioration in the context of febrile illness. Both patients exhibited a reversible leukoencephalopathy and a markedly increased and persisting over time urinary excretion (260–1120 mmol/mol creatinine; nl: < 82 (>5Y)) of alpha-ketoglutarate (aKG). In one patient, increased CSF NAA and DCA concentrations were also observed.
Methods: After extended work-up in order to exclude classical leukoencephalopathy etiologies, our two groups performed whole exome sequencing (WES) on patient DNA. Our two teams were connected through Genematcher.
Results: WES analysis revealed biallelic variants in *SLC13A3*. A missense mutation (p.A254D) in the homozygous state was found in the first patient. The second patient was heterozygous for another missense mutation (p.G548S) and a mutation of a potential splice intronic site (c.1016+3A>G). Mutations and segregations were confirmed by Sanger sequencing. Functional studies performed on HEK293T cells transfected with wild-type and mutant recombinant NaDC3 indicated that the mutations caused a marked reduction in the capacity to import aKG (> 10-fold), succinate (> 10-fold), and NAA (> 2.5-fold).
Discussion: NaDC3 deficiency causes a marked hyperexcretion of aKG in urine and favors the development of acute and reversible leukoencephalopathy. Urine organic acids and *SLC13A3* mutations should be screened in patients presenting with unexplained acute leukoencephalopathy.
NaDC3 deficiency is a novel differential diagnosis of reversible leukoencephalopathy that should be searched for in the setting of increased aKG concentrations.

O-045

Dichotomous effect of mutations causing X-linked sideroblasmic anaemia and protoporphyria explained by the first human ALAS2 structure

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Background: Erythroid-specific aminolevulinate synthase (ALAS2) catalyses the first, rate-determining step in haem biosynthesis. Gain-of-function *ALAS2* mutations lead to X-linked protoporphyria (XLPP) characterized by photosensitivity and accumulation of haem precursors. Loss-of-function *ALAS2* mutations lead to X-linked sideroblastic anemia (XLSA) characterized by haem-deficient red blood cells. These two disorders are associated with entirely opposing effects on ALAS2 enzyme activity, although the underlying molecular mechanism is unknown.
Methods: To decipher the molecular dysfunctions caused by ALAS2 mutations, we determined the 2.9 Å resolution structure of human ALAS2, the first for a higher eukaryotic aminolevulinate synthase.
Results: The structure displays a conserved catalytic core harbouring the enzyme active site and attachment for its cofactor, pyridoxal phosphate (PLP). Mapping of XLSA-causing missense mutations, largely clustered in the catalytic core, reveals the molecular basis for their loss of function arises from protein destabilization or reduced enzyme activity. XLPP-causing mutations, however, are frameshift/indels found exclusively within a 33-amino-acid extension that follows the catalytic core. In the structure, this extension folds back onto the catalytic core, acting as a gatekeeper for the active site. Combined with site-specific mutagenesis, our structure suggests that disrupted gating of the ALAS2 active site by this extension leads to a constitutive enzyme, resulting in a gain of function.
Discussion: Determination of the first ALAS2 structure is of notable importance to the field of metabolic medicine, as it offers the first explanation for the dichotomous effect of XLPP and XLSA variants. This work also uncovers the enzyme's regulatory features evolved to limit metabolic flux into haem biosynthesis, and as such there is potential of ALAS2 inhibition for the treatment of XLPP and other porphyria disorders.

O-046

Expanding the neurological spectrum of seipin deficiency (*BSCL2*), a complex lipid defect

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Background: *BSCL2* mutations disrupt seipin, an endoplasmic reticulum membrane protein that modulates lipid droplets. Clinical phenotypes include congenital lipodystrophy 2, progressive encephalopathy with or without lipodystrophy (recessive), distal motor neuropathy type V, and spastic paraparesis (SP) (dominant).
Methods: Clinical, electrophysiological, biochemical, neuroimaging and genetic data were reviewed in a cohort of *BSCL2* patients.
Results: Seven patients from five families (mean age: 7.6 years; range: 1–14; 3 females/4 males) were identified. Onset age was before 6 months in all but one starting at 11 years (SP group). Onset symptoms include: hypertriglyceridemia, hyperglycemia and hepatomegaly (1); seizures (4); psychomotor delay (3) and abnormal upper limb postures (1). Three patients showed homozygous *BSCL2* variants presenting as lipodystrophy and neurological deterioration (1), psychomotor delay, spastic-dystonic quadriparesis and epilepsy (2). Four patients showed heterozygous *BSCL2* presenting SP (2) and epileptic encephalopathy (2; new *de novo* mutation). Peripheral neuropathy was found in all patients with nerve conduction studies (4). In one patient CSF analysis found low levels of homovanilic and 5-hydroxy-indolacetic acid. One patient showed pallidus hypointensity. Neuropathology showed brainstem and basal ganglia astrogliosis. Intranuclear ubiquitin inclusions were not identified.
Discussion: Seipin defects disturb lipid droplet biosynthesis and belong to the recently described category of complex lipid disorders. Prominent motor and cognitive symptoms, neurodegeneration, early refractory epilepsy (dominant de novo variant described for the first time), axonal neuropathy and the abnormal neurotransmitter study point towards an important role of synaptic vesicle function in the pathophysiology. Hypertriglyceridemia as biomarker was found only in lipodystrophy.

PARALLEL SESSION 3D: Disorders of fatty acid and keytone metabolism

O-047

Structural insights into drug therapy of mitochondrial fatty acid oxidation disorders

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Background: Fatty acid oxidation (FAO) is essential for many tissues including heart and skeletal muscles and is critical during physiologic stress. Deficiency of FAO enzymes causes serious diseases that are often lethal. Most patients with FAO disorders are identified through NBS. Symptoms of long-chain FAODs include fasting or stress-related hypoketotic hypoglycemia, cardiomyopathy, and stress-induced rhabdomyolysis. Current treatment is focused on alleviating acute symptoms by IV glucose infusion and carnitine and, except in MCADD, bypassing long chain fatty acids oxidation with MCT oils. Patients are nevertheless, at risk for symptoms including late onset cardiomyopathy and rhabdomyolysis and significant morbidity and mortality persist. To treat the heterogeneous symptoms caused by FAODs, candidate drugs that address various aspects of the cell pathophysiology were tested.
Methods: Pathway analysis, molecular modeling, enzyme presence and activity, and mitochondrial energetics and metabolite analyses were performed to assess the potential use of various drug therapies to alleviate the phenotype of FAOD.
Results: Stabilizing chaperone-based treatment using phenylbutyrate, trimetazidine, and cardiolipin binding peptide enhanced enzyme presence and activity in patient cells with FAODs. Transcription activators, PPARδ agonists, enhanced the production of defective FAO proteins and level of activity and combining them with chaperone-based therapy demonstrated synergistic effect. JP4-039, a novel mitochondrially targeted free radical scavenger, significantly reduced ROS levels in cells with FAODs. Anaplerotic agents replenishing the Kreb's cycle also provided relief for the energy deficit.
Discussion: The results suggesting significant improvements in key markers using the drugs tested give the impetus for their immediate development towards clinical trials. Our proposed drug therapy solutions bring hope for effective therapies for patients in the foreseeable future.

O-048

Impact of UX007 and dietary management on major clinical events in a 78-week single-arm open-label phase 2 LC-FAOD study

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Background: LC-FAOD are autosomal recessive disorders caused by defects in mitochondrial fatty acid oxidation enzymes. UX007, a pharmaceutical grade, purified seven-carbon medium-chain triglyceride (MCT), is being investigated for treatment of LC-FAOD. A single-arm, Phase 2 study was conducted to prospectively evaluate safety and efficacy of 78 weeks of UX007 treatment (Tx) in 29 pediatric and adult subjects with severe LC-FAOD, compared with a retrospective pre-UX007 period during which subjects were optimally managed under current dietary guidelines by metabolic clinics.
Methods / case report: Dietary reports were collected to examine the relationship between diet, UX007, and their impact on major clinical events (MCE; rhabdomyolysis, hypoglycemia, and cardiomyopathy).
Results: Subjects followed standard FAOD diet recommendations during pre-UX007 period, usually including a source of MCT. For Tx period, subjects switched from MCT to UX007 with a targeted dose of 25-35% of daily caloric intake (DCI). Subjects received mean 17.4% DCI from MCT prior to entering the study and per protocol, received and overall tolerated mean 27.5% DCI from UX007 during the study, while protein, long-chain fat, and carbohydrates remained relatively consistent. Total DCI modestly increased from ~62 to 72 kcals/kg driven by energy requirements for growth and activity level. Following 78 weeks of UX007 Tx, the mean annualized MCE rate decreased from 1.69 to 0.88 events/year (48.1% reduction, p=0.021), and a 50% reduction in median event rate from 1.33 to 0.66. The mean annualized MCE duration rate decreased from 5.96 to 2.96 days/year (50.3% reduction, p=0.028), and a 77% reduction in median hospital days from 5.33 to 1.24.
Discussion: Dosed at 25-35% DCI, UX007 demonstrated significant reduction in MCEs compared with subjects' prior treatment regimens. It is important to consider that the transition from MCT to UX007 and UX007's anaplerotic effect may have also contributed to the improvement.
Conflict of Interest declared.

O-049

Metabolomics profiling in dried blood spots differentiates clinical phenotypes in VLCADD

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Background: Inclusion of very long-chain acyl-CoA dehydrogenase deficiency (VLCADD) in newborn screening (NBS) programs worldwide has led to identification of more patients including pre-symptomatic individuals. It is not yet clear which patients will develop severe symptoms and which not, so the need for early phenotype prediction is high. We previously reported a correlation between long-chain fatty acid oxidation (lc-FAO) flux in fibroblasts and clinical outcome. Disadvantages of this method are the need for skin biopsies and long turnaround time. Here, we developed a rapid, as less invasive as possible, prognosis prediction method for VLCADD.
Methods: DBS from VLCADD patients from regular visits to the outpatient clinic (n=25) and from the Dutch NBS (n=13) were used. A metabolic fingerprint was generated by direct infusion high-resolution mass-spectrometry. Before metabolomics profiling, patients were divided in phenotypic groups based on lc-FAO flux and clinical features. Data analysis in MetaboAnalyst (http://metaboanalyst.ca/) included supervised multivariate statistics (Partial Least Squares Discriminant Analysis; PLS-DA) and hierarchical clustering.
Results: The biomarker for NBS, C14:1-carnitine, was elevated in all. In addition, C16:2-carnitine was elevated in all VLCADD patients. Based on untargeted metabolite profiles by PLS-DA, patients with a severe phenotype could be discriminated from patients with a mild phenotype. Results were confirmed by blinded NBS bloodspot analysis of 13 patients and 35 age matched NBS DBS controls. Of those 2 had a metabolic profile fitting the severe phenotype. One of the patients was deceased, the other had severe VLCADD related symptoms. Both had the lowest lc-FAO flux of the VLCADD patients.
Discussion: State-of-the-art DBS metabolomics showed different metabolic profiles between VLCADD patients with the severe and phenotypes. This method may accelerate prognosis prediction and improve rapid initiation of individualized therapy.

O-050

3-hydroxybutyrate (3-HB) treatment in MADD: a systematic literature review and international retrospective cohort study

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Background: Multiple acyl-CoA dehydrogenase deficiency (MADD) is a rare disorder of mitochondrial fatty acid oxidation and amino acid metabolism. Few case reports describe successful

sodium-D,L-3-hydroxybutyrate (3-HB) treatment in severely affected MADD-patients.
Methods: To investigate efficacy and safety of 3-HB, we performed (1) a systematic literature review, and (2) an international, retrospective questionnaire study on clinical presentation, 3-HB treatment method and (long-term) outcome in MADD(−like)-patients diagnosed through biochemical, molecular and/or enzymatic methods.
Results: Our systematic review identified 15 MADD(−like)-patients treated with 3-HB. Our questionnaire study summarizes 19 patients, including 14 new cases. Median age at first clinical presentation was 4 months (0d–26y). Molecular analysis was abnormal in 16 patients (homozygosity in *ETFA* (n=3) and *ETFDH* (n=4); compound heterozygosity in *ETFA* (n=1), *ETFDH* (n=4) and *SLC52A3* (n=2); compound heterozygosity in both *ETFA* and *ETFB* (n=1); one *ETFDH* mutation (n=1)). Median age at start of 3-HB treatment was 9 months (9d–29y). Doses ranged from 100 to 2600 mg/kg/day in one to six times/day. 3-HB resulted in clinical improvement (CI) in 12 patients for leukodystrophy (4/5), cardiac (3/3), muscle- (10/14) and/or liver pathology (4/5). Three patients received 3-HB to prevent complications. Time till first CI ranged from 2 days to 6 months. Reported side effects included dehydration, vomiting/nausea, diarrhea, constipation and abdominal pain. 3-HB treatment was terminated in nine patients at median age of 4 years (10d–29y) due to death (n=3),CI un-necessitating 3-HB treatment (n=2), no CI (n=2), costs (n=2) and/or side effects (n=1). Median 3-HB treatment duration was 2 years (1d–10y).
Discussion: This is the largest international cohort study on 3-HB treatment in MADD(−like)-patients. Based on our retrospective data, 3-HB can be efficacious and safe in MADD-patients.

O-051

Restoration of VLCAD in null mice and mutant human fibroblasts using novel mRNA technology: Model to treat fatty acid β-oxidation disorders

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Background: Very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency is an autosomal recessive disorder identifiable by newborn screening. VLCAD patients present with severe cardiomyopathy, hypoketotic hypoglycemia, or intermittent recurrent rhabdomyolysis. VLCAD catalyzes the initial dehydrogenation step of fatty acid β-oxidation in mitochondria. The current treatment circumvents the metabolic block by avoiding fasting and maintaining a low-fat diet that includes medium-chain fats. To eliminate the hypoglycemia and reduce pathologic metabolites, we have restored liver VLCAD protein expression through targeted mRNA treatment in mice.
Methods: Initially, mRNA coding for human VLCAD was transfected into cultured human cells using Lipofectamine 2000 or Mirus Trans-It. Protein expression and activity, restitution of β-oxidation flux, and metabolite reduction by acylcarnitine quantitation were determined. Mitochondrial localization was assessed using immunofluorescence microscopy. Mice deficient in VLCAD were injected with mRNA encapsulated in a liver-targeting lipid nanoparticle. Isolated livers were examined for protein expression and enzyme activity.
Results: VLCAD mRNA transfected well and expressed enzymatically active protein in HeLa cells and VLCAD mutant fibroblasts. The translated VLCAD trafficked to mitochondria where it was clipped to the correct size product. VLCAD transfection reduced accumulation of C16-carnitine and improved β-oxidation flux. Expressed protein was stable for 144 hours. Finally, when VLCAD liver-targeting constructs were injected into tail veins of VLCAD knockout mice, VLCAD was expressed and persisted for 116 hours.
Discussion: VLCAD liver-targeted mRNA treatment results in the expression of protein in vitro and in vivo. A human VLCAD mRNA construct produced abundant active protein in mutant human cells. Future mouse studies will determine whether VLCAD mRNA transfected into liver rescues the phenotype, especially in response to stress.
Conflict of Interest declared.

O-052

Targeting cardiolipin: a new therapeutic approach to treat LCHAD and mitochondrial TFP deficiencies

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Background: Replacing energy in long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) and trifunctional protein (TFP) deficiencies does not prevent rhabdomyolysis, retinal disease or peripheral neuropathy, underscoring the need to investigate other mechanisms of disease. TFP is a hetero-octamer encompassing LCHAD activity. Studies in our lab indicate that it physically interacts with respiratory chain supercomplexes (SC) through complex I. The C-terminal end of TFPα also acts as a monolysocardiolipin acyl transferase (MLCLAT) in cardiolipin (CL) remodeling. CL sustains the integrity of OXPHOS by maintaining SC stability. We hypothesized that 1) LCHAD/TFP mutations lead to abnormal MLCLAT activity and CL remodeling, SC instability, and increased ROS production; and 2) stabilizing CL will improve TFP/OXPHOS interactions, and decrease ROS production.
Methods / case report: Fibroblasts deficient in LCHAD/TFP were studied before and after treatment with a CL stabilizing peptide. Oxygen consumption was measured using Seahorse Analyzer. CL content was studied using MS/MS. Protein levels were quantitated by western blot and immunofluorescence. MLCLAT was measured by TLC. Flow cytometry was used to measure ROS and mitochondrial proliferation.
Results: Fibroblasts from patients with LCHAD deficiency showed increased ROS production, mitochondrial proliferation, and reduction in MLCLAT compared to control and TFP deficient cells. Lysocardiolipin levels varied with mutations. TFP interaction with SCs was destabilized in LCHAD and TFP deficient cell lines. Treatment with a CL stabilizing peptide reduced ROS production in LCHAD deficient cell lines, and increased immunofluorescence signal of both TFP subunits.
Discussion: Studying FAO-OXPHOS-CL interactions improves our understanding of pathophysiologic mechanisms involved in TFP/LCHAD deficiencies. The CL stabilizing peptide used in this study is promising as a therapeutic agent for these disorders.

PARALLEL SESSION 4A: Amino acid disorders and organic acidurias

O-053

Interplay of enzyme replacement therapy, diet and betaine in murine cystathionine beta-synthase-deficient homocystinuria

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Background: Cystathionine beta-synthase (CBS)-deficient homocystinuria (HCU) is an autosomal recessive inherited metabolic disorder managed with combination of low methionine (Met) intake, pyridoxine and/or betaine often with poor compliance. We developed an enzyme replacement therapy (ERT) for HCU and assessed its efficacy in face of current treatment in HCU mice.
Methods: HCU mice were on a standard diet (STD, 5 g/kg Met) or put on either high Met diet (HMD, 8.2 g/kg Met) or Met-restricted diet (MRD, 0.5 g/kg Met). At week 3, 2% betaine water was provided to all mice and ERT was initiated at week 6 with 3 weekly subcutaneous injections (7.5 mg/kg). At week 9, betaine was discontinued, but ERT administration continued until the end of study, while diet was switched in 2-weeks interval among groups. Blood was collected 1–3 times per week throughout the study and plasma levels of sulfur amino acid metabolites were determined.
Results: Plasma total homocysteine (Hcy) levels in the HCU mice were strongly responsive to diet increasing 3.6-fold (to ~450 μM) on HMD and decreasing 4.8-times (to ~26 μM) on MRD compared to the STD (125 μM). Betaine was effective particularly in combination with MRD, but did not markedly improve Hcy levels in mice on STD and HMD where ERT showed substantial efficacy by dramatically decreasing plasma Hcy levels (to ~35 μM). The suppressed Hcy levels, including the normalized values in mice on MRD (< 5 μM), were maintained by the ERT treatment in all groups, even after betaine withdrawal and dietary switching. Plasma Met levels in mice on HMD were elevated (up to ~220 μM) and further increased with betaine (up to ~720 μM), while ERT decreased them < 100 μM and stably maintained throughout the study. In addition, ERT increased or fully normalized plasma total Cys levels in mice on STD and HMD.
Discussion: Data suggest that ERT alone represents an effective treatment for HCU even under high Met load as well as in combination with current therapies.
Conflict of Interest declared.

O-054

Surrogate biomarkers for clinical trial design in methylmalonic acidemia (MMA): A bench to bedside approach.

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Background: Liver and/or kidney transplantation (LT/KT/LKT) are surgical approaches used to stabilize severely affected MMA patients. Although serum methylmalonic acid provides a disease-specific marker of biochemical improvement, the significant intra-patient variability of this metabolite, as well as influence by diet and renal function, underscore the need for additional markers of therapeutic efficacy in MMA.
Methods: We used a mouse model to discover new hepatic biomarkers, and then examined candidates in MMA patients. MMA mice expressing the *Mut* gene under a muscle-specific promoter ($Mut^{-/-}$;$Tg^{INS\text{-}MCK\text{-}Mut}$) were used to profile hepatic transcriptional adaptations, identify candidate biomarkers, and confirm response after systemic gene therapy. In parallel, 1-^{13}C-propionate oxidation, clinical and metabolic parameters were examined in 72 patients (*N*=58 *mut*, 8 *cblA* and 6 *cblB*), including before and after LT/KT/LKT (*N*=14), as well as in age, gender, height, BMI-matched controls.
Results: Fibroblast growth factor 21 (FGF21) was: validated as a hepatic biomarker responsive to systemic liver-directed AAV8 MUT gene therapy in $Mut^{-/-}$;$Tg^{INS\text{-}MCK\text{-}Mut}$ mice; markedly elevated in MMA patients compared to controls ($p< 0.0001$); and correlated with 1-^{13}C-propionate oxidation ($p=0.03$) and height for age *z*-score ($p=0.04$). L(K)T recipients (*N*=10) experienced a significant drop in plasma FGF21 (from 13,953 ±12,611 to 467.8±522.9 pg/ml, $p< 0.002$) that was accompanied by restored 1-^{13}C-propionate oxidation (p=NS vs *N*=19 healty controls). Patients with isolated KT (*N*=4) had minimal improvement in 1-^{13}C-propionate oxidation and variable responses in FGF21.
Discussion: After L(K)T in MMA patients or liver-directed AAV8 gene therapy in MMA mice, 1-^{13}C- propionate oxidation is restored and plasma FGF21 concentrations are reduced. These biomarkers, studied in concert with canonical metabolites, should be useful to examine therapeutic effects of experimental hepatic interventions in MMA.

O-055

Identification of a multi-protein complex for mitochondrial cobalamin and methylmalonyl-CoA processing

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Background: Isolated methylmalonic aciduria may be caused by autosomal recessive mutations resulting in disturbed function of proteins involved in production (MMAA, MMAB, MMADHC) or utilization (MUT) of adenosylcobalamin and processing of methylmalonyl-CoA (MCEE, MUT). Continuous sequestration of the highly reactive but scarce adenosylcobalamin cofactor as well as the need for enzymatic tunneling of L-methylmalonyl-CoA have been predicted to avoid loss of pathway function. Despite this, no published evidence of a multi-protein complex within these pathways exist and only the direct interaction between one human protein pair (MMAA-MUT) has been confirmed so far.
Methods: We used immunoprecipitation and immunoblot of endogenous and over-expressed proteins in HEK293 cells following cross-linking to identify the complete protein complex. We used CRISPR-Cas9 technology to knock-out individual proteins in order to map which proteins directly interact with each other.
Results: Investigations in wild-type HEK293 cells revealed a protein complex consisting of at least MUT, MMAA, MMAB, MMADHC and MCEE. We therefore expect these proteins to constitute the core of a multi-protein complex. To study interactions between individual complex members we used HEK293 cells knocked-out for MUT or MMAB. This revealed direct interactions between the cobalamin pathway protein MMAB with MMAA, MCEE and MUT as well as direct interactions between MUT and MMAA, MMADHC and MCEE. We were further able to confirm and visualize individual interactions with fluorescence microscopy by using a proximity ligation assay.
Discussion: Combination of different methods and knock-out cells allowed us to characterize the network of protein interactions involved

in mitochondrial cobalamin and methylmalonyl-CoA processing. As mutation in all proteins involved leads to methylmalonic aciduria, disruption of this protein complex may be a direct cause of disease.

O-056

Newborn screening, a disease-modifying intervention for glutaric aciduria type 1

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Background: Untreated individuals with glutaric aciduria type 1 (GA1, OMIM #231670) commonly present with a mostly dystonic movement disorder (MD) following acute or insidious onset of striatal injury. Implementation of GA1 into national newborn screening (NBS) programs has improved the neurologic short-term outcome. However, it is unclear whether NBS changes the long-term outcome and which outcome variables are predictive.
Methods: This prospective, observational, multi-centre study includes 87 patients identified by NBS, four patients missed by NBS and three mothers with GA1 identified by positive NBS results of their unaffected children.
Results: The study population comprises 98.3% of patients with GA1 identified by NBS in Germany between 1999–2016. Overall, cumulative sensitivity of NBS is 95.6%, but is lower (84%) for patients with low excreting phenotype. Neurologic outcome of patients missed by NBS is as poor as in the pre-NBS era, while the clinical phenotype of diagnosed patients depends on the quality of therapeutic interventions rather than non-interventional variables (gender, biochemical subtype, migration). Presymptomatic start of treatment according to recent guideline recommendations (Boy et al. *J Inherit Metab Dis.* 2017), clearly improves the neurologic outcome (MD: 7% of patients), while delayed emergency treatment results in acute onset MD (100%), and deviations from maintenance treatment increase the risk of insidious onset MD (50%). Non-adherence to recommended emergency treatment has a major negative impact on survival (p=0.0036). Independent of the neurologic phenotype, kidney function tends to decline with age, a non-neurologic manifestation not predicted by any variable included in this study.
Discussion: NBS is a beneficial, disease-modifying intervention for GA1. However, improved neurologic outcome critically depends on adherence to recommended therapy regimen while kidney dysfunction does not appear to be impacted by recommended therapy.

PARALLEL SESSION 3B: Disorders of vitamins, co-factors, trace elements and miscellaneous disorders

O-057

Biochemical and molecular characterization of neurological Wilson disease

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Background: Wilson disease (WD) is a disorder of copper metabolism caused by the ATPase copper transporting beta (ATP7B) deficiency. The clinical courses of patients with neurological manifestations (nWD) were suggested as surreptitiously progressive and less favorable. The aim of this study was to identify biochemical and genetic features that characterize nWD as a distinct disease subgroup
Methods: A total of 86 nWD and 233 hepatic WD (hWD) patients from 368 unrelated, non-consanguineous Korean families were enrolled. Their detailed clinical, biochemical, and genetic characteristics were reviewed.
Results: The age at presentation and diagnosis was older in nWD than in hWD patients. At diagnosis, nWD patients also showed lower serum aspartate aminotransferase, alanine aminotransferase, gamma glutamyltransferase, and total bilirubin levels, and prothrombin time than hWD. Kayser-Fleischer ring, liver cirrhosis, unfavorable outcome(62% vs 80%, $P < 0.016$), and higher serum creatinine levelwere more common in nWD patients. Regarding copper (Cu) metabolism, nWD patients showed lower serum ceruloplasmin (3.1 ± 2.1 vs. 4.2 ± 3.2 mg/dL, $P < 0.001$), Cu (26.4 ± 13.8 vs. 35.8 ± 42.4 μg/dL, $P = 0.005$), free Cu (17.2 ± 12.5 vs. 23.5 ± 38.2 μg/dL, $P = 0.038$), and 24-h urinary Cu (280.9 ± 162.9 *vs.* 611.1 ± 1124.2 μg/day, $P < 0.001$) levels. Especially, low serum free Cu levels< 10 μg/dL were common in nWD patients with unfavorable outcome (62.5%). Frameshift, nonsense, or splice-site mutations in at least one allele of *ATP7B* were less frequent in nWD patients. Additionally, both mutations in the transduction and/or ATP hinge domain (2.4% vs. 23.1%, $P = 0.006$) were identified less frequently in nWD patients.
Discussion: Biochemical profiles representing hepatic dysfunction or Cu metabolism, *ATP7B* mutation spectrums, and clinical outcome were distinct in nWD patients. These results suggest that unknown, environmental and genetic factors may underlie the distinct features of nWD.

O-058

Structural basis of severe 5,10-methylenetetrahydrofolate reductase (MTHFR) deficiency

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Background: Severe 5,10-methylenetetrahydrofolate reductase (MTHFR) deficiency is inherited in an autosomal recessive manner and is the most common inborn error of folate deficiency, with >200 patients described harbouring >100 clinically relevant mutations. The current

repertoire of bacterial MTHFR structures do not provide any information for many of these mutations, nor any mechanistic insights into the enzymatic regulation of MTHFR by phosphorylation and SAM inhibition, because these features are absent in prokaryotes.
Methods: To decipher the molecular dysfunctions caused by mutation of MTHFR, as well as better understand enzymatic inhibition caused by SAM-binding and phosphorylation, we have determined the structural properties of MTHFR by crystallography, small angle x-ray scattering and mass spectrometry.
Results: The 2.5 Å resolution structure of human MTHFR reveals an architecture distinct from previous bacterial structures, whereby a eukaryote-only SAM-binding regulatory domain is appended to the well-conserved catalytic TIM-barrel via a 25-amino-acid linker. Mapping of known patient mutations onto this structure reveals 38 separate mutation sites in the catalytic domain, as well as 20 in the regulatory domain and 6 in the linker region. Surprisingly, many severe mutations, previously found to ablate enzymatic activity in patient fibroblasts, map to the conjunction of the linker and the catalytic domain, away from the active-site. Consistent with this, solution and X-ray data demonstrate that the linker allows for conformational plasticity, and is likely responsible for conveying the inhibition signal upon SAM-binding, which is sensitized by the presence of phosphorylation.
Discussion: These data provide the first atomic structure of eukaryotic MTHFR, reveal a novel mechanism for severe MTHFR deficiency and pave the way to a better understanding of disease causing mutations and to exploit rational design of new structure-based therapeutics.

O-059

New insights in the regulation of vitamin B6 metabolism by pyridoxal 5'-phosphate binding protein PLPBP

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Background: Recently, several patients with early-onset vitamin B6-dependent epilepsy harboring loss of function PLPBP mutations have been identified. The responsiveness to treatment with B6 vitamers (pyridoxine or pyridoxal 5′-phosphate (PLP)) suggested that PLPBP may be involved in the regulation of intracellular vitamin B6 homeostasis. Our aim was to investigate in detail the function and mechanism of action of PLPBP.
Methods: Control and PLPBP-deficient patient skin fibroblast and HEK 293 cell lines were used as the model systems. B6 vitamer profiles were determined with UPLC-MS/MS. Stably labeled ^{13}C4-pyridoxine HCl was used to assess the dynamics of vitamin B6 pathway. Protein levels of enzymes involved in vitamin B6 metabolism were measured by immunoblotting.
Results and discussion: We showed that independent of PLP precursor, PLPBP deficiency led to lower intracellular PLP levels. Strikingly, intracellular pyridoxine 5'-phosphate (PNP) or pyridoxamine 5'-phosphate strongly accumulated when cells were cultured with pyridoxine or pyridoxamine, respectively, suggesting that PLPBP deficiency affected pyridox(am)ine phosphate oxidase (PNPO) activity. However, the effects were much milder than in PNPO-deficient cells. PNPO protein was upregulated in PLPBP-deficient cells, indicating a compensatory response. In agreement with the steady state measurements of B6 vitamers, ^{13}C4-pyridoxine labeling experiments revealed increased labeling of PNP and decreased labeling of PLP pools in cells cultured with pyridoxine. Fractional turnover rates of pyridoxine, PLP and pyridoxal but not PNP were increased in PLPBP-deficient cells, confirming the effect on the PNPO activity. Taken together our data show that some aspects of PLPBP deficiency resemble PNPO deficiency, suggesting that PLPBP may regulate PNPO activity.

O-060

A mild case of molybdenum cofactor deficiency defines an alternative route of MOCS1 protein maturation

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Background: Molybdenum cofactor deficiency is an autosomal recessive inborn error of metabolism, which results from mutations in genes involved in Moco biosynthesis. MoCD is clinically characterized by intractable seizures and severe, rapidly progressing neurodegeneration.
Case report: Here we report a patient with an unusual late disease onset and mild phenotype, characterized by a lack of seizures, normal early development, but a decline triggered by febrile illness. Genetic analysis revealed a homozygous c.1338delG *MOCS1* mutation causing a frameshift (p.S442fs) with a premature termination of the MOCS1AB translation product at position 477 lacking the entire MOCS1B domain. Surprisingly, urine analysis detected trace amounts (1% of control) of the Moco degradation product urothione, suggesting a residual Moco synthesis in the patient.
Results: *In silico* analysis of the patient's mutated *MOCS1* transcript revealed a potential Kozak-sequence downstream of the mutation site providing the possibility of an independent expression of a MOCS1B protein. Following the expression of the patient's *MOCS1* cDNA in HEK293 cells we detected two proteins: a truncated MOCS1AB protein and a 22.4 kDa protein representing MOCS1B. Functional studies of both proteins confirmed activity of MOCS1B, but not of the truncated MOCS1AB. Based on these results subsequent experiments investigating WT-MOCS1 proteins revealed that MOCS1 proteins are translocated to the mitochondrial matrix. During this reaction, the WT-MOCS1AB protein is proteolysed creating an active MOCS1B protein as well.
Discussion: Our findings demonstrate an unusual mechanism of translation re-initiation in the *MOCS1* transcript in the patient, which results in trace amounts of functional MOCS1B protein being sufficient to partially protect the patient from the most severe symptoms of MoCD. Furthermore these findings uncovered the unusual, so far unknown maturation of MOCS1AB proteins.
Conflict of Interest declared.

O-061

The first viable mouse model of *cblC* deficiency

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Background: Combined methylmalonic acidemia and homocysteinemia, cblC type (cblC), is the most common inborn error of intracellular cobalamin metabolism and caused by mutations in the MMACHC gene.

METHODS:We used genome editing to create pathogenic Mmachc mutations, and further studied two alleles: c.163_164delAC p.Pro56Cysfs*4 and c.162_164delCAC p.Ser54_Thr55delinsArg.
Results: At E18.5 Mmachc$^{-/-}$ embryos were present in predicted ratios but had intrauterine growth retardation. A decreased number of homozygous mutant pups were noted at birth ($p< 0.0005$). The median survival of the Mmachc$^{-/-}$ mice was less than 7 days, with complete lethality by 1 month (n=42; $p< 0.0001$). At 2 weeks, the weights of Mmachc$^{-/-}$ mice (n=9) were reduced relative to controls (n=94; $p< 0.0001$). Mutants (n=6) displayed elevated plasma [MMA], homocysteine, cystathionine and decreased methionine vs controls (n=18) ($p< 0.02$). Systemic and ocular pathology was variable, and limited by the severe mutant phenotype.To explore systemic gene therapy as a treatment for cblC, we generated two AAVs, rAAVrh10-CBA-mMmachc and rAAV9-CBA-hMMACHC, that were delivered by a single neonatal intrahepatic injection (1 x 10^{11} GC/pup) and compared with weekly hydroxocobalamin (OHCbl) injections. Mmachc$^{-/-}$ mice treated with AAVrh10 (n=9), AAV9 (n=11) and OHCbl (n=9) displayed dramatically improved clinical appearance with increased survival ($p< 0.0001$), with the oldest treated mutants living beyond 1 year. We also observed improved growth ($p< 0.05$) and reduced [MMA] ($p=0.03$) at 2 weeks following AAV9 treatment.
Discussion: In summary, this mouse model recapitulates the disease manifestations seen in humans with cblC including intrauterine growth retardation, decreased survival, poor growth and metabolic abnormalities. A single neonatal injection of an AAV vector, expressing either the mouse or human MMACHC gene, produces equivalent metabolic and phenotypic effects as chronic, injectable OHCbl treatment in the Mmachc$^{-/-}$ mice.

O-062

Self-sufficient recycling of uncommitted cobalamin by aortic endothelial cells

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Background: Cobalamin (Cbl) deficiency causes megaloblastic anemia and a variety of neuropathies. However, homeostatic mechanisms of CNCbl and other cobalamins by vascular endothelial cells are poorly understood.
Methods: We investigate whether cultured bovine aortic endothelial cells (BAEC) carry out transcytosis of Cbl bound to transcobalamin (holo-TC). We examine the role of the Cbl processing protein MMACHC (cblC), the Cbl form, and the specificity toward the holo-TC receptor, CD320.
Results: Cultured BAEC endocytose [^{57}Co]-CNCbl-TC (source material) via the CD320 receptor. The bound cobalamin is bidirectionally transported across the cell both via exocytosis in its free form, [^{57}Co]-CNCbl, and via transcytosis as [^{57}Co]-CNCbl-TC. A portion of the endocytosed [^{57}Co]-CNCbl is enzymatically processed by cblC with subsequent formation of hydroxocobalamin ([^{57}Co]-HOCbl), methylcobalamin ([^{57}Co]-MeCbl) and adenosylcobalamin ([^{57}Co]-AdoCbl), which were transported across the cell in a bidirectional manner. Thus, transport mechanisms for cobalamin in vascular endothelial cells do not discriminate between different b-axial ligands of the vitamin. Competition studies with apo- and holo-TC and holo-intrinsic factor (holo-IF) showed that only holo-TC was effective at inhibiting transcellular transport of cobalamin. Incubation of BAEC with a blocking antibody against the cytosolic domain of the CD320 receptor inhibited Cbl uptake and transcytosis by approximately 40%.
Discussion: This study reveals that endothelial cells recycle uncommitted intracellular Cbl for downstream usage by other cell types and suggests that the endothelium is self-sufficient for the specific acquisition and subsequent distribution of circulating B_{12} via the CD320 receptor. We posit that the endothelial lining of the vasculature is an essential component for the maintenance of serum-tissue homeostasis of B_{12} and that it contributes to distinct phenotypes in inherited disorders of Cbl.

PARALLEL SESSION 4C: Metabolic disorders in adults

O-063

Changes in cornstarch dosing in adults with glycogen storage disease type Ia

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Background: Cornstarch has been the primary treatment for glycogen storage disease type Ia (GSD Ia) for over 30 years. When cornstarch was first described as a treatment for this disorder, few people survived to adulthood. Treatment of this disease early in life is well documented, but there is a paucity of literature about treatment of adults.
Methods / case report: Records from 114 patients (10 – 62 years of age) with GSD Ia seen at the GSD program at the University of Florida and Connecticut Children's Medical Center between 2015 and 2017 were reviewed. Upon admission, an intravenous catheter was placed and glucose and lactates were drawn hourly for at least 24 hours while titrating cornstarch doses.
Results: Data demonstrate that adult and adolescent treatment needs vary greatly, and the required cornstarch support decreases with age ($p< 0.01$). The 10–17 year-old age group (n=44) was prescribed 5.7 g/kg/day with a daily mean of 337 ± 39 grams. The 18–25 year old age group (n=31) was prescribed 4.6 g/kg/day with a daily mean of 332 ± 48. The 26–35 age group (n=22) was prescribed 4.6 g/kg/day (315 ± 48 grams/day) and the 36+ age group (n=17) was prescribed 3.5 g/kg/day (285 ± 63 grams/day). Males were prescribed more cornstarch than the females to maintain euglycemia in all groups over 18 years of age. The number of doses, however, did not change with a mean of 6 doses per day in all age groups.
Discussion: With aging, the basal metabolic rate decreases, and adults were found to require less cornstarch particularly after 35 years of age. Failure to lower the cornstarch doses contributes to over-treatment in adults with GSD Ia. Not only does this lead to worsening hepatomegaly and extra weight, but over-treatment contributes to relative hyperinsulinism and rebound hypoglycemia. This knowledge is essential in designing nutritional therapies for the aging GSD population.

O-064

Retrospective observational study on 53 adult patients with suspected metabolic myopathy

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Background: At our specialist centre in last three years a total of 53 adult patients were referred with suspected metabolic myopathy. The

predominant symptoms were single or multiple episodes of rhabdomyolysis, exertional muscle pain, cramps and fatigue with raised CK. We have next generation gene sequencing (NGS) for rhabdomyolysis (total 32 genes) is available to us now. This study reviews the management approach for this cohort of patients.
Methods: Retrospective data was collected from this cohort on demographics, presenting symptoms, diagnostic tests performed and the results and the management. The patients had routine blood with CK, acyl carnitine profile and NGS performed as first line. Further tests like fatty acid flux studies on skin fibroblasts and muscle biopsy were performed where NGS was inconclusive.
Results: There were 39 male and 14 female patients with age 17 – 72 years at presentation. 34 patients had episode of rhabdomyolysis and the remaining presented with myalgia/cramps. NGS confirmed diagnosis on 13 patients: CPT2- five, GSD5- two, GSD6- one, GSD9- two, VLACD- one, TK 2 - one and polyglucosan myopathy one patient. Single pathogenic mutations were identified in 14 other patients, with RYR-1 mutation identified in 4. Acyl carnitine was suggestive of a diagnosis in 2 patients (CPT2 and MADD). Baseline CK was raised in 38 patients. Studies on skin fibroblasts helped with the diagnosis in 4 patients. Identification of RYR-1 mutation led to referral for malignant hyperthermia studies. Total 24 patients subsequently had muscle biopsy. Patients were offered physio and dietetic input when appropriate. MCT in 29, riboflavin in 7 and ubiquinone in 6 patients were tried with variable response.
Discussion: NGS has avoided further tests where the diagnosis was confirmed. Identification of RYR1 has led to referral for further tests. Further specialist biochemical tests were required where single pathogenic mutation was identified.

O-065

Does a period of restricted phenylalanine intake influence the decision to be on PKU diet? An audit on maternal phenylketonuria experience

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Background: Although diet for life is recommended for patients with Phenylketonuria (PKU), a significant number of adult patients are off diet. There are multiple reasons for this decision, which vary between individuals. It has been postulated that these patients, as adults, might never experience the benefit of being on PKU diet with associated lower blood phenylalanine (phe) and whether a period on diet may encourage them to remain on diet long-term. Our objective was to explore if that is the case in our maternal cohort.
Methods: We carried out a retrospective review of maternal PKU cases from preconception to 12 months post-partum over a 6 year period. Patients were broadly classified as on or off diet prior to pregnancy. At conception and during pregnancy the aim is to keep bloodspot phe below 300umol/L. All patients were on standard phe-free amino acid supplements and their natural protein intake was adjusted accordingly.
Results: All patients followed a restricted diet during pregnancy. There were 22 pregnancies; 15 were off PKU diet prior. Two thirds [10/15] of those off diet planned their pregnancy, compared with 28% of those on diet [2/7]. Post-partum, 86% of patients reverted to their pre-pregnancy dietary status. 13% of patients who were off diet prior to pregnancy remained on PKU diet post-partum and 28% of patients who were on diet prior to conception decided to be off diet.
Discussion: The majority of patients reverted to their pre-pregnancy diets post-partum. This may suggest that the neurocognitive benefits associated with lower blood phe may not be a strong enough factor to influence the decision to be on or off PKU diet. However, smaller cohort number, more restrictive maternal PKU diet and personal motivation are other confounding factors which may influence the outcome. Further qualitative research would be useful to understand reasons for adult patients not following a restricted protein diet to allow us to address these barriers.

O-066

Adult patients with hereditary fructose intolerance are characterized by an increased intrahepatic triglyceride content

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Background: Hereditary fructose intolerance (HFI; OMIM: #229600) is caused by a deficiency in aldolase B. Treatment with a fructose-restricted diet leads to complete resolution of symptoms. The present study was conducted to investigate the long-term consequences of aldolase B deficiency on the liver in adult HFI patients treated with a fructose-restricted diet.
Methods: In this case–control study, 15 confirmed HFI patients and 15 age-, sex-, and BMI-matched control subjects underwent proton magnetic resonance spectroscopy (1H-MRS) and transient elastography to detect intrahepatic triglyceride (IHTG) content and liver stiffness (as a measure of hepatic fibrosis), respectively. All individuals filled out a three-day food diary to determine their daily fructose intake. Plasma transferrin glycosylation patterns were measured with high-resolution mass spectrometry.
Results: IHTG content was higher in HFI patients when compared to healthy controls (median IHTG content: 2.5% and 0.6% respectively, p=0.001). Liver stiffness did not differ between both groups (median stiffness: 5 kPa and 4 kPa, p=0.09; corresponding to fibrosis stage F0-1 in both groups). The most fructose-intolerant patients – indicated by the lowest dietary fructose intake – had the highest IHTG content (Spearman's rho=− 0.77, p=0.001). Hypoglycosylated transferrin was more abundant in HFI patients when compared to controls ($p< 0.001$) and tended to be higher in HFI patients with clinically relevant IHTG content (n=5) compared to those without (n=10) (p=0.09).
Discussion: This study demonstrates that adult HFI patients on a fructose-restricted diet accumulate more hepatic fat without any sign of hepatic fibrosis. High-resolution mass spectrometry is able to detect abnormal transferrin glycosylation patterns even on a background of fructose-restriction, which may be associated with the severity of hepatic fat accumulation.

O-067

Late-onset diagnosis of urea cycle disorders: results from an adult French cohort of 49 patients

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Background: Urea cycle disorders (UCD) are a group of inherited metabolic diseases caused by a deficiency of enzymes or transporters involved in the urea cycle, which is necessary to epurate organisms from ammonium. In most cases, the diagnosis is made in the first months of life, but this can sometimes appear as a late-onset revelation. Several cases are reported but there are no cohorts describing the late-onset revelation in adulthood. The aim of this paper is to describe a French UCD cohort whose diagnoses were made in adulthood.
Methods: This was a multicentre retrospective observational study done in France. Data were collected by answering a questionnaire about clinical, biological and therapeutic information. Inclusion criteria were having a UCD and being diagnosed after the age of sixteen.
Results: 49 patients were included, 31 women and 18 men. The diagnosis was made during the first hyperammonemic decompensation in 29 patients, after a screening because of a familial proband for 18 patients, and after chronic symptoms for 2 patients. The mean patient age was 37 years. 42 patients had OTCD, 3 CPSI deficiencies, 2 HHH syndromes, 1 argininosuccinic aciduria, and one with no clear diagnosis. Patients had neurological, digestive and psychiatric symptoms. 78% of the patients diagnosed because of a familial proband, were already symptomatic. During decompensations, all diagnosed patients had hyperammonemia (average level of 395μmol/L), and a triggering factor was found in 76% of cases. 5 patients died, 3 of them during their first decompensation.
Discussion: We present the largest cohort of late-onset UCD patients all diagnosed during adulthood. We confirmed the clinical spectrum previously described based on three types of symptoms (neurological, abdominal and psychiatric). The mortality rate of the cohort was 10%. UCD are life-threatening conditions that can lead to death and clinicians need to be aware that hyperammonemic crises could occur at any age.

O-068

Treatment with chenodeoxycholic acid in cerebrotendinous xanthomatosis: clinical, neurophysiological and brain structural outcomes

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Background: Cerebrotendinous xanthomatosis (CTX) is a rare neurodegenerative disease related to sterols metabolism. It affects both central and peripheral nervous systems but treatment with chenodeoxycholic acid (CDCA) has been reported to stabilize clinical scores and improve nerve conduction parameters. Few quantitative brain structural studies have been conducted to assess the effect of CDCA in CTX.
Methods: We collected retrospectively clinical, neurophysiological, and quantitative brain structural data in a cohort of fourteen patients with CTX treated by CDCA over a mean period of 5 years.
Results: Plasma cholestanol levels normalized under treatment with CDCA within a few months. We observed a significant clinical improvement in patients up to 25 years old, whose treatment was initiated less than 15 years after the onset of neurological symptoms. Conversely, patients whose treatment was initiated more than 25 years after neurological disease onset pursued their clinical deterioration. Eleven patients presented with a length-dependent peripheral neuropathy, whom electrophysiological parameters improved significantly under CDCA. Volumetric analyses in a subset of patients showed no overt volume loss under CDCA. Moreover, diffusion weighted imaging showed improved fibre integrity of the ponto-cerebellar and the internal capsule with CDCA. CDCA was well tolerated in all patients with CTX.
Discussion: CDCA may reverse the pathophysiological process in patients with CTX, especially if treatment is initiated early in the disease process. Besides tendon xanthoma, this study stresses out the need to consider plasma cholestanol measurement in any patient with infantile chronic diarrhoea and/or jaundice, juvenile cataract, learning disability and/or autism spectrum disorder, pyramidal signs, cerebellar syndrome or peripheral neuropathy.

Poster Presentations

01. Inborn Errors of Metabolism in Adults

P-001

Arterial stiffness in adults with phenylketonuria

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Background: While a negative correlation has been observed between plasma cholesterol and phenylalanine concentrations cardiovascular risk may be increased in phenylketonuria patients (PKU) due to higher prevalence of overweight/obesity and higher systolic blood pressure. The aim of this study was to assess arterial stiffness measured by applanation tonometry in adult PKU patients compared to healthy controls.
Methods / case report: We carried out a cross-sectional study in 20 PKU patients (range age: 18–50 years old) and 21 age and gender-matched healthy controls. Evaluated data included clinical and biochemical parameters. Aortic stiffness was assessed noninvasively by applanation tonometry measuring central blood pressure, aortic augmentation index (Aix), augmentation pressure (AP) and pulse wave velocity (PWV).
Results: As stated, there were no statistical significant differences between PKU patients and their matched controls regarding age or gender. We did not find any difference between groups for systolic and diastolic blood pressure (BP) means, body mass index (BMI), waist perimeter or lipid profile. AP was increased among adult PKU patients when compared to healthy controls (6.95 mmHg vs 2.8 mmHg; p: 0,043). We also found higher PWV in PKU patients (7.23 m/sec vs 6.0 m/sec; p: 0.022).
Discussion: Our data show increased aortic stiffness in adult PKU patients, measured by applanation tonometry when compared to healthy controls, independent from age, sex, BP level, obesity or lipid profile. The prognostic value of these differences in terms of cardiovascular disease still remains unclear but these results could have marked effects in

both research and clinical daily practice for a proper evaluation of cardiovascular risk in adult PKU subjects.

P-002

A combination treatment with a histidine-rich peptide and Alglucosidase alfa reduces cytoplasmic glycogen storage in liver of GSD III mice

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Background: M6PR-mediated endocytosis of recombinant human GAA (rhGAA, Alglucosidase alfa), an approved therapy for Pompe disease, limits the enzyme delivery to lysosomes. A method that can extend rhGAA delivery to the cytosol would be a feasible treatment approach for clearing cytoplasmic glycogen storage in GSD III. Recent studies showed that histidine-rich, pH-responsive LAH4 family peptides can promote cytoplasmic delivery of DNA and protein cargos in cultured cells through induction of endosomal escape.
Methods: LAH6-L1-80 (a peptide of the LAH4 family) was pre-mixed with rhGAA at a ratio (w/w) of 1:1. Ten-week-old GSD III mice were intravenously injected with rhGAA, LAH6-L1-80, or the combination of the two at a dose of 20 mg/kg twice weekly for 4 weeks. Age-matched untreated mice were used as controls. Mice were sacrificed 48 h after the last injection. Uptake of rhGAA and glycogen content were analyzed in different tissues.
Results: GAA activity was extremely high in liver but slightly increased in heart of both the rhGAA-treated and the rhGAA+LAH6-L1-80-treated mice. Western blot showed markedly increased the 110 kDa full-length rhGAA band (unprocessed cytoplasmic form) in liver lysates of rhGAA+LAH6-L1-80-treated mice, but not the rhGAA-treated mice, indicating the peptide-induced release of rhGAA from endosomes. The combination treatment reduced glycogen content only in liver (−48%), and significantly lowered the liver/body weight ratio. In contrast, treatment with rhGAA or LAH6-L1-80 alone had no effect on glycogen storage in any tissues. In addition, LAH6-L1-80 showed no significant toxicity as indicated by the unchanged plasma levels of ALT and AST (liver functions), and BUN and Creatinine (kidney functions).
Discussion: This is the first study to demonstrate the therapeutic potential of the LAH4 family peptides in an animal model. This novel treatment approach addresses the unmet need for treatment of GSD III and other cytoplasmic GSDs.
Conflict of Interest declared.

P-003

Cardiac magnetic resonance imaging and spectroscopy in patients with long-chain fatty acid β-oxidation deficiency

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Background: Cardiomyopathy can be a severe complication in patients with inborn errors of long-chain fatty acid β-oxidation (lcFAO), particularly during episodes of metabolic derangement. It has not been established yet whether functional or structural myocardial abnormalities, if any, are present from birth or develop later in life. Studies of mouse models of lcFAO deficiency and autopsies of patients suggest that myocardial lipid accumulation and diffuse fibrosis contribute to the etiology of cardiomyopathy. In this observational case–control study, we used magnetic resonance imaging (MRI) and spectroscopy (MRS) to characterize the heart of adult patients with lcFAO deficiency.
Methods: Thirteen lcFAO-deficient patients (1 female LCHADD; 4 male CPT2D; 8 VLCADD, 2 females/6 males) and thirteen age-, gender-, and BMI-matched control subjects were examined using a 3 Tesla MR system. The protocol included cinematographic MRI for the quantification of left-ventricular (LV) volumes and myocardial mass, T_1 relaxometry for the assessment of diffuse myocardial fibrosis, and proton MRS for the quantification of myocardial lipid content.
Results: In CPT2D and VLCADD patients, LV mass was 19% higher compared to matched controls. In one female VLCADD patient, this was accompanied by a low LV ejection fraction (41%) and an elevation of the myocardial T_1 relaxation time constant, indicative of diffuse fibrosis. In other patients, LV ejection fraction and other LV parameters were essentially similar to those in control subjects. Myocardial lipid content was similar in patients and controls.
Discussion: We found no *in vivo* evidence of overt lipid accumulation in the hearts of adult lcFAO-deficient patients in this cross-sectional study. Mild LV hypertrophy was present in the CPT2D and VLCADD patients. Cardiac MRI and MRS during metabolic derangement may shed light on the role of lipid accumulation and diffuse fibrosis in the development of cardiomyopathy in lcFAO-deficient patients.

P-004

Pharmacodynamic results from a randomized, multicenter, open-label study of asfotase alfa in adults with pediatric hypophosphatasia

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Background: Hypophosphatasia (HPP) is a rare inherited disease with low tissue-nonspecific alkaline phosphatase (TNSALP) activity leading to elevated plasma inorganic pyrophosphate (PPi) and pyridoxal 5'-phosphate (PLP). Adults with HPP can have osteomalacia, fractures, pain, and functional impairment. Asfotase alfa (AA) is a recombinant human TNSALP replacement therapy approved for pediatric-onset HPP.
Methods: We report pharmacodynamic and safety results of a 13-wk, randomized, open-label study of AA in 27 adults (≥18y) with a pediatric-onset HPP diagnosis, Screening PPi ≥3.9 μM, and documented TNSALP gene mutation(s) or low Screening alkaline phosphatase with elevated PLP. Pts were randomized 1:1:1 to a single dose of AA 0.5, 2.0, or 3.0 mg/kg at Wk 1, then 3x/wk starting Wk 3 for 7 wk. Doses were selected to bracket the recommended dose of 2.0 mg/kg 3x/wk.
Results: Most demographics were similar between groups (median age: 45 [18–77] y; mean weight:82 [20] kg; 96% white). Least squares

mean (LSM) changes from Baseline (BL) to Wk 9 (last predose value/ Day 61) in PPi (μM; primary outcome measure) were significant for AA 3.0 vs 0.5 mg/kg (LSM [SE]: −4.5 [0.2] vs −2.6 [0.2]; LSM difference estimate: −1.9; $P< 0.0001$) and 2.0 vs 0.5 mg/kg (−3.8 [0.2] vs −2.6 [0.2]; −1.2; $P=0.0008$). LSM changes from BL to Wk 9 in PLP (ng/mL) were significant for AA 3.0 vs 0.5 mg/kg (−338 [8.5] vs −304 [9.4]; −34; $P=0.0128$) and 2.0 vs 0.5 mg/kg (−333 [8.2] vs −304 [9.4]; −29.5; $P=0.0239$). Adverse events (AEs) of interest were injection site reactions (138 events/n=21); lipodystrophy (2/n=1); ectopic calcifications (4/n=4: kidney [n=2], eye, liver); and hypersensitivity (4/n=1). Most AEs (480/ 485) were mild/moderate. No serious AEs or withdrawals due to AEs were reported.
Discussion: Adults with pediatric-onset HPP treated with AA at or above the recommended dose had significantly decreased PPi and PLP from BL vs the lower than recommended dose. AA was well tolerated at all doses.
Conflict of Interest declared.

P-005

Case report: Pregnancy in a patient with Abetalipoproteinaemia

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Background: Abetalipoproteinaemia (ABLP) is a rare autosomal recessive disorder characterised by the inability to absorb dietary fat, fat-soluble vitamins and cholesterol. Fat-soluble vitamin requirements in ABLP are documented. However, management in pregnancy is limited to few case reports with varying vitamin doses. Balancing vitamin supplementation to prevent risks associated with hypovitaminosis whilst avoiding fetal teratogenicity or high dose complications for the mother remains challenging.
Case report: A 26 year old female, diagnosed at birth with ABLP, presented to our clinic after being lost to follow up. She was on a vegetarian, fat restricted diet (20g/day), 12,000mg vitamin E (219mg/kg), 10mg vitamin K, 31μg vitamin D and 3308μg vitamin A plus folic acid 5mg. Despite supplementation, biochemistry indicated vitamin K, A and E deficiency. Vitamin D, folate and vitamin B12 were in range. Ferritin was low. There was acanthocytosis on blood film. In response, daily doses were titrated up to vitamin E 18,000mg (328mg/kg), vitamin K 20mg, vitamin D 36μg and vitamin A 4108μg plus omega-3 supplement was added, and contraception advised. She reported an unplanned pregnancy (6-7/40 weeks). Fat-soluble vitamins were monitored and titrated regularly. However, maternal plasma vitamin E and A remained low throughout pregnancy. Pivka II improved. Vitamin D remained in range. Primary Hypothyroidism was diagnosed in the first trimester and Gestational Diabetes at 26 weeks. Both requiring drug treatment.
Results: Baby was born at 29/40 gestation and no abnormalities reported. Between 19–22 days of life, plasma fat-soluble vitamins and lipid profile levels were within range. Life-long monitoring for baby and mother was advised.
Discussion: Positive outcomes of pregnancies in ABLP have been reported with differing fat-soluble vitamin doses. Biochemical monitoring remains unreliable, particularly of vitamin E and A, in the context of very low levels of circulating lipoproteins.

P-006

Clinical situation of patients with glycogen storage disease type I in adulthood followed in Spanish referral centers.

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Background: Glycogen storage disease (GSD) is a well-known congenital error of metabolism in childhood. The purpose of this study was to analyze the clinical characteristics and complications related to the disease in adult patients with GSD.
Methods: We conducted a cross-sectional study, in which patients over the age of 18, with a diagnosis of GSD type 1, were followed in Spanish referral centers.
Results: Eleven patients were enrolled (66% male) with a mean age of 33 (ranging between 18 and 48), 64% were glycogenosis type 1a and the rest 1b. All but one of the patients were diagnosed during the first year of life. One patient was diagnosed at age 32. Main symptoms at diagnosis were hypoglycemia, hepatomegaly, and growth retardation. In all patients who were diagnosed in childhood, diagnosis was determined by liver biopsy and enzymatic study. 82% had a height below the third percentile. Six patients had hepatocellular adenomas, two of them having more than ten adenomas. 54% had proteinuria, 18% had triglycerides greater than 1000 mg/dL and 72% percent had hyperlactacidemia. Although all patients of infantile age had enteral nocturnal nutrition, only two maintained it in adulthood. All of them performed at least one night-time shot with a maximum fasting time of 6 hours. Half of the patients performed a regular dietary regimen. 57% of patients had osteoporosis, with half of these being serious.
All patients with glycogenosis 1b were treated with granulocyte colony-stimulating factor and they all had a history of infections. Three patients had chronic liver disease with portal hypertension. All of the patients had a quality of life partially limited by the disease. Three patients had formed a family and only the patient with the late diagnosis had children.
Discussion: Glycogenosis type 1 requires adequate metabolic control. The majority of patients in adulthood have a reduced quality of life and present with significant clinical complications.

P-007

An adult with late onset ataxia

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Background: Neuronal Ceroid Lipofuscinosis (NCLs), are a group of inherited neurodegenerative disorders mostly affecting children. These are characterized by the intralysosomal accumulation of cytoplasmic lipopigments in both neural and peripheral tissues. Clinically these patients present with progressive epilepsy, cerebellar ataxia, visual impairment, behavioral disturbances, mental deterioration and early death in childhood.
Methods / case report: A 45 year old man presented with history of slurred speech and unsteady gait for three years. MRI brain showed cerebellar atrophy and no cerebral atrophy. He had no

cognitive decline, no visual symptoms and no history of seizures. Genetic testing revealed a novel homozygous mutation in *CLN5*: c.562T>C; p.F188L, confirming adult onset neuronal ceroid lipofuscinosis (CLN5).
Results: We report an adult patient with Neuronal Ceroid Lipofuscinosis who presented late in the fourth decade of life.
Discussion: Adult onset cerebellar ataxia may pose a diagnostic challenge due to genetic heterogeneity. We identified a novel pathogenic mutation in *CLN 5* in our patient. Although most *CLN5* mutations cause the disease during childhood, this case indicates that NCL should be suspected in adult onset neurodegenerative diseases.

P-008

Management of PKU during pregnancy case study – the use of anti-emetics for hyperemesis gravidarum

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Background: Unplanned PKU pregnancy complicated by hyperemesis gravidarum.Management of PKU during pregnancy (mPKU) is highly challenging and of critical importance. Phenylalanine levels are tightly controlled in pregnancy to minimise mPKU syndrome and associated foetal abnormalities. At CDMU our phenylalanine target range is 120-300umol/L, with an ideal range of 120-250umol/L.
Methods / case report: There are no published PKU specific guidelines on the management of nausea and vomiting in pregnancy. A step by step approach of the 2017 NICE guidelines on hyperemesis gravidarum was adapted to exhaust management options, including diet, thrice weekly bloodspots and medications.
Results: The unit was made aware of unplanned pregnancy on 28/12/2017 at 3–4 weeks gestation, with a baseline phenylalanine of 466umol/L. Phenylalanine at perceived conception: 722umol/L. The patient was reduced to 0 exchanges and commenced on four amino acid supplements with 20g protein equivalent and prescribed low protein foods. Nausea, poor oral intake and 2.5kg weight loss was reported on 09/01/2018 with phenylalanine levels of 471umol/L. First trial of anti-emetics prescribed a day later. Three different medications were trialled and aggressive dietary intervention was commenced. On 03/02/2018 phenylalanine levels were within target range of 120-300umol/L for the first time.
Discussion: Hyperemesis gravidarum can cause catabolism, urinary ketones and poor nutritional status, which can impact phenylalanine control and prevent optimum foetal outcomes. A combination of aggressive dietary input and the use of antiemetics can aid phenylalanine control by managing symptoms of nausea and vomiting.
Conflict of Interest declared.

P-009

Diagnostic Difficulties in Late Onset Pompe Disease: The Importance of a Multi-Disciplinary Approach

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Background: Pompe disease is an inherited multisystemic disorder caused by deficiency of lysosomal alpha-glucosidase (GAA), leading to lysosomal glycogen accumulation. Enzyme replacement therapy is available, with early treatment being vital to prevent irreversible complications. However, diagnosis may be delayed by up to several years in in late onset disease
Case report: A 72 year old lady was admitted for investigation of breathlessness and recurrent lower respiratory tract infections. She had been well up until her mid 50s when she presented with walking difficulties and progressive muscular weakness which was attributed to limb girdle muscular dystrophy. On admission, type 2 respiratory failure with significant nocturnal desaturation was noted and her swallow was impaired. Her recent presentation prompted reassessment of her diagnosis and GAA analysis was requested in leukocytes and dried blood spots
Results: In leukocytes, the results were inconclusive- lysosomal GAA activity was normal and the ratio of lysosomal to total GAA activity was only slightly lower than the unaffected range. However, in dried blood spots the lysosomal GAA activity and the ratio of lysosomal to total GAA activity were both markedly reduced– consistent with Pompe disease. Urinary glucose tetrasaccharide was elevated (12 umol/mmol creatinine; normal < 5) which helped confirm the diagnosis. Subsequent genetic analysis revealed heterozygous mutations in the GAA gene- one known pathogenic mutation and one novel missense mutation
Discussion: This case demonstrates some of the difficulties encountered in establishing a timely diagnosis in late onset Pompe disease including large variability in phenotypic presentation, relatively high residual enzyme activity and genetic heterogeneity. Good communication between the lab and clinical team is essential to interpret results in context. Urinary glucose tetrasaccharide is a simple and non-invasive test that can be helpful in corroborating a diagnosis

P-010

Education and training in adult metabolic medicine: results of an international survey

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Background: Adult metabolic medicine is a new branch of medical science, progressively expanding due to the increasing prevalence of adult patients with inborn error of metabolism (IEM). However, a formal academic education in this field is still lacking in the majority of countries, and adult metabolic specialists (AMS) have different backgrounds.The aim of this survey was to assess the state of the art about education and training in adult metabolic medicine worldwide.
Methods: All the AMS included in the mailing list of the SSIEM adult metabolic group (89 members) were asked to take part in this survey, carried out between July and December 2017.Data were collected by a questionnaire, consisting in 11 questions, divided in 3 sections (personal curriculum, existing education and training, competence of an adult metabolic specialist).
Results: Forty-two AMS from 18 different countries completed the questionnaire. Of whom, 47.6% were working in this field for more than 10 years, 35.7% for 5–10 years, 16.7% for less than 5 years. The more common specializations were internal medicine (38.1%), endocrinology (26.2%), genetics (21.4%) and neurology (21.4%). Practical experience in their own Unit was indicated as important or very important in their personal curriculum as AMS by 95% of participants, while formal academic education only by 27%. The current state of available education and training was judged as generally poor or fair by 73%. The best voted (reported) ways to improve it were in order: facilitate international internships (considered very important or important

by 97%), implement courses on Adult-IEM (85%), and create a formal academic education (78%).
Discussion/conclusions: Worldwide, current available education and training in adult metabolic medicine is considered insufficient. This survey may represent a basis for developing new programmes to improve the knowledge and the competencies in this field in the next future.

P-011

Unraveling a Neurometabolic Hereditary Spastic Paraplegia: B4GALNT1 Deficiency affecting Glycosphingolipid Biosynthesis

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Background: Hereditary spastic paraplegias (HSPs) comprise a complex and heterogeneous group of neurological disorders. Although majority of cases of HSPs are due to genes involved in axonal growth or vesicular trafficking, there is an overlooked group of HSPs that are caused by inborn errors of metabolism (IEMs).Adrenomyeloneuropathy, late-onset biotinidase deficiency, cerebrotendineous xanthomatosis are among relatively well known metabolic causes of HSPs. Objective: To report a new hereditary metabolic cause of HSP in a Brazilian family caused by enzyme deficiency of beta-1,4-N-acetyl-galactosaminyl transferase 1 (B4GALNT1), involved in ganglioside biosynthesis.
Methods: After excluding the traditional IEMs associated with HSPs and molecular analysis of SPG11 and SPG15 genes, whole exome sequencing (WES) was performed.All sequencing results were imported and analyzed by the GEnomes Management Application (GEM.app).
Results: Mutations in the B4GALNT1 gene (in the SPG26 locus) were identified in all affected patients in the family; parents were heterozygous carriers of the mutation. Patients affected by this disease have early onset spastic paresis, mild intellectual disability, cerebellar ataxia, strabismus and some develop psychiatric symptoms. Male hypogonadism was also noticed. Brain MRI showed non specific white matter changes in older patients.
Discussion: Although there are many IEMs involved in ganglioside catabolism presenting as neurodegenerative disorders, this enzyme deficiency is the second human disorder identified in the pathway of ganglioside biosynthesis, suggesting that other human diseases can be caused by metabolic errors in this biochemical pathway.

P-012

Neurodegenerative markers and structural brain atrophy in adult PKU patients

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Background: Adult PKUpatients might have an increased risk of neurodegenerative disorders given the co-presence of risk of phenylalanine aggregation, metabolic abnormalities and oxidative damage. The aim of the study was to evaluate neurodegenerative markers and cortical thickness in adult PKU patients.
Methods / case report: Inclusion criteria: classical PKU (Phe levels >1200 μmol/l) patients older than 30 years and age-matched controls (Ctrl, n=30). Assessment: neurological evaluation, neuropsychological testing, 3T structural and functional MRI (analysed on single-subject, by voxel-based morphometry-VBM and using cortical thickness technique), sensory and motor evoked potentials, blood and urine analyses. Cerebrospinal fluid (CSF) concentrations of neurodegenerative markers were also evaluated in a subset of patients who underwent lumbar puncture.
Results: A total of21 early treated PKU patients (mean age 38.5 + 5.7 y, range 30–46 y) with different dietary treatment regimens entered the study. Motor, autonomic and cognitive abnormalities were more common in adult PKU patients compared with age-matched controls. This early features and the Parkinson's disease (PD) prodromal risk correlated with blod Phe levels. CSF analyses in 12 patients showed no Alzheimer specific alteration (Tau/Abeta42 ratio). VBM and cortical thickness analyses showed grey matter atrophy in putamen, bilateral frontal and temporal structures which correlated with Phe blood levels.
Discussion: The pilot study argues for an increased risk of a non-AD brain damage in adult patients with phenylketonuria. The findings suggested a possible relationship between poor metabolic control and neurodegenerative markers. Larger studies are pivotal in order to understand the role of life-long metabolic control, genetic variability and environmental factors as modulators of brain damage in adult PKU patients.
Conflict of Interest declared.

02. Novel diagnostic/laboratory methods

P-013

Diagnosing mitochondrial disease by high resolution respirometry

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Background: Mitochondrial diseases are an eclectic collection of disorders, and to use the cliché can affect any organ, with any severity, at any age. Diagnosing these disorders has been a huge challenge, owing to their overlapping symptoms with many other disorders, irregular genetics, and difficulty in accessing affected tissues (primarily brain, skeletal muscle, liver). The golden approach of old to diagnosing these disorders has been to analyse the activity of the individual oxidative phosphorylation (OXPHOS) complexes by spectrophotometric methods. While this technique has served the field well, it can be invasive, costly, time consuming, and even in primary tissues is prone to generating false negative results. Here, we describe an alternative biochemical test for this disease – high resolution respirometery.
Methods: Genetically defined mitochondrial disease patient and control fibroblasts were cultured in DMEM media with 10% foetal calf serum containing 1g/L glucose. We then accessed the respiratory capacity of these cells under different conditions to measure the activity of the OXPHOS system.
Results: In 5 patients with an isolated OXPHOS deficiency (4 Complex I (CI), and 1 Complex IV (CIV)), respiratory capacity was clearly reduced compared to controls in all patients. By comparison, of the 4 patients with an isolated CI deficiency, only 2 showed reduced activity by classical spectrophotometric analysis. Of the 5 patients with a combined OXPHOS deficiency, 4 showed clearly reduced respiratory capacity compared to controls,

while 1 patient (with mild very symptoms) appeared normal. However, of significance, multiple measures (≥3) were required (performed over different days) to reduce the intra-sample variation.
Discussion: If intra-sample variation can be reduced, this technique proffers a new path to diagnosing mitochondrial diseases that is quicker, cheaper, less invasive, and more sensitive than the established spectrophotometric methods.

P-014

Hydrodynamic transfection of porcine hepatocytes in newborn (weaned) pigs with non-viral naked-DNA vectors

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Background: Hydrodynamic gene delivery to hepatocytes is an experimental approach that is established to treat mouse models for hepatic diseases but is also efficient for vector delivery in larger animal models. We have previously established hydrodynamic intraportal (retrograde) injection in newborn (domestic) pigs with transduction of up to 60% of liver hepatocytes. Here we improved this procedure and developed a surgical method that allows vector administration via the bile duct as a potential alternative.
Methods / case report: For intraportal and intrabiliary (hydrodynamic) injection of vector solution in at least 6 pigs, median laparotomy was performed, including transient clamping of V. portae, V. cava caudalis, and Aa. hepaticae. For intraportal injection, incision of V. portae was required to insert a catheter. For intrabiliary injection, access via the hepatic duct was established by anti-mesenterial enterotomy at the level of the papilla duodeni major and insertion of a catheter through the papilla that was moved forward to the common hepatic duct. In this procedure, common bile duct and cystic duct were clamped additionally. For both procedures, pressure conditions for injections were 10 ml/sec of a total volume of 100 ml containing between 2–12 mg of DNA of a luciferase-expressing vector. All vessels remained clamped for one minute after injection.
Results: Increasing the vector dose to up to 12 mg resulted in 100% transfection of hepatocytes upon intraportal injection. In comparison, we found with the biliary injection method thus far a transduction rate of 85% by delivering 2 mg of vector DNA.
Discussion: While further optimization of hepatocyte transfection via the biliary access is still needed, the here described intrabiliary injection procedure is superior or safer over the intraportal access. An intrabiliary approach in addition has the potential for (non-pressurized) injection and would be even less invasive with an endoscopic retrograde access.

P-015

Analysis of urinary free catecholamines, metanephrines and its end-products vanillylmandelic acid and homovanillic acid by UPLC-MS/MS

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Background: HVA (homovanillic acid) represents the end-product of dopamine metabolism, whereas VMA (vanillylmandelic acid) is the end-product of norepinephrine and epinephrine metabolism. Analyses of urinary HVA, VMA, catecholamines [epinephrine (E), norepinephrine (NE), and dopamine (D)] and their O-methylated metabolites [3-methoxytyramine (3-MT), normetanephrine (NME) and metanephrine (ME)] are commonly requested as a first-line investigation and therapy control for neuroblastoma. In addition, analysis of these metabolites is requested for detecting abnormalities in dopamine metabolism.
Methods:After dilution and filtration of the urine, HVA and VMA were analysed using UPLC-MS/MS with a 0.025% formic acid/acetonitrile gradient (5.5 min). Catecholamines and metanephrines in urine were analysed using a 0.1% formic acid/ methanol gradient (4 min) aftersample clean up using solid phase extraction (OASIS WCX). Chromatographic separation for both methods was achieved using a Waters Acquity UPLC HSS T3 column. 550 μl urine is sufficient for quantification of all compounds.Both developed methods were compared with other methods (HVA and VMA: GC/MS and HPLC, catecholamines and metanephrines: HPLC-Fluorescence and UPLC-MS/MS).
Results:Accuracy (based on reference material) was between 88 and 113%. LOQ of all metabolites were far below reference values. Within run precision ranged from 2.3-17.2% and between run precision ranged from 5.6-11.7%. Correlation between methods was good although for some metabolites a small but significant difference exist. Age related reference values of all metabolites were established.
Discussion: Developed UPLC-MS/MS methods are simple, rapid with excellent sensitivity and accuracy allowing incorporation into a routine clinical setting.

P-016

Earwax – a potentially useful medium to identify inborn errors of metabolism (IEM)?

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Background: Post mortem (PM) diagnosis of metabolic disease in sudden infant death syndrome (SIDS) patients based upon the levels of characteristic metabolic markers in tissues is complicated by PM changes. We have considered the potential for earwax as a source to gain a reliable biochemical insight into SIDS patients suspected of having an IEM.
Methods: Acylcarnitines, amino acids and guanidino metabolites were measured in ear wax from 29 treated patients with 12 different metabolic disorders including 3 organic acidaemias, 2 fatty acid oxidation defects, 6 amino acid disorders and 1 peroxisomal abnormality.
Results: The ratio of different acylcarnitine species relative to free carnitine enabled discrimination of isovaleric acidaemia, methylmalonic acidaemia and long chain hydroxyacylCoA dehydrogenase deficiency from the other disorders.
For amino acids neither creatinine nor alternative amino acid proved suitable reference standards against which results could be expressed. However argininosuccinate and alloisoleucine were present in significantly elevated concentrations in 2 patients with argininosuccinate lyase deficiency and 2 patients with branched chain ketoacid dehydrogenase deficiency.
Discussion: Although diagnostically raised for specific disorders the correlation of the characteristic markers present in ear wax with those in blood or urine was poor. This may be due to (1) blood and urine reflecting the time of sampling concentration whereas ear wax represents longer term changes (2) the absence of suitable reference standard for expression of results (3) the limited number

of patients investigated here and (4) influence of transportation of metabolites into secreted ear wax.
In conclusion ear wax has potential to be of significant use in PM studies where diagnosis of an IEM is suspected. In view of its limited invasiveness, earwax also may have potential as a diagnostic material in living patients and to monitor treatment responses and compliance.

P-017

Diagnostic exome sequencing in neurometabolic disorders

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Background: Molecular diagnoses of inherited neurometabolic disorders (NMDs) are challenging due to their genetic and clinical heterogeneity. Especially in inborn errors of metabolism an early identification is crucial for treatment decisions. Though next generation sequencing-based genetic testing is scientifically established in European countries, concerted efforts of researchers and clinicians are needed to promote the potential benefit of whole exome / genome sequencing (WES/WGS) as a first line diagnostic tool. We here report on the results of WES-based diagnostics of a cohort of 97 index cases to evaluate the diagnostic utility of WES.
Methods: Inclusion criteria were suspected NMDs based on biochemical or radiological findings. Coding genomic regions were enriched for sequencing as 2x125 bp/2x100 bp paired-end reads on a Illumina HiSeq2500/NovaSeq6000 system. Generated sequences were analyzed using the megSAP pipeline. Clinical variant prioritization included filtering steps according to an in-house operating procedure.
Results: In 44 (46%) cases, we identified likely pathogenic or pathogenic variants in genes that have been associated with NMDs (35) or other neurodevelopmental disorders (9). In 36% of these targeted therapy was available; in 33% the established diagnoses implicated a modification of clinical management. In 31% of cases however the disorders were not amenable to targeted therapy.In 29 cases (30%) we identified variants of unknown significance were follow-up studies for functional analyses are pending. Moreover we newly identified 5 candidate genes. However 18 cases (19%) remain unsolved.Ongoing investigations of the latter in a research setting include WGS and transcriptome sequencing.
Discussion: WES is established as a potent first line diagnostic tool for neurometabolic disorders. Although presenting with a wide phenotypic spectrum WES facilitated a definite diagnosis in 46% and a possible diagnosis pending follow-up in 30% of the cases.

P-018

Quantification of mono-unsaturated very long chain fatty acids in plasma of patients with peroxisomal disorders by LC-MS/MS

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Background: Saturated very long chain fatty acids (VLCFAs) accumulate due to defects in peroxisome functions. Analysis of these markers in plasma is the primary method for investigating a multitude of peroxisomal disorders. While mono-unsaturated VLCFAs (i.e. C26:1, C24:1) are also relevant to peroxisomal disorders, these metabolites are not routinely measured. We present a rapid and specific LC-MS/MS method for the simultaneous measurements of saturated and mono-unsaturated VLCFAs markers of peroxisomal diseases.
Methods: C26:1, C24:1, C26:0, C24:0, C22:0, pristanic and phytanic acids in plasma (20 microliter) were extracted and derivatized with DAABD-AE. Derivatization was undertaken to improve the chromatographic and mass spectrometric properties of these analytes. DAABD derivatives of fatty acids were separated on a reversed phase column and detected by positive ion electrospray ionization tandem mass spectrometry with a 5 min run time. Deuterium labelled stable isotopes were used as internal standards.
Results: C26:1 and C24:1 were quantified using calibration curves prepared with standard material of C26:0 in the range of 0–15 micromol/L and C24:0 in the range of 0–245 micromol/L, respectively. Using control plasma (n=115), the 95%ile reference interval (median) of C26:1 was 0.1-4.4 (1.7) micromol/L whereas that of C24:1 was 54.1-142.8 (93.7) micromol/L. In a patient with classical Zellweger phenotype, C26:1 and C24:1 were at 13.5 and 51.2 micromol/L, respectively. In another patient with an attenuated peroxisomal presentation, C26:1 was elevated at 6.3 micromol/L whereas C24:1 was within normal at 70.7 micromol/L.
Discussion: This method allows for detecting mono-unsaturated VLCFAs C26:1 and C24:1 in the analytical run that measures simultaneously C26:0, C24:0, C22:0, pristanic and phytanic acids in plasma. Further evaluation of the diagnostic utility of these markers is under investigation.

P-019

Persistent hypoglycemia in children: targeted gene panel improves the diagnosis of hypoglycemia due to inborn errors of metabolism

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Background: To evaluate the impact of Next Generation sequencing in genetic diagnosis of pediatric patients with persistent hypoglycemia due to inborn errors of metabolism.
Methods / Case report: Sixty-four patients investigated through an extensive workup were divided in 3 diagnostic classes: a) single candidate gene (9/64), b) multiple candidate genes (43/64) and c) no candidate gene (12/64). Subsequently, patients were tested through a custom gene panel of 65 targeted genes, which included five disease categories: i) hyperinsulinemic hypoglycemia (HI), ii) fatty acid-oxidation (FAOD) and ketogenesis defects, iii) ketolysis defects, iv) glycogen storage diseases (GSDs) and other disorders of carbohydrate metabolism, and v) mitochondrial disorders. Molecular data were compared with clinical and biochemical data.
Results: A proven diagnosis was obtained in 78% of patients with suspicion for a single candidate gene, in 49% with multiple candidate genes and in 33% with no candidate genes. The diagnostic yield was 48% for HI, 66% per FAOD and ketogenesis defects, 59% for GSDs and other carbohydrate disorders, and 67% for mitochondrial disorders.
Discussion: This approach provided a diagnosis in about 50% of patients in whom clinical and laboratory evaluation did not allow to identify a single candidate gene**.** Biochemical tests are pivotal for the diagnosis of inborn errors of metabolism as they can orientate genetic testing. Remarkably, a molecular diagnosis was established in 33% of patients belonging to the no candidate gene class. In conclusion, our study shows that NGS technique is cost-effective compared to Sanger sequencing of multiple genes, and represents a powerful tool for the diagnosis of inborn errors of metabolism presenting with persistent hypoglycemia.

P-020

Evaluation of NMR analysis of urine for the diagnosis of organic acidurias and aminoacidopathies

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Background: Biochemical diagnosis of inborn errors of metabolism (IEM) involving small molecule metabolism, is based on detection of specific metabolites using GC-MS and HPLC. Because it is based on the capacity to make a more global analysis of metabolites, NMR Spectroscopy (^{1}H-NMR), is an alternative for an initial diagnostic approach for IEMs. This work provides evidence on specific profiles for patients with organic acidemias and aminoacidopathies compared to controls, using NMR-400 MHz.
Methods: 36 controls and 17 pathological urine samples (180μL KH_2PO_4 buffer 1,5M pH 7.0; 540μL of urine sample with internal standard TMSP-d4 0,5Mm) were studied using a 400 MHz Bruker Avance III. Data was analyzed using MESTRENOVA[10] and SIMCA P-14. Finally, assignment of a metabolite's chemical shift was done by comparing with literature and databases like HMDB, BMRB and others.
Results: NMR-400 MHz, urine analyses allowed clear discrimination between healthy and affected individuals. Characteristic metabolites were observed for most pathologies evaluated and even some metabolites that are not detected by GC-MS or HPLC like isovaleryl-alanine and ketoleucine in IVA and MSUD, respectively, which only appear in the acute state of these diseases. Additionally, we described the ^{1}H-NMR profile of holocarboxylase synthetase deficiency, a disease for which in our knowledge, there are no reports of ^{1}H-NMR specific profiles.
Discussion: Our results evidenced that, although NMR-400 MHz analyses of urine samples were able to detect profiles highly suggestive of IEM and even distinguish among different states of the disease, it may be insufficient to establish a specific diagnosis in cases where ^{1}H-NMR profile may be inconclusive. Based on our observations, we consider that these analyses may be useful to strengthen the diagnostic impression before using gold standard techniques, which can save time and money, especially when differential diagnoses include various groups of IEM.

P-021

Interference of urinary bile acid profiling by propofol

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Background: Urinary bile acid analysis is routinely used to screen for cerebrotendinous xanthomatosis (CTX), an autosomal recessive disorder caused by CYP27A1 mutations. Recently, we identified a urinary bile acid profile suggestive of CTX in a boy with surgery for an epidural hematoma. Plasma was not available. Although not symptomatic, therapy with chenodeoxycholic acid was started. However, bile acid profile was normal in a second pre-medication sample and no pathogenic CYP27A1 mutations were found. Similarly, a CTX bile acid profile was identified in a boy undergoing endoscopy for failure to thrive and diarrhea. Bile acid excretion and plasma cholestanol were normal in previous samples from this patient. Both boys underwent propofol (2,6-diisopropylphenol) sedation prior to urinary sampling and because propofol is metabolized via cytochrome P450 enzymes, we hypothesized that the bile acid profiles were caused by pharmacological inhibition of CYP27A1 activity by propofol.
Methods / Case report: We analyzed bile acid profiles in urinary samples from 5 patients after sedation with propofol 8-12mg/kg/hour for at least 4 hours during surgery for idiopathic scoliosis.
Results: Similar to our cases, patients with propofol sedation showed bile acid profiles suggestive of CTX.
Discussion: Although many pharmacological agents are known to affect cytochrome P450 enzyme activities, we are not aware of any prior study showing that propofol inhibits CYP27A1, resulting in a diagnostic footprint for CTX. The clinical impact is clearly illustrated by our cases. Propofol is one of the safest and most commonly used anesthetic agents and urethral catheterization during anesthesia offers an opportunity for urinary sampling for metabolic screening. Misdiagnosis with CTX has significant impact. CTX is a severe progressive disorder that requires lifelong expensive treatment. To prevent further misdiagnoses, we suggest to add propofol to the list of known interferences of bile acid profiling.

P-022

New technologies in a clinical setting: metabolic diagnostic screening using untargeted metabolomics

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Background: The recent evolvement of untargeted metabolomics creates the opportunity to profoundly advance diagnostics of inborn errors of metabolism (IEM). We investigated the use of direct-infusion high-resolution mass spectrometry (DI-HRMS) in metabolic diagnostics.
Methods: Samples were analysed by DI-HRMS, collecting mass over charge (m/z) 70–600 in positive and negative mode. A workflow was designed, reducing ~186,000 unidentified m/z peaks to an easily interpretable sorted list of ~3,800 endogenous metabolites. To demonstrate the diagnostic value, 91 plasma samples of 40 patients and 114 dried blood spots (DBS) of 46 patients, with 24 different IEM that are generally diagnosed on altered concentrations of hydrophilic small-molecule metabolites in blood, were included. Non-metabolic patients were included to assess whether these could be distinguished from IEM patients. All patients were assigned a "most probable diagnosis" in a prospective fashion.
Results: 46/46 patients were prospectively assigned the correct diagnosis based on DBS and 35/40 on plasma samples. 6/8 non-metabolic patients were correctly assigned "no IEM" as diagnosis based on DBS and 2/7 on plasma samples. Prediction based on supervised clustering analysis, performed to better distinguish IEM from "no IEM", resulted in negative predictive values of 85% for DBS and 82% for plasma.
Discussion: We demonstrate that next to solely recognizing IEM biochemical patterns in untargeted metabolomics data, also prospectively diagnosing IEM is readily achievable. Therefore, our DI-HRMS untargeted metabolomics workflow for plasma and DBS

can be implemented as an initial screening tool in the diagnostic work-up of patients suspected of IEM, requiring only limited confirmational analyses.

P-023

In vivo Raman micro-spectroscopy: a new way to screen Fabry disease

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Background: Fabry disease is a multisystemic X-linked lysosomal storage disorder caused by a deficiency of α-galactosidase and resulting in the accumulation of glycosphingolipids in several organs such as skin. Some screening methods are available to determine the activity of this enzyme from a variety of sources such as blood and urine. However, besides to be expensive, these conventional techniques are time-consuming and can be invasive.
Methods: Thus, our study aims to use Raman micro-spectroscopy, coupled to a confocal micro-probe, as a non-invasive and label-free technique in order to diagnose the pathology directly on the skin of Fabry patients. To do so, 20 healthy volunteers and 21 Fabry patients participated in this study. *Stratum corneum* thickness was evaluated on protected arm site by using a confocal Raman micro-probe.
Results: Principal Component Analysis was used and permitted to separate the healthy volunteers from the Fabry patients. Moreover, the principal components were analyzed and showed the spectral vibrations responsible of this discrimination. Thereafter, a variable selection algorithm, called *randfeatures*, was used to determine the most spectral discriminant vibrations between two groups of spectra collected respectively from healthy volunteers and Fabry patients. Finally, specific spectroscopic markers associated to the lipid compacity and secondary structure of proteins were assessed.
Discussion: This pilot study allowed to assess the efficiency of the remote confocal Raman micro-probe for the *in vivo* diagnosis of Fabry disease. In the future, this tool would permit a better detection of the prevalence and the severity of Fabry disease.

P-024

Next-generation metabolic screening (NGMS): a diagnostic tool in the functional genomics laboratory

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Background: The implementation of 'Next Generation Sequencing' (NGS) in clinical routine generates an increasing need for functional data to evaluate the pathogenicity of variants discovered. To provide the required functional information, we have developed a metabolic complement to NGS, which we have termed '*Next Generation Metabolic Screening*' (NGMS).
Methods: Plasma samples of individual patients comprising 46 different Inborn Errors of Metabolism (IEMs) and controls were analyzed by time-of-flight mass spectrometry (UHPLC-QTOF-MS) analysis. Automated chemometric analysis identifies significant features (signals) in the data and metabolite annotation is performed using the HMDB database. For diagnostic evaluation, a dataset filter was created consisting of a targeted panel of 340 diagnostic metabolites associated with IEMs. This filter was applied to the untargeted dataset to obtain the semi-quantitative intensities of these 340 metabolites.
Results: The individual patient samples analysed against reference samples and the complete dataset was filtered for the 340 metabolites. We were able to establish the correct diagnosis in 42 out of 46 different IEMs tested, that comprised e.g. amino acid disorders, fatty acid oxidation defects, organic acidurias and purine/pyrimidine disorders. In addition, this dataset could be evaluated for metabolite perturbations to provide diagnostic information after identification of genetic variants of uncertain significance (VUS) in metabolic pathways.
Discussion: We present a single-platform, untargeted, high-resolution, LC-QTOF, metabolomic profiling method, termed NGMS, which can be successfully applied for diagnosing a substantial spectrum of IEMs in individual patients. We demonstrate that it bridges genetic and metabolic diagnostics, provides complementary information, which illustrates that NGMS is a promising novel technique in the modern, functional genomics laboratory.

P-025

Detection of large genomic deletions by massive parallel sequencing in inherited metabolic disorders

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Background: Recent developments in high-throughput sequence capture have made next generation sequencing (NGS) feasible for use in routine genetic diagnosis. The improvement of the depth coverage allows the detection of large genomic deletions in addition to single-nucleotide variants. The aim of this work is to test the application of NGS for detection of genomic deletions in metabolic disorders.
Methods/Case Report: Two NGS panels were used in this study. Panel 1 is an in-house, targeted, customized exome sequencing panel to capture the exome of 120 genes involved in metabolic disorders. This panel includes the entire sequence of the genes *PAH, ALDOB, OTC, SLC22A5, GLDC and PCCA*. Panel 2 is an extended panel that includes all the known (in 2013) disease-associated genes described in the OMIM database (Mendeliome panel).
Results: Using these two panels we were able to detect large deletion in 20 patients by quantification of the coverage and comparison between controls and cases. The analysis detected 17 different large deletions in 15 different genes (*SLC7A2, GK, ALG1, MLYCD, PHKA2, BCKDHA, GCSH, IVD, ASS1, PAH, GLDC, ACADM, DBT, PDHX and BCAT2*), among which ten are novel genomic deletions. Further characterization by long range PCR was conducted and the results showed that 30% of deletions detected involved *Alu* sequences. Furthermore, two cases presented in addition an *Alu* insertion in *SLC7A2* or *PHKA2* not identified by either panels used because of the incorrect alignment of the inserted *Alu* with the reference genome.
Discussion: In conclusion, NGS is able to detect genomic deletions but further methods are needed for precise characterization

P-026

Changes in plasma acylcarnitine concentrations in children upon fasting

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Background: Childhood fasting intolerance is a common, life-threatening problem associated with various inborn errors of metabolism. Plasma acylcarnitine (AC) profiles reflect fatty acid oxidation status and help determine fasting intolerance etiology. Fasting-induced changes in pediatric AC concentrations have not been described before, complicating the interpretation of stress samples.
Methods: Retrospective analysis of 48 children who underwent a supervised clinical fasting study between 01/2005 and 12/2012, but who could otherwise be defined as apparently healthy. Children were grouped according to age: group A, aged 0–24 months (n=13; mean 16m); group B, aged 25–84 months (n=23; 49m); and group C, aged ≥85 months (n=12; 131m). Median and 2.5th to 97.5th percentiles of fasting parameters and per AC were determined at the start and end of testing and analyzed for significant differences ($p< 0.05$).
Results: Hypoglycemia occurred in 21%, mostly in group A and B. In all groups, ketonemia and 'glucose x KB' significantly increased upon fasting, while FFA/3-HB and FFA/KB significantly decreased. End ketonemia, 'glucose x KB' and end FFA levels were significantly lower in older children. Upon fasting, all groups showed a significant decrease in free carnitine and C3, while C2, C6, C12:1, C12, C14:1, C14, C16:1 and C16 significantly increased. End fasting concentrations of C6, C12:1, C12, C14:1, C14, C16:1, C16 and C18:1 were significantly higher in younger children.
Discussion: We described fasting-induced changes in plasma AC concentrations. The course of counter-regulatory mechanisms upon fasting seems age-dependent with younger children demonstrating an earlier FFA mobilization and ketogenesis. This influences the degree of accumulation per AC. Our data enables improved interpretation of stress samples to distinguish normal from abnormal fasting responses. In patients, it can refine the assessment of the minimal safe fasting time or treatment response.

P-027

Development and evaluation of an LC-Orbitrap MS method for untargeted metabolomics of dried blood spots

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Background: Reliable analysis of biomarkers is essential for correct diagnosis and monitoring of IEMs, requiring thorough method development and evaluation. We have previously developed an LC-Orbitrap MS method for untargeted metabolomics of dried blood spots (DBS). Methods: This method has been substantially improved and simplified using only one DBS punch, extraction with 80% aqueous methanol with formic acid (mix at 700rpm, 45°C, 45min). A mobile phase gradient and analysis time of 27,5 min ensures sufficient separation while maintaining good signal intensity (scan range *m/z* 50–750, resolution 70 000, electrospray 3.5 kV).The method is included in research protocols and will be used to detect differences between healthy controls and patients with various IEMs to evaluate existing biomarkers and possibly identify new and better ones. For assessment of the DBS method's sensitivity in detecting metabolic changes, we conducted an experiment with controlled diet and 36 hours of fasting in six healthy volunteers.
Results: Analytical evaluation revealed excellent results (retention time variation 0.2 % and peak area variation 1–5 % for all analytes). The controlled diet experiment showed that fasting induced changes in the metabolome as well as clustering of results in Principal Component Analysis plots from healthy volunteers when changing from a free to a controlled diet. This demonstrates that the DBS-metabolome is significantly affected by diet and that the method developed is suitable to identify metabolic changes.
Discussion: The DBS metabolomics method showed excellent analytical performance and ability to identify changes in the blood metabolome reflecting altered physiologic states induced by dietary intervention. The method will be used in research to characterize metabolic states and changes in disease, controlled intervention and during normal daily life activities in order to identify better biomarkers for diagnosis and monitoring of patients with IEMs.

P-028

A novel method to measure heparin sulphamidase activity in plasma and CSF.

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Background: Mucopolysaccharidosis IIIA is caused by the deficiency of the enzyme Heparin Sulphamidase (N-sulphoglucosamine sulphohydrolase, SGSH), due to mutations in the SGSH gene, and resulting in storage of heparin and heparan sulphate. Because MPS IIIA is a neurological condition the current treatment options are limited, with significant challenges in delivering treatment to the brain. A potential therapeutic approach is to deliver replacement enzyme through gene therapy. As part of treatment efficacy assessments SGSH activity in leucocytes, plasma and CSF should be measured in patients. We present a novel validated assay to measure SGSH activity in plasma and CSF.
Methods / Case Report: SGSH activity was measured with the fluorogenic substrate4-Methylumbelliferyl-alpha-D-N-sulphoglucosaminide using a modification of the published protocol. Briefly the substrate buffer was modified and samples were de-salted prior to analysis.
Results: Measured activity of SGSH in all samples increased by ~60-80% following de-salting prior to analysis. There was significant loss of SGSH activity in CSF samples following multiple freeze-thaw but not in plasma. The assay showed acceptable repeatability (< 10%) and reproducibility (< 15%) in plasma and CSF. Activity in CSF in unaffected individuals is relatively low but detectable (1.3-5.0 nmol/ml/17hours, n = 11). Activity measured in plasma from patients with I-Cell disease is much higher (96–318 nmol/ml/17 hours, n = 15).
Discussion: The assay has been fully validated to ISO15189 standards. Removal of phosphates and sulphates by de-salting enhances activity significantly. SGSH activity is not stable in CSF; samples must be frozen and only thawed once for analysis. SGSH activity is normally present at low levels in plasma and CSF; however the assay remains functional at supranormal levels.
Conflict of Interest declared.

P-029

Biomarker discovery using Next Generation Metabolic Screening (NGMS) and multistage fragmentation analysis

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Background: High quality biomarkers are key to effective diagnosis and personalized treatment modalities in Inborn Errors of Metabolism (IEMs). We have established Next Generation Metabolic Screening (NGMS) as a new analytical tool in the diagnosis of IEMs in which we analyze body fluids of IEM patients by (1) targeted evaluation of panel of metabolites, and (2) untargeted analysis ("open the metabolome") in the search for new biomarkers and/or diseases.
Methods: Plasma samples of IEM patients and controls were analyzed by UHPLC-QTOF-MS analysis. The features obtained were analyzed by XCMS online data software. Univariate and multivariate techniques were applied to the metabolome data sets of patients with either PKU, HAL deficiency, MCAD and HMG-CoA lyase deficiency in the search for potential biomarkers. In addition, multi-stage fragmentation MS analysis was applied to potentially interesting features in further characterization and annotation of these signals.
Results: In the diseases listed, the well-established biomarkers could be detected. In addition, in PKU we detected several new potential biomarkers: glutamyl-Phe, Phe-glucose, N-lactoyl-Phe and the tripeptide Glu-Glu-Phe. In MCAD, we observed signals that could be attributed to capryloylglycine, heptanoyl- and nonanoylcarnitine as potential new biomarkers. In histidinemia, we observed a significantly increased signal (m/z 157.0768, RT 0.71) that we ultimately could attribute to $[M+H]^+$ adduct of imidazole lactic acid. In HMG-CoA lyase deficiency we were able to annotate 3 chromatographically separated signals (m/z 288.1440) to 3 positional isomers of 3-methylglutaconylcarnitine.
Discussion: By applying untargeted analysis to metabolome data of IEM patients we detected potentially new biomarkers for well-known and well-studied IEMs. These newly found biomarkers may provide additional insights in pathophysiology of diseases and may contribute to clinical decision making and personalized treatment.

P-030

Improved method for the determination of succinylacetone in plasma

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Background: The measurement of succinylacetone (SA) in plasma is important for diagnosis and follow-up of patients with tyrosinemia type I. The method employed in our laboratory is based in the inhibition of porphobilinogen synthase (PBG synthase). The main advantage compared to the direct measurement of free SA by GC-MS is that it takes into account the contribution of other inhibitory compounds present in the plasma of patients, like SA bound to lysine and other amino acids, as well as several 1,3 dicarbonyl compounds, namely maleyl-, fumaryl- and succinylacetoacetate.
Methods / Case Report: The method is a modification of the one published by Collier et al. (Clin. Biochem., 1971) for the measurement of PBG-synthase in which a preincubation of a hemolyzed blood pool with the plasma samples is used to evaluate the inhibition extent when compared with calibrators made of a plasma pool with a known added concentration of SA. This preincubation step has been extended from 15 min to 22 ± 2 hours with a reduction of the temperature from 30°C to 0°C to preserve the activity of the enzyme.
Results: The changes made have reduced >3 times the limits of detection and quantitation that now are 0,031 and 0,062 μmol/L, respectively.
Discussion: SA forms adducts by reacting with the amino groups of all amino acids via Schiff base. This adduct formation can be reversible as demonstrated by the fact that SA can be released from the proteins simply by boiling the plasma. Nevertheless, during the preincubation process at lower temperature, SA reacts slowly with the active site of PBG-synthase and this covalent binding can be considered as irreversible at 37°C, or at least like an equilibrium that is very much displaced to the production of the adduct.The elongation of the pre-incubation time at low temperature dramatically improves the performance of this method.

P-031

Targeted next generation sequencing in five selected groups of inborn errors of metabolism

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Background: We implemented next-generation sequencing (NGS) for establishing the diagnosis and exploration of complex biochemical traits in patients with a suspicion for 5 groups of inborn errors of metabolism.
Methods: A total of 200 genes were analyzed using a custom-designed oligo capture probe set(Roche NimbleGen) and MiSeq sequencer (Illumina).
Results: The glycogen storage disease panel was used in 28 probands with the diagnostic yield of 43%; 1, 3 and 9 patients were diagnosed with GSD III, GSD VI and GSD IXA, respectively. On the contrary, the diagnostic yield for MSUD was 100% (1x *BCKDHA*, 6x *BCKDHB*). Three out of 5 patients were diagnosed within the panel for peroxisomal disorders (mutations in *PEX1*, *PEX12*, *HSD17B4*).The hyperhomocysteinemia panel revealed 1 patient with CblD defect, 1 patient with formiminoglutamic aciduria, and 3 patients with the MTHFR deficiency. In 10 patients the cause of hyperhomocysteinemia was not identified.In the panel of urea cycle disorders we diagnosed 1 patient with argininosuccinic aciduria and 1 patient with CPS I deficiency. In 10 patients with the mild form of orotic aciduria (5–50 mmol/mol creat.) we detected heterozygous *UMPS*-mutations while in 9 patients the cause of orotic aciduria has not been established.
Discussion: Relatively low diagnostic yield in hyperhomocysteinemia panel (33%) may be explained by the fact that only non-CBS, non-vitamin deficient hyperhomocysteinemia patients are enrolled in this cohort. Similarly, patients without typical clinical and/or biochemical findings are enrolled in GSD panel. The correlation of NGS results with biochemical findings and clinical data is essential for completing the diagnosis.
Support: MZ CR – RVO VFN64165 and GAUK 580716

P-032

A NGS panel for acute rhabdomyolysis

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Background: Rhabdomyolysis is a pathological condition resulting from skeletal muscle injury with myoglobinuria (>1000 μg/mL) and hyperCKemia (>1000 IU/L). It can result from a variety of acquired and inherited causes. In children, viral infection is the mainly suspected cause. Rhabdomyolysis can also be the first presentation of an underlying inborn error of metabolism (IEM) that can be triggered by exercise, fever or viral infection. It is mandatory to consider an IEM in cases of recurrent rhabdomyolysis or rhabdomyolysis with positive family history. The diagnosis of IEM underlying rhabdomyolysis is important since most IEMs are life-threatening conditions, however, some are treatable and can benefit from an early diagnosis. Metabolic investigations in the acute phase (plasma acylcarnitines, urinary organic acids) can show specific alterations of fatty acid beta-oxidation (FAO) defects (e.g. *CPT2, VLCAD, LCHAD*). Increased TSH concentrations indicating hypothyroidism associated with laboratory findings suggestive of impaired FAO have been documented in patients with *TANGO2* gene mutations. The etiological diagnosis of rhabdomyolysis can be difficult; CK levels are often normal between attacks and metabolic samples are not often collected during the acute phase or not evocative of a specific defect.
Methods: The availability of a next generation sequencing (NGS) panel of 29 genes causative of rhabdomyolysis can be useful in patients whose investigations did not allow the specific diagnosis and whose clinical history was suggestive of IEM.
Results: We identified gene mutations in 6 patients who presented recurrent rhabdomyolysis. We diagnosed 3 FAO defects (2 *LCHAD*, 1 *CPT2*), 1 *TANGO2*, 1 glycogen storage disease (McArdle syndrome) and 1 Lipin-1 deficiency.
Discussion: The aim of the study is to underline the importance of molecular analysis by NGS panel that, in association with clinical features, allows to identify the genetic base of recurrent rhabdomyolysis.

P-033

Development of a LC-MS/MS method for the quantitation of sulfur-containing amino acids in human plasma and CSF

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Background: Homocysteine (Hcy) and its closely related metabolite methionine (Met) play a central role is several biochemical processes. Met, via its interconversion into S-adenosylmethionine (SAM), acts as a methyl group donor for the synthesis of many essential compounds. Moreover, Met and Hcy are a main source of sulphur necessary for the synthesis of sulphur-containing amino acids. Several analytical methods exist for the measurement of Met, Hcy and cysteine; however only a few laboratories are performing the measurement of SAM and its related compound S-adenosylhomocysteine (SAH), which are markedly increased in some inborn errors of metabolism.
Methods / Case report: Analytes were extracted via protein precipitation. After addition of the internal standards, samples were measured by LC-MS/MS.
Results: To screen for such disorders, a method based on LC-MS/MS has been established to quantify SAM, SAH, Hcy and Met in plasma and CSF. Overall, good precisions were obtained for all four analytes (inter-day CV< 10%). Moreover, the sensitivity of the method enabled the quantification of all pathophysiological concentrations. To assess the accuracy of the measurements of SAM and SAH an inter-lab comparison was performed, which revealed a good agreement in the measured concentrations of SAM (slope=0.90, r^2=0.994, n=20) and SAH (slope=0.90, r^2=0.982, n=20) in plasma. The stability of the samples was also investigated: in particular, Hcy, SAM and SAH seem to be very instable in whole blood and plasma, which requires a very careful sample preparation and storage. However, when samples are stored immediately after collection at −80°C, they are stable over a prolonged time.
Discussion: When the validated method was used to measure samples from patients with known or suspected disorders in the methylation cycle (homocystinuria, MTHF-reductase deficiency, SAH-hydrolase deficiency, CblE and CblG deficiency), all pathological samples could be correctly identified.

P-034

NMR analysis of 6000 urine samples from a metabolic lab for inborn errors of metabolism: preliminary results

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Background: NMR analysis in urine is a new approach for quantitative measurement of a high number of analytes of different substance classes in the metabolic laboratory. Minimal sample preparation, short analysis time, high reproducibility, single analytical platform, and ease of use are additional advantages over conventional methods. Even though NMR application for identifying various metabolic diseases have been demonstrated, there is still no practical experience using routine NMR measurements in the metabolic laboratory.
Methods: Quantitative NMR analysis was tested for 250 metabolites in 7 different disease categories. Over 3 years, a rest volume of 6000 urine samples were collected to perform additional NMR analyses. Samples had been sent to the metabolic lab either for excluding metabolic disease in a symptomatic patient, for confirming diagnoses, or for treatment monitoring in treated patients. In addition, 600 urine samples of healthy children were analyzed to generate reference values to compare with the ones obtained from the non-healthy group.
Results: For 150 of the 250 metabolites, reference value cut-off decisions could be determined, which gave a good congruency with the age-matched control group. Targeted analysis provided the key metabolites found in 56 different diseases and, in 4 lysosomal storage diseases, metabolic profiling gave a preliminary hint for the underlying disease. All together 60 diseases of 7 different substance classes identified by conventional methods could also be identified by NMR.
Discussion: NMR analysis is an excellent tool for the fast quantification of a wide range of different metabolites which up until now

required different quantification methods. Our results show the practicality of NMR in the metabolic laboratory. Participation in ERNDIM proficiency testing program will give further experiences and improvement of the method by analyzing samples with an unknown diagnosis.

P-035

Selective Screening in urine for inborn errors of metabolism using NMR analysis linked to METAGENE knowledgebase

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Background: NMR analysis in urine is a new approach for highly quantitative and reproducible measurement of a high number of analytes with different substance classes running on one platform. Because of the huge number of information provided by the NMR report an automatic evaluation showing out of normal range results and their interpretation for possible diagnoses is desirable.
Methods / Case report: Quantitative NMR analysis (Bruker Biospin Avance IVDr, B.I.Quant-UR™) was performed automatically for 152 metabolites of 12 substance classes. Metagene, a knowledgebase for Analysis support of inborn errors of metabolism (www.metagene.de) was adopted for direct interpretation of the NMR reports. Ranking of potential diagnoses explainable by the metabolic findings in the report is done by comparison to the disease database in Metagene containing 209 diseases and differential diagnoses.
Results: In 60 known metabolic diseases the diagnosis was made by conventional analysis and data based automatic ranking. Comparison to the NMR based method showed high concordance. Using click boxes to add additional information as age and clinical symptoms to the quantitative results, rational ranking was highly improved. Diseases which may be well-defined by one characteristic metabolite (e.g. L-Alloisoleucine in MSUD) show the best rankings.
Discussion: NMR analysis provides an excellent tool for using automatic analysis to further enable high throughput screening of urine samples and to improve yield of genetic metabolic diseases in the metabolic laboratory.

P-036

HR-MAS NMR reveals specific metabolomic profile under galactose stress in OXPHOS deficient fibroblasts

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Background: Defects in the mitochondrial oxidative phosphorylation (OXPHOS) lead to an extremely heterogeneous group of disorders. Laboratory screening methods are prone to misinterpretations and do not allow for unambiguous diagnosis.
Methods / Case Report: We used human skin fibroblasts (FB) from four controls, two patients with complex I deficiency (CI, namely ND6 and NDUFAF2), and from two patients with pyruvate dehydrogenase deficiency (PDH, namely 1Eα defects). Cells were grown under standard culture condition (glucose) or galactose stress test. We investigated extracellular metabolic flux using Seahorse XF24 live cell Analyzer and assessed in parallel the metabolome fingerprint using NMR spectroscopy, which allows rapid profiling of over 40 metabolites.
Results: Separation of specific defects from controls were clearly improved under galactose stress test. Metabolomes revealed specific defect dependent changes from glucose to galactose based media and were clearly separated under galactose. While PDH deficient cell lines showed distinct upregulation of glutaminolytic metabolism, no upregulation of glutaminolysis was observed in metabolomes of CI deficient cell lines. CI deficient cell lines showed significant upregulation of antioxidants such as GSH and Taurine under galactose condition, which is in agreement with observed increases in reactive oxygen species in these defects.
Discussion: These preliminary results show that untargeted fingerprinting NMR has potential to discriminate between control and OXPHOS deficient cells and allows important insight in the adaptation of cells to different culture conditions. Further selective culture method may greatly improve discrimination of normal from metabolic deficient cells.

P-037

The evolving role of enzymology and metabolomics in the diagnosis of metabolic disorders in the post genomic era.

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Background: In recent years there has been an exponential increase in genetic testing, in particular the use of gene panels and whole exome/genome sequencing. With this wealth of data we have seen an increase in the finding of genetic variants of unknown significance and identification of patients with genetic diagnosis but atypical phenotypes or clinical diagnosis but no mutations found. There is increasingly a need for diagnostic tests which reflect in-vivo gene function as an adjunct to molecular genetics. This is leading to a change in the way many patients are investigated.
Case Report:Here we discuss recent experience in the Enzyme Unit at Great Ormond Street Hospital, of a number of cases where extensive molecular genetics have been performed but further tests have been required to elucidate genotype phenotype correlations and/or confirm the diagnosis.
Results: Patients discussed include:
Unexpected genetic diagnosis of mucopolysaccharidosis type III from whole genome testing in 2 patients seen in an ophthalmology setting.
The identification variants of unknown significance in genes for mucopolysaccharidosis type IV and glycogen storage disease type III, in patients with atypical clinical presentation.
Patients with mutations in the gene for β-enolase identified from a myopathy gene panel.
Patients with clinically suspected orthinine transcarbamylase deficiency and GM2 gangliosidosis with no mutations or a single mutation identified, respectively.
In all cases, enzymology and the measurement of biomarkers and metabolites has helped to confirm diagnosis
Discussion: In the post genomic era the role of the specialised biochemistry laboratory is evolving. The increasing use of molecular genetics as a screening tool has inevitably led to the identification of atypical patients. The appropriate investigation of such patients requires support from experienced and responsive specialised laboratories using sensitive enzyme assays and biomarkers.

P-038

Broad-scale untargeted metabolomic profiling improves the diagnosis of inherited metabolic disorders

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Background: Clinical metabolomics provides small molecule metabolic profiling to identify >900 metabolites in a single test for the analysis of individual patients. This method has provided insight into the spectrum of phenotypic expression for rare inborn errors of metabolism (IEMs), provided new diagnoses for disorders that do not otherwise have clinical tests available, and provided a functional assay to assess the pathogenicity of genetic variants.
Methods: Metabolomic profiling of plasma was performed in 1302 individuals with diverse ethnicity and wide clinical spectrum with 90% having neurological phenotypes. Metabolites were analyzed by high-resolution UPLC-MS/MS, and data were generated as Z-scores by comparing to a normal reference population.
Results: Of the total 1302 samples, metabolomic analysis contributed to confirm the diagnosis in 91 cases by detecting the biochemical abnormalities that were consistent with the identified conditions, achieving a 7% diagnostic rate. Further, 21 cases were also confirmed by targeted biochemical tests supporting the reliability of metabolomics. Totally, we have identified 33 known conditions including but not limited to aminoacidopathies, organic acidurias, fatty acid disorders, vitamin deficiencies, pentose pathway disorders, peroxisomal disorders, purine and neurotransmitter abnormalities, indicating that metabolomics can be utilized as an initial screening tool for diagnosis of IEMs. Using this method, we were able to monitor therapies, detect disorders at the mild end of the phenotypic spectrum, identify novel biomarkers, and provide strong functional evidence in variant interpretation.
Discussion: Diagnosis of IEMs is challenging due to the complexity of metabolic disorders. We have demonstrated that metabolomic profiling can improve the diagnosis of rare IEMs and demonstrates the power of integrating clinical metabolomics with other molecular genetic data to provide a definitive diagnosis for metabolic disorders.

P-039

Plasma biomarkers for the screening of Niemann-Pick type C disease: 2-year experience in a clinical setting in France.

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Background: The diagnosis of Niemann-Pick type C (NPC) disease has long relied on time-consuming filipin staining test on fibroblasts, associated with genetic study of *NPC1* and *NPC2*. The emergence of sensitive plasmatic biomarkers in the early 2010s, e.g. cholestane-triol, 7-ketocholesterol and the analogue 509 of lysosphingomyelin (LysoSM509) coupled to lysosphingomyelin, has changed the paradigm of NPC diagnosis and now allows an easier and more systematic screening of this disease.
Methods / Case Report: Measurement of the 4 above biomarkers was performed by LC-MS/MS for about 800 patients referred to our laboratory in 2016 and 2017 for NPC screening purpose, including adolescents/adults (70%) and children (30%).
Results: Thirty-nine patients displayed pathological levels for at least one marker. Depending on the biomarker profile, suitable complementary investigations were completed for 33 patients (in progress for the remaining 6). Sixteen patients were genetically confirmed as NPC index cases, and 6 classified as NPC heterozygotes. Five patients were diagnosed with sphingomyelinase deficiency (NPA/B), and one adult patient with oxysterols increase only displayed cerebrotendinous xanthomatosis. Only 6 cases currently remain as unresolved false-positives and no false negative has been encountered so far.
Discussion: Implementation of plasmatic biomarkers as the first-line screening test for NPC allows a larger scale screening for this disease, especially in adult patients with atypical/incomplete clinical picture. Nevertheless, confirmation of the diagnosis is always mandatory. The current French guidelines for NPC diagnosis strategy are (i) biomarkers measurement as the first line step (ii) *NPC1* and *NPC2* gene sequencing as the confirmation step (iii) filipin test for difficult cases. New biomarkers are needed to better discriminate NPC patients from carriers.
Note: CP and MP contributed equally to the study.
Conflict of Interest declared.

03. Newborn screening

P-040

Reducing False-Positive Screening results for GALT on the Perkin-Elmer GSP for G6PD Deficient Newborns

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Background: The Metabolic Laboratory in Qatar seeks to provide early detection of babies affected by metabolic disorders while minimizing false positives. Determination of galactose-1-phosphate uridyltransferase (GALT) activity in dried blood spots (DBS) detects classical galactosemia caused by GALT deficiency. The Perkin-Elmer GSP Neonatal GALT assay is an adaptation of the semi-quantitative assay of Beutler and Baluda. The assay reaction sequence relies on endogenous glucose-6-phosphate dehydrogenase (G6PD) activity. Since G6PD deficiency is common in the Middle East, newborns with G6PD deficiency often falsely screen positive for GALT deficiency. This increases false positives, unnecessary recall of patients for confirmatory tests, and anxiety of parents. The goal of the present study is to determine whether supplementing the DBS with G6PD enzyme will minimize false positives for GALT for newborns with G6PD deficiency.
Methods / Case Report: GALT activity was assayed according to the recommended protocol. Lyophilized chromatographically purified G6PD enzyme was diluted to 2mU/μL with GSP GALT assay buffer.
Results: Addition of 2 μL (4mU activity) of G6PD to the DBS of samples with low GALT significantly increased the GALT result within the normal range for newborns with G6PD deficiency. Adding G6PD enzyme to the DBS of newborns with normal G6PD activity had no effect on the GALT result. Addition of G6PD to the DBS of newborns with confirmed GALT, galactokinase, or UDP-galactose-4 epimerase deficiencies had no effect on the GALT result. Increasing the G6PD to 20 mU/DBS or reducing to 2 mU/DBS did not affect GALT activity of G6PD competent or deficient newborns. Diluted G6PD reagent maintains activity for at least 9 months storage at −80°C which permits storage until needed.
Discussion: The procedure reported here is a rapid and simple method for eliminating interferences caused by G6PD deficiency in the GSP Neonatal GALT assay and reduces the false positive rate.

P-041

Development of a HILIC-LC-MS analytical method to screen pyridoxine dependent epilepsy from dried blood spot

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Background: Pyridoxine dependent epilepsy (PDE) is a metabolic disorder where the affected newborns have prolonged seizures within the first month of life. These seizures will not respond to anticonvulsant therapy. However, if identified earlier, patients can be treated prophylactically with pyridoxine and a severe brain damage can be prevented. Pipecolic acid (PA), Alpha amino adipic semialdehyde (AASA) and Piperidine-6-carboxylic acid (P6C) are known to be the markers for this condition. Development of an analytical method for the simultaneous quantification of these markers from dried blood spot (DBS) is reported here.
Methods:Dried blood spots were prepared from blood samples of neonates of 3–5 days of age born at Kasturba Hospital, Manipal after obtaining ethical clearance (MUEC/010/2017 dtd 08/05/2017). The chromatographic conditions and sample extraction procedure for DBS were optimized by varying different experimental parameters. The optimized method was validated as per the guidelines.
Results:The separation of biomarkers was optimized on the iHILIC column with the following conditions. Mobile phase (acetonitrile: formic acid buffer, 80:20 v/v ratio), flow rate 0.5 mL/min, injection volume 10 μL, and run time 3 min. The retention time of PA, AASA/P6C and d9-PA were 1.79, 2.59 and 2.20 min respectively. MS conditions were optimized for spray voltage, vaporizer temperature, sheath gas flow and for collision energy. For extraction of sample, the spot size was optimized to 3.2 mm DBS. Extraction was affected with 100μL of methanol with vortexing for 30 min at room temperature. The LOQ of the method was found to be 10 ng/mL and 50 ng/mL respectively for AASA/6PC and PA respectively.
Discussion:The iHILIC column facilitated the retention of PA and AASA without derivatization and has a low run time of 3 min. The validation of the method demonstrated a good linearity, accuracy and precision. The method can be applied for newborn screening of PDE.

P-042

Determination of 2OH-glutaric acid, 3OH-glutaric acid, glutaric acid, 2-methylsuccinic acid, and EMA in blood spots and plasma by LC-MS/MS

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Background: To improve the specificity of newborn screening (NBS) we developed a method for simultaneous determination of 2-OH glutaric acid (2-OHGA), 3-OH glutaric acid (3-OHGA), glutaric acid (GA), ethylmalonic acid (EMA) and methylsuccinic acid (MSA) in dried blood spot (DBS). These analytes are considered more specific for conditions screened by elevation of C4-acylcarnitine (C4) such as short-chain acyl-CoA dehydrogenase deficiency (SCAD), isobutyryl-CoA dehydrogenase deficiency, glutaric acidemia type II and ethylmalonic encephalopathy (EE) or C5DC for glutaric acidemia type I (GA-I).
Methods: The analysis is performed in 1/8-inch DBS punch using d5-3OH GA, d4-GA, d3-EMA, and d6-MSA as internal standards. Samples are analyzed by LC-MS/MS and results are interpreted using post-analytical web tools of Collaborative Laboratory Integrated Reports (CLIR).
Results: The 99%iles of the reference population (in nmol/mL), determined from 156 NBS controls, were as follows: EMA: < 3.55; MSA: < 0.69; 3-OHGA: < 1.41; 2-OHGA: < 25.2; GA: < 1.74. 71 SCAD cases with known genotype, 1 EE, and 4 GA I were also analyzed. Elevated concentrations of the markers diagnostic for the particular disease were observed in all cases. This approach allows also for discrimination between genotypes within the same condition in case of SCAD: 2 pathogenic mutations vs. carrier status or polymorphisms.
Discussion: We describe a new LC-MS/MS method for the determination of 2-OHGA, 3-OHGA, GA, MSA and EMA as a 2nd tier test in the original NBS sample. An improved algorithm for disorders associated with abnormal C4 and C5-DC concentrations is based on: 1) interpretation of the complete profile; 2) application of 2nd tier tests; 3) regular use of CLIR post-analytical tools. Routine application of this approach leads to further reduction of the false positive rate and in case of SCAD allows better genotype/biochemical phenotype correlation. This assay can also be applied to plasma and postmortem samples.

P-043

Recommendations for newborn screening for galactokinase deficiency: A systematic review and evaluation of Dutch newborn screening data

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Background: Galactokinase (GALK) deficiency is a cause of cataract leading to severe developmental problems unless treated early. The Dutch Health Council advised to include GALK deficiency in the Dutch newborn screening program, because of the easy prevention and rapid reversibility of cataract with treatment. We aim to establish the optimal screening method and cut-off value (COV) for GALK deficiency screening by performing a systematic literature review of screening strategies and total galactose (TGAL) values and by evaluating TGAL values of screened newborns in the Netherlands.
Methods: Systematic literature search strategies were developed and study selection, data collection and analyses were performed by two independent investigators. TGAL values measured in the first week of life by the Quantase Neonatal Total Galactose screening assay in a cohort of Dutch newborns in 2007 were evaluated.
Results: Four studies describing screening strategies used TGAL as the primary screening marker combined with galactose-1-phosphate uridyltransferase (GALT) measurement that is used for classical galactosemia screening. TGAL COVs of 2200, 1665 and 1110 μmol/L blood resulted in positive predictive values of 100%, 82% and 10% respectively. For 39 GALK deficiency patients TGAL values measured in the newborn period were reported. Individually reported values ranged from 3963 to 8159 μmol/L blood. Dutch newborn screening data of 72,786 newborns from 2007 provided a median TGAL value of 110 μmol/L blood (range 30–2431).
Discussion: Based on TGAL values measured in GALK deficiency patients reported in literature and TGAL values in the Dutch newborn

screening cohort, we suggest to perform GALK screening with TGAL as a primary marker with a COV of 2500 μmol/L blood, combined with GALT enzyme activity measurement as used in the classical galactosemia screening. This will ensure detection of GALK deficiency patients and keep the amount of false positive referrals to a minimum.
Conflict of Interest declared.

P-044

Newborn screening for VLCAD deficiency: risk assessment of positive subjects by genetic and enzymatic study

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Background: There are several challenges to providing definitive diagnoses for newborns screened positive for VLCAD deficiency. In our experience, distinct severe cases with low enzymatic activity (< 10%) or cases with bi-allelic nonsense mutations, are rare. We introduce what we have learned from the genetic diagnosis of screened positives especially in patients whose residual activity ranging 10-50%.
Methods: We characterized 48 newborns requested to be diagnosed genetically and enzymatically from other institutions after 2013. Enzymatic activities were assessed by comparing dehydrating activity of C18CoA with normal controls. Subsequently, we analyse the *ACADVL* gene of each case with low residual activity**.**
Results: Of 48 total cases 8 had < 10% residual activity and 37 had 10-50% residual activity. Among the 37 cases with 10-50% residual activity, 20 cases carried bi-allelic mutations and 15 of them had 10-24% residual activity. 11 out of the 14 cases with a mono-allelic mutation preserved over 24% of their residual activity. The p.Cys607Ser mutation (only found among newborn screening positives) existed in 13/96 alleles, and the p.Ala416Thr mutation (prevalent in Japanese patients) was found in 8/96 alleles.
Discussion: Hoffmann *et al.* had reported similar results in 2012. Among the screened positives, there were four times more patients with 10-50% residual activity than those with < 10% activity. Most cases carrying bi-allelic missense mutations retained less than 25% residual activity. Like Western countries, mutations found among Japanese screened positives were scattered in the whole coding sequence and there were not many common mutations. We found nearly 40% residual activity in carriers (parents and siblings of symptomatic patients) and concluded that the extent of residual activity could possibly result in the absence of symptoms throughout life. However, there was a report of a sudden death of a compound heterozygote whose mutations had been found only among screen positives. We therefore need to examine compound heterozygotes with 10-50% residual enzyme activity more carefully.

P-045

Carnitine uptake disorder identified by expanded newborn screening in Thailand

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Background: Carnitine uptake disorder (CUD) is one of the targeted conditions of expanded newborn screening (NBS) in several developed countries. However, there have been emerging debates whether this condition should remain in the NBS panel due to detection of many asymptomatic adults with CUD identified through NBS.
Methods: In Thailand, a pilot expanded NBS program was implemented in Bangkok since 2014, and 119,664 newborns were screened in a 3-year-period. CUD was among 40 inherited metabolic disorders (IMD) screened by MS/MS. Newborns who had free carnitine (C0) in dried blood spots (DBS) < 5 μmol/L would be recalled for repeats in babies and their mothers. Then individuals with positive results were confirmed with C0 clearances calculated from plasma and urine C0 levels. Molecular analyses of the *SLC22A5* gene were performed in confirmed cases. Echocardiography and blood chemistry tests were performed in all affected newborns and most adult cases.
Results: We identified 13 cases of CUD including 5 newborns, 7 mothers, and 1 older sibling, with an incidence in newborns of 1:24,000 and a prevalence in mothers of 1:17,000. All newborns were asymptomatic at diagnosis and supplemented with carnitine. Six mothers reported nonspecific symptoms such as fatigue or reduced exercise tolerance. Echocardiography showed normal results in all babies but dilated cardiomyopathy (DCM) was identified in one affected mother. Another mother had ECG changes but normal echocardiogram and cardiac MRI. Serum creatine kinase levels were elevated in 4/10 cases.
Discussion: CUD is the most common IMD identified by NBS in Thailand. All identified cases were not seriously affected except one mother who had DCM. However, several affected individuals had minor symptoms and changes in ECGs or blood chemistry. Inclusion of this condition in the national NBS panel remains to be elucidated.

P-046

Expanded Newborn Screening. Six-year experience in a single centre

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Background: To describe our experience since the implementation of the Expanded Newborn Screening program in our region.
Methods / Case Report: Observational and retrospective study in a tertiary pediatric centre with long term follow-up of neonatal metabolic screening. All the patients studied in the Metabolic Department, since October 2011 to December 2017, for an abnormal screening, were included. In all of them, the altered metabolite, the confirmation in reference laboratory, the clínical data of the patients, the biochemical and clinical diagnosis and the follow-up, were analysed.
Results: During the period of time 83 newborn were studied in our centre for an abnormal screening, 48 of them (57.8%) were girls. Altered metabolite was confirmed in 44 patients (53%). 23 Inborn Errors of Metabolism (IEMs) were diagnosed: 8 hyperphenylalaninemia, 3 MCAD deficiency, 3 VLCAD deficiency, 3 methylcrotonylglycinuria, 2 methylmalonic acidemia with homocystinuria, 2 propionic acidemia and 1 primary deficiency of carnitine. Other diagnoses were: vitamin B 12 deficiency (12 patients), transitional alterations without any pathology identified (5 patients), heterozygous mutations of fatty chain oxidation (4 patients). 15 disorders in newborns' affected parents were detected: 11 maternal vitamin B 12 deficiency, 1 maternal systemic primary carnitine deficiency, 1 maternal carnitine transporter deficiency, 1 maternal glutaric aciduria type 1 and 1 paternal benign hyperphenylalaninemia. Genetic diagnosis was conclusive in all the IEMs and 6 new mutations were found. None of the patients died due to his metabolic disorder.

Discussion: Neonatal metabolic screening detects metabolic and non-metabolic diseases in patients and relatives, especially vitamin B 12 deficiency secondary to maternal deficiency. There is a high correlation between biochemical and genetical diagnosis in IEMs.

P-047

Six years of expanded newborn screening for metabolic diseases in Norway

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Background:In 2012 the national Norwegian newborn screening program (NBS) was expanded from two conditions (PKU and congenital hypothyroidism) to 23 diseases. We present the results after 6 years of NBS for 19 metabolic diseases excluding PKU.
Methods:Screening samples were collected on filter cards 48–72 hours after birth. Amino acids and acylcarnitines were determined by LC-MS/MS (Waters Quatro Permier XE or Xevo TQS) using the NeoBase kit from Perkin Elmer. Biotinidase activity was determined using the GSP Biotinidase kit from Perkin Elmer. DNA assessments were performed using the screening samples and Sanger sequencing (3500Dx, Applied Biosystems).
Results: From 2012–2018, 357.436 children were screened and 60 non-PKU true positive cases were identified (1:5940 newborns): 31 fatty acid oxidation defects, 22 organic acidurias, and 7 aminoacidopathies. 45/57 (79%) of genetically confirmed cases were identified by rapid DNA analysis in bloodspots. 15/60 affected children (25%) were symptomatic at the time of the NBS result. The incidence of Medium chain acyl-CoA dehydrogenase deficiency increased from 1:74.573 to 1:25.197 before and after NBS, respectively. False negatives: Intermittent Maple Syrup Urine Disease (n=2) and mild Carnitine-palmitoyl- transferase type II deficiency (n=1). The positive predictive value (PPV) increased from 26% in 2012 to 68% in 2017. The incidence of metabolic diseases included in the NBS panel increased by 40 % compared to clinically confirmed patients in the preceding decade.
Discussion: Our findings are in accordance with other expanded NBS programs including increased incidence of milder phenotypes and severe cases presenting early with persistent morbidity despite treatment. However, the majority of cases benefited from pre-symptomatic early diagnosis and the PPV improved significantly to 68%. The expanded NBS program in Norway has enabled better national overview, awareness of and follow-up programs for the metabolic diseases.

P-048

Gene panel study for target metabolic diseases in newborn mass screening

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Background: Nationwide newborn screening (NBS) using tandem mass spectrometry has been conducted in Japan since 2014. We have performed a study in which mutations are identified using a gene panel and were evaluated by research examination and have been conducting genetic diagnosis for target metabolic diseases (TMDs) since January 2014. Then, in order to overcome the problem of examination fee, from November 2017, we changed to a system that can examine several diseases using Japanese public medical insurance.
Methods: We designed a gene panel covering the TMDs and the related diseases. Gene panel analysis was performed at Kazusa DNA Research Institute using the MiSeq or NextSeq (Illumina®). If patient and physician's consent is obtained, we can provide mutation reports by experts. (This research was partially supported by Japan Agency for Medical Research and Development.)
Results: More than 240 cases were enrolled in this gene panel study during four years (January 2014 to April 2018). Among them, the number of patients with TMDs detected by NBS were 183 cases, as follows: Propionic acidemia (36), Hyperphenylalaninemia (23), VLCAD deficiency (17), Methylcrotonylglycinuria (16), Methylmalonic acidemia (15), MCAD deficiency (13), Galactosemia (12), primary systemic carnitine deficiency (9), Maple syrup urine disease (9), Citrullinemia type 1 (6), Glutaric acidemia type 1 (4), Glutaric acidemia type 2 (4), CPT2 deficiency (4), Others (14). In most cases, we could find the gene mutations in their corresponding genes. We have also started clinical follow up studies for some cases.
Discussion: In some TMDs, clinical course and severity may vary from patient to patient. One major factor determining the clinical phenotype is, of course, the genotype. It is therefore important to follow up on patients who have defined mutations to evaluate the effectiveness of treatment and management. In the near future, we plan to personalize clinical guidelines by genotyping of several TMDs.

P-049

Second tier test in the first DBS in NBS of methylmalonic and propionic acidurias and, homocystinurias: Catalonia experience

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Background: Methylmalonic and propionic acidurias, as well as homocystinurias, are an extensive group of autosomal recessive diseases included in the expanded newborn screening (NBS) program in several countries. Their detection can be performed through the analysis of primary markers [propionylcarnitine (C3), propionylcarnitine/acetylcarnitine ratio (C3/C2), C3/methionine ratio (C3/Met), Met or heptadecanoilcarnitine (C17)] on dried blood spots (DBS). However, specificity and predictive positive value (PPV) is highly improved by the measurement of methylmalonic acid (MMA), total homocysteine (HCY) and methylcitric acid (MCA) in DBS as second-tier test.

Methods: Our study included 207,310 newborns analyzed from 2015 to 2017. Second tier-tests were performed on the first DBS in 7,748 newborns that had alterations in primary markers above described. MMA, HCY and MCA concentration were normal in 80.5 % (6,241 cases) and, in 17% (1,328 cases) a second sample was requested.
Results:109 cases were directed to the clinical unit: 1 methylmalonic aciduria with homocystinuria (CblC), 1 methylmalonic aciduria type B (CblB), 3 methylmalonyl-CoA mutase (MUT) deficiencies, 3 propionic acidemias and, 3 cystathionine-β-synthase (CBS) deficiencies were genetically diagnosed, besides 1 case of suspected MUT/CblA or CblB. Moreover, 87 maternal or newborn vitamin B12 deficiencies were also detected (44 confirmed and 43 suspected), and other 7 cases had biochemical alterations although vitamin B12 data was not available. Furthermore, 3 transient cases were also detected.
Discussion: In summary, our data strengthens the importance of second-tier tests on the first DBS. They increase specificity and PPV (80%), reduces number of unnecessary second requested samples, as well as, of children directed to the clinical unit to be further studied.

P-050

Characterization of the significance of newly identified missense mutations in newborn screening: A novel approach for faster analysis

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Background: Newborn screening (NBS) by mass spectrometry is now common in many countries and follow up confirmation of diagnosis is often by DNA sequencing. When variants of unknown significance (VUS) are identified, a firm diagnosis remains in question as specific functional testing for each disorder is often unavailable or difficult to obtain and usually leads to a significant delay to final diagnosis. Thus, a general approach to confirmation of significance of VUSs would be of great value to NBS programs. This model will serve as a general platform for any NBS disease identified by acylcarnitine profiling.
Methods / Case Report: CRISPR/Cas9 genome editing was used to create knockouts (KOs) of genes relevant to NBS in HEK293 cells and transfected with expression vectors harboring VUSs. As proof of principle, IVD^{KO} cells transfected with a pcDNA3.1 vector expressing wild-type IVD or a missense mutation, *e.g.*, IVD Arg53Pro, or IVD Ala314Val will be tested for rescue of the knockout phenotype by acylcarnitine profiling.
Results: In three cell linesPCR has confirmed the homozygosity of deletions in *IVD*, *ACAD9* and *VLCAD*, and western blotting and enzyme analysis showed loss of the desired enzyme of these genes, respectively. Mass spectrometry of cellular media in an IVD-deleted cell line identified a characteristic altered acylcarnitine profile. Creation of additional KO cell lines and rescue with expression plasmids is in progress.
Discussion: We have developed a series of mutant cell lines to allow high throughput expression analysis of plasmids containing VUSs, identified by NBS, in order to assess functional significance. Our method will allow rapid functional significance of any mutation or VUS identified in a gene that leads to an abnormal acylcarnitine profile in NBS.

P-051

Evaluation of prospective and parallel assessments of cystic fibrosis newborn screening protocols in Eastern Andalusía

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Background: In June 2011, our region began cystic fibrosis (CF) newborn screening (NBS). It adopted the IRT/IRT protocol, which leads to a high rate of false positives. The objective of this study was to evaluate preliminary results of a prospective, sequential, and parallel IRT/PAP strategy compared to our NBS center's current IRT/IRT strategy.
Methods: From June 2017 to March 2018, the IRT/IRT strategy with cutoffs of IRT1≥61 ng/dL and IRT2≥40 ng/dL was used to screen 28,750 newborns. In parallel, PAP was assayed using a MucoPAP-F kit (DYNABIO, Marseille) in newborns with IRT1≥50 ng/dL. When IRT1=50-60.9 ng/dL, 2.1 μg/L was set as a provisional PAP cutoff for requesting a second sample to evaluate if PAP measurement could improve sensitivity in this range of IRT1 levels. A PAP concentration above 3.8 μg/L when IRT1≥50 and IRT2≥35, was considered a positive screen. Newborns with positive screening results according to one or both protocols were referred for further confirmatory testing (sweat test and/or identification of biallelic pathogenic variants in the *CFTR* gene).
Results: 440 newborns had IRT1 levels above 50 ng/dL and a second dried blood spot sample was requested for 302 newborns (1.05%). Of these, 8 were lost to follow-up due to early death (*n*=5), parental refusal (*n*=1), or wrong address(n=2).CF was detected in 6 newborns. All of them would have been detected by both strategies as they had high PAP values (median 5.96 μg/L, range 2.93-96.2). A ROC analysis of PAP determined a sensitivity of 100% and specificity of 89.9% for the point 2.91 μg/L. Using this cutoff, the number of sweat tests would have been 33 instead of 31 with the IRT/IRT strategy.
Discussion: Sequential measurement of IRT/PAP provides good sensitivity and specificity and allows for more reliable and cost-effective CF NBS than the IRT/IRT strategy.

P-052

Mutation spectrum of neonatal citrin deficiency diagnosed in Singapore

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Background: Citrin deficiency is caused by mutations in the *SLC25A13* gene and can present as neonatal intrahepatic cholestasis (NICCD). The common abnormalities include prolonged conjugated hyperbilirubinemia, recurrence of jaundice, elevation in liver enzymes, and multiple amino acidemia. Here we report the mutation spectrum and the clinical and biochemical characteristics of NICCD diagnosed in Singapore.

Methods: Citrin deficiency (CD) is included as a secondary target in Newborn Screening by tandem mass spectrometry (MS/MS). In this study we reviewed babies in Singapore who were eventually diagnosed with CD.
Results: From 2006–2018, there were 18 babies diagnosed with CD. Of these, 14 had MS/MS based newborn screening while 4 did not. Only 2 of 14 babies had a positive screening result and were subsequently diagnosed with CD (citrulline: 62 and 88 μmol/L; normal< 40). The remaining 16 infants (12 missed by MS/MS, 4 unscreened) were investigated after the onset of symptoms (age range: 1–5 months). Prolonged conjugated hyperbilirubinemia was the most common presentation and ~ 50% of them also had galactosemia. DNA analysis was performed for 14 infants. Most of the mutations identified were common pathogenic changes known to occur in the Asian population: c.852_855delTATG (p.M285Pfs*2), c.615+5G>A, 1638_1660dup23, c.851_854delGTAT and del Exon 14 of *SLC25A13*. A variant c.1064G>A(p.R355Q) was predicted by SIFT and PolyPhen-2 computer algorithms to be pathogenic. Five of 14 infants tested have homozygous c.852_855delTATG mutation and 4 have compound heterozygous c.852_855delTATG/ c.615+5G>A mutations.
Discussion: The incidence of NICCD is @ 1:26,259. However, the overall incidence of CD may be underestimated due to poor recognition of CD as a cause of prolonged jaundice. CD patients can also present later in childhood or adulthood with diagnostic challenges. Two common mutations (c.852_855delTATG, c.615+5G>A) constitute 71% (20/28) of mutations identified.

P-053

Newborn screening for MCAD deficiency: Experience of the first 8 years in Eastern Andalusia, Spain

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Background: Medium-Chain Acyl-CoA Dehydrogenase Deficiency (MCADD) is an autosomal recessive fatty acid oxidation disorder with a potential fatal outcome. It is caused by mutations in the *ACADM* gene; the most prevalent mutation is c.985A>G. The objective of this study was to evaluate the prevalence, clinical course, and biochemical and molecular phenotype of MCADD cases detected in the first 8 years of newborn screening in our centre.
Methods: From April 2010 to March 2018, the acylcarnitine profile, including C6, C8, C10, and C10:1, of 341,152 newborn DBS cards was measured by tandem mass spectrometry. Newborns with screen positive results were referred to physicians for further confirmatory testing (plasma acylcarnitine analysis and identification of biallelic pathogenic variants in the *ACADM* gene) and follow-up care.
Results: 21 newborns were referred for confirmatory testing for having C8 values above the screening cutoff of 0.17 μmol/L. They had a mean level of 5.83 μmol/L (range 0.37-32.38). All 21 had elevated C6 levels and 15 also had elevated C10 values. 20 of the screen positive infants had an MCADD diagnosis confirmed by plasma acylcarnitine analysis. Molecular testing was available for 12 confirmed cases: 6 were homozygous for the common c.985A>G mutation, 2 were compound heterozygous for c.985A>G, and 4 had other mutations. The average follow-up period was 3.5 years. One patient was lost to follow-up during the first year. Two patients had a metabolic crisis; both were homozygous for the c.985A>G mutation. The estimated prevalence of MCADD is 1:17,157 live births.
Discussion: MCADD frequency in our centre is comparable to reports from other newborn screening programs. Early detection and treatment have successfully prevented adverse health outcomes in our MCADD patients.

P-054

Abnormal results in newborn screening for lysosomal disorders: the role of laboratory follow-up

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Background: The increasing availability of treatments and the importance of early intervention have stimulated newborn screening (NBS) for lysosomal storage diseases(LSDs). We present our experience of lysosomal newborns screening in North East Italy by LC-MS/MS and the role of biomarkers in the laboratory follow-up.
Methods: Activities of acid β-glucocerebrosidase (ABG; Gaucher), acid α-glucosidase (GAA; Pompe), acid α-galactosidase (GLA; Fabry), and acid α-L-iduronidase (IDUA; MPS-I) were determined in dried blood spots (DBS) in one single assay at age of 48 hours by multiplexed tandem mass spectrometry(MS/MS) using the NeoLSD® kit (Perkin Elmer). Biochemical confirmatory tests including assays of the following biomarkers: plasma Glucosylsphingosine (LysoGb1) and Lyso-Globotriaosylceramide (LysoGb3) (Polo et al., 2017), urinary tetraglucoside (Glc4) adapted from Young et al. 2003, and urinary glycosaminoglycans(GAGs) after methanolysis (Zhang H 2013) were considered in positive screening patients. All these metabolites were measured by LC-MS/MS.
Results: Since September 2015 84.631 newborns were screened. 94 neonates (0.11%) were recalled for a second DBS. Low enzyme activities were confirmed in 43 patients, who subsequently had the following confirmatory tests: LysoGb1 for Gaucher, Glc4 for Pompe, LysoGb3 for Fabry and urinary GAGs for MPS I. 15/43 had abnormal biomarkers values and mutational analysis confirmed the disease. In 28/43 the biomarkers were normal with mutation analysis data showing pseudodeficiency or carrier status. Of note, the incidences for Pompe and Gaucher diseases were 1/21.158, Fabry disease 1/14.105 and MPS-I 1/42.316. The combined incidence of the four disorders was 1/5.289 births.
Discussion: Simultaneously determining multiple enzyme activities by MS/MS adding specific biochemical markers as confirmatory tests, significantly reduce the need for unnecessary follow-up of newborns with pseudodeficiency enzyme activities or carrier status.

P-055

Epidemiology of cobalamin deficits detected through newborn screening in Portugal

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Background: Cobalamin (Cbl)-related defects may be caused by nutritional deficiency or genetic defects. Inherited disorders of intracellular

Cbl metabolism cause methylmalonic aciduria (MMA) and/or homocystinuria (HC), which may both be present, combined or isolated, depending on the affected metabolic step. Specific diagnosis of these inborn errors of metabolism is not straightforward and, based on complementation analysis, nine complementation groups were defined (CblA-G, J and X). Defects in *MMACHC* gene are the major cause of combined MMA and HC, and are especially frequent among Southern European (Portugal, Spain and Italy) and Chinese patients. Birth prevalence of Cbl C defect in Portugal is one of the highest worldwide (1: 85,000), although the frequency of this disease has been rising in other populations due to newborn screening (NBS) identification of milder forms.
Methods: Cbl deficits can be identified through NBS due to elevated C3 and C3/C2, usually associated with low methionine. The same alterations may be observed due to nutritional Cbl deficiency of the newborn, associated with maternal disease.
Results: Since the implementation of expanded NBS in Portugal, in 2004, twelve Cbl C, one Cbl D and one Cbl B cases have been identified. Mutation c.271dupA was found in 96% of Cbl C patients, a much higher frequency than the one identified among patients diagnosed before NBS (61%).
Discussion: This fact raised the possibility of no identification through NBS of patients with mutations associated with late onset forms, but recently one Cbl C case with a mutation associated with the late onset form was identified. The implementation, in 2017, of second-tier tests (MMA and HC) will surely also contribute to change this picture and already allowed the identification of several cases of nutritional Cbl deficiency due to maternal disease, which otherwise would have been missed.

P-056

Mass spectrometry meets NGS: Molecular confirmation of abnormal TMS in emergency setting

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Background: Diagnosis of treatable inborn errors of metabolism (IEM) in a country like Brazil, with continental dimensions and scarce resources, is a huge challenge. Tandem mass sprectrometry (TMS) allows for presymptomatic diagnosis in newborn screening (NBS), as well as recognition of abnormal biochemical patterns in at-risk babies. Using a combination of screening by TMS and molecular confirmation of abnormal results with a NGS panel for treatable IEM, we developed a pilot study for speeding up diagnosis of babies in the ICU as well to confirm abnormal findings in NBS, called *Projeto Pé-de-Molec*.
Methods/Case Report: Abnormal results of TMS, detected in NBS were further evaluated using a NGS panel for 123 treatable IEM, which included all clinically manageable disorders recognized by amino acids and acylcarnitine analysis by TMS. After informed consent, an oral swab sample for DNA was collected and used for molecular analysis of the 23 individuals with abnormal screening. Turn-around time for molecular analysis varied from 14 to 21 days.
Results: Final diagnoses were established in 14 of the 23 individuals (60%): 7 aminoacidopathies, 4 fatty acid oxidation defects and 3 organic acidemias. TMS false positive results occurred in 8 cases, mostly with C3, medium and very long chain acylcarnitines, mostly due to diet artefacts. One case with typical biochemical profile of HMG-CoA lyase (filter paper AC and urine OA) had a normal molecular analysis.
Discussion: After one year of the pilot *Projeto Pé de Molec* using molecular method to confirm abnormal TMS, the results are very stimulating, with improvement of the quality of diagnosis, discrimination of false positives from true positives, and allowing adequate treatment. This strategy is feasible and cost effective and can be replicated in other countries with similar biochemical diagnostic infrastructure limitations.
Conflict of Interest declared.

P-057

Targeted metabolomic analysis of dry blood spots from patients suffering from SCADD

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Background: SCADD is an autosomal recessive disorder of mitochondrial β-oxidation of fatty acids (FA). It is caused by mutations in the ACADS gene resulting in FAD-deficient short-chain acyl-CoA dehydrogenase FA deficiency (EC 1.3.8.1). Clinical symptoms are very heterogeneous, including no symptoms and severe metabolic decompensation. Implementation of NBS for SCADD is still the subject of discussion with different views. Expansion of known biomarkers (butyryl carnitine in blood and urinary ethylmalonic acid) may lead to increased selectivity for possible seizures of this disease.
Methods / Case Report: The aim of the study was to compare metabolic profiles of blood specimens of 165 patients and 122 controls. Samples were obtained as part of the neonatal screening of the Slovak population on the basis of informed consent. The sampling and sampling procedure was the same as the classic NBS preparation. Samples were analyzed by liquid chromatography with the TipleQuad 6500 mass spectrometer (SCIEX, Framingham, MA, USA) in MRM mode. Statistical evaluation was carried out in the R program.
Results: A total of 127 metabolites were detected by the targeted metabolomic method in the dry blood spots. Unsupervised principal component analysis showed patient separation from control samples. Based on the least squares orthogonal discrimination analysis (OPLSDA), the most discriminating metabolites were found: butyryl carnitine, oxalacetate / glutarate / ethyl malonate, inosine, hypoxanthine, inosine monophosphate and medium chain acylcarnitines.
Discussion: In SCADD patients, the results of targeted metabolomic analysis suggest not only changes in butyryl carnitine and ethylmalonate but also the influence of purine metabolism and medium chain acylcarnitines. Next, the data will be compared in terms of mutations in the ACAD gene and non-target lipidomic analysis will be performed.
GRANT SUPPORT:
GACR 18-12204S a NPU I (LO1304)

04. Dietetics and nutrition

P-058

Ketogenic Diet: Impact of C10 and beta-hydroxybutyrate on sirtuins in cultured murine neurons

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Background: Ketogenic Diet (KD) is a clinically well-established therapeutic option in pharmacoresistant epilepsy; however the mechanism of antiepileptic action is still elusive. Sirtuins (SIRT), a group of NAD dependent deacetylases, are important regulators of cellular energy metabolism. The link of ketone bodies to energy metabolism prompted us to hypothesize that KD may have an impact on sirtuins, mediating its antiepileptic therapeutic effect.
Methods: HT-22 neurons (hippocampal mouse cell line) were incubated with decanoic acid (C10) & beta hydroxybutyrate (BHB), the key metabolites accumulating under KD, for 1 week in DME medium under hypoglycaemic conditions. Enzyme assays, Western Blot and qRT-PCR were performed for SIRT 1, 2 & 3. Monocarboxylate Transporters (MCT) 1, 2 responsible for the uptake of BHB and transport of lactate in myelinated axons, were measured at gene expression level.
Results: A consistent Sirtuin induction was seen with BHB at the enzyme, protein and gene expression levels. C10 treatment showed variable results on the Sirtuins. Although both MCT1 and MCT2 expression was increased in KD component treated cells, MCT2 showed a more prominent change.
Discussion: Sirtuin and MCT 2 induction in HT-22 cells by key components of KD may result in alterations of cellular metabolism which may play a role in the antiepileptic effect of KD.

P-059

Predictors of micronutrient deficiencies in paediatric patients with PKU

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Background: Phenylketonuria (PKU) diet is protein-restricted, and therefore daily micronutrient-enriched protein substitutes are prescribed to PKU patients. Despite of this, micronutrient deficiencies are commonly reported in PKU patients. Therefore, this study aims to evaluate the micronutrient status of children with PKU, and to investigate predictors of micronutrient deficiencies in this group of patients.
Methods: This was a retrospective analysis of clinical data obtained from PKU children (≤16 years) attending metabolic medicine clinic at the Glasgow Royal Hospital for Children between 1990 and 2013. The study included 81 patients who provided a total of 512 blood samples for their routine annual micronutrient screening.
Results: Status of vitamin B12, E, and serum and erythrocyte folate measurements were above laboratory reference ranges (RR) in 27%, 54%, 46% and 35% of the blood samples, respectively. However, 44 % of selenium and 14% of zinc measurements were below the RR. Multivariate regression analysis revealed that poor metabolic control (higher % of measurements with raised PHE concentrations), severe PKU (low number of prescribed protein exchanges), and low adherence to PKU protein substitutes were predictors associated with low selenium status. On the other hand, low zinc status was solely predicted by low adherence to PKU protein substitutes. Nevertheless, collectively these predictors explained a small proportion of (5.8 – 8.8 %) variation in the micronutrient levels in patients with PKU. Both selenium and zinc showed reduced concentrations in blood samples of patients with high or normal status of vitamins E, B12 and folate.
Discussion: Selenium and zinc deficiencies are common in PKU patients. The results suggest that selenium and zinc deficiencies reported in patients with PKU may be due to other factors which we were unable to measure in this retrospective study such as low bioavailability of those nutrients from the PKU protein substitutes.

P-060

Contribution of Thermic Effect of Feeding and Fat Oxidation to Body Fatness and Prevalence of Obesity in Patients with PKU

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Background: Prevalence of overweight and obesity is increasing among patients with PKU. We have previously shown in healthy individuals that consumption of a PKU special low protein food (SLPF) may increase risk of obesity by diminishing thermic effect of feeding (TEF) and postprandial fat oxidation (FO). This study aimed to evaluate the resting metabolic rate (RMR), TEF and body composition in patients with PKU.
Methods: Thirteen PKU patients and ten healthy controls matched for gender, age and BMI were recruited. PKU participants received a PKU SLPF-based meal and the healthy controls an isocaloric meal (Controls). RMR was measured at baseline and TEF and FO over a period of 3 hours postprandially. Body composition was measured using deuterium dilution technique.
Results: RMR (kJ/min/kg) of fat-free mass, median (IQR), 0.10 (0.04) vs 0.09 (0.02) was not significantly (P>0.05) different between the PKU and the Control groups. TEF calculated as increase in EE, relative to the energy provided by breakfast meal, was not significantly (P>0.05) different between PKU 5.8% (7.9) and Control 6.7% (8.6) groups. Similarly, amount of fat oxidised postprandially was not significantly (P>0.05) different between the two groups, PKU, 9.7 g (6.8) vs Control 10.6 g (4.8). PKU patients tended (P=0.08) to have a higher body fatness PKU 35% (13) vs Control 25% (13), despite no differences in fat-free mass.
Discussion: In this pilot study, no differences in RMR, TEF and postprandial FO were observed between patients with PKU and matched controls. These findings need to be verified by larger studies.

P-061

Low plasma zinc and selenium in adult patients with Maple Syrup Urine Disease- A single centre experience.

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Background: Maple Syrup Urine Disease (MSUD) is a rare inherited metabolic disorder. The mainstay of long-term therapy is a synthetic formula devoid of leucine, isoleucine, and valine and adding appropriate amounts of these BCAAs in the patient's regular diet. Essential amino acid deficiency may result in epidermal dysfunction because keratinocytes growth is arrested, which is especially apparent in isoleucine deficiency (Shipley 1987). We present a review of selected micronutrient deficiencies and possible associated complications in adult patients with MSUD.
Methods: Known patients affected with MSUD attend Adult Metabolic Clinics at Salford and have their trace elements routinely measured in blood 6-monthly as a part of a nutritional

assessment. Zinc and selenium were analysed using ICPMS method. Reference ranges for zinc and selenium were: 10–21 μmol/L and 0.55-1.2 μmol/L respectively.
Results: RESULTS from 12 patients were extracted (8/12 females). Median age was 30 years (range 19–40). MSUD control was variable with median leucine concentration of 277 μmol/L (83–424) and isoleucine 171 umol/L (42–760). All patients took synthetic protein daily, with some taking extra multivitamins. Median zinc was 9.4 μmol/L (range 5.4-12). Median selenium was 0.65 μmol/L (range 0.3-0.97). There was no correlation between trace element concentrations and the reported natural-protein or synthetic-protein intake. 3/12 patients had BMI =< 18 kg/m^2. 8/12 (66%) patients reported dryness of the skin, eczema or hair loss.
Discussion:Zinc, selenium and isoleucine deficiencies, common in our MSUD population, may contribute to dermatological complications and warrant further review of nutritional status. Potential causes include inadequate food intake, poor compliance with supplements, malabsorption, conditionally increased requirements and/or excess losses of these trace elements.
Conflict of Interest declared.

P-062

Individual dietary intervention in adult patients with mitochondrial disease due to the m.3243A>G mutation: the DINAMITE study

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Background:To evaluate the effect of an individually tailored dietary intervention on nutritional intake, body composition (BC), functioning, and quality of life (QoL) in adult patients with mitochondrial disease (MD) due to the m.3243A>G mutation.
Methods: In this explorative randomized controlled trial a total of 39 MD patients were included. The intervention group (n=20) received an individually tailored dietary intervention for six months. The control group (n=19) received usual care during six months (control period) followed by an individually tailored dietary intervention for the next six months (intervention period). Nutritional assessment and QoL measurements were performed at three-month intervals. Personalized treatment goals of the MD patients were evaluated at 3 and 6 months during dietary intervention. Linear mixed models were used to test the effect of the dietary intervention on continuous outcomes. Achievement of the personalized goals was assessed using descriptive statistics.
Results: Individually tailored dietary intervention increased handgrip strength (HGS) (p=0.037), the vitality component of QoL (p=0.026), and decreased fatigue score (p=0.024) after three months of treatment, but this effect did not last. No significant effect was observed for protein intake and BC. After 3 months 56% of personalized goals were achieved and 97% of the patients achieved at least one goal. Most goals were achieved for BC, HGS, and gastro- intestinal complaints.
Discussion: An individually tailored dietary intervention is promising to achieve personalized goals of patients with MD, especially with regard to BC, HGS, and gastro-intestinal complaints. The intervention also improves handgrip strength, QoL, and decreases fatigue.
I would suggest the authors to report more data about:
a) The dietary intervention on nutritional intake concerning to diabetes, gastrointestinal issues, seizures, stroke-like episodes
b) The phenotypic heterogeneity they observed in their patients.
Conflict of Interest declared.

P-063

Transition from paediatric to adult care in phenylketonuria (TRANS-PAC-PKU): the 2 year's impact on metabolic control and adherence

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Background: In PKU, transition to adult care (TAC) is challenging and information on adults follow-up is limited. We aimed to see how TAC affects metabolic control and adherence.
Methods: 55 PKU patients (55% females; 5 HPA, 26 mild PKU and 24 classical PKU) in whom TAC occurred between 2011 and 2015, were analysed in two different study periods: 2 y pre and post-TAC (SP1 and SP2, respectively). Mean age at TAC was 23.3 ± 4.3 y. None of the patients received sapropterin, but there was one pregnancy in SP2. Retrospective data on metabolic control (median blood [Phe], number of blood spots and % of blood [Phe] < 8 mg/dl) and number of clinic visits was collected for SP1 and SP2. Natural protein (NP, g/kg), protein equivalent (PE, g/kg), total protein (TP, g/kg) and Phe (mg/day) intakes closest to TAC were compared with those recorded on the first appointment after SP2.
Results: In SP2, 3 patients (2 females) were lost in follow-up (6%) resulting in a final sample of 52 patients. Median number of analysed blood spots significantly increased in SP2: 22 [13–30] vs. 29 [15–41]; p=0.002. Mean (SD) of the median blood [Phe] remained stable from SP1 to SP2 (8.7 ± 4.1 vs. 9.1 ± 3.7; p=0.100) while the median % of blood [Phe] < 8 mg/dl significantly decreased in SP2 (51.5 [3.7-95.7] vs. 36.5 [4.6-84.6]; p=0.041). Median number of total clinic visits significantly increased in SP2 (5 [4–6] vs. 11 [8–13]; p< 0.001). NP, PE, TP and Phe remained similar between SP1 and SP2: 0.46 [0.35-0.88] vs. 0.46 [0.28-0.94], p=0.873; 0.85 [0.47-1.10] vs. 0.83 [0.43-1.05], p=0.066; 1.51 [1.26-1.66] vs. 1.34 [1.07-1.54], p=0.194; 1210 [830–2311] vs. 1318 [763–2935], p=0.278, respectively.
Discussion: TAC had limited impact on metabolic control and few patients were lost in follow-up. Maintenance of similar dietary patterns, increase of clinical visits and the inclusion of the same experienced nutritionists in paediatric and adult care multidisciplinary teams may have contributed to our results.
Conflict of Interest declared.

P-064

Early feeding in Phenylketonuria infants across Europe

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Background: In infants with phenylketonuria (PKU), dietary management is based on lowering and titrating phenylalanine (Phe) intake from breast milk or standard infant formula in order to maintain blood Phe levels within target range. Different methods are used to feed PKU infants and our survey aimed to assess practices across Europe.

Methods / Case Report: We sent a cross sectional, survey monkey® questionnaire to IMD European health professionals. It contained 31 open and multiple-choice questions. The results were analysed according to different geographical regions.

Results:Ninety-five centres from 21 countries responded. Over 60% of centres commenced diet in infants by 10 days of age, with 58% of centres performing newborn screening by day 3 post birth. At diagnosis, infant hospital admission occurred in 61% of metabolic centres, mainly in Eastern, Western and Southern Europe. Breastfeeding fell sharply following diagnosis and only 30% of women still breast fed at 6 months. 53% of centres gave pre-measured Phe-free infant formula before breast feeds to satiety and 23% alternated breast feeds with Phe-free infant formula. With standard infant formula feeds, measured amounts of this was followed by Phe-free infant formula to satiety in 37% of centres (n=35/95), whereas 44% (n=42/95) advised mixing both formulas together. Weaning started between 17–26 weeks in 85% centres, ≥26 weeks in 12% and < 17 weeks in 3%.

Discussion:This is the largest European survey completed on PKU infant feeding practices. It is evident that practices varied widely across Europe, and this aspect received little focus in the PKU European Guidelines (2017). There are few reports comparing different feeding techniques with blood Phe control and its stability and growth. Controlled prospective studies are necessary in order to assess how different practices may influence these outcomes in order to define the optimal PKU infant feeding practices.

P-065

Nutritional management of maternal PKU in CHP (NMMPKU): retrospective study and offspring outcome

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Background: Optimal nutritional management during pregnancy is essential to prevent maternal phenylketonuria (MPKU) syndrome. We retrospectively studied the nutritional management practices compared with infant outcome in a series of MPKU case reports followed at our centre.
Methods: Data on metabolic control, nutritional intake, anthropometry and biochemical markers of 5 pregnancies, 4 single and 1 multiple, from 2007 to 2017 was collected. A total of 4 early diagnosed PKU women, 1 was pregnant twice, had an age range at conception of 25–31 y. Data was collected before (BP), during (DP) and post pregnancy (PP). Body composition BP and PP was interpreted. Intrauterine development and neonatal anthropometry was recorded for the 6 offspring (2 females and 4 males).
Results: BP median blood [Phe] was 2.6-11.4 mg/dL (20-95% of the values within target range). Two pregnancies were planned. DP median blood [Phe] range reduced into 2.5-5.5, 1.2-3.7 and 1.0-4.0 mg/dL by the end of trimester 1, 2 and 3, respectively. PP median blood [Phe] range increased to 6.7-20.2 mg/dL. All women were treated with a Phe-restricted diet. In 4 cases Phe-free L-amino acids mixtures (AAM) (28–84 g/d) and in 1 pregnancy a combination of GMP and AAM were used. Phe intake BP was between 414–1415 mg/d and, by the end of trimester 1, 2 and 3, was 460–1018, 469–1259 and 1336–1520 mg/d, respectively. Protein equivalent intake DP was 0.9-1.3 g/kg/d. Maternal weight gain was 13.5-21 kg. Body fat % increased from 25.8-32.7% (BP) to 30.9-37.7% (PP). No significant biochemical deficiencies were found. Four were eutocic births with one elective caesarean. Infants born at 36–39 w, with 2420–3410 g (weight), 45–49 cm (length) and 31.5-34 cm (head circumference) and were breastfed until 1–7 months. Only one woman stopped diet PP.
Discussion: Although sub-optimal metabolic control BP, excellent blood Phe control was achieved throughout all pregnancies, allowing normal neonatal outcomes in all 6 children.
Conflict of Interest declared.

P-066

How Europe weans their babies with Phenylketonuria

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Background: In phenylketonuria (PKU), weaning is more challenging than in normal infant feeding. The primary aim is to replace natural protein from breast milk or standard infant formula with solids containing equivalent phenylalanine (Phe). In addition Phe-free solids are given and a second stage Phe-free protein substitute (PS) from 6 months. Our aim was to assess weaning approaches across Europe.
Methods / Case Report:A cross sectional questionnaire (survey monkey®) composed of 31 questions was sent to IMD European colleagues. Centres were grouped into geographical regions for result analysis.
Results:Weaning started at 17–26 weeks in 85% (n=81/95) of centres, >26 weeks in 12% (n=11/95) and < 17 weeks in 3% (n=3/95). Infant's age and infant interest in foods were important factors influencing age of weaning. First solids included mostly low Phe vegetables (59%, n=56/95) and fruit (34%, n=32/95).51% (n=48/95) of centres introduced Phe containing foods at 17–26 weeks and 48% (n=46/95) at >26 weeks. A Phe exchange system was used by 52% (n=49/95) of centres mainly from Northern and Southern Europe and 48% (n=46/95) counted all Phe containing food sources (all centres in Eastern Europe and majority from Germany and Austria).A second stage/higher protein equivalent containing PS was introduced by 41% (n=39/95) of centres at infant age 26–36 weeks (mainly from Germany, Austria, Northern and Eastern Europe) and 37% (n=35/95) at age >1y mainly from Southern Europe. 53% (n=50/95) of centres recommended the second stage PS as a gel, paste or semi-solid.
Discussion:Weaning strategies vary widely according to different regions. No reports are available assessing how different strategies may influence adherence to the PKU diet and acceptance of PS during childhood, so prospective long-term studies are important. It is important to define the most acceptable weaning strategy for infants associated with least caregiver burden.

P-067

Dietary management and outcome of patients with Citrin deficiency from one UK centre

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Background:Citrin deficiency (CD), caused by mutations in SLC25A13, typically presents with neonatal cholestasis or faltering growth and dyslipidaemia in older children. A low sugar, high fat and protein (LS) diet is often prescribed. We describe management in 7 CD patients (9y, 6–14).
Methods: All children had Pakistani origin, first cousin consanguineous parents except Case7.
Results: Family1[homozygous c.1763G>A(p.Arg588Gln)]: Case1; presented at 6m but diagnosed at 6y with coeliac disease and CD. Presented with recurrent ketotic hypoglycaemia. On fasting she had sluggish ketone production, which led to whole exome sequencing. She started a gluten free, LS diet with MCT oil and 1 night feed. Z scores: height (H) -3.04, weight (W), −1.34. Case2 and 3 were diagnosed by sibling screen. Case2: diagnosed at 8y, sibling screen, H, −2.34; W, −1.44. Case3; asymptomatic with normal growth. Diet commenced at diagnosis (6y). Family2[homozygous, c.1763G>A (p.Arg588Gln)]: Case4; diagnosed at 7y, had GSD type IX. H, 1.29; W, 1.04. Diagnosed with CD by whole exome sequencing. Case5: sibling screen at 5y, asymptomatic. H, −1.13; W, −0.98. Both have normal diet but fed 4 hourly. Case6 [homozygous, 1610–1612 delTA Gins AT]: presented with neonatal cholestasis and diagnosed in the first year. Given LS diet with MCT oil but still eats sugar at 11y. Has abdominal pain with constipation. H, −1.97; W, −2.74. Case7 [homozygous 1465T>C (C489R in cDNA but heterozygous in genomic DNA]: presented with neonatal cholestasis. LS diet (with LCT emulsion) started at 2y. Carbohydrate limit of 30g/day at 8y due to recurrent admissions with abdominal pain which resolved with overnight gastrostomy feeds. H, 0.86; W, 0.66.
Discussion: Treatment is an individualised LS diet with emergency feeding plan. Patients had variable tolerance to carbohydrate. Growth was poor in 4/5 children, diagnosed after 1y. Data is required assessing different approaches in relationship to clinical symptoms and outcome.

P-068

Testing maximum natural protein tolerance in patients with Phenylketonuria

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Background:Phenylalanine (Phe) tolerance is highly variable in phenylketonuria (PKU) and rarely described in patients aged ≥12y. In Portugal, the upper target blood Phe level ≥12y is 480 μmol/l. As part of a sapropterin (BH4) loading test protocol, we systematically challenge with additional natural protein (NP) until Phe levels reach 480μmol/l. Prior to BH4, we report additional Phe tolerance.
Methods / Case Report:We studied 40 well-controlled PKU patients (11 HPA, 21 mild and 8 classic PKU) with mean age 17±5y (12-29y). NP tolerance was calculated at baseline and a median of 6m after systematic challenge with NP, during 2014. The aim was to maintain blood Phe at 480μmol/l. Anthropometry was assessed at both times. Routine blood Phe results were collected.
Results:Although, NP did not significantly increase (0.91vs.0.77g/kg, p=0.083), 26 patients (11 HPA, 11 mild and 4 classic PKU) were

able to increase in median NP intake by 12g (2-42g)/day. In fact, 20 patients increased NP by at least 20% and 5 (3 HPA, 1 mild and 1 classic PKU) tolerated a 50% increase prior introduction of BH4. 20 patients increased NP from animal sources like dairy products, fish and meat but 6 patients (3 mild and 3 classical PKU) only increased NP from non-animal foods (bread, pasta, potatoes). Protein equivalent from protein substitute intake decreased from 1st to 2nd assessment (0.77vs. 0.69g/kg, p=0.002) and blood Phe levels did not change (276±96vs.288±96 μmol/l, p=0.06).
Discussion: Many older patients with PKU tolerated additional Phe when challenged. It is possible that some patients may be over treated, and it is important to assess maximum Phe tolerance routinely. Extra dietary Phe may positively influence quality of life. Moreover, different sources of NP may have a different impact on rate of Phe absorption and blood Phe and this needs systematic study.

P-069

Nutritional status in children with PKU: glycomacropeptide compared to phenylalanine free AA

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Background: In children with PKU, nutritional status is an important consideration particularly, with the primary nutrient supply being provided by protein substitutes (PS), supplemented with vitamins and minerals. Glycomacropeptide (CGMP-AA) has many suggested biological advantages. It is possible that it may enhance vitamin and mineral status, but no studies have compared the efficacy of CGMP-AA compared with conventional amino acid substitutes (L-AA) on nutritional status in children with PKU.
Methods: 48 children with PKU, median age 9.2y (5-16y), 28 boys, were divided into two groups; CGMP-AA (n=29) or L-AA (n=19)..A fasting morning venous blood sample was taken at baseline (when all the children were on L-AA) and after 6m intervention, and analysed for: zinc, selenium (plasma and whole blood), calcium, magnesium, phosphate, C-reactive protein, haemoglobin, mean cell volume, ferritin, vitamin B12 and 25 hydroxy vitamin D.
Results: At the end of 6m, median % of PS provided by CGMP-AA was 75% and L-AA, 25%. Whole blood and plasma selenium significantly increased from baseline to 6m (p=0.0002, p=0.0007) in the CGMP-AA compared to L-AA group. Within the CGMP-AA group from baseline to 6m whole blood selenium (p=< 0.0001) and plasma selenium (0.0005) significantly increased, but ferritin decreased (p=0.0006), but median values all remained within reference ranges (RR). Comparable changes were not observed in the L-AA group. All other nutritional parameters measured at baseline and 6m were within the RR. One exception in both groups was vitamin B12, which was above the RR at 6m. No differences were observed at baseline between groups.
Discussion: Both protein substitutes are efficacious in providing adequate nutritional status. There was a marked significant increase in plasma and whole blood selenium with CGMP-AA. It is possible the bioactive antioxidant properties of CGMP-AA and absorption in the gut may have a selenium sparing effect compared to a non-peptide-based feed.
Conflict of Interest declared.

P-070

Variability of Phenylalanine concentrations over 24 hours using two different protein substiutes and changing dietary phenylalanine intake.

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Background: In PKU, there is evidence that glycomacropeptide (GMP-AA) may improve protein utilisation, which may stabilise blood phenylalanine (Phe) variability. In children with PKU, we studied the impact of GMP-AA on 24 hour blood Phe variability. A 6 week randomised controlled crossover study assessing blood Phe over 24 hours in children with PKU under three controlled dietary regimens, using 2 different protein substitutes CGMP-AA and Phe-free amino acids (L-AA).
Methods: We recruited 18 children with PKU, median age 10y (6–16), 7 boys. Each subject was randomised to 3 different regimens(R): R1, CGMP-AA and usual dietary Phe (CGMP+Phe); R2, CGMP-AA minus Phe from diet (CGMP minus Phe); R3, L-AA and usual Phe intake. Each regimen was for 14 days; on the last 2 days, Phe blood spots were collected 4hrly for 48 hr (08h,12h,16h,20h,24h,04h). Isocaloric intake was maintained.
Results: There was a consistent significant difference in median Phe concentrations over 24 h between each group: median Phe μmol/L; R1 v R2: 290 (30–580), 220(10–670) p< 0.0001; R1 v R3: 290 (30–580); 165 (10–640) p< 0.0001; R2 v R3: 220(10–670), 165 (10–640) p=0.0009. There was a significant difference in median Phe at each time point between R1vR2, p=0.0027 and R1vR3 p< 0.0001, but not between each time point for R2 v R3. Two groups show a reduction in Phe over time R2 = −1.67 Phe/hour and R3 = −2.05 Phe/hour, and R1 an increase over time+1.32 Phe/hour. Differences in the rate of change do not reach statistical significance. Of the 18 patients, 8 in the R1 group (44%) maintained target Phe levels for the study period, 10 in the R2 group (56%) and 7 in the R3 group (39%) [p = 0.052 – Chi-Square test]
Discussion: The residual Phe in CGMP-AA increases blood Phe control in children. Difference in the rate of change of Phe does not reach statistical significance although there is some evidence of GMP minus Phe giving the most consistent profile.
Conflict of Interest declared.

P-071

Dietary management of PKU during pregnancy – survey of practice at specialist centres in the UK and Ireland

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Background: Management of phenylketonuria (PKU) during pregnancy is highly challenging and of critical importance. The recent publication of the European PKU guidelines aims to standardise care, including maternal PKU (mPKU) care, but little is known about current variations in practice within the UK and Ireland.
Methods / Case Report: An online survey was sent to centres in the United Kingdom and Ireland that manage PKU pregnancies. Reponses were received from 10 centres. The survey was conducted prior to the publication of European guidelines.
Results:There was no single target range for phenylalanine in pregnancy used by all of the centres. 5 centres reported that ≤50% of PKU pregnancies were planned (levels in range prior to conception) and 5 centres reported that 75-100% of pregnancies are planned. 7 centres reported that dietary treatment would be commenced within 1 day of being informed of an unplanned pregnancy. Achieving phenylalanine levels in range if pregnancy was unplanned varied from 3–28 days. Recommendation of additional folic acid varied, and the majority of centres supplement with additional L-tyrosine during pregnancy. All centres discussed the importance of planning a pregnancy with female patients at routine clinic visits.
Discussion:There is variation in current mPKU practice in the UK and Ireland. Following the publication of the European guidelines, it will be important to assess what impact these may have on standardising care within the UK and Ireland.
Conflict of Interest declared.

P-072

Various aspects of quality of life in parents of phenylketonuria pediatric patients in Anatolia, Turkey

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Background: Early dietary treatment of phenylketonuria (PKU), results in normal cognitive development. Therefore, caring for these children requires a great deal of attention and effort. This study was planned to evaluate the quality of life (QOL) of parents having children with PKU residing in Anatolia, Turkey.
Methods/Case Report: This cross-sectional descriptive study comprised of a study group of parents of 62 PKU patients and 62 age- and sex-matched healthy controls. The 36-Item Short Form Health Survey questionnaire (SF-36) was used to assessed the health-related quality of life (QOL) and explore the eight domains of the SF-36 to examine physical, mental, social and environmental health.
Results: Out of all participants in the study, 54,8% were mothers. The mean age of the PKU patients was 9.65±4.92 years (50,1% were under 10 years of age) and 90.3% were diagnosed by the National Neonatal Screening Programme and were treated early. The mean total QOL scores of the mothers and the fathers in the study group were found to be 46.96±13.37 and 70.39±8.84, respectively (p< 0,05). The lowest QOL scores were found in the mothers of PKU patients and highest QOL scores in the fathers of healthy controls. Various aspects of QOL have been analyzed depending on gender, age, and educational levels of the subjects. QOL was not only influenced by gender, but also greatly by the educational level of the parents. Moreover, among all groups, vitality scores were significantly higher in parents with 1-3-year-old PKU patients (73 ±5.7) compared to all other groups.
Discussion: According to the findings of this study, the QOL of mothers of PKU patients were more affected than their fathers. It could be recommended that special attention be given to the improvement of the social and psychological support for parents (especially mothers) of sick children.

P-073

Swallowing problems in patients with a mitochondrial disease

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Background: Dysphagia for solid food is reported in patients with mitochondrial disease. In 2017 the Test of Mastication and Swallowing Solids (TOMASS) was published, a test to quantitatively assess solid bolus ingestion. The aim of the current study was to objectify the problems of solid food ingestion in patients with mitochondrial disease due to the m.3243A>G mutation, using the TOMASS.
Methods: 60 patients (17 men and 43 women, age range 19–67 years) carrying the m.3243A>G mutation were included. Sex, age, body mass index and heteroplasmy percentage of the mutation were gathered as personal characteristics. Patients were asked to eat a standardized cracker (Albert Heijn Basic^{TM}), following the protocol. The number of bites, masticatory cycles, swallows and total time were scored from videotape. For reliability measurements Intra Class Correlation Coefficients (ICC) were calculated. Three speech language therapy students scored 10 videos for inter-rater reliability. After two weeks, the same videos were scored again for intra-rater reliability. Data were compared with (Dutch) normal values using independent t-tests. Multiple linear regression analyses (on the normally distributed factor total time) and MANOVA (on number of bites, masticatory cycles and swallows) were used to test the influence of personal characteristics.
Results: Patients need more mastication cycles (p=.00), more swallows (p=.01) and more time (p=.00) for the cracker. Inter-rater reliability was excellent to good: 1.00 (bites), .969 (masticatory cycles), .976 (time), .741 (swallows). Intra-rater reliability was also excellent to good: 1.00 (bites), .970 (masticatory cycles), .964 (time), .798 (swallows).There were no statistically significant influencing personal characteristics.
Discussion: The TOMASS objectified dysphagia for solid food in patients with a mitochondrial disease. Assessment and management should be considered in this group.

P-074

Successful dietary management of infantile onset lysosomal acid lipase deficiency (LAL-D)

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Background: Lysosomal acid lipase deficiency (LAL-D) is a rare condition of impaired fat metabolism characterised by malabsorption, faltering growth and hepatosplenomegaly. Until recently treatment was palliative with minimal dietetic intervention. We present a case of successful nutritional intervention in an infant diagnosed with LAL-D and commenced on enzyme replacement therapy (ERT).
Case Report: The infant presented at 2 months with diarrhoea and faltering growth despite adequate oral intake. Weight (wt): 3.09kg (< 0.4th centile), birth wt: 3.12kg (25th centile), length 51cm (0.4th centile). Parenteral nutrition (PN) along with trophic feeds of an amino acid

formula via nasogastric tube (NGT) was initiated. A diagnosis of LAL-D was confirmed and ERT commenced, wt: 4.66kg (< 0.4th centile) length: 58cm (2nd centile). A modular fat-free feed using an amino acid powder, glucose polymer, electrolytes, vitamins and minerals was used. From 5 weeks post-ERT, oral feeding was introduced. At 8 weeks post-ERT, Medium chain triglyceride (MCT) fat was added at 1g/kg, to the enteral feed. At 8 months of age, fat free solids were introduced and PN subsequently stopped. Gradual replacement of each feed with equivalent calories and fat from solids is planned using 1g LCT fat exchanges. The fat intake will be increased by 1g per month.
Results: By the age of 15 months, wt is between 50th-75th centile, mid arm circumference < 50th centile, length: 9th centile. Most feeding is oral, consisting of 3 meals daily along with 4 bottle feeds with occasional NG top ups.
Discussion: Few patients are reported as taking full oral diet. Knowledge surrounding the tolerance of LCT/MCT fat in this patient group is sparse. Our patient has no aversion to oral feeding, making progression with solids a challenge. Close monitoring of introduction of LCT fat to 1g/kg will provide information to help the management of this very challenging condition.
Conflict of Interest declared.

P-075

The successful use of C8 oil in Carnitine-acylcarnitine Translocase deficiency (CACTD)

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Background: CACTD is an extremely rare inherited metabolic disease caused by the inability to transfer long chain fats into the mitochondrial matrix. There is a high risk of mortality and cardiac involvement in surviving patients. Experience is limited and describes the use of medium chain triglyceride (MCT) formulas (84% MCT: 60% C8, 40% C10) and long chain triglyceride (LCT) restriction. However, Parini *et al*. (1999) recommends limiting the use of MCT as they suggest that 50% of enteral MCT is not oxidized in CACTD, resulting in energy deficiency and tissue toxicity of non-oxidized C10-12 fatty acyl-CoA. We present a novel dietary regimen using C8 triglycerides (TG) and avoidance of C10 TG.
Case Report: A female patient born to consanguineous Kurdish parents was identified prenatally. Her sibling died at 36 hours of age and was subsequently found to be homozygous for a mutation in the *SLC25A20A* gene, associated with severe CACT deficiency. Our patient was born in good condition with normal biochemistry. Cardiac assessment showed a supraventricular arrhythmia, responsive to medical therapy, and no cardiomyopathy. A modular feed containing a minimal fat formula, C8 oil and essential fatty acids (per 100ml: 76kcal, 14.9% carbohydrate, 1.6% protein, 0.9% C8, 0.2% LCT) was commenced. Topical C8 oil (2ml 6-hourly) was also used. On discharge, a feeding regimen of 3-hourly boluses and continuous overnight feeds was achieved. An emergency regimen of 10% carbohydrate with enteral and topical C8 was advised.
Results: Plasma C10 levels decreased following treatment. Development and growth are age-appropriate at 7 months. She has not had any metabolic decompensations and has commenced low fat weaning.
Discussion: We have demonstrated the use of C8 oil as an alternative fat source to supplement a minimal LCT feeding regimen in an infant with severe CACT deficiency. This challenging treatment scenario was compounded by a non-English speaking family with limited education.

P-076

Systematic review of the effect of ketogenic or high-fat diet in mitochondrial diseases

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Background: The reason to start a ketogenic diet (KD) or high-fat diet (HFD) in patients with a mitochondrial disease differs among centers in the Netherlands as there is no clear guideline. The objective was to review the evidence and collect published cases to assess the clinical benefit.
Methods: We searched Pubmed (April 2018) for English publications reporting patients with a mitochondrial disease (MD) using a KD or HFD. The following search terms were used: mitochondrial, disease, disorder, MELAS, Leigh, complex deficiency, respiratory chain defect, ketogenic diet, ketone bodies, modified Atkins, high-fat diet. Cases without a reported outcome and cases diagnosed with PDHC deficiency were excluded. Reference lists were also reviewed. Two authors independently screened and selected the papers. Discrepancies were resolved through discussion and consensus. One author extracted data, the second author checked data for completeness.
Results: Out of 777 papers we identified 16 papers reporting 41 cases with MD (19 genetically confirmed). The major reason for initiation of the KD was intractable epilepsy (IE) (n=29) with a reported effect similar to KD in IE without MD. In 12 cases KD was initiated for other reasons than epilepsy. Two of them (2/12) showed some clinical improvement, possibly due to the KD or due to the fluctuating course of the disease. In addition 1 cohort (MD patients with IE n=24) and 3 trials (MD patients without IE n=10) were found. Side effects as hypoglycemia, metabolic acidosis and hyperlipidemia were reported. One trial reported muscle damage in myopathic patients.
Discussion:KD in MD might be beneficial in the treatment of IE, for other signs and symptoms there is no evidence. KD or a HFD should not be standard initiated after the diagnosis mitochondrial disease as the clinical effect seems very limited and side effects can be serious.
Conflict of Interest declared.

P-077

Impact of liver transplantation on protein tolerance and biochemical parameters in children with inborn errors of protein metabolism

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Background: Liver transplantation (LT) represents a therapeutic approach for a growing number of metabolic diseases, allowing better quality of life by reducing risk of metabolic decompensation and need of dietary restrictions.
Methods / Case Report:We report a retrospective analysis on 24 children undergoing LT in the period 2008–2018: 7 with urea cycle defects (4 ASLD, 2 OTC, 1 CPS), 6 with tyrosinemia type 1-HT-1 (5 with HCC,

1 with chronic liver failure), 4 with methylmalonic aciduria (MMA), 2 propionic aciduria (PA) and 5 with a maple-syrup disease (MSUD).
Results:Median age at transplant was 51 months (33–134), patients and graft survival was 100% and all children had normal graft function after a mean follow up of 30+/−25 months. Protein tolerance increased after LT allowing reaching RDA for age in a mean time of 2.8 months (range 1–5 months) in all patients. Plasma levels of target metabolites (μmol/L) resulted significantly reduced after LT. ASA in ASLD 445+/−45 vs 112+/−7, p< 0.001. BCAAs in MSUD: leucine 265+/−32 vs 206+/−7; p: 0.009; alloisoleucine 136+/−12 vs 15+/−1.5; p< 0.0001. MMA 606+/−414 vs 206+/−180; p< 0.0001. Tyrosine in HT-1 437+/−35 vs 190+/−43, p:0.0005.
Discussion:Normalization of protein tolerance and significant improvement of biochemical parameters after LT are achievable goals in patients with inborn errors related to aminoacid metabolism.

P-078

WITHDRAWN

P-079

WITHDRAWN

P-080

BMI trends of Adult Phenylketonuria Patients- Irish findings

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Background: Obesity and weight related disease is increasing worldwide.Adults with Phenylketonuria (PKU) in Ireland have a higher incidence of obesity when compared to the Irish population. This is contributing to other health concerns for this patient group as reported previously [1,2].
Methods: A retrospective audit was completed on PKU adults, at the Mater Hospital Dublin between 2016–2017. Body Mass Index (BMI) data was collected retrospectively from dietetic records assessed at annual clinic visits. BMI was compared with historical data collected in 2009 for the same population, and with the current incidence reported in the general population as per 'Healthy Ireland' (HI)Survey 2015[3]. Gender, BMI and age were recorded, anonymised, coded and compared. Maternal PKU adults actively pregnant were excluded. Data on 230 patients was collected compared to 195 patients in 2009.
Results: Data was analysed on 230 patients (37%male, 63%female), average age 36.7 years, median 37 years, range 21-73years. 70% were overweight or obese, 33% and 37% respectively. In 2009, 58% were overweight or obese, 32% and 26% respectively. 27% PKU females had a normal BMI (20-25kg/m2) compared to 44% from HI Survey. 29% PKU males had a normal BMI compared to 31% from HI Survey. Data showed a marked increase in BMI from 35years onwards in both PKU males and females.
Discussion: BMI has increased in the Irish PKU adult population in the last 8 years, with a greater number now classified as obese. Female patients appear to be more at risk of developing obesity than males and this may be associated with the PKU diet during pregnancy. Priority in PKU clinic is often patient's blood levels with limited time to focus on weight loss at annual reviews. Moving to group sessions for additional support on weight loss in this cohort and further investment in resources could help this population avoid developing co-morbidities associated with obesity.

P-081

Nutritional assessment and metabolic profile in children affected by phenylketonuria: a case–control study.

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Background: Phenylketonuria (PKU) is a well-known inborn error of phenylalanine (Phe) metabolism due to defects in phenylalanine hydroxylase enzyme activity. Main-stay treatment currently consists of a low Phe diet managed with the use of special low protein products and natural protein restriction replaced with Phe-free protein substitutes. As previously reported, PKU children have a higher daily glycemic index (GI) and glycemic load (GL) than healthy children. The aim of the present study was to evaluate the body composition and the metabolic profile in PKU compared to healthy children.
Methods/Case Report: 25 well-compliant-to-diet PKU children (aged 5–14 years), sex- and age-matched to 25 otherwise healthy children, were enrolled. Anthropometric measures, body composition analysis, dietary data (including GI and GL) and laboratory assessments were collected.
Results: Fat mass was significantly lower in PKU subjects (19,85%) compared to controls (22,74%), while total body water, fat-free mass (kg) and carbohydrate intake were significantly higher. Daily GI (including lunch and dinner GI) were significantly higher in PKU (p=0.001) and the same results was found for lunch and dinner GL (p= 0.05 and 0.02, respectively). Lipid profile, however, differed to healthy controls only for total and LDL cholesterol, which were lower in PKU. None of the glucose homeostasis indices (glucose, insulin, QUICK index, Homa, Homa-β) were significantly different. No difference was observed for anthropometric measurements.
Discussion: Although no adiposity increase or metabolic profile alteration was observed, PKU patients are consuming a diet with a overall dietary GI and GL higher than in healthy children. It is desirable for the future to improve carbohydrate quality in order to obtain a diet possibly enhancing protective aspects in the prevention of non-communicable diseases

P-082

Energy expenditure in a group of italian patients affected with argininosuccinic aciduria

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Background: Argininosuccinic Aciduria (ASA) patients (pts) often show central adiposity, despite adequate dietary intake. ASA expenditure and energy requirement information is limited.

Methods : We studied 13 ASA patients (pts) (9 females) age 2–60 years. Anthropometric variables (Height, weight, BMI and Waist Circumference to height ratio (WCHr)), body composition (BC) with Dual X-Ray Absorptiometry, and basal metabolic rate (BMR) through indirect calorimetry (IC) were assessed. BMR obtained through IC was compared to BMR calculated with the commonly used predictive equations (PE) FAO and Harris-Benedict. We also evaluated blood pressure (BP), insulin resistance (IR) markers and lipid profile.
Results: Overweight was detected in 31% of pts. Most pts showed hypertriglyceridemia (HT) (92%) and reduced HDL cholesterol levels (92%). WCHr was increased in 60% of subjects. Elevated BP and increased HOMA index were found in 38% and 27% of pts respectively. Metabolic Syndrome (MS) was diagnosed in 23% of pts. BMR measured by IC was significantly different from that estimated by PE ($p< 0.05$) both with or without adjustment by Fat-Free Mass data ($p< 0.05$). Most ASA pts presented increased cardiovascular risk (elevated BP, increased WCHr, HT, and IR) and 23.1% of pts met diagnostic criteria for MS.
Discussion: When compared to the golden standard IC, PE overestimated the energy need (average plus 15% and 17% respectively). PE are to be used with caution when estimating individual energy requirements in ASA. The use of IC is advisable in ASA pts because excessive energy prescriptions might aggravate their increased cardiovascular risk. A similar study is ongoing in other pts with different urea cycle disorders.

P-083

Parental difficulties in daily life and anxieties about raising children with inborn errors of metabolism who need diet therapies.

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Background: Dietary therapy for inborn errors of metabolism (IEM) is burdensome on patients and their parents. Generally, parents *manage a diet therapy* for their child until their transition has been completed. In order to support dietary adherence, it is important to understand parent difficulties in daily life and anxieties about raising a child with IEM.
Methods: This study was done through a questionnaire survey for those in the voluntary Japanese registry system for patients with IEM in 2015. Predictable causes of difficulties and anxieties were created by reviewing the previous studies focused on parents' quality of life. Descriptive statistics were reported.
Results: Nightly, three parents answered the above-referenced items. The mean age of children was 9.6 years old (range, 0–20), and mean parent age was 42.7 years old and 89.2% were mothers. Reported daily life difficulties included the heavy burden of the daily diet therapy for children (40.9%), and a lack of information about their child's disease and treatment (50.5%). Parental anxieties of raising a child with IEM included 75.3% of parents having anxiety about new symptom occurrences and 53.8% about symptom occurrences in deconditioning.
Discussion: Our results identified strategies to provide better qualitative care for parents raising a child with IEM who need diet therapy. Medical professionals should regard parents' burden and effort for their child's diet therapy as daily care in the outpatient department. Also, provision of new information about treatment and support resources would contribute to reduction of difficulties in their daily life. In addition, based on the high rate of anxiety about a child's symptom occurrences in deconditioning or future symptom reoccurrences, parents and *medical* professionals should discuss whether to go to emergency, outpatient, or regular clinical visits in case of each possible symptom occurrence.

P-084

A retrospective review of inpatient admissions to stabilise elevated phenylalanine levels

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Background: PKU is an inherited metabolic disorder affecting phenylalanine metabolism. The Irish incidence is 1:4500. Currently there are 500 patients under the care of National Centre for Inherited Metabolic Disorders (NCIMD) in Temple Street Children's University Hospital (TSCUH). Practice at present is to admit PKU patients with phenylalanine (Phe) levels that are consistently out of range despite intensive multidisciplinary team (MDT) input on an outpatient basis. The aim of this current study was to evaluate changes in PKU levels pre, during and post admissions and to examine of there was a sustained impact post discharge.
Methods: Ward admission record books from 2003–2013 were used to identify patients admitted for stabilisation only. Demographic data and the reason for admission was supported by the patient's medical and dietetic notes. Patient's individual Phe level records were used to collect their Phe levels 6 months prior to admission, during admission and at respective time points 1-6months & 7–12 months post discharge.
Results: Fifty six patients were admitted from Jan 2003-December 2013. Patients were all < 18 years of age. Greater than 70% (n=39) of the reasons for admission were due to multiple issues. Average admission time was 5 days. There was a significant decrease in median Phe levels from prior to the admission to during the admission. However, there was a significant increase in median Phe levels from during the admission (505 μmol/L) to both the 1–6 months and 7–12 months' time points(618 and 651μmol/L respectively).
Discussion: The results highlight that while inpatient admissions can stabilise levels within the acute setting this is not sustained long term. The ward environment does not accurately replicate home circumstances. Moreover this study highlighted that admissions are more likely to be multifactorial which are less likely to be possible to resolve during an admission period.

P-085

L-Isoleucine (ISO) supplementation in neonates at risk of Maple Syrup Urine Disease (MSUD) improves diagnostic procedures.

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Background: Babies at risk of MSUD require prospective birth plans and rapid diagnosis. BIMDG guidelines (www.bimdg.org) recommend 50% BCAA-free formula and 50% breastmilk/infant formula

with plasma BCAA measurements from 12–24 hours post birth. Allo-isoleucine (AI) is pathognomonic for MSUD. Our methodology only reliably detects AI in plasma samples at > 5μmol/L and dried blood spot (DBS) >10μmol/L. Limiting normal feeds may mask early diagnosis. We propose ISO supplementation from birth increases formation of AI in affected babies and decreases time to confirm or exclude a diagnosis. It also allows LEU intake to be restricted and plasma to remain low in affected cases.
Methods: To compare AI and LEU results in plasma and DBS in 6 neonates (born 2015–2018) with or without ISO supplementation.
Results: All were managed as BIMDG guidelines. Four had ISO supplements. MSUD was confirmed in 3 cases based on blood AI. **Case 1** - DBS results: day (D)1, AI < 10μmol/L, LEU 166μmol/L, D5, AI 11μmol/L, LEU 343μmol/L, D6, AI 26μmol/L, LEU 283μmol/L. Supplemented with ISO on D4-200mg. **Case 2** - DBS results: D2, AI 14 μmol/L, LEU 305 μmol/L, D3, AI 33 μmol/L, LEU 399 μmol/L. Supplemented with ISO on D1-50mg, D2-100mg, D3-150mg. **Case 3** - was diagnosed prenatally. DBS results: D1, AI 13 μmol/L, LEU 442 μmol/L, D3, AI 48 μmol/L, LEU 374 μmol/L. Supplemented with ISO from D5 as part of MSUD feed regimen. **Non-MSUD cases (n=3)** - blood results D1, AI in plasma < 5μmol/L and DBS < 10μmol/L. Blood monitoring was discontinued on D14, 6 (no ISO) and 4 (75mg ISO) as AI remained non-detectable and LEU normal.
Discussion: Case 2 received ISO supplementation from D1 with diagnostic blood concentrations of AI by D2. Case 1 had diagnostic AI only after ISO supplementation on D4. With ISO supplementation in non-affected cases we were more confident to stop blood monitoring and return to normal diet earlier. Our results suggest ISO supplementation increases AI and decreases time to confirm or exclude MSUD.

P-086

Management of Glycogen storage disease type IIIa with a ketogenic, high protein diet

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Background: The conventional treatment for Glycogen storage disease type IIIa (GSD IIIa) involves a high carbohydrate diet but this may worsen any cardiomyopathy due to accumulation of limit dextrin. Recently there have been a few reports of improvement with a high fat diet.
Case report: Our patient presented with a seizure aged 5 months and with unresponsiveness and hypoglycaemia at 7 months of age. Continuous feeds provididng 8.5mg glucose/kg/minute were required to maintain normoglycaemia and hypoglycaemia occurred whenever this was interrupted. Investigations showed hepatomegaly and hypertrophic cardiomyopathy with raised ALT and triglycerides. A diagnosis of GSD IIIa was supported by deficient debrancher activity in leukocytes and confirmed by identifying *AGL* mutations [c.2590C>Tp.(Arg864Ter) and c.4162-1G>A]. In view of her presentation and difficulty maintaining normoglycaemia we opted to manage her with a high fat (60% energy), high protein (20% energy), low carbohydrate (20% energy) diet, aiming to provide ketone bodies as an alternative fuel to glucose. Illnes is managed, where possible, with a high fat, low glucose emergency regimen.
Results:The high fat diet was well tolerated and allowed the interval between feeds to be increased to 5 hours without symptomatic hypoglycaemia developing. Blood ketones have ranged from 0.5 - 2.4mmol/l with glucose >2.8mmol/l. Echocardiography showed no deterioration after 4 months of dietary treatment.The cardiomyopathy fully resolved after 7 months of treatment and has not recurred.
Discussion: During sustained ketosis ketone bodies are used by most tissues, including the brain, reducing the glucose requirement. Our case demonstrates that this can prevent symptomatic hypoglycaemia and increase fasting tolerance in GSD IIIa. Moreover the heart uses ketone bodies, in preference to glucose, allowing resolution of the cardiomyopathy.

P-087

Branched-chain amino acid profiling in management of branched-chain ketoacid dehydrogenase kinase deficiency.

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Background: Branched-chain ketoacid dehydrogenase kinase (BCKDK) is a key regulatory enzyme in the metabolism of the branched-chain amino acids (BCAA). It inhibits branched-chain ketoacid dehydrogenase complex (BCKDH) activity by phosphorylation. A deficiency of BCKDK causes increased BCKDH activity with enhanced BCAA catabolism. We describe our experience managing two paediatric patients with BCKDK deficiency.
Methods / Case Report: Case 1 presented aged 33 months with autism and acquired microcephaly. BCAA were decreased in plasma and CSF. Protein load showed an appropriate increase in BCAA, thus ruling out a problem with intestinal absorption and transport. Case 2 was referred age 14y for investigation of underlying developmental delay, epilepsy, autism and microcephaly. He had persistently low BCAA in plasma. Genetic mutation analysis confirmed diagnosis of BCKDK deficiency in both.
Results: BCAA supplementation resulted in improvements in head growth, development and in neurocognitive scores. The rates of decay constants for all amino acids were calculated in both cases and were raised for BCAA, ranging from 5.0 to 5.9 fold in case 1 and from 1.9 - 2.1 fold in case 2. Based on repeat profiling and pre-dose, pre-prandial home dried blood spot BCAA monitoring we established that frequency rather than amount of supplement was important in maintaining stable (avoiding peak and trough) levels of plasma BCAA over 24hrs. Case 1 - total 30g BCAA/day divided 4 hourly over 24hr provided 14g leucine, 9g isoleucine and 8g valine. Case 2 – total 60g BCAA/day divided 3–4 hourly over 24 hours providing 27g leucine, 18g isoleucine, 15g valine.
Discussion: BCKDK deficiency is an important differential diagnosis in children with neurocognitive problems. A standardised approach to establish the optimum dosing and frequency of BCAA is required to maximise outcome. 24 hour BCAA profiling helps the calculations of the BCAA decay constant and assists in diagnosis and monitoring.

P-088

Breastfeeding in Multiple-Acyl CoA Dehydrogenase Deficiency (MADD) - two cases studies

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Background: Recommended dietary treatment for MADD is a protein and long chain fat (LCT) restricted, high carbohydrate

(CHO) diet with an energy distribution: 65-70% CHO, 8-10% protein, 20-25% LCT. Breast milk contains around 50% energy from LCT. There are no published studies of breast feeding (BF) in MADD. We describe two infants who were partially BF.
Case Report: Both were term babies with final diagnosis of MADD due to homozygous mutations in ETFDH. **Case 1** (wt 3.1kg) was prospectively managed with riboflavin (100mg/d) and diet due to history of previous deceased sibling with MADD. Delayed newborn screening (NBS) was abnormal and further biochemical testing consistent with MADD. **Case 2** (wt 4.7kg) had transient hypoglycaemia on D2. NBS was abnormal suggestive of MADD and they presented on D11 with metabolic acidosis and hypoglycaemia. Treatment commenced with riboflavin (100mg/d) and carnitine (50mg/kg/d). Biochemical testing was consistent with MADD. Both mothers opted to BF.
Results: Feed composition was based on combined feeds providing 96-100kcal/kg and 150ml/kg. A set volume of an LCT free, modular feed (MF) was given before BF to reduce LCT from normal of 50% to 25-30% of daily energy intake. Half of daily feeds were provided from BF. MF comprised - glucose polymer, protein powder, electrolytes, vitamin and mineral supplement. Combined feeds energy ratio - 9% protein, 62% CHO, 27-29% fat. Due to poor feeding case 2 had NG top-up feeds. Case 1 BF to 24wks; Case 2 to 11wks, then expressed breast milk until 22wks. Feeds were well tolerated with no use of ER. Case 1 tracked 9th- 25th centile, case 2 tracked 50th centile for growth. At home feeds were adjusted based on growth.
Discussion: Optimal dietary treatment for MADD is unknown. We successfully combined BF and MF with no episodes of decompensation in the initial follow up period. Both mothers were committed to BF. Close monitoring and regular feed adjustment ensured nutritional adequacy and normal growth.

P-089

Dietary adherence in pyridoxine nonresponsive homocystinuria patients: Coping with noncomplaince.

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Background:Classical homocystinuria (HCU) is a recessively inherited disorder of methionine metabolism caused by cystathionine b-synthase deficiency. There are two types of HCU: pyridoxine-responder milder form and severe pyridoxine nonresponsive form. Lifelong low protein/methionine diet with supplementation with methionine-free cysteine supplemented aminoacid mixtures (MFAAM) with/without betaine is recommended in pyridoxine nonresponsive form. In the absence of newborn screening programmes, diagnosis is commonly delayed which is related with significant long-term morbidity and mortality.
Methods: This retrospective study aims to describe the clinical and biochemical phenotypes of pyridoxine nonresponsive and pyridoxine responsive HCU patients and their compliance with the dietary treatment on the long term follow-up.
Results: 19 pyridoxine nonresponsive and one pyridoxine responsive HCU patient were enrolled to the study. 9 of the patients were female (43%), and 12 male (57%). All of the patients but one were late diagnosed. The current mean age was 15.3±7.9 years (range 5–34 years) with a mean age at diagnosis of 81±41months (range 1–156 months). 19 of the nonresponsive group received both diet and betain treatment. Although the mean natural protein intake described in the diet was 21.9±13.1 gr/day (min 6–49 gr/day) and the protein from MFAAM was 1.27±0.56 gr/kg/day; the dietary recall revealed a natural protein consumption of 30.8 ±23.8 gr/day natural protein and 0.9±0.6 gr/kg/day protein from MFAAM. The mean tHcy was 222.5±96.7mmol/L at the time of diagnosis while the mean plasma tHcy was found to be high 90,1±68,5 mmol/L (min: 3.79, max:299.6) during the follow up, despite dietary treatment.
Discussion:Lifelong adherence to this low protein diet is recommended but is challenging and compliance issues are often encountered especially in the late diagnosed patients. Poor adherence increases with the age even in the patients diagnosed in the newborn period.

P-090

Maple syrup urine disease (MSUD) Nutrition management: assessment of clinical, biochemical and anthropometric parameters

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Background:Guidelines for MSUD dietary treatment were recently published. Higher BCAAs plasma cutoff values are recommended aiming to improve metabolic homeostasis, reduce decompensations and achieve better nutritional status.
To evaluate the impact of these recommendations in the outcome of patients followed in our Paediatric Units.
Methods / Case Report: A cohort of 11 classical MSUD patients with a median age of 4.1 years (2 m – 14 y) were enrolled.Higher doses of Ile and Val were initiated and clinical, anthropometric and biochemical data were collected at baseline (T0), 3 months (T1) and 6 months (T2). Biochemical tests included: hematologic parameters, liver and renal function biomarkers, vitamin status, pre-albumin, albumin, retinol-binding protein, micro-elements, specific metabolites and complete plasma amino acid profile. Statistical analysis was performed by SPSS® 24 version.
Results:Median plasma Val and Ile levels raised from 217.8 μM to 392.8 μM (p=0.033) and from 164.4 μM to 235.9 μM (p=0.013), respectively, and comparing T0 with T2. A significant decreased number of decompensations, from 2.64 to 1.59 (p=0.002) was observed in the same period. The patients who did not decompensate (n=7), when compared to the ones that underwent decompensations (n=4), showed a significantly better BMI z-score (p=0.042) as well as a better weight z-score (p=0.036) when T2 period was compared with T1. Moreover, reversion of skin, hair and nail damages was observed. Biochemical parameters were within reference values except for selenium values, for which half of patients were below or on the low side of control range.
Discussion:The introduction of the recommendations, namely higher intake of Val and Ile, had a positive impact in the studied MSUD population. Therefore, a longer period of follow-up and further metabolic correlation studies will be done to ascertain insights for the better management of MSUD patients.

05. Phenylketonuria: general

P-091

Single-residue variants of phenylalanine hydroxylase help to observe multiple structural isoforms

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Background: Phenylalanine hydroxylase (PAH) catalyzes hydroxylation of phenylalanine (Phe). In humans, hydroxylation of Phe prevents its toxic accumulation. Persistently high Phe is the hallmark of phenylketonuria (PKU), the most common inborn error of amino acid metabolism. In nearly all cases, PKU is caused by dysfunctional PAH. Elevated Phe triggers allosteric activation of normally functioning PAH, while a drop in Phe returns PAH to its resting state. PKU-associated PAH is not properly tunable to changes in Phe. Therefore, we recently proposed that the structural basis of PKU lies, at least in some part not previously appreciated, in a disequilibrium of PAH structures. Our rat PAH structure showed, for the first time, the structure of resting-state PAH. Based on measurements in solution, that crystal structure differs significantly in shape to activated PAH. Structure determination of activated PAH remains a challenge yet is essential to being able to predict the effect on function and structure of any of the hundreds of PKU-associated mutations.
Methods / Case Report: We used X-ray crystallography to determine the structure of resting-state human PAH, using a single-residue substitution to make it more amenable to characterization, mutagenesis to engineer single-residue PAH variants that have a perturbed structure equilibrium, and a variety of biophysical and biochemical tools to characterize the proteins' properties in solution.
Results: We will present the first full-length structure of human PAH. We will describe our recent work towards isolating activated rat and human PAH using engineered single-residue variants that more readily sample the activated form.
Discussion: Single-residue PAH variants result in different defects in structure and function. Some of our engineered variants help us isolate the activated form in order to study its shape and flexibility. These variants promise to help determine the structure of activated PAH at higher resolution.

P-092

A population with an extremely high incidence of PKU

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Background: Phenylketonuria (PKU) is an inherited disease caused by mutations in the *PAH* gene. Different *PAH* mutations are spread in different ethnic groups with various frequencies, and the incidence of the disease itself varies from country to country. In the Caucasus region of Russia there are some ethnoses which are geographically and culturally isolated. The tradition of monoethnic marriages may cause the decrease in genetic variation in these populations.
Methods: DNA of 63 unrelated probands with PKU and hyperphenylalaninemia (HPA) from Karachay-Cherkess Republic (KCR, Russia) was analysed for the presence of *PAH* mutations. DNA of 12 siblings with PKU, 58 healthy relatives and 774 healthy KCR residents was also analysed. Analysis was performed with allele-specific MLPA and Sanger sequencing.
Results: In KCR the a PKU frequency of 1:850 newborns, the highest in the world, was detected. Karachays are the titular nation and constitute 41% of the population. In the PKU cohort from KCR, Karachays constitute 79%. PKU incidence among Karachays is 1:332. The phenomenon of the high incidence of PKU among Karachays is due to the widespread p.Arg261* mutation. Its allele frequency among Karachay patients is 67.3% with a carrier frequency of 1:16 healthy individuals. By the *PAH* haplotype analysis, the common origin of p.Arg261* was shown. The founder haplotype and mutation age were estimated by the analysis of linkage disequilibrium between p.Arg261* and extragene STR loci. P.Arg261* mutation occurred in Karachays 10.2±2.7 generations ago (275±73 years).
Discussion: The rapid accumulation of p.Arg261* can be explained by two mechanisms. First, the accumulation occurred in a small population spead further to the growing population through predominantly monoethnic marriages. Second, compensation of the severe variant p.Arg261* by common mild variants results in milder HPA phenotype and the patients lead a full life, transmitting *PAH* mutations to 100% of their descendants.

P-093

Identification of helpful factors to gain good metabolic control before and during pregnancy in phenylketonuria (PKU)

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Background: Maternal PKU syndrome affects the unborn child of PKU-mothers with insufficient metabolic control during pregnancy. We sought to identify factors favoring good metabolic control to design a tailored training program.
Methods: In a retrospective survey, 53 PKU-mothers from 12 metabolic centers completed a 38-item questionnaire for 74 pregnancies. We asked which circumstance or measure was regarded supportive to reach good metabolic control. We now present final analysis of the data. Pregnancies were divided in two groups (ideal metabolic control: all phe-values < 360 μmol/l, n=15; or suboptimal; n=59). There were no significant differences regarding phe tolerance, frequency of phe testing and dietary management, while metabolic control was significantly better in group "ideal" than "suboptimal" (mean phe 151 vs 250 μmol/l, $p< 0.001$; range phe during pregnancy 6–353 vs 6–1195 μmol/l, $p< 0.001$).
Results: Seven items were significantly more often indicated as helpful; the groups did not differ in their preference. "Support by partner" was most important (indicated by 69%), followed by "support of family and friends" (55%), "informative conversation with current physician" (55%), "…dietician" (54%), "contact to metabolic center before pregnancy" (54 %), "unlimited fruit and vegetable consumption" (53%) and "information brochure" (47%). Potential support group "suboptimal" predominantly indicated "group meetings with PKU-mothers" (29%, $p< 0.001$).
Discussion: It seems to be most important to develop a tailored training program for young PKU women, which includes partners and install

group-meetings with other PKU-mothers, to help reaching sufficient metabolic control during pregnancy.

P-094

Effect of Blood Phenylalanine Levels on Oxidative Stress in Classical Phenylketonuric Patients

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Background: Mental retardation, which occurs in phenylketonuric patients, is associated with increased levels of phenylalanine (Phe), increased oxidative stress, and an imbalance of amino acids in the brain. Recent studies have shown that oxidative stress plays a role in the pathogenesis of phenylketonuria. In this work, we aimed to compare the influence of blood Phe levels on oxidative stress parameters in phenylketonuric patients.
Methods: The study included 20 classical phenylketonuric patients. Considering adherence to diet, the study patients were classified in two groups as good adherence and poor adherence. The mean blood Phe level was 300±156 μmol/L and the mean age was 4.2±2.8 years in good adherence patients. The mean blood Phe level was 828±156 μmol/L and the mean age was 5.4±2.4 years in poor adherence patients. The control group was composed of healthy individuals of similar ages (5.8±3.2 years).
Results: Results showed significant differences in gluta thioneperoxidase (GSHPx), coenzyme Q10 (Q10), Q10/cholesterol, and L-carnitine levels in phenylketonuria patients and the control group (p=0.039, p=0.017, p=0.048, p=0.029, respectively). GSHPx, Q10, and Q10/cholesterol levels were significantly lower in poor adherence patients than in the control groups (p=0.036, p=0.03, p=0.015, respectively). L-carnitine levels were significantly increased in good adherence patients than poor adherence patients (p=0.034) and decreased in poor adherence patients than healthy controls (p=0.026). No significant differences were observed in paraoxonase 1, total antioxidant status, total oxidant status and oxidative stress index levels.
Discussion: In this study it was found that poor adherence patients are prone to oxidative stress. Although the patients may have the same diagnosis, patients have different clinical characteristics and different prognosis. Antioxidants can be used as an adjuvant therapy in order to avoid neurological damage in these patients.

P-095

Burden of illness and associated comorbidities in adult phenylketonuria patients - A German retrospective database study

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Background: The aim of this study was to investigate the burden of illness (BOI) in adult phenylketonuria (PKU) patients in Germany, compared with a matched non-PKU cohort.
Methods: This analysis used the Institut für angewandte Gesund heitsforschung Berlin (InGef) research database. This retrospective matched cohort analysis compared PKU patients to matched controls from the general population (1:10 ratio). PKU patients were included if they were aged ≥18 years on 01/01/15 and were continuously enrolled from 01/01/10 to 31/12/15. Selected comorbidities using ICD 10 GM codes (determined by medical expert review) and also 50 most commonly reported comorbidities in the PKU population were analyzed. Selected concomitant medications were included in the analysis. Differences between groups were tested using 95%CI of prevalence ratio (PR) values.
Results: The analysis included 377 adult patients with PKU (< 5 were receiving sapropterin) and 3,770 matched controls. The selected comorbidities that were significantly more prevalent in PKU patients (PR; 95%CI) included intellectual disabilities (21.02; 9.97,44.30), developmental disorders (12.21; 5.09,29.27), psychosis/schizophrenia (2.81; 1.35,5.85), and behavioral conduct (2.59; 1.14,5.91). Selected medications that were significantly more common in PKU patients were gastrointestinal agents, statins, vitamin D, analgesics & antipyretics, and antidepressants. Of the 50 most common comorbidities in the PKU population, those with a PR>1.5 vs controls included, major depressive disorders, reaction to severe stress & adjustment disorders, other anxiety disorders, chronic ischemic heart disease, infectious gastroenteritis & colitis, unspecified diabetes mellitus, and asthma.
Discussion: In this population, overall BOI in PKU is exacerbated by a significantly higher prevalence of numerous comorbidities and associated medications, both for recognized and more unexpected comorbidities (e.g. ischemic heart disease).
Conflict of Interest declared.

P-096

Liking of phenylalanine-free protein substitutes in patients affected by Phenylketonuria: a case study

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Background: A low and controlled dietary phenylalanine intake is the mainstay of phenylketonuria (PKU) therapy. Even if many aminoacid mixtures have been developed, these products are really poor liked by PKU patients. In this context, casein Glycomacropeptide (GMP) could be an interesting alternative protein source to usual amino acid mixtures. The aim of the present study was to evaluate sensory properties and liking of L-amino acid and GMP samples comparing patients from North and South of Italy.
Methods / Case Report:67 PKU patients (age range: 14–40 years), 34 admitted to San Paolo Hospital (Milan) and 33 admitted to A.O.U. Federico II of Naples, were recruited. Patients evaluated 8 low protein samples: 4 L-amino acid base samples and 4 GMP base samples, flavored with the same aromas (neutral, chocolate, strawberry and tomato) and with an equivalent protein intake of 5g/100ml. For each sample, participants were investigated to indicate which sensory attributes characterize the formulations and to score the overall liking.
Results: The mean blood phenylalanine (SD) was 675,2 (307) μmol/L and mean Body Mass Index was 23,4 (4,5) Kg/m2. Significant differences were found between samples regarding liking scores in both groups (Milan: F= 42.48; p < 0.001; Naples:

F=23.59, p < 0.001). GMP samples were generally preferred than habitual amino acid mixtures, especially for samples flavored with chocolate and strawberry, described as sweets, with a mild and natural taste and odor. Patients from South of Italy gave significant higher liking scores to the amino acid base samples compared to patients from North suggesting different attitude toward products.
Discussion: The results of this study demonstrated that GMP-based formulas showed a greater acceptability compared to amino acid mixtures. In this context, different products could be developed using this cheese whey derived protein in order to improve compliance to dietary treatment of PKU patients.

P-097

Efficacy & safety of sapropterin in PKU patients

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Background: Following on from the 26-wk SPARK study period in children (< 4 yrs of age) with BH_4-responsive PKU or mild hyperphenylalaninemia, the SPARK extension study evaluated the long-term safety, dietary Phe tolerance (the amount of dietary Phe ingested while keeping blood Phe levels 120–360 μmol/L), and Phe levels over an additional 36 mos of sapropterin (Sap) treatment.
Methods: Subjects previously treated with a Phe-restricted diet only were initiated on Sap at 20 mg/kg/day ("Sap EXT") and those initially treated with Sap + diet remained on this regimen ("Sap CONT"). Key differences vs the 26-wk study period: dietary Phe intake adjustments followed clinical practice standards of participating centers vs algorithm driven and Phe & Sap dose were adjusted every 3 mos vs every 2 wks. Analyses were performed in the Intention-To-Treat Extension population (randomized subjects who continued into extension period).
Results: Phe-tolerance increased significantly vs Baseline (BL; start of Sap in 26 wk study) in the "Sap CONT" group (n=25) and significant increases were maintained throughout the study (p< 0.001); dietary Phe tolerance at the end of study (EOS) increased by 38.74 mg/kg/day vs BL (p< 0.0001). In the "Sap eEXT" group (n=26), significant differences vs BL (start of Sap in extension study) were only observed between mos 9 & 21 (p≤0.0264); dietary Phe tolerance at EOS increased by 5.48 mg/kg/day vs BL (p=0.1929). Blood Phe levels in the "Sap CONT" group remained stable over time; in the "Sap EXT" group, decreases in blood Phe levels vs BL were observed at all visits (significant at mos 21, 30 & 33). No subjects withdrew due to adverse events.
Discussion: These data demonstrate that long-term treatment with Sap + diet in subjects < 4 yrs old maintains increased dietary Phe tolerance over 3.5 yrs & supports the favorable risk/benefit profile for Sap in patients < 4 yrs with BH_4-responsive PKU.
Conflict of Interest declared.

P-098

Prevalence of comorbidities among phenylketonuria patients - A retrospective study of US health insurance claims data

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Background: To investigate the full burden of phenylketonuria (PKU) we evaluated the prevalence of various comorbidities in adult PKU patients vs matched controls.
Methods: This retrospective, insurance claim-based study utilized 3 US MarketScan® databases (all include inpatient, outpatient, and outpatient prescription drug claims). The study included patients with PKU at any time between 01/01/98 to 31/10/14 (Commercial & Medicare) or 01/01/00 and 30/06/14 (Medicaid). This analysis compared patients ≥20 years of age with a diagnosis of PKU (ICD-9-CM code 270.1) with matched controls (1:5 ratio). Demographic and clinical characteristics were examined in the pre-index period. Prevalence ratios (PR) for 15 selected conditions were developed for PKU patients vs controls during the follow-up period (≥30 days post-index) using multiple regression analysis.
Results: The analysis included 3,691 adult patients with PKU and 18,455 matched control subjects (mean age 35 years, 63.7% female). After adjusting for baseline characteristics, prevalence of the 15 selected comorbid conditions were significantly higher for PKU patients vs controls; the highest adjusted PRs (PR; 95%CI) were for renal insufficiency with hypertension (2.20, 1.60-3.00) and overweight (2.06, 1.85-2.30). The majority of concomitant medications were used more frequently in the PKU vs control groups; analgesics and antipyretics (19% vs 14%) and antihypertensives (15% vs 9%) were the most commonly used concomitant medications. Mean pre-index healthcare costs among PKU patients were ~4-fold that of controls ($4,141 vs $1,283; p< 0.0001).
Discussion: PKU patients may experience higher rates of numerous systemic comorbidities vs matched non-PKU controls. Management of PKU patients may need to include regular screening for systemic comorbidities (e.g. renal insufficiency). Further prospective studies are required to validate these findings.
Conflict of Interest declared.

P-099

The burden of illness in adults with phenylketonuria (PKU): Interim analysis of a cross-sectional study

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Background: To assess the relationships between blood phenylalanine (Phe) control and executive function, mood, health-related quality of life, and health care resource use in PKU adults.
Methods: A cross-sectional patient-reported outcomes survey was conducted at 10 US and 2 Canadian clinics, followed by a retrospective chart review of PKU adults ≥18 years of age. RESULTS from the Behavioral Rating Inventory of Executive Function Adult version (BRIEF-A) and the Cambridge Neuropsychological Test Automated Battery (CANTAB) were assessed relative to blood Phe concentrations in this interim analysis performed February 2018.
Results: Thus far 48 subjects, mean (SD) age 34 (12.23) years, 57% female, have enrolled; 11 have blood Phe concentrations < 360 μmol/L, 8 have 360–600 μmol/L, 10 have 601–900 μmol/L and 19 have ≥900 μmol/L. Respective data for the four blood Phe groups (< 360, 360–600, 601–900, and ≥900 μmol/L) are as follows: median (interquartile range, IQR) BRIEF-A t-scores were 47.0 (35–73), 50.5 (35–62), 58.5 (37–70), and 56.0 (36–88); median (IQR) CANTAB results for Rapid Visual Information Processing time (RVP) were 447.0 (389–645), 471.0 (334–674), 503.0 (413–661.5), and 573.5 (356–1205); median IQR for Stop Signal Go Reaction Time (SSG) were 568.0 (467–600), 549.2 (438–621), 552.5 (472.5-643), and 567.0 (482–737); median (IQR) Spatial Working Memory number of errors (SWM) were 4.0 (0–35), 11.0 (0–26), 14.5 (0–32), and 15.0 (2–31). Significant Pearson Correlation Coefficient (PCC) relationships were seen between blood Phe and RVP (r= −0.43, p=0.0024) and SWM (r=0.29, p=0.0463), but not SSG (r=0.2, p=0.1627) or BRIEF-A t-scores (r=0.22, p=0.1418).
Discussion: Preliminary results show that as blood Phe concentrations increase, there is a trend for greater impairments in processing speed and working memory, but not in impulse control or BRIEF-A t-scores.
Conflict of Interest declared.

P-100

Living with PKU in the UK – Experiences of Reproductive Health

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Background: Women's experience of maternal PKU starts prior to preconception and extends beyond childbirth. We explored the perspectives of adult women with PKU
Methods: A sub-section of an online survey conducted by the NSPKU (National Society for Phenylketonuria) was aimed at women with PKU aged >18 years in the UK. They completed 9 multiple choice questions about reproductive health
Results: 300 women responded, with 36% (n=111) having ≥ one child. 55% (n=71) of pregnancies were planned. Most women (73%, n= 200) expressed concerns, fears and distress about pregnancy. 60% (n=164) were concerned about harm they may cause to a baby, 54% (n=147) had anxiety about their ability to maintain blood Phe within target, and 48% feared unplanned pregnancy. Some were concerned that it may be unsafe to have a baby as a woman with PKU (39%, n=107); some worried about their parenting skills (16%, n=43), and women even described how they avoided sexual relations. 8% (n=22) of women were too embarrassed to discuss pregnancy in clinic; 9% (n=23) said they had a pregnancy termination due to PKU, 14% (n=36) had a miscarriage and 8% (n=21) had more than one miscarriage.In the post-natal period, of 93 women, 48% (n=45) had low mood or sadness, 41% (n=38) were depressed, 25% (n=32) felt unable to cope, 33% (n=31) said they could not care for their PKU as well as their baby, 14% (n=13) struggled with child care needs and 4% (n=4) worried they might hurt themselves or their baby. 14% (n=14) thought that child health or developmental problems were linked to PKU
Discussion: There are unmet needs relating to sexual and reproductive health of women with PKU. Interventions are needed to reduce the psychological impact of the risk of maternal PKU syndrome and assist with safe pregnancies. Sapropterin may help women (who respond) have a safe and positive experience of pregnancy
Conflict of Interest declared.

P-101

Living with Phenylketonuria (PKU) in the UK – Experiences of Children and Adults

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Background: A low phenylalanine diet (LPD) is well established in PKU. We report practical, social and psychological issues of living with PKU in 631 adults (n=338) and children (n=293) with PKU
Methods: People with PKU in the UK were surveyed online by the NSPKU (National Society for Phenylketonuria) using 49 multiple choice and 3 open questions about how PKU affected their lives
Results:
Children with PKU: 89% of n=293 children followed a supervised LPD, 79% were prescribed ≤10g natural protein/day. 72% said protein substitute was unpleasant and food choices too restrictive. 48% (n=144/236) had difficulty with maintaining focus, 28% (n=67/236) had educational difficulties, 29% (n=68/236) experienced anxiety or depression. 53% (n=125/236) had digestive problems, and 27% (n=64/236) reported headaches. 51% (n=120/236) described exclusion in social settings; 17% (n=41/236) had relationship issues with friends or family.
Adults with PKU 57% of n=323 adults followed a supervised LPD, 53% were prescribed ≤10g natural protein/day. 50% said LPD was too time consuming, and 49% said protein substitute was unpleasant. 65% (n=187/286) had depression or anxiety, 55% (n=180/327) reported low mood and 54% tiredness (n=178/327). 54% (n=154/286) had difficulty maintaining focus, 52% (n=148/286) work related anxiety, 46% (n=131/286) headaches, 28% (n=79/286) struggled to gain qualifications, and 15% (n=42/286) had difficulties with career progression. Other issues were: difficulty with relationships (34%, n=96); exclusion from social settings (44%, n=126); digestive problems (44%, n=125), and difficulty managing weight (55%, n=158). Common medications included: antidepressants (40%) and anxiolytics (21%).
Discussion: Living with PKU is challenging: there are many 'hidden' clinical, social, and psychological issues which need attention by PKU health professionals
Conflict of Interest declared.

P-102

Psychological wellbeing of early and continuously treated phenylketonuria patients

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Background: Phenylketonuria (PKU)-patients depend on a lifelong Phe-restricted diet to reach good metabolic control and optimal cognitive outcome. Former studies showed heterogeneous impact of PKU on psychological wellbeing. We present a cross-sectional study assessing emotional functioning and psychological distress.
Methods: Inclusion of 49 PKU-patients (26f, 2-17y). Behavioral attributes were evaluated by Strengths and Difficulties Questionnaire (SDQ; 5 scales: hyperactivity, emotional symptoms, conduct problems, peer problems, prosocial behavior; 2 versions: self-report 11-17y; parent-report 2-17y) in relation to metabolic control. Total difficulties- and impact score (impact of difficulties on social life) were analyzed. Comparison to healthy controls (n=98; 46f).
Results: Independent of age and sex, all PKU patients revealed a median total difficulties score within the normal range (< 9; normal 0–13) by parent-report which was not significantly different from controls (< 8.5; P>0.6). However, 18.5% of 2–10 old and 4.5% of adolescent patients were rated as borderline/abnormal in this score by parents. For all of these patients difficulties in daily life were reported by impact score. 11–17 year old patients more often rated themselves as borderline/abnormal compared to their parents (total difficulties score: 13.6% vs. 4.5%; P=0.607). Adolescent girls mainly reported emotional problems (27.3% abnormal). Median mean dried blood phenylalanine concentration in all patients was 231 μmol/l (Q1: 199; Q3: 437). No relation between insufficient metabolic control and higher SDQ values was detected.
Discussion: PKU-patients mostly revealed normal behavioral attributes as judged by SDQ. A borderline or abnormal total difficulties score shows a negative impact on daily life. SDQ in the standard care of PKU patients is a suitable screening tool for psychological problems Special attention should be paid on adolescent girls who seem to be at risk to develop emotional problems.

P-103

Does large neutral amino acid supplementation in PKU need the phenylalanine-restricted diet?

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Background: Large neutral amino acid (LNAA) treatment has shown promising results in PKU patients. Research in the PKU mouse model has further elucidated its working mechanisms in relation to LNAA treatment composition. Moreover, we showed that LNAA supplementation to either an unrestricted diet or liberalized phenylalanine (Phe)-restricted diet could improve brain amino acid and monoamine biochemistry in PKU mice, not knowing whether the diet is still necessary.
AIM: To elucidate if LNAA could be applied as a treatment on its own or should be combined with a liberalized Phe-restricted diet.
Methods: We studied the effect of LNAA treatment to either an unrestricted or liberalized Phe-restricted diet on brain monoaminergic neurotransmitter synthesis in relation to brain and plasma amino acid biochemistry in young BTBR *Pah-enu2* PKU mice, and compared to a severe or liberalized Phe-restricted diet or unrestricted diet in PKU mice as well as to WT control mice.
Results: Brain concentrations of monoamines, Phe, and other LNAA did not significantly differ, while plasma Phe concentrations were significantly higher when LNAA supplementation was added to an unrestricted than to a liberalized Phe-restricted diet. Compared to an unsupplemented liberalized Phe-restricted, LNAA supplementation either with or without a liberalized Phe-restricted diet was at least as effective with respect to all brain biochemical treatment objectives. Compared to an unsupplemented severe Phe-restricted diet, brain monoamines on LNAA supplementation either with or without a liberalized Phe-restricted diet brain monoamines were comparable, but brain Phe concentrations were significantly higher.
Discussion: The present results indicate no additional value of a liberalized Phe-restricted diet to LNAA treatment with respect to brain amino acids or monoamines. Our results also show that, with further liberalization of the Phe-restricted diet, LNAA supplementation becomes increasingly important.
Conflict of Interest declared.

P-104

PKU from blood to brain: a systems biology approach

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Background: Systems biology becomes increasingly important in the further understanding of the pathophysiology of inborn errors of metabolism. In PKU, despite extensive investigations, the relationship between plasma amino acids and brain biochemistry in PKU remains largely speculative. This issue also applies to other inborn errors of amino acid metabolism in which abnormal plasma amino acid is the primary consequence of the enzymatic defect as well as the principal target for (dietary) treatment, while the secondarily induced brain biochemical disturbances underlie brain dysfunction as one of the core symptoms.
Methods: To better understand PKU pathophysiology and, in particular, the relationship between plasma amino acid, brain amino acid and monoamine biochemistry, we used a systems biology approach to model the transport of large neutral amino acids across the blood–brain barrier as well as cerebral amino acid and monoamine metabolism.
Results: The model accurately describes uptake of amino acids into the brain, as validated by direct measurements of brain amino acid concentrations in PKU mice at various dietary treatments. In addition, control analysis shows strong control of the monoaminergic neurotransmitter system from both TH and TPH2, according to current knowledge. Apart from previously suggested key players like LAT1, TH, and TPH2, some other systems (e.g. system Y) have been identified that seem to show an important role in brain amino acid and neurotransmitter biochemistry in PKU as well.
Discussion: In conclusion, the model provides a tool for developing novel dietary interventions to optimize brain amino acid and neurotransmitter concentrations by predicting brain biochemistry in result to variable plasma amino acid profiles. Moreover, the model aims to contribute to the

identification of new treatment targets and better biomarkers for treatment monitoring to further optimize neurocognitive and psychosocial outcome of PKU patients.
Conflict of Interest declared.

P-105

Our experience in molecular characterization of patients with hyperphenylananinemia

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Background:Most forms of phenylketonuria (PKU) and hyperphenylalaninaemia (HPA) are caused by mutations in the *PAH* gene on chromosome 12q23.2. *PAH* molecular characterization benefits from the integration of exon and intron- exon boundaries sequencing analyses with the MLPA (Multiplex Ligation-dependent Probe Amplification) analyses, eventually employed to detect gross gene rearrangements.
Methods:Here we report the molecular characterization of about 150 patients affected by PKU/HPA referred to our Metabolic Centre.
Results:Unless we performed both sequencing analyses and, when necessary, MLPA analyses, we still did not identify the second disease causing allele in several patients. We identified the new p.Leu48Trp PAH missense variant. We performed *in silico* structural analyses of this new variant in order to classify it as disease-associated or neutral, based on phylogenetic conservation of amino acids and/or functional algorithms. The *silico* data underline that this variant can lead to disease causing allele. The analysis of our cohort identified several unreported *PAH* variant combinations.We also underline that 26% of our patients carry the c.1208 C>T (p.A403V) mutation and 28% carry the c.1066-11 G>A mutation in the *PAH* gene.
Discussion: We would like to stress the importance of the accurate characterization of patients at molecular level. The here reported data elaboration allow us to obtain new genotype-phenotype correlations, useful for patients' management.

P-106

Pah^{R261Q}: a new mouse model for phenylketonuria

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Background: Phenylketonuria (PKU) is the most common inborn error of metabolism, characterized by the inability of the body to break down the amino acid phenylalanine (Phe), which, ultimately, builds up in the brain of untreated patients leading to neurodevelopmental problems and impaired cognitive functions. PKU arises from mutations in phenylalanine hydroxylase (PAH), a tetrameric, non-heme iron enzyme responsible for the conversion of Phe into tyrosine using molecular oxygen as additional substrate and the cofactor (6*R*)-5,6,7,8-tetrahydrobiopterin (BH4).
Methods: In order to better understand the disease PKU through animal experimentation, three transgenic mouse lines were created back in 1989 by phenotype-driven N-ethyl-N-nitrosourea (ENU) germline mutagenesis, namely *Pah*^{enu1}, *Pah*^{enu2}, and *Pah*^{enu3}. Since that time, the field of genome engineering has progressed greatly and thus, we took advantage of the recent methodological advances to develop a novel PKU mouse model by the use of CRISPR/Cas9 technology. Notably, this new transgenic mouse harbors the more relevant, disease-like point mutation p.Arg261Gln. The conversion of an arginine to a glutamine at the residue 261 leads to an unstable, misfolded conformation of PAH, which resembles the pathogenic determinant of PKU. In addition, the mutation p.Arg261Gln is one of the most abundant in the registries with an allele frequency of 5.5 %.
Results: The characterization of the *Pah*^{R261Q} mouse revealed normal behavior and neurotransmitter profile, residual hepatic PAH levels and activity (compared to WT) of 23.8 % and 16.9 %, respectively, a basal blood Phe concentration of approx. 100 μmol/L, and hepatic intracellular BH4 levels significantly decreased respect to WT.
Discussion: Altogether, these findings demonstrate the improved features of this novel mouse model for the study of PAH conformational status and proteostasis regulation, to further investigate pathophysiological mechanisms in PKU and develop new therapies.

P-107

The effect of phenylalanine restricted diet on anthropometric parameters in classical phenylketonuria patients

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Background: Although, there are publications that evaluate the effect of phenylalanine (Phe) restricted diet on the anthropometric parameters in classical phenylketonuria (PKU) patients in the literature, the results are contradictory. In this study, we aimed to compare the anthropometric status of PKU patients who were followed up with Phe restricted diet and patient with mild hyperphenylalaninemia (HPA) patients who were followed up without phenylalanine (Phe) restriction to determine the effect of Phe restricted diet on anthropometric parameters.
Methods / Case Report: Forty patients with PKU and 78 patients with HPA were enrolled in the study. At birth, 6th month, 12th month, 18th month, 24th month, 30th month and 36th month of life, weight, height, weight standard deviation score (SDS), height SDS, body mass index (BMI) SDS were retrospectively analyzed for all patients.
Results: Twenty two (55%) patients in PKU group and 36 (46.2%) patients in HPA group were female. Between two groups (PKU and HPA) there was no difference in terms of gender. In the analysis of anthropometric parameters of patients at birth, 6th month, 12th month, 18th month, 24th month, 30th month and 36th month of life there was no differences in weight gain (p=0.516). In contrast, height growth rate was higher in HPA group than PKU group (p=0.037). No statistically significant differences were detected in BMI SDS, weight SDS and height SDS between PKU and HPA groups.
Discussion: We can conclude that the height growth rate is lower in PKU patients than HPA patients. We believe that the lower height growth rate of PKU patients is the result of Phe and natural protein-restricted diet.

P-108

Cognitive functions in children with PKU

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Background: Phenylketonuria (PKU) is an inherited metabolic disorder caused by the failure of the phenylalanine hydroxylase enzyme. Untreated or poorly treated PKU can lead to intellectual disability, seizures, behavioural problems and mental disorders. The aim of our study was to examine the cognitive functions of children with PKU and comparing them with age-related control patients. The control group do not have any diseases associated with brain damage. We examined the correlation between cognitive functions and serum phenylalanine (Phe) levels.
Methods/Case Report: 48 children with PKU and 99 controls were examined with 5 nonverbal tests from the Cambridge Neuropsychological Test Automated Battery (CANTAB) including tests of working memory, learning and executive function; visual, verbal and episodic memory, attention, information processing, and reaction time. Data were analyzed using Microsoft Excel, Graphpad Prism and R statistical packages. A Mann–Whitney test was used to compare the two groups. The results of each task were normalized and a 0–10 score system was used to characterize mean cognitive functions.
Results: We found the most explicit difference between patients and controls in stocking of Cambridge (SOC) subsequent thinking time where PKU children with were 3.2 times slower. Our results show a correlation between cognitive function with age and serum Phe. The mean normalized cognitive function was 5,77 in the group of children with PKU, and 7,52 in the control group.
Discussion: Some CANTAB tests showed significant differences between PKU and control patients, the most significant being SOC subsequent thinking time. According to our results, nonverbal computer tests can be used to examine the fine cognitive dysfunctions caused by PKU. Cognitive dysfunctions also have a correlation with serum Phe. These results confirm the importance of adherence to a strict diet for PKU patients.

P-109

Is there a need for phenylketonuria (PKU) group clinics? A result of an electronic survey.

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Background: PKU patients on a phenylalanine (phe) restricted diet attend appointments at our tertiary metabolic centre. Patients over 1 year of age are reviewed individually by the multi-disciplinary team (MDT- consultant, clinical nurse specialist and dietitian). Patients under 1 year of age are offered weaning sessions in a group clinic format. Review by a psychologist is prioritised for those with adherence issues. This survey reviewed PKU patients and their family's satisfaction with the current individual clinic format and assessed if there was a need to change to a group clinic format.
Methods: An electronic survey was emailed to 108 parents or carers of children on phe restricted diets. Questions targeted the content, structure and delivery of clinic appointments and how we could improve the service. Replies were anonymised. A reminder email and a newsletter article was sent.
Results: Thirty seven parents of children with PKU completed the survey (37%). RESULTS showed that parents were happy with the existing clinic format. Although, when asked about the opportunity for group sessions, 76% favoured this. Replies to preference of education topics were ranked from highest to lowest as research and development in treatment of PKU, meal ideas, food tasting sessions, importance of taking supplements, holiday tips, reading food labels, cooking sessions and blood testing tips. 78% requested access to a psychologist for support with managing and living with PKU.
Discussion: This survey suggests that responders favoured a group clinic format with psychology support. Since this we have developed an age banded education programme for group clinics. However, the development of group clinics require frequent meetings of the MDT and administrative time (invites, lesson plans, questionnaires, evaluation forms and organisation of a suitable venue). Ongoing evaluation will assess the effectiveness of this service.

P-110

Living with a Patient with Phenylketonuria Affects Household Nutrition

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Background:Lifelong adherence to a low-phenylalanine(Phe) diet is essential in order to prevent neurological damage in phenylketonuria (PKU) patients.A strict diet of an individualmay change the eating habits of all family members sharing the same house.We examined the eating attitudes and habits of family members living with a PKU patient.
Methods: Diet records of 62 PKU patients was reviewed.Thirty patients who could consume more than 700 mg Phe in a day,patients with BH4 treatment or patients with large neutral aminoacid supplementation were not included in the study.Sociodemographic characteristics of the patients and the families were recorded.Food preference questionnaire for adults was used to detect food category preferences scores.
Results:Parents of 32 patients and 32 healthy children completed the face-to-face questionnaires. Three parents were never eating their meals together with their PKU children. Twenty six (%89.7) parents were eating their meals together with PKU children and they were choosing their foods compatible with the PKU diet. We also examined the food consumption types and preferences of family members at home. 84.5% of the families were eating vegetables each day of the week. 37.5% and 18.8% of the families consumed meat once a week and once a month, respectively. There were no statistically significant differences between family members of PKU patients and parents of healthy children regarding mean food category preferences scores (vegetables 3.79±0.79 and 4.02±0.60, fruit 3.79±0.79 and 4.59±0.52, meat 3.54±0.89 and 3.95±0.72, dairy products 3.65±0.95 and 4.07±0.55, snacks 3.39±0.84 and 3.82±0.85, starches 3.08±1.03 and 4.11±0.73, respectively, $p>0.05$).
Discussion:Sharing the same house with PKU patients may be a burden for family members in many ways.While some parents don't eat their meals on the same table with PKU patiens, most of them share same table and adapt their meals with PKU compatible foods, such as vegetables instead of meat.

P-111

Growth and Nutritional Status in PKU Children from Birth to Adulthood

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Background: We assessed the nutritional and growth status, using serial anthropometry, of PKU patients attending a single tertiary centre from birth to adulthood.
Methods / Case Report: Demographics, disease characteristics and anthropometry (n= 5,339) were retrieved for 137 PKU children (1980 to 2012). Controls were 414 healthy children and the Scottish NDNS data. Demographics, number and % raised PHE measurements and PKU severity were explored as over- nutrition (BMI>85th) associates. Timing to over-nutrition was assessed using survival analysis. Associates of BMI Z-score were explored with the Generalised Additive Mixed Model.
Results: PKU children were shorter (height Z-score: −0.20 vs 0.15; p< 0.001) and had higher BMI Z-scores (0.56 vs 0.42; p=0.05) than controls. In PKU girls, but not boys, over-nutrition prevalence was higher than the NDNS data. Female gender and number of PHE tests per year were positively associated with BMI Z-scores and negatively with age. Median time to over-nutrition onset was 12 and 15 months for boys and girls respectively. PKU boys were taller than girls. Height Z-score declined over the first two years of life, then improved until age 12 and declined thereafter. BMI Z-score increased dramatically for both genders from birth to two years, then declined and stabilised by age 12. Thereafter, boys continued to decline whereas girls increased. Over-nutrition was more common in classical than moderate PKU (23% vs 2%). BMI Z-scores were not associated with PHE control.
Discussion: Prevalence of over-nutrition is high in PKU children, particularly girls. Factors predisposing to obesity invite further research.

P-112

Living with phenylketonuria in adulthood: the PKU-ATTITUDE study

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Background: Dietary treatment is the cornerstone of therapy for phenylketonuria (PKU), but adherence to low-phenylalanine diet progressively decreases after adolescence. No studies have addressed the issue from the point of view of the patient. The aim of this Italian multicenter survey (Analysis of the mosT relevanT and predIctive facTors inflUencing aDherence to PKU diEt [ATTITUDE]) was to collect information on the subjective perceptions of patients.
Methods: Participants (n=111; response rate 94%) were adults diagnosed with PKU at birth, who had attended a metabolic clinic regularly ever since. Theywere asked to complete a structured questionnaire designed to identify psychological factors associated with poor compliance to dietary treatment. We selected metabolic centers where a dedicated psychologist, trained in following PKU patients, could administer the questionnaire.
Results: Patients appeared to have an altered perception and awareness of the disease. About 40% of them did not consider PKU a disease and, despite declaring regular monitoring of phenylalanine levels (85%), 48% reported a high plasma value over the last 6 months (>600 μmol/L); 31% were unable to specify it. Adherence to PKU diet was unsatisfactory, with increased consumption of natural protein sources and reduced daily use of amino-acid supplements (< 4–5 times/day in 82% patients). The most important factor influencing their consumption was the increased social pressure associated with their use (55%).
Discussion: To our knowledge, we have carried out the first survey focused on the subjective perceptions of patients. The survey has thus provided unique information on how patients actually experience PKU and related dietary treatment. It uncovers altered patient perception of diseasefor the first time and shows how important it is to implement educational measures, as well as structured transitional care processes.

P-113

Neurocognitive functioning in adults with phenylketonuria: results of a ten-year-follow-up

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Background: A controlled long-term study was performed to assess the neurological and neuropsychological performance in adult patients with early-treated phenylketonuria (PKU).
Methods:We investigated 35 patients with early-treated classical PKU aged 19 to 41 years (mean age 32 years) and 46 matched healthy controls, matched for age and socioeconomic status. Patients and controls were assessed for their intelligence quotient (IQ), and attention and information-processing abilities. Magnetic resonance imaging (MRI) of the brain was performed in all patients. Neuropsychological assessments and MRI were repeated at a ten-year-follow-up.
Results:In the ten-year interval IQ, information processing and attention of patients and controls remained constant. At both assessment times the IQ scores were significantly lower in patients compared to controls. Older adult patients (>32 years) showed poorer information processing and attention at both assessment times compared to young adult patients (< 32 years) and controls. IQ, information processing and attention showed no correlation to imaging results but were significantly correlated to blood phenylalanine (Phe) levels in patients' childhood and adolescence, and Phe levels had been higher in the adolescent years of older adult patients.
Discussion:Cognitive performance in adult patients with early-treated PKU does not seem to be subject to deterioration observable in a ten-year interval. Neuropsychological assessment in adults with PKU revealed neurocognitive impairment particularly in older adult patients. This seems to refer to an early relaxation of diet that was recommended when the older patients were adolescents. RESULTS indicate a benefit of dietary control during adolescence in PKU.

P-114

Fluctuations of blood phenylalanine levels in children and adolescents with phenylketonuria

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Background: The stability of blood Phe levels may be related to cognitive outcome in early and continuously treated PKU.
Methods: We investigated 49 patients (28 boys, 21 girls) with early-treated PKU aged 6 to 18 years (mean age 11.2 years, SD 4.1 years). Patients were on a phenylalanine (Phe) restricted continuous diet. Of the patients, 28 (18 boys, 10 girls, mean age 11.7 years, SD 4.3 years) had classical PKU, and 21 (10 boys, 11 girls, mean age 10.6 years, SD 3.8 years) had mild PKU. For 26 weeks, patients were assessed weekly for their blood Phe levels. Data were analysed for fluctuations indicated by the standard deviation of the individual blood Phe levels. Additionally, we assessed the concurrent Full Scale IQ (FSIQ) of the patients.
Results: In patients with classical PKU FSIQ was correlated negatively with blood Phe levels, but not with level fluctuations. In patients with mild PKU FSIQ was correlated not with blood Phe levels, but - negatively - with level fluctuations.
Discussion: In patients with mild PKU the blood Phe levels show minor interindividual differences that presumably that may allow the fluctuations to exert a negative effect on the FSIQ.

P-115

Growth in children with Phenylketonuria – diet and protein – have we got it right?

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Background: Adherence to a special phenylalanine-restricted diet in PKU (Phenylketonuria) patients is key to prevent cognitive impairment. For children on dietary restriction an optimal protein to energy ratio is important to allow normal growth in childhood. We aimed to evaluate stature in children with PKU in a large tertiary center.
Methods / Case Report: We identified children with PKU within our local database. Cross sectional data were retrieved for children attending the clinic during 2017–2018. The most recent height was recorded and converted to a z-score using UK reference data.
Results: Among 80 children with PKU and no other co morbidities 60 children aged 4–18 years (median 10.17 years) were identified (boys n=29). There was a tendency for children aged younger than 12 years to have elevated z-scores for height (boys n=17, median z-score 0.77, girls n=21, median z-score −0.11) compared to in their teenage years (boys n=12, median z-score −0.35, girls n=9, median z-score −0.44). For boys this observation was statistically significant (p=0.03). By age 14.4, 16.5, 16.2 and 16.3 years 4 boys (14% of boys) had attained final height. Two had a parental target height available and one was just above whilst the other was just below mid parental height target.
Discussion: Our data suggest that children with PKU are relatively taller in childhood compared to in adolescence. The results may be biased due to improved dietetic input, management and compliance over the years. Infancy and puberty are both phases with rapid growth, but only in infancy are children routinely treated with an increased protein amount. Increased protein requirements may need to be considered during pubertal growth. Finally we speculate if PKU itself may affect onset and intensity of pubertal growth. Further studies are needed to evaluate these findings.

P-116

Hyperphenylalaninemia due to mutations in the *DNAJC12* gene

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Background: Mutations in *DNAJC12* have recently been identified as a tetrahydrobiopterin (BH4)-responsive cause of hyperphenylalaninemia (HPA), clinically and biochemically mimicking BH4 metabolism disorders. *DNAJC12* encodes a co-chaperone for phenylalanine hydroxylase (PAH), tyrosine hydroxylase and tryptophan hydroxylase. The phenotypic spectrum ranges from mild autistic features or hyperactivity to severe intellectual disability, dystonia and parkinsonism. We report the case of two sisters with a homozygous mutation in the *DNAJC12* gene.
Case Report: Two sisters aged 1 and 7 years, born to consanguineous Pakistani parents, presented with moderate HPA. The younger patient was born in Italy and was diagnosed by newborn screening. Pterins and DHPR activity on dried blood spot was normal. Molecular analysis of the *PAH* gene did not show any mutations and a next generation sequencing panel of genes causative of HPA (*GCH1*, *PTS*, *QDPR*, and *PCBD1*) also resulted negative. We performed a molecular analysis of the *DNAJC12* gene. In both sisters, CSF analysis showed normal values of prolactin and pterins, but very low levels of the biogenic amines (5HIAA and HVA).
Results: The patients were homozygous for the new c.58_59del mutation in the *DNAJC12* gene. This mutation causes the deletion of two exonic nucleotides leading to frameshift and premature termination codon p.Gly20Metfs*2. The parents were both heterozygous for the mutation.
Discussion: Mutations in *DNAJC12* were identified to be causative of a rare form of HPA. *DNAJC12* gene sequencing analysis should be considered in newborns positive for HPA without a *PAH* gene mutation. We have now included *DNAJC12* in our gene panel for HPA.

P-117

International best practice recommendations for evaluation of responsiveness to sapropterin in phenylketonuria patients

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Background: International guidelines recommend lifelong control of blood phenylalanine (Phe) in phenylketonuria (PKU) patients to prevent detrimental effects on the brain. Sapropterin (Sap) has been shown to reduce blood Phe and increase Phe tolerance in responsive PKU patients. Protocols to establish Sap responsiveness vary widely.
Methods: Two meetings were held with an international panel of clinical experts in PKU management to develop recommendations for Sap response testing. Regional similarities/differences in testing practices were discussed, based on a systematic literature review, outcomes of a global physician survey, and case reports. Statements developed from these discussions were sent to all participants for consensus evaluation and further fine-tuning.
Results: A Sap trial (20 mg/kg/day) is recommended in patients with blood Phe 360–2000 μmol/L, except in those with two null mutations. For neonates, a 24-h loading test is recommended and a ≥30% decrease from baseline blood Phe indicates response. For the majority of older infants, children, adolescents, and adults, a test duration of ≥48 h to 7 days is recommended, with a decrease in blood Phe as the main outcome. This may be extended to ~4 wks, with improved Phe tolerance as the main outcome, to identify additional responders; this duration may not be possible in some countries (e.g. access to Sap, public health regulation). A 48-h Sap test should be considered for PKU pregnant women who cannot achieve blood Phe ≤360 μmol/L. Relevant medical, nutritional, and social history data and baseline information should be collected for all patients before Sap testing, and long-term durability of response and clinical benefits of treatment should be assessed.
Discussion: Harmonization of sapropterin response testing protocols may improve determination of sapropterin responders and comparison of test results worldwide, and help ensure that prescriptions for this therapy are for those that would benefit most.
Conflict of Interest declared.

P-118

Assessment of anxiety and depression in adult patients with phenylketonuria

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Background: Phenylketonuria (PKU) is an inborn error of metabolism resulting from deficiency of phenylalanine hydroxylase activity. Approximately 1/4,500 babies born in Ireland have PKU or hyperphenylalaninaemia. Anxiety and depression are thought to be increased in people with PKU but, to date, only limited studies have been undertaken to estimate their prevalence. Phenylalanine (Phe) level variation has been correlated to IQ rather than to anxiety/depression in previous studies.
Aim: To estimate anxiety/depression scores in adult patients with PKU attending the NCIMD, Mater Hospital.
Methods: Individuals attending the PKU clinic completed the Hospital Anxiety and Depression Scale (HADS) questionnaire. Anxiety/depression scores were calculated and correlated with concurrent Phe levels.
Results: N=199 individuals; Male = 69, Females = 130; Mean age = 35.8 +/− 9.0; Range = 21 – 58 yrs. Concurrent Phe levels Mean: 640 mmol/L; Median: 566 mmol/L; Range = 121 – 1703 mmol/L. Eighty six of 199 (43%) had Phe levels >600mmol/L.
Aggregated depression scores: Abnormal 6/199 (3.0%), Mean Phe Level (MPL) 553 mmol/L; Borderline 13/199 (6.5%), MPL 755 mmol/L; Normal 180/199 (90.5%), MPL 615 mmol/L.Aggregated anxiety scores: Abnormal 30/199 (15.1%), MPL 638 mmol/L; Borderline 37/199 (18.5%), MPL 742 mmol/L; Normal 132/199 (66.4%) MPL 588 mmol/L.
Discussion/Conclusions: • Anxiety is more common than depression in adults with PKU and 48 patients were identified for review with clinical psychology.
• These initial results indicate when considering truly abnormal scores neither levels of anxiety (P = 0.3669) nor depression (P= − 0.1012) are significantly higher in adults with PKU compared to the general population (Mental Health Foundation. Fundamental Facts About Mental Health 2016).
• A prospective study to analyse cognitive and executive function, mood, work productivity and quality of life in this patient cohort is ongoing.

P-119

Impact of prevailing phenylalanine levels on mental health symptoms: a retrospective clinical audit

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Background: Accumulating evidence links elevated phenylalanine (Phe) levels to suboptimal neuropsychiatric outcomes in adults with phenylketonuria (PKU). Current European Guidelines advise more stringent metabolic control beyond historical practice in adulthood. Application currently remains controversial pending further clinical data.
Methods: A registered retrospective clinical audit of the Mark Holland Metabolic Unit adult PKU patient population, UK, was undertaken during June-July 2017. Records of patients attending in the previous two years were reviewed as well as mean Phe levels over two years preceding the last clinic appointment. Contemporaneous mental health status was reviewed by searching for the terms 'mood swings', 'low mood', 'anxiety' and 'depression' in the electronic patient record (EPR). Symptom prevalence was measured across the entire cohort, per quartile of Phe control, and, using the Odds Ratio (OR), compared symptoms in those above versus below current European Guidelines of < 600μmol. Pregnant patients were excluded from the final analysis.
Results: Of 244 PKU patients, 220 patients' records, with available Phe levels, were reviewed. Overall, respective prevalences of mood swings, low mood, anxiety and depression were 10%, 15%, 19% and 14%. Quartiles of Phe control (Q1-4) were Q1:162-574mmol/l, Q2:575-938mmol/l, Q3:939-1287mmol/l and Q4:1288-2003mmol/l. Increases in all above-defined mental health symptoms were seen progressively across Q1-Q3. Compared with European Guidelines target of < 600mmol/l, OR for mood swings, low mood, anxiety, and depression were 4.3, 3.1, 2.2 and 2.9 respectively, where mean Phe levels were >600mmol/l.
Discussion: Findings from this 'real world' retrospective clinical audit of mental health symptoms in a single centre PKU population provide

evidence supporting the current European Guidelines. Prospective and multi-centre studies are needed to confirm these findings.
Conflict of Interest declared.

06. Phenylketonuria: treatment, BH4

P-120

Long-term follow-up and Clinical findings of BH4 deficiency patients in Korea

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Background: A deficiency of BH4 (tetrahydrobiopterin) not only causes the classical phenylketonuric phenotype, but also is the source of neurological signs and symptoms due to impaired syntheses of L-Dopa and serotonin. The treatment of BH4 deficiency usually consists of replacement with BH4 and the neurotransmitters. We performed this study to find out long-term follow-up clinical symptoms and prognosis of BH4 deficiency.
Methods: Clinical and biochemical, genetic analysis were done retrospectively from January 1999 to March 2018 in Soonchunhyang University Hospital.
Results: In our study, total 211 patients were confirmed to classic phenylketonuria (PKU), BH4 responsive PKU. Among them, thirteen patients were BH4 deficiency. 11 patients were 6-pyruvoyl-tetrahydropterin (PTPS) deficiency, one patient was dihydropteridine reductase (DHPR) deficiency and one patient was GTPCH deficiency. The patients who received delayed treatment, most of our patients suffered from severe psychomotor retardation, hypotonia and seizure. C.259C>T mutation was identified most commonly in PTPS gene analysis. A patient with DHPR deficiency had a mental retardation, dystonia, seizure. His seizure semiology was dialeptic feature. His EEG showed generalized spike wave patterns. Valproic acid had a good effect for his symptoms. All patients had treated with tolerate BH4, L-Dopa and 5-hydroxytryptophan. Most of the early treated (newborn) patients have a good tolerance for drugs well. But some patients had a neurologic symptoms, despite early detection and treatment.
Discussion: In Korea, the incidence of BH4 deficiency among hyperphenylalaninemia is 6.2%, is higher than Europe. 85% of BH4 deficiency is PTPS deficiency. Patients who has unknown seizure, mental retardation and hypotonia can be suspicious of BH4 deficiency.

P-121

A Genotyping program of phenylketonuria patients in Russia

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Background: Phenylketonuria (PKU) caused by PAH gene mutations is a common metabolic disease (1:7000 newborns in Russia) that leads to mental retardation. The phenomenon of BH4-sensitive PAH deficiency is confirmed by the number of studies. Nowadays in Russia the BH4-treatmetment of PKU patients starts. Data on the genotype of PKU patients make it possible to predict their sensitivity to the cofactor therapy.
Methods: DNA of 1254 unrelated probands with PKU and hyperphenylalaninemia (HPA) from 50 Russian regions were examined for the presence of 25 common mutations of the PAH gene. The allele-specific MLPA method was used.
Results: Pathogenic variants are revealed on 86,3% of investigated chromosomes. In 75.3% of patients both pathogenic alleles are identified and the diagnosis of "phenylketonuria" caused by mutations in the PAH gene is confirmed. Only one pathogenic variant is found in 22.1% of probands, 2.6% is not reveal mutations in the PAH gene. Allelic frequencies of 25 common mutations of the PAH gene are determined. Summary allele frequencies of severe and mild mutations are 74.6% and 11.7% respectively. The most common severe mutation is R408W (51.8%). Severe phenotypes occur more frequently: classic PKU is observed in 71.2% of patients, moderate PKU in 19.8%, mild HPA in 8.3%. Severe genotypes also prevail among Russian patients. According to the results of the study, 56.9% of patients are non-responders to BH4 therapy, 21.8% - are potential responders.
Discussion: Within the program of genotyping patients with PKU and HPA, an unique scope of the research was performed. Physicians in the regions of the Russian Federation use data obtained during the study for therapy and family counseling of patients. Genotype data contributed to the launch of BH4 loading tests in several regions.

P-122

Phase 3 PRISM studies evaluating efficacy and safety of pegvaliase for the treatment of adults with phenylketonuria

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Background: Pegvaliase, PEGylated recombinant *Anabaena variabilis* phenylalanine ammonia lyase, is a potential enzyme substitution therapy to lower blood phenylalanine (Phe).
Methods: Phase 3 studies evaluated subcutaneous pegvaliase to lower plasma blood Phe in adults with phenylketonuria (PKU). PRISM-2 enrolled subjects from parent study PRISM-1 and includes an ongoing long-term extension that allows pegvaliase dosing of 5–60 mg/day.
Results: 261 subjects had mean (SD) of 18.5 (11.7) months of pegvaliase treatment. Mean (SD) blood Phe decreased from pre-treatment baseline of 1233 (386) μmol/L (n=261) to 565 (531) μmol/L at month 12 (n=164) and 311 (427) μmol/L at month 24 (n=51). More subjects reached >20% blood Phe reduction from baseline (initial signal of pharmacologic effect) over time: 41% to 56% by 12 and 24 weeks, respectively, on 20 mg/day. Subjects not initially reaching >20% blood Phe reduction that increased dose to 40 mg/day had mean (SD) blood Phe decrease from 1252 (339) μmol/L (n=30) to 830 (387) μmol/L (n=37) over 8 weeks. Per Kaplan-Meier estimates, 44% and 61% of subjects achieved blood Phe ≤360 μmol/L by months 12 and 24, respectively, and 55% and 68% of subjects achieved blood Phe ≤600 μmol/L by months 12 and 24, respectively. Common AEs were arthralgia (70%), injection-site reaction (62%), injection-site erythema (48%), and headache (47%). Twelve subjects

had 17 anaphylactic AEs that were non-IgE mediated and resolved without sequelae; 6 subjects discontinued after an anaphylactic AE. Two of the remaining 6 subjects had subsequent events and continued treatment.
Discussion: Pegvaliase treatment was associated with sustained and substantial blood Phe reduction. The increased proportion of subjects achieving blood Phe efficacy was due to a combination of increased time on treatment and ability to adjust dose. Pegvaliase has a manageable safety profile for most subjects with arthralgia and injection site reactions as the most common AEs.
Conflict of Interest declared.

P-123

Long-term safety of induction, titration, and maintenance dosing of pegvaliase treatment in adults with phenylketonuria

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Background: Phenylketonuria (PKU) is caused by phenylalanine hydroxylase deficiency resulting in phenylalanine (Phe) accumulation. Pegvaliase converts Phe to trans-cinnamic acid and ammonia and is a potential enzyme substitution therapy to lower blood Phe in adults with PKU.
Methods: Subcutaneous pegvaliase was administered to adults with PKU using an induction, titration, and maintenance dosing regimen with doses up to 60 mg/day. Safety was comprehensively assessed in phase 2 and 3 studies.
Results: 285 subjects received pegvaliase treatment for up to 7.6 years, with a mean (SD) of 24.40 (15.46) months and total exposure of 579.6 person-years. Mean (SD) blood Phe decreased from pre-treatment baseline level of 1227 (379) μmol/L to 294 (398) μmol/L at month 24. Most adverse events (AEs) were mild (74.8%) or moderate (24.5%) in severity and resolved without dose interruption. The most common AEs were arthralgia (73%), injection-site reactions (65%), headache (51%), and injection-site erythema (50%). Exposure-adjusted rate of AEs (52.4 vs 19.0 event rate per person-year), serious AEs (0.24 vs 0.10), and hypersensitivity AEs (15.1 vs 4.0) were higher in the induction/titration period than in the maintenance period, respectively. Thirteen subjects had 21 externally-adjudicated acute systemic hypersensitivity events: eight subjects continued pegvaliase after the event, four of which had subsequent events; 6 of the 8 subjects have remained on therapy. Drug-specific IgE was not detected near the time of the events and all 21 events resolved without sequelae. There is no evidence of immune complex related end organ damage.
Discussion: Long-term pegvaliase treatment was associated with substantial blood Phe reduction. Acute systemic hypersensitivity events were important events consistent with type 3 hypersensitivity. Pegvaliase treatment has a manageable safety profile for most subjects, with rate of AEs decreasing in maintenance period compared to induction/titration period.
Conflict of Interest declared.

P-124

Characterization of hypophenylalaninemia in pegvaliase treated adults with PKU

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Background: Pegvaliase converts phenylalanine (Phe) to trans-cinnamic acid and ammonia, and is a potential enzyme substitution therapy to lower blood Phe in adults with PKU.
Methods: Subcutaneous pegvaliase was administered to adults with PKU using an induction, titration, and maintenance dosing regimen with doses of 5–60 mg/day in phase 2 and 3 studies. Modifying dietary protein intake and pegvaliase dose were allowed in the event of hypophenylalaninemia (hypoPhe). Adverse events (AEs) that occurred in subjects during hypoPhe periods, defined as ≥2 consecutive blood Phe levels < 30 μmol/L, were compared to subjects not experiencing a hypoPhe event.
Results: 285 subjects received pegvaliase for mean (SD) of 24.40 (15.46) months with total exposure of 579.6 person-years. A total of 100 subjects had ≥1 event of hypoPhe. Mean (SD) time to first hypoPhe event was 347 (280) days after initiating pegvaliase and mean (SD) duration of all events was 204 (188) days. Mean baseline blood Phe (1236 vs. 1223 μmol/L) and protein intake (66.4 vs. 64.1 grams/day) were similar between those with and without a hypoPhe event, respectively. AE rate was 25.90 events/person-years in subjects with hypoPhe and 33.02 in subjects without hypoPhe. The incidence of AEs leading to drug discontinuation (2% vs 22%) and AEs that were serious (7% vs. 20%) were lower in subjects with a hypoPhe event compared to subjects without, respectively. Alopecia occurred more frequently in subjects with hypoPhe (27.0% of subjects with hypoPhe and 6.5% without hypoPhe). Alopecia events were mild or moderate severity and 95% of subjects with alopecia remained on pegvaliase with no dose change. The majority of alopecia AE (72%) resolved while subjects were hypoPhe.
Discussion: About 1/3 of subjects administered pegvaliase developed hypoPhe. With the exception of alopecia, incidence and exposure-adjusted AE rates were generally lower in subjects with hypoPhe compared to subjects without hypoPhe.
Conflict of Interest declared.

P-125

An interim analysis of the KAMPER and PKUDOS registries: Efficacy and safety of sapropterin before and during pregnancy

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Background: To describe the efficacy and safety of sapropterin dihydrochloride (sapropterin) during 68 pregnancies in women with phenylketonuria (PKU).
Methods: Efficacy and safety data on sapropterin use prior to and/or during pregnancy in women with PKU were collected from the Kuvan® Adult Maternal Paediatric European Registry (KAMPER) and Phenylketonuria Developmental Outcomes and Safety (PKUDOS) maternal sub-registries.
Results: Data were provided from 12 pregnancies (10 women) from KAMPER, and 55 pregnancies (43 women) from PKUDOS; mean maternal age was ~30 years in both. Prior to pregnancy, 58% of KAMPER and 61% of PKUDOS women were on phenylalanine (Phe)-restricted diets. In KAMPER vs PKUDOS the mean±SD dose of sapropterin during pregnancy was lower (11.4±5.8 vs 18.5±3.2 mg/kg/day), and mean±SD duration of sapropterin use during pregnancy was 268.9±14.2 vs 242.7±70.2 days. Mean blood Phe levels per trimester were maintained ≤ 360 μmol/L, except for 3 pregnancies in KAMPER and 14 in PKUDOS. Six women in KAMPER experienced adverse events (AEs) during pregnancies; all were mild, except 1 serious AE of arrhythmia. In total, 5 pregnancies (all in PKUDOS) were spontaneously or electively terminated. In KAMPER and PKUDOS, respectively, 10/10 and 36/40 (10 missing outcomes data) pregnancies that were carried to term resulted in infants reported to be normal at birth. Of the 3 major abnormal outcomes in PKUDOS, 2 were associated with Phe levels >360 μmol/L during pregnancy (microcephaly, cleft palate).
Discussion: In this population of pregnant women with PKU, a Phe-restricted diet and/or sapropterin therapy during and/or prior to gestation demonstrates efficacy in maintaining blood Phe within the targeted range. Of the 50 pregnancies with birth outcome data, over 90% (46/50) were reported to be normal at birth.
Conflict of Interest declared.

P-126

A meta-analysis of growth outcomes in phenylketonuria patients treated with phenylalanine-restricted diet + sapropterin

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Background: There is evidence that young children with phenylketonuria (PKU) treated with diet only may exhibit sub optimal growth. Increasing natural protein/Phe intake has been shown to increase patient height and suggests that maximizing natural protein intake over time is an important factor for optimal growth. Treatment with sapropterin dihydrochloride allows many patients to increase natural protein intake, while maintaining blood Phe levels within range (120–360 μmol/L). Herein, we assess growth outcomes in PKU patients (aged 0–4 years) treated with sapropterin.
Methods: Growth data on children (aged 0–4 years) treated with sapropterin were derived from one registry in which patients were followed in a real-world setting (PKU Demographic, Outcomes, and Safety Registry [PKUDOS; NCT00778206]), one open-label phase 3b trial (PKU-015 [NCT00838435]), and one randomized controlled trial (SPARK [NCT01376908]). The pooled data captured measurements (height, weight, head circumference) at irregular intervals over a period of ≤2 years. Z-scores (Centers for Disease Control reference values) for height-for-age, weight-for-age, and head circumference-for-age were assessed in the pooled PKU population.
Results: This analysis included 249 children with PKU; Mean (range) age was 25.8 (13.6-38.8) months and 53.3% were female. Mean (range) amount of prescribed Phe increased from 360.2 mg/day (326.4-394.0) to 633.6 mg/day (516.8-750.1), while blood Phe remained within range. Baseline (first visit) z-scores (SD) for all three growth parameters (height 0.21 ± 1.01, weight 0.30 ± 1.05, and head circumference 0.13 ± 1.15) were above the 50th percentile vs reference values and were maintained throughout 2 years of follow-up (except head circumference at 2 years – 48th percentile).
Discussion: In this meta-analysis, children (aged 0–4 years) treated with sapropterin increased dietary Phe intake and exhibited normal growth parameters over 2 years of follow-up.
Conflict of Interest declared.

P-127

Preliminary indications of metabolic modulation by provision of amino acids engineered to allow physiological absorption

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Background: A pharmaceutical technology applied to free amino acids (AA) - Physiomimic Technology- has shown ability to mask AA odor and taste. Intestinal AA release and absorption kinetics also seem to be modified, mimicking intact dietary proteins. We evaluated the metabolic impact of 3 nitrogen sources: a Physiomimic Technology based product (P1); casein, a slow-release protein (CAS); a marketed product with modified release kinetics (P2). Each product was also compared with its free AA mix.
Methods: A single dose (0.7 g AA/kg bw) of each product + glucose 20% was given to healthy rats (n=7-12/group). Glycemia was measured at 0, 15, 30, 45, 60 and 90 min and other metabolic markers [blood urea nitrogen (BUN), ghrelin, gastric inhibitory polypeptide (GIP), glucagon-like peptide-1 (GLP-1), glucagon and insulin] at 90 min.
Results: BUN‡ (μM/L) was significantly reduced in rats fed P1 vs its free AA mix (14.27±0.83 vs 16.41±0.46; p=0.044§), but the same was not found with P2 vs its free AA mix (15.95±0.75 vs 14.17±0.92; p=0.149§). When compared with CAS, BUN increased in rats fed P2 (12.45±0.84 vs 15.95±0.75; p=0.006§) but not when fed P1 (12.45±0.84 vs 14.27±0.83; p=0.171§). Glycemia trend of P1 did not differ significantly from CAS (p=0.399†), while P2 did (p=0.044†). Also, if compared with the respective free AA mix, glycemia trend in rats fed P1 seemed to go in the same direction of glycemia of rats fed CAS (p=0.074†; p=0.061†, respectively). This trend was not visible comparing P2 with its free AA mix (p=0.356†). The other metabolic markers did not differ in rats fed CAS, P1 or P2 at 90 min.
Discussion: In rats, the Physiomimic Technology (P1) was able to modulate BUN and glycemia in the same direction of CAS. The BUN results

suggest a potential benefit towards a better AA utilization pattern with P1, although further research is warranted.
‡Mean ± SEM; §Unpaired T test; †two-way ANOVA
Conflict of Interest declared.

P-128

The extended tetrahydrobiopterin loading test in PKU patients, a Dutch pilot cohort-study

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Background: Tetrahydrobiopterin (BH4) is a treatment option for some patients with PKU. The 48-hr BH4 loading test was developed to assess BH4 responsiveness in an efficacious way. In order to find out if PKU patients (late-responders) are missed with the 48-hr test, patients with 20-30% decrease of blood Phe concentration during the 48-hr BH4 loading test and patients with at least one favorable mutation underwent an extended BH4 loading test of 168 hrs.
Methods: BH4 was administered orally once daily for 7 days (20 mg/kg/day). Blood samples on filter paper were collected at T= −8, 0, 8, 16, 24, 32, 40, 48, 72, 96, 120, 144 and 168 hrs. Potential BH4 responders [≥20% decrease at ≥1 moment within the first 48 hrs or ≥30% at ≥1 moment during the entire test] underwent a treatment trial to assess true long-term responsiveness (≥30% decrease of Phe concentration compared to baseline and/or ≥4g or ≥50% increase in natural protein tolerance in accordance with the Dutch guidelines before 2017).
Results: Of the 23 patients who completed the 168-hr loading test, 2 were excluded, 9 were negative and 12 were considered to be potential BH4 responders. Of these 12 potential BH4 responsive PKU patients, so far 10 have completed the treatment trial. Five turned out to be negative, 4 turned out to be a true-responder and 1 was undecided. Two of the true-responders and 3 of the non-true-responders had a positive genotype according to the BIOPKU database. The mutation R261Q was apparent in 2 true-responders and 4 of the non-true-responders.
Discussion: A cut-off of ≥20% decrease at ≥1 moment within the first 48-hrs is not a good parameter in predicting true-responsiveness. An extended BH4 loading test can be helpful to detect true BH4 responsive PKU patients if they have either a decrease of Phe of 20-30% within 48-hrs or have one mutation associated with BH4 responsiveness. However, predicting true BH4 responsiveness in patients with the R261Q mutation seems especially difficult.
Conflict of Interest declared.

P-129

Tetrahydrobiopterin responsiveness and phenotype prediction by BIOPKU database in a cohort of PKU patients

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Background:genotype is associated with phenylketonuria (PKU) severity and tetrahydrobiopterin responsiveness (BH4-r), however not without conflicting results; the international database BIOPKU (http://www.biopku.org) could help in characterization and management of PKU patients (pts). The aim of the study is to compare the genotype and BH4-r of a group of PKU pts diagnosed in our Centre in the last 30 years to the phenotype prediction obtained by BIOPKU.
Methods / Case Report:BH4-r was evaluated in 20 out of 105 pts with PKU diagnosed and managed at our Centre. In all cases *PAH* gene mutations were evaluated. The pts were classified on the basis of BH4-r: responders (>30%); slow responder (20-30%) not responders (< 20%). By BIOPKU we evaluated the allelic phenotype value (APV) for each allele (0–2.7 is classic PKU; 2.8-6.6 is mild PKU; 6.7-10.0 is mild hyperphenylalaninemia) and the predicted BH4-r. APV max is the highest APV value in every patient.
Results:BH4-r: 16/20 pts were responders, 1/20 pt slow responder, 3/20 pts not responders. Overall we detected 22 different allele mutations, the more frequent were IVS10-11 G>A (n.7) and R408Q (n.4) and R261Q (n.4). According to APV max, 8 pts have classic PKU, 4 pts mild PKU, 3 mild hyperphenylalaninemia; in 5 pts APV max was not available. BH4-r prediction was available for 14 alleles combinations and confirmed our results in all cases but one: this responder pt has APV max 0 (homozygous for G352fsX c.1055 delG mutation).
Discussion:PKU phenotype depends on several factors, mainly the type/ site of mutations and the interplay between alleles. Our results seems to confirm that prediction of phenotypes and BH4-r in PKU patients has been highly improved; BH4-r should not be predicted only on the basis of APV max and it should be tested not to exclude any patient that would benefit from BH4 treatment.

P-130

Body mass index and nutritional biomarkers after 5 years of BH_4 treatment in phenylketonuria patients

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Background: The introduction of BH_4 (prescribed as sapropterin dihydrochloride) has resulted in an increased intake of natural protein in a subset of phenylketonuria (PKU).The objective of this study was to assess the long-term effects of BH_4 treatment on body mass index (BMI) and nutritional biomarkers in PKU patients.
Methods: A retrospective cohort study was conducted using data from 21 BH_4-treated and 21 birth date- and gender-matched PKU patients. Study parameters were BMI, various nutritional biomarkers, and prescribed vitamin/mineral supplements from baseline and after 5 years of BH_4 treatment. Parametric and non-parametric statistical analyses were performed for continuous data, and McNemar's and Chi-squared tests were used for dichotomous outcomes.
Results: Prescribed natural protein intake increased following BH_4 treatment ($p < 0.001$), while amino acid supplement intake decreased ($p < 0.001$). While no differences were observed in BMI, serum concentrations of cholesterol, hemoglobin, phosphate, and MMA had changed significantly after 5 years of BH_4 treatment. These changes are however probably age-related, as no differences compared to the control group were seen. A tendency to an increase

in calcium supplementation was observed in BH_4-treated patients ($p = 0.063$), which was not the case in the control group.
Discussion: The dietary changes following BH_4 treatment did not affect BMI and nutritional biomarkers when compared to a control group. However, calcium was supplemented more often, indicating that at least dietary follow-up of BH_4-treated PKU patients is still warranted.
Conflict of Interest declared.

P-131

Studies on the p.L249F phenylalanine hydroxylase variant: insights into the mechanism of the associated BH_4 non-responsive phenotype

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Background: Response to administration of the human phenylalanine hydroxylase (hPAH) cofactor (tetrahydrobiopterin; BH_4) is more prevalent in milder phenylketonuric (PKU) phenotypes. However, inconsistent BH_4 responses have been reported. The p.L249F variant, associated with mild PKU and a 51% residual activity of eukaryotic expressed protein (Trunzo et al. Gene, 2016; 594:138), has been found in BH_4 non-responsive phenotypes. Data from our group supported those findings as among 1 homozygous and 7 compound heterozygous p.L249F patients evaluated, 7 in 8 were non-responders irrespective to the underlying genotype.
Methods: Wild-type (WT) and p.L249F proteins were produced in *E. coli*, purified and characterized to elucidate the molecular mechanism of pathogenesis and BH_4 response in the p.L249F variant.
Results: The p.L249F was expressed in high yields but was mainly found as soluble aggregates (43%; WT:11%), with lower levels of tetramers (36%; WT:60%) that presented ≈46% residual activity. Thermostability assays indicated a lower stability of the catalytic domain ($\Delta T_{m2} \approx -3°C$) and L-Phe did not change T_{m1} ($\Delta T_{m1} \approx 0°C$; WT=6°C).
Discussion: Prokaryotic and eukaryotic expressed p.L249F presented similar residual enzyme activities, namely 46 and 51%, supporting the mild PKU phenotype. The L249 residue localizes in the hPAH binding pocket interacting with BH_4. Our data indicate a change in p.L249F conformational stability (higher content of aggregates and decreased thermal stability of the catalytic domain) resulting from the change to a more hydrophobic residue (Phe). Movement of the N-terminal regulatory has been proposed as an important event for BH_4 inhibition/stabilization. We postulate that the observed decrease stability of the regulatory domain in the presence of L-Phe indicate that in this variant N-terminal motions are impaired thus contributing to the unresponsive phenotype.
Funding:PEst-OE/SAU/UI4013/2011; NPKUAlliance; SPDM

P-132

Secondary BH4 salvage pathway alterations due to chemotherapy may provoke phenylalanine hydroxylase deficiency

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Background: Hyperphenylalaninemia results from either primary phenylalanine hydroxylase (PAH) deficit or disorders of biopterin metabolism. The imbalance of levels of this and related metabolites leads to neuro-psychiatric symptoms. We describe several cases of secondary BH4 deficiency related to onco-haematological chemotherapy exploring the possible pathogenic mechanisms.
Case Report: Seven patients, age (4–15 y) with acute leukemia were submitted to several rounds chemotherapy (5/7 patients received methotrexate (MTX)). Plasma aminoacids, urine organic acids and urine pterins were analyzed by standard methods.
Results: PKU-like metabolite profiles were observed with increased plasma phenylalanine (mean: 308 (109–701) μmol/L, N:34–101) as well as elevated phenolic compounds in urine. Total pterin levels in urine were analyzed in 1 patient and found elevated (biopterin: 5,9 mmol/mol creat, N:0,3-3,0; %biopterin: 85%, N:18–80).
Discussion: Hyperphenylalaninemia were found in accordance with a previous report (Blau, 1989). MTX works by dihydrofolate-reductase (DHFR) inhibition and it has been suggested that could also inhibit dihydropterin-reductase (DPHR), through BH4 recycling pathway. However, the pterin salvage pathway depends mainly on DHFR, and its inhibition is probably the cause of secondary hyperphenylalaninemia by lowering the effective BH4 level. In 1 studied patient the urine total pterins were surprisingly elevated. This may result from the influence of the chemotherapy agents on the BH2/BH4 cycle, provoking low activity towards BH4 and accumulation of BH2 and derivatives. Two of our patients did not receive MTX suggesting similar action mechanisms by other drugs of the chemotherapy mix. By this way all patients included cotrimoxazol which interferes with the folate metabolism. The preventive administration of BH4 in chemotherapy treatment may avoid neuro-psychiatric symptoms since it can stimulate the hydroxylase activities.

07. Sulphur amino acid disorders

P-133

Metabolism of hydrogen sulfide in patients with two types of homocystinurias

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Background: Hydrogen sulfide is an important signalling molecule which is synthesized endogenously from cysteine and homocysteine by several enzymes including cystathionine beta-synthase (CBS) and gamma-cystathionase. We explored whether the severely perturbed sulfur amino acid (SAA) metabolism in patients with homocystinurias affects hydrogen sulfide homeostasis.
Methods: We studied 10 treated patients with CBS deficiency (CBSD), and 6 treated patients with remethylation defects (RMD). Control groups for CBSD and RMD patients consisted of 22 patients with phenylketonuria on low protein diet and of 12 healthy controls, respectively. Plasma and urine concentrations of selected sulfur compounds were analysed by HPLC and LC-MS/MS.
Results: Patients with CBSD exhibited plasma concentrations of monobromobimane-detected sulfide similar to appropriate controls (median 0.67 vs 0.54, and 0.15 vs 0.19 μmol/L by two methods, respectively). Urinary homolanthionine and thiosulfate in CBSD were increased significantly 1.9 and 3-times indicating possibly higher hydrogen sulfide synthesis and detoxification, respectively. Surprisingly, patients with RMD had significantly lower plasma sulfide levels (53% and 64% of controls) with lower sulfite

concentrations and higher taurine and thiosulfate levels indicating possibly enhanced cysteine oxidation and hydrogen sulfide catabolism, respectively.
Discussion: This study suggests that grossly deranged flux of SAA may be accompanied by only moderately perturbed hydrogen sulfide metabolism and indirectly suggests that enzymes in the transsulfuration pathway may not be the major direct contributors to the endogenous hydrogen sulfide pool.
Acknowledgement: This work was supported by the grant Nr. 16-30384A from the Czech Health Research Council, and grants No. KH17_126766 and K 109843 from the National Research, Development and Innovation Office. Institutional support was provided by the projects RVO-VFN 64165 and Progres Q26.

P-134

JP4-039 prevents alterations in enzymatic antioxidant defenses induced by sulfite in striatum of rats

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Background: Sulfite oxidase (SO) deficiency is an autosomal recessive disorder characterized by severe seizures and progressive neurological damage that often result in early death. Affected individuals present accumulation of sulfite in tissues, including brain. We evaluated the effects of sulfite administration on antioxidant defenses, creatine kinase (CK) activity and the p38 MAPK pathway in rat striatum, as well as the influence of the mitochondrial-targeted electron scavenger JP4-039 on sulfite-induced toxicity.
Methods: Thirty-day-old rats were intrastriatally administered with sulfite (2 μmol) or NaCl (2 μmol; control). Pre-treatment with JP4-039 (doses ranging from 3.5 to 5 μg/g) was performed with two intraperitoneal injections of this compound 12 and 2 h before sulfite administration. Thirty minutes after sulfite administration, rats were euthanized and had their striata homogenized for the evaluation of the parameters.
Results: Sulfite administration decreased reduced glutathione (GSH) concentrations and glutathione peroxidase (GPx), glucose-6-phosphate dehydrogenase (G6PDH), glutathione S-transferase (GST) and glutathione reductase (GR) activities in the striatum. Sulfite also decreased CK activity and p38 phosphorylation. JP4-039 treatment prevented sulfite-induced decrease of GPx, G6PDH, GR and GST activities. However, alterations in GSH concentrations, CK activity and p38 phosphorylation induced by sulfite were not affected by JP4-039.
Discussion: Our findings demonstrate that sulfite impairs antioxidant defenses, energy transfer and p38 pathway in rat striatum. Furthermore, the data showing that some of the toxic effects elicited by sulfite are prevented by JP4-039 suggest that this protective compound may be a promising candidate for treatment of SO deficient patients. Financial support: CNPq, CAPES, Propesq-UFRGS, FAPERGS, INCT-EN.

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WITHDRAWN

P-136

WITHDRAWN

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Treatment of CBS deficiency in mice using a minicircle-based naked DNA vector

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Background: Individuals with loss of function mutations in the cystathionine β-synthase (*CBS*) gene have classical homocystinuria, which is characterized by extremely elevated total homocysteine (tHcy) in plasma and a variety of clinical phenotypes. Mice lacking *Cbs* die in the neonatal period, but can be rescued by expression of a transgene expressing patient-derived mutant human *CBS*. Here we use two mouse models, *Tg-R336C Cbs*$^{-/-}$ and *Tg-I278T Cbs*$^{-/-}$ to evaluate the potential of minicircle-based naked DNA gene therapy to treat CBS deficiency.
Methods: A 2.3 Kb DNA minicircle containing the liver specific P3 promoter driving the human *CBS* cDNA (MC.P3-hCBS) was delivered into *Tg-R336C Cbs*$^{-/-}$ (n=5) and *Tg-I278T Cbs*$^{-/-}$ (n=7) mice via a single hydrodynamic tail vein injection (HTV). Serum tHcy was measured before injection and every week for a month after injection. After 28 days mice were sacrificed and liver was examined for DNA copy number by qPCR and for CBS protein expression by western blot and enzyme activity.
Results: Both *Tg-R336C Cbs*$^{-/-}$ and *Tg-I278T Cbs*$^{-/-}$ miceinjected with MC.P3-hCBS showed a significant decrease in serum tHcy compared to control treated animals. For *Tg-R336C Cbs*$^{-/-}$ mice we observed a 64% reduction in tHcy (326 to 121 μM, P< 0.0001) after 8 days and a 34% reduction after 21 days (326 to 217 μM, P< 0.028). For *Tg-I278T Cbs*$^{-/-}$ mice we observed a 51 % reduction (351 to 176 μM, P< 0.0007) after 8 days and a 67% reduction (351 to 120 μM, P< 0.0001) after 21 days. Analysis of liver lysates of *Tg-R336C Cbs*$^{-/-}$ mice showed presence of MC.P3-hCBS DNA at a level of 1–44 copies per genome. Western blot and enzyme activity analysis reveal significant vector-directed CBS expression.
Discussion: The data shows that hydrodynamic tail vein injection of a CBS-expressing naked DNA minicircle vector can express CBS sufficiently to lower tHcy in CBS-deficient mice. These findings suggest that minicircle gene therapy may be feasible to treat CBS deficiency.

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Impaired mitochondrial maturation of sulfite oxidase in a patient with severe sulfite oxidase deficiency

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Background: Sulfite oxidase (SO) catalyzes the terminal step of cysteine catabolism. The two-electron oxidation of sulfite to sulfate is dependent on two prosthetic groups of SO, a heme b_5-type cytochrome and the molybdenum cofactor (Moco) the later forming the catalytic site of sulfite oxidation. SO is localized to the intermembrane space of mitochondria where electrons derived from sulfite oxidation are passed to cytochrome

c. Both, cofactor insertion and dimerization are critical determinants of SO maturation in mitochondria. Within the folded dimeric enzyme, Moco is coordinated by evolutionary conserved residues, which densely bury the cofactor inside the folded enzyme by electrostatic interactions towards the terminal phosphate of Moco. In humans, two of these residues (Arg366 and Lys379) have been reported to be affected by mutations causing isolated SO deficiency (iSOD), an inborn error in metabolism causing severe neurodegeneration and early childhood death.
Methods / Case Report: Here, we describe a SO variant of a patient with a homozygous mutation at c.1084G>A replacing Gly362 to serine. To understand the molecular mechanism of the disease we expressed the patient-derived G362S SO variant in *E. coli* and investigated patient fibroblasts.
Results: We found full activity of the recombinant enzyme while in extracts of patient fibroblast no SO activity could be detected. Moco reconstitution of apo-G362S SO was approximately 90-fold reduced in comparison to apo-WT SO providing strong evidence for an impaired maturation of the G362S variant in patient mitochondria. Addition of molybdate to the culture medium increased Moco content of *E. coli* expressed G362S SO and partially restored the SO activity in patient fibroblast.
Discussion: This study demonstrates the importance of the orchestrated maturation of SO by Moco incorporation and provides a possible option for treatment.

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Mitochondrial-targeted JP4-039 improves mitochondrial homeostasis in ETHE1 and molybdenum cofactor deficient fibroblasts

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Background: ETHE1 and molybdenum cofactor deficiency are inborn errors that affect the metabolism of sulfur amino acids. Patients affected by both disorders present with progressive encephalopathy and severe seizures, whereas ETHE1 deficiency is also characterized by chronic diarrhea, petechiae and orthostatic acrocyanosis. Since the pathophysiology of these diseases is not yet elucidated and treatment options are limited, we evaluated mitochondrial dynamics and bioenergetics, superoxide production and cell death in fibroblasts from patients, as well as the potential beneficial effects of the mitochondrial antioxidant JP4-039.
Methods: Content of proteins involved in mitochondrial dynamics (MFN1, MFN2, OPA1 and DRP1), mitochondrial oxygen consumption, superoxide levels and apoptosis were measured in primary human dermal fibroblasts obtained from four patients with ETHE1 deficiency and one with molybdenum cofactor deficiency type A (MOCS1 deficiency).
Results: Increased MFN1, MFN2, OPA1 and DRP1 was verified in one ETHE1 deficient cell line, whereas MOCS1 deficient cells showed reduced MFN1 and MFN2, and increased DRP1 content. Moreover, all cell lines presented lower basal and maximal mitochondrial respiration rate, which was ameliorated by JP4-039 in the MOCS1 cell line and in two ETHE1 cell lines. High superoxide levels observed in all fibroblasts were also decreased by JP4-039. Finally, apoptosis induction was seen in all cell lines.
Discussion: Taken together, these findings show reduced mitochondrial respiration and changes in mitochondrial dynamics, as well as increased reactive oxygen species production and cell death, that might be involved in the pathophysiology of ETHE1 and MOCS1 deficiencies. Furthermore, since JP4-039 improved mitochondrial respiration and decreased superoxide production, it is presumed that this compound may be considered as a novel therapeutic approach for these disorders.
Financial support: CAPES

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Ethylmalonic encephalopathy: Can liver transplantation be a treatment option?

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Background: Ethylmalonic encephalopathy (OMIM: 608451) is an autosomal recessive, rapidly progressive disorder caused by *ETHE1* mutations presenting with neurologic and vascular manifestations due to the toxic accumulation of hydrogen sulphide and its metabolites. Dionisi-Vici *et al.* reported improvement in neurologic outcome in a case of ethylmalonic encephalopathy after liver transplantation.
Results: The aim was to evaluate the efficacy of liver transplantation in an infant with ethylmalonic encephalopathy not responding to treatment.
Case Report: 6 month-old boy, with delayed psychomotor development, hypotonia and convulsions was referred. Diarrhoea was present since birth. He had cutis marmorata, petechiae purpura and a developmental age of around age zero. Urinary organic acid analysis by GC-MS showed elevated ethylmalonic acid excretion (2200 mmol/mol creatinine) (normal range:1.7-14.6). Dried blood spot acylcarnitines showed elevated butyryl and isovaleryl carnitines;2.34(0–1.2) and 0.78(0–0.6) respectively and a homozygous c.554T>C (p.Leu185Arg) mutation on *ETHE1* gene was found. He wasn't responsive to anticonvulsants, N-acetylcysteine or metronidasole. He received a liver transplant at the age of 7.5 months from his living donor aunt. The last evaluation at the age of 17 months, one year after liver transplantation, showed remarkable clinical, neurologic and biochemical improvement. Treatment resistant convulsions were under control with anticonvulsant drugs. He has acquired the ability to stand up with aid, to say words and to pass objects from one hand to the other. Urinary ethylmalonic acid excretion decreased to 50%, elevated butyryl and isovalerylcarnitines were normalised. He is still on metronidasole, N-acetylcysteine and tacrolimus treatment.
Discussion: We suggest that liver transplantation is a successful treatment option for ethylmalonic encephalopathy; a neurologically devastating and potentially lethal disorder.

P-141

A New Model for Isolated Sulfite Oxidase Deficiency (ISOD) Shows Organ-Specific Deregulation of Hydrogen Sulfide Pathways

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Background: Isolated sulfite oxidase deficiency (ISOD) and molybdenum cofactor deficiency (MoCD) are rare inherited metabolic disorders characterized by severe neurological abnormalities and early death. MoCD is caused by mutations in genes involved in molybdenum cofactor (Moco) biosynthesis while ISOD is based on mutations in the *SUOX* gene encoding for sulfite oxidase (SO). SO, a mitochondrial enzyme, is responsible for the detoxification of sulfite to sulfate formed within the

catabolism of cysteine and H_2S, and SO is considered to be the most important Moco-dependent enzyme. At biochemical level, sulfite, thiosulfate, S-sulfocysteine, and taurine are highly accumulated, while glutathione, cystine and sulfate levels are below normal range in both diseases.
Methods / Case Report: Recently, we have established a $SUOX^{KO/KO}$ mouse line using the Crispr/Cas9 system in order to study the underlying changes in cysteine and H_2S metabolism caused by ISOD.
Results: Lifespan of $SUOX^{KO/KO}$ mice was reduced to ~ 9 days, with weight and growth arrest at ~ 3 g. Using MRI, a decrease in brain volume and a general change in brain morphology was detected. While brain damage is generally established as the predominant cause of death in ISOD and MoCD, no changes of cysteine metabolism enzymes were detected in the brain. Differential expression of cysteine dioxygenase (CDO) and aspartate aminotransferase 2 (GOT2) was observed in livers and kidneys, but not in brains of $SUOX^{KO/KO}$ mice. Additionally, GOT1 and 3-mercaptopyruvate sulfurtransferase (MST) were also found to be deregulated. Sulfide:quinone oxidoreductase (SQR) was found to be negatively impacted by ISOD. We analyzed thiosulfate levels in blood and urine of $SUOX^{KO/KO}$ mice and ISOD patients as well as organ-specific persulfidation levels.
Discussion: We present a novel disease model translated to mice, displaying alterations in the hydrogen sulfide pathway and potential effects for sulfite generation through hydrogen sulfide oxidation.

P-142

Hydrocephalus and Dandy-Walker-like malformations associated with MTHFR deficiency

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Background: Hydrocephalus has been reported in MTHFR deficiency with a Dandy-Walker-like cyst (Baethmann, 2000). A mouse model of MTHFR deficiency showed reduced cerebellar size & disruption of the normal trilaminar structure (Chen, 2001). Hydrocephalus also occurs in the cblC defect, another remethylation disorder, but there are no malformations and it is caused by microangiopathy.
Case Report: We report hydrocephalus with Dandy-Walker-like malformations in 5 MTHFR patients. 4 presented aged 6–10 weeks & the 5th at 11 months (with previous developmental delay). All had poor feeding, drowsiness and truncal hypotonia +/− spasticity, hypothermia, abnormal breathing or eye movements or sun-setting. In all cases, MRI showed dilatation of the lateral, third and fourth ventricles. Four patients had periventricular oedema; 3 received VP shunts & the 4th died. One infant did not need a shunt. MTHFR deficiency was diagnosed presurgery in one patient and subsequently in others. They were treated with betaine and mefolinate or calcium folinate. All are severely handicapped.
Results: In addition to tetraventricular dilatation, 4 patients had abnormalities resembling the Dandy-Walker malformation, with severe cerebellar hypoplasia, an enlarged posterior fossa and a posterior fossa cyst communicating with the fourth ventricle. The 5th patient only had ventricular dilatation and cerebellar hypoplasia.
Discussion: The severity of the Dandy-Walker malformation varied & did not correlate with the severity of the raised pressure. Thus, our patient with isolated cerebellar hypoplasia required a VP shunt. This suggests that hydrocephalus in MTHFR deficiency may be due to microangiopathy, as in cblC, and not due to malformations obstructing CSF flow. MTHFR deficiency causes cerebral folate deficiency, which may lead to malformations as 5,10-methylene-tetrahydrofolate is a cofactor in converting dUMP to dTMP. This would explain why malformations are not seen in cobalamin defects.

08. Other amino acid disorders

P-143

WITHDRAWN

P-144

Brain imaging in classic nonketotic hyperglycinemia: quantitative analysis and relation to phenotype

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Background: Patients with severe classic nonketotic hyperglycinemia (NKH) have absent psychomotor development and intractable epilepsy, whereas attenuated patients have variable psychomotor development and absent or treatable epilepsy. Differences in brain MRI between these phenotypes have not been reported.
Methods: We reviewed 38 MRI studies from 24 molecularly proven NKH patients, and two transient NKH patients. Quantitative analyses included corpus callosum size, apparent diffusion coefficient, automated brain volumetric analysis, and glycine/creatine ratio by spectroscopy.
Results: All patients age < 3 months had restricted diffusion in the posterior limb of the internal capsule, anterior brain stem, posterior tegmental tracts, and cerebellum, a pattern not seen in transient NKH. In older infants, the diffusion restriction evolved and included generalized diffusion restriction in the supratentorial white matter, which quantitatively peaked between 3 and 12 months. No patient had absent corpus callosum or gyral abnormality. The corpus callosum was more shortened in severe than in attenuated, and thin in severe cases only. The corpus callosum growth rate differed by severity; age-matched Z-scores of thickness worsened in severe cases only. Cerebral volume was decreased in the hippocampus, globus pallidus, cerebral cortex, thalamus, and cerebellum. Severe patients had greatest glycine/creatine ratio on spectroscopy.
Discussion: In NKH, brain malformations are limited to rare hydrocephalus. The growth failure of the corpus callosum is worse in severe NKH, whereas the diffusion restriction pattern, reflecting microspongiosis, does not discriminate by phenotypic severity. This identifies NKH as a disorder of brain growth best noted in the corpus callosum, whereas spongiosis poorly relates to outcome.

P-145

Functional analysis of missense variants in glycine transporter 1 gene (*SLC6A9*)

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Background: Glycine encephalopathy (or non-ketotic hyperglycinemia - NKH) is a severe metabolic disorder, recently also associated with mutations in the *SLC6A9* gene encoding the glycine transporter 1 (GlyT1). To date, 5 NKH patients are described harbouring homozygous mutations in this gene. Recently, one patient displaying clinical similarities to the published cases came to our attention. Whole-exome sequencing (WES) revealed two missense variants (p.(Phe322Leu) and p.(Pro567Leu)) in the *SLC6A9* gene, strongly suggesting SLC6A9 deficiency. Any further WES data analysis was suspended. To confirm/refute this diagnosis we developed an overexpression system to characterize glycine uptake of the missense alleles.
Methods: The *SLC6A9* ORF was cloned into pCMV6-AN-GFP vector, and recombinant constructs were created using site directed mutagenesis. HEK293 cells overexpressing the missense alleles were incubated in Earle's Balanced Salt Solution containing [D2]Glycine, and L-Alanine and ALX-1393 as inhibitors for the non GlyT1 mediated glycine transport. GlyT1 activity was expressed as ratio of [D2]Glycine/endogenous glycine levels measured by LC-MS/MS.
Results: The intracellular [D2]Glycine levels in the HEK293 SLC6A9 transfectants were measured after incubation in the presence of 0.5mM [D2]Glycine, 0.037μM ALX-1393 and 5mM L-Alanine. Overexpression of each of the novel missense alleles displayed activities in the range of the wild type transfectants, whereas the published missense variant, p.(Ser407Gly), resulted in complete impairment of glycine uptake. This ruled out the pathogenicity of both novel variants.
Discussion: The newly developed glycine uptake assay in HEK293 SLC6A9 tranfectants lead to re-evaluation of WES data and an alternative confirmative diagnosis of a patient suspected of SLC6A9 deficiency. This illustrates that functional tests of missense variants are of utmost importance for proper diagnosis and are desired for all genes suspected to be associated with IEM.

P-146

Human skin-derived mesenchymal stromal cells do not rescue hereditary tyrosinemia type 1 mice after permanent nitisinone withdrawal

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Background: Hereditary tyrosinemia type 1 (HT1) is caused by a defective fumarylacetoacetate hydrolase (FAH) enzyme that leads to the accumulation of toxic metabolites in the liver causing a severe loss of hepatocytes. In this study, a murine HT1 transplantation model was established and used to evaluate the potential therapeutic effects of human skin-derived mesenchymal stromal cells (sMSCs).
Methods: $Fah^{-/-}/Rag2^{-/-}/Il2rg^{-/-}$ mice (FRG) were withdrawn from nitisinone and the optimal time point (7 days) for transplantation was established and biochemically evaluated by LC-MS/MS. Next, luciferase-positive sMSCs were transplanted by intrasplenic injection and nitisinone was re-administered for twelve weeks in order to allow liver regeneration, providing all the necessary cues for the *in vivo* expansion and hepatic differentiation of sMSCs. At defined time points, bioluminescence imaging (BLI) was performed. Twelve weeks post transplantation, FRG mice were permanently withdrawn from nitisinone.
Results: LC-MS/MS analyses showed that nitisinone withdrawal was complete after seven days, accompanied by a strong increase in succinylacetone (SA). Microarray analyses of whole livers confirmed the acute onset of severe liver damage and predicted the active state of the NRF2-mediated oxidative stress response pathway in nitisinone-deprived FRG mice. After transplantation, BLI revealed a strong variation in the amount of engrafted sMSCs per mouse liver. Importantly, sMSC-transplanted mice did not exhibit lower SA blood levels than SHAM-transplanted mice and acquired no gain in survival time after permanent nitisinone withdrawal.
Discussion: Our data suggests that the regeneration capacity of human sMSCs is low in the HT1 disease model. This might be due to their low initial engraftment in the liver, their inability to sufficiently expand *in vivo* and/or their immature hepatic differentiation state. Therefore, follow-up studies with hepatic predifferentiated sMSCs will be performed.

P-147

Need for optimizing NTBC doses in patients with HT1

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Background: Treatment of Hereditary tyrosinemia type I (HTT1) includes phenylalanine -tyrosine restricted diet and NTBC. Recommended average dose of NIBC is 1mg/kg/day. Despite benefits, there are concerns about its adverse effects when used long term.
Methods / Case Report: We diagnosed 53 patients with HT1 in last 16 years of which 15 are under follow-up. 6 monthly monitoring included measurement of tyrosine, succinylacetone (SA) and NTBC in blood. USG and MRI of abdomen were monitored yearly.
Results:NTBC is given in two divided doses orally (1 mg/kg/day). All of our patients are on a protein restricted diet but no special diet. SA levels in blood and urine of all patients remained undetectable after starting NTBC. Average NTBC levels in 4 patients were maintained within expected range of 30–50 umol/L (43.42 ±4.71). 3 patients had levels less than 30 uMol/L (18.89±4.25). However compliance in these adolescent patients can be uncertain, however SA was still undetectable. 5 patients had levels of NTBC above the expected ranges (72.41±14.63), without any adverse clinical effects. 3 patients have been recently diagnosed and started on NTBC therefore followup levels are not available.
Discussion: Patients with HT1 are treated with NTBC and a low phenylalanine-tyrosine diet. There is significant improvement in liver and kidney functions. NTBC increases blood tyrosine levels, which may lead to neurologic problems and patients need to be kept on phenylalanine- tyrosine restricted diet. Also some patients on this therapy have been reported to develop HCC if treatment is started late. In some of our patients we have documented persistently higher levels of NTBC while being treated with same recommended dose. Therefore it may be possible that they may achieve therapeutic level with a lower dose (e.g. 0.7 mg/kg/day). Hence a lower than 1 mgm/kg/day dose may be explored in some of the patients. We ropose monitoring levels of blood NTBC to titrate the dose and ensure compliance.

P-148

A very rare etiology of hypotonia and seizures: Congenital glutamine deficiency

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Background: Mutations in the human *GLUL* gene encoding for glutamine synthetase cause congenital glutamine deficiency. The disease was first described in 2005 and only three patients have been reported since that time. We report the fourth patient suffering from congenital glutamine deficiency with some different clinical findings compared to those in previous case reports.
Methods / Case Report: A 2.5-year-old girl was referred to us with seizures and developmental delay. Seizures had been started when she was 5 months old. She had microcephaly and axial hypotonia. She was seizure free for 5 months with valproic acid and vigabatrin. Plasma glutamine level was near normal and she had mild hyperammonemia. Cranial magnetic resonance imaging was normal. Whole exome sequencing (WES) was performed on the patient and the homozygous pathogenic c.121C>T (p.Arg41Cys) variant was found in the *GLUL* gene.
Results: Oral glutamine powder supplementation and nicotinamide riboside treatments were commenced but an ammonia lowering agent was not started initially. In a three month follow-up period, the patient had only one seizure attack lasting two days triggered by a fever. After three months of treatment, serum and cerebrospinal fluid glutamine levels were within normal limits. The serum ammonia level was 140 μmol/L (N: 35–65) with only glutamine supplementation, an ammonia lowering agent was not added to treatment.
Discussion: This patient underlines the importance of paying attention to marginally low concentrations of glutamine levels in body fluids. L-glutamine alongside nicotinamide treatments were commenced in the patient. Outcome of this patient may provide additional knowledge about the effectiveness of glutamine and nicotinamide treatment.

P-149

WITHDRAWN

P-150

Lysinuric protein intolerance: analysis of blood nitric oxide and redox marker

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Background: Lysinuric protein intolerance (LPI) is caused by dysfunction of y+LAT-1, a light chain of system y^+L for dibasic amino acid transport. Oral supplementation of L-citrulline is beneficial to prevent postprandial hyperammonemia caused by secondary urea cycle disorder in LPI. However, L-citrulline can increase the intracellular synthesis of arginine, further stimulating the immune cascade in multi-organs including kidney and lung through the involvement of nitric oxide (NO) and oxidative stress systems. We evaluated NO levels and oxidative stress markers in LPI patients during L-citrulline treatment.
Methods: This study included 13 patients aged as 30.0 ± 10.8 years, 7 males and 6 females, with LPI diagnosed by biochemical and genetic testings. Measurements were hydroperoxides (dROMs), NOx, and asymmetric dimethylarginine (ADMA) in serum and 8-OHdG (8-hydroxy - 2 '-deoxyguanosine) in urine. In addition, exhaled NO concentrations were evaluated.
Results: Data were shown as average ± S.D., serum dROMs; 363 ± 112 U, reference range (r.r.): 200–300 U, serum NOx; 62 ± 43 μmol / l, r.r.: 30.9 ± 10 μmol / l, serum ADMA; 0.49 ± N.D. μmol / l,r.r.: 0.06-0.59 μmol / l, urinary 8-OhdG; 7.27± N.D., r.r.: 1.38-9.50 ng / mgCre, exhaled NO, 21.4 ± N.D. ppb, r.r.: 11–39 ppb.
Discussion: LPI patients were evaluated in NO system and oxidative stress markers during L-citrulline treatment. The results suggested some disturbances of NO system and showed increases of stress markers in the LPI patients.

P-151

Mitochondrial branched-chain aminotransferase deficiency in three asymptomatic cases identified in newborn screening

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Background: Increase of plasma BCAA may be caused by prolonged fasting, diabetes mellitus or by an inherited defect in either mitochondrial branched-chain α-ketoacid dehydrogenase complex or in mitochondrial aminotransferase (BCAT2) recently described.
Methods: We present clinical, biochemical and molecular data of three children with hypervalinemia and hyperleucine-isoleucinemia detected at birth in the Spanish expanded newborn screening and carrying pathogenic variants in the *BCAT2* gene.
Results: Biochemical signatures were similar in the three cases showing a persistent increase of plasma branched-chain amino acids (BCAA) levels (Leu 150–1060 μmol/L; Ileu 100–850; Val 150–2600) with no increase of alloisoleucine and close-to reference values for branched-chain α-ketoacids levels (BCKAs). Targeted exome sequencing of BCAA-related genes using Mendeliome panel revealed three different novel *BCAT2* genotypes: two were homozygous for changes with predictable effects of loss of function (a small insdel c.1154_1160delinsTGGATGCCCTCT or a large deletion c.1-?_300+?); and the third one was a heterozygous compound of a predictable missense (c.762G>C) and nonsense (c.923G>A) changes. Decreased leucine oxidation rate and severe decreased of BCAT2 protein levels by Western blot analysis in patients' fibroblasts supported an impaired BCAA catabolism. The three infants; 3, 4 or 8 years-old on protein-controlled diet from birth present a correct body-mass index and normal psychomotor development.
Discussion: These results render inconclusive data to understand the involvement of BCAT2 activity in sustaining a protein turnover or metabolite shuttling. A wide analysis of clinical phenotypes might be necessary to understand the implication of BCAT2 deficiency in human disease.
MINECO-FEDER (PI16/00573). Fundación Isabel Gemio. Fundación La Caixa (LCF/PR/PR16/11110018)

P-152

The effect of phenylalanine supplementation on metabolic control in Tyrosinemia Type 1 patients, an ongoing study.

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Background:Tyrosinemia type 1 (HT1) is treated with NTBC and a phenylalanine (Phe) tyrosine (Tyr) restricted diet. Low Phe concentrations are sometimes found, possibly related to growth and developmental problems, especially in early life. Phe supplementation may prevent these low Phe concentrations, but may also cause too high Tyr concentrations. Therefore, we investigated the effect of different amounts of Phe supplementation on Phe and Tyr concentrations.
Methods: The study was divided in one baseline period without Phe supplementation and 2 periods with different dosages (20 and 40 mg/kg/day) of Phe supplementation. In each period, home blood spots were taken 3 times daily for measuring Phe and Tyr concentrations. Statistical analyses were performed by using Friedman tests, Spearman and Pearson correlation tests.
Results: Five patients are included in the study so far. Mean (± SD) Phe concentrations were 38.94 ±3.89, 48.39 ±6.06 and 54.17 ±14.40 μmol/L while receiving 0, 20 and 40 mg/kg/day Phe respectively (p=0.074). Low Phe concentrations (< 30μmol/L) were found in 27.8% of the samples without Phe supplementation, and in 2.8% and 0.0% when respectively 20 and 40 mg/kg/day Phe supplementation was given. A significant positive correlation was found between Phe supplementation and Phe concentrations (ρ=0.575, p=0.025) and Tyr concentrations (ρ=0.640, p=0.010). Mean (± SD) Tyr concentrations were 364.79 ±144.52, 433.82 ±84.78, and 575.35 ±143.41 μmol/L when 0, 20 and 40mg/kg/day Phe was given respectively (p=0.015).Tyr concentrations did not rise above upper target level of 600 μmol/L that much when 20 mg/kg/day of Phe was given, but did in 38.0% when 40 mg/kg/day was given
Discussion:Preliminary results show that Phe concentrations tend to increase with Phe supplementation as do Tyr concentrations especially when 40mg/kg/day Phe supplementation is given. A larger sample size is required to draw more specific conclusions for example on the dosage of Phe.
Conflict of Interest declared.

P-153

Metabolic consequences of a new defect in the malate aspartate shuttle

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Background: Recently we discovered a new defect of the malate aspartate shuttle, the mitochondrial glutamate oxaloacetate transaminase (GOT2) deficiency. The affected patient had increased concentrations of citrulline and lactate and decreased serine in plasma.
Methods: To elucidate the pathogenesis of this new inborn error of metabolism, we investigated the intracellular metabolome of GOT2-knockout HEK293 cells generated by CRISPR/Cas9. Whole cell lysates of knockout and wild type cells were analysed by direct-infusion high resolution mass spectrometry. Findings were validated by targeted metabolomics.
Results: In GOT2-deficient cells, we observed decreased concentrations of the TCA cycle intermediates (especially fumarate and pyruvate) and amino acids (alanine, aspartate). Glutamine, glutamate and ornithine were increased, as was the lactate/pyruvate ratio. Reasoning that supplementation of pyruvate would lead to formation of lactate and potentially to correction of the $NADH/NAD^+$ ratio, we added different concentrations of pyruvate to the culture medium. This resulted in (partial) correction of most abnormalities.
Discussion: This new defect of the malate aspartate shuttle clearly results in a disbalance of $NADH/NAD^+$ ratio in the cytosol, as reflected by the increased lactate/pyruvate ratio. In addition, mitochondrial metabolism is affected, as the Krebs cycle intermediate fumarate was decreased. The direct product of GOT2, aspartate, was low, demonstrating the cells dependence on GOT2 for aspartate provision. Adjustment of the severely decreased pyruvate level, which is a consequence of hampered glycolysis, corrected the pyruvate-related metabolic aberrations.

P-154

Hypervalinemia due to branched chain aminoacids aminotransferase deficiency (BCAT2): newborn screening and clinical challenges

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Background: Only recently an adult patient has been described with hypervalinemia and two different mutations in the branched chain aminoacids aminotransferase(BCAT2) complaining headache and loss of memory. Treatment with pyridoxine made a difference.
Case Report and Results: we here describe a child who is now three years old who was picked up by expanded newborn screening(NBS). The first dried blood spot(DBS) showed mild elevation of branched chain aminoacids and alloisoleucine slightly above normal values on second tier test. Following specimens (DBS and plasma) confirmed a peculiar pattern showing a greater elevation of valine compared to leucine and isoleucine. Normal urine organic acids and DBT, BCKDHA and BCKDHB genes analysis ruled out maple syrup disease (MSUD). An NGS panel found an homozygous mutation on gene BCAT2 who was confirmed with Sanger: NM_001190.3:c.600C>A. This mutation produces a stop codon which generates an altered function protein. A low natural protein intake diet with a special infant formula was started from first weeks. An oral thiamine(B1) trial did not show changes in aminoacid levels but a trial with pyridoxine (B6) did. Now he is on a 1.6-1.8 g/kg/day natural protein regimen and pyridoxine, 200 mg twice a day, orally. Branched chain plasma aminoacids levels are well controlled. Recent cerebral MRI shows normal a T1 pattern while T2 images show incomplete myelination on fronto-parietal white matter. Normal EEG and neurological development.
Discussion: our report is the first pointing the attention to NBS interpretation on branched chain aminoacid results in relation to this new defect.

Newborns with even mild elevations of branched chain aminoacids but with higher levels of valine should not be discarted and mutation of BCAT should be kept in mind. Possibile treatment options are available and other clinical data are needed to better understand the course of this disease.

P-155

A novel urine and plasma amino acids tandem mass spectrometry profiling for diagnosing prolidase deficiency

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Background: Prolidase deficiency is a rare autosomal recessive disorder characterized by severe skin ulcerative lesions.Usually, increased urine Glycine-Proline (Gly-Pro) dipeptide in aminoacids profiles (AAP) is used to screen for iminodipeptiduria. We aim to describe a new tandem mass spectrometry specific method for proline (X-Pro) and hydroxyproline (X-Hyp) iminodipeptides screening and characterization.
Methods / Case Report: We report the case of a five months old girl presenting with hepatomegaly, splenomegaly, dysmorphic facial features and nonspecific eczematous skin lesions. Gly-Pro dipeptide was highly increased in the urinary AAP suggesting Prolidase defciciency. Diagnosis was confirmed by decreased prolidase activity and genetic investigation of the *PEPD* gene.We developed a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for Gly-Pro quantification, associated with a second method allowing the detection and identification of all (X-Proline) and (X- hydroxyproline) iminodipeptides using precursor ion scan mode.
Results: In 3 unrelated prolidase samples, Gly-Pro concentration ranged from 1016 to 1498 μmol/mmol creatinine in urine (0–62 in controls) and from 25 to 41 μmol/L in plasma (0 – 1 in controls) versus. Precursor ion scan mode highlighted the presence of 15 X-Pro and 11 X-Hyp dipeptides in urine and plasma samples of patients. These dipeptides were not detectable in controls.
Discussion: We develop a rapid and easy method for the detection, quantification of Gly-Pro and the confirmation of prolidase deficiency by X-Pro andX-Hyp dipeptides detection. Our results strongly advocate for the systematic incorporation of Gly-Pro quantitation in both urine and plasma LC-MS/MS method for AAP, followed if necessary by screening of X-Pro and X-Hyp peptides in urine and plasma that allows the confirmation of iminodipeptiduria/eamia.

P-156

3-MCC deficiency: Seven cases with different clinical severity ranging from an asymptomatic mother to a bedridden course

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Background: 3-Methylcrotonyl-CoA carboxylase deficiency (3-MCC) is the most common autosomal recessively inherited inborn error of leucine metabolism, characterized by a very heterogeneous clinical course ranging from asymptomatic individuals to rapidly progressive classical disease. After the introduction of expanded newborn screening programs (NBS), the number of patients, especially asymptomatic individuals increased. Developmental delay, hypotonia, seizures, hypoglycemia, metabolic acidosis, cardiomyopathy, and Reye like syndromes are the typical clinical signs. Mutations of *MCCC1* and *MCCC2* genes are the causes of the disease.
Case Report: 7 patients from 5 families were included in this retrospective study. Both the age of diagnosis (1 month-31 years), and the clinical severity of our cases were extremely heterogeneous. There were different clinical presentations even in the same family. Out of 3 asymptomatic individuals two were detected by family screening, and one was diagnosed after the NBS result of her baby indicated carnitine deficiency. One had only mild hepatomegaly and liver dysfunction. One had severe mental retardation and epilepsy. The youngest patient had West syndrome. The remaining patient was diagnosed as 3-MCC with the etiological evaluation of dilated cardiomyopathy.
Results: None of them had the history of acute metabolic decompensation. 6 different mutations were detected; one was compound heterozygous and only one of them was in the *MCCC1* gene.
Discussion: After NBS, 3-MCC has become one of the most common inherited metabolic disease in some countries. This disease is not in the NBS program in Turkey. Like the other cases reported in the literature, except for one mother and two siblings, all of our patients diagnosed as 3-MCC presented with various clinical symptoms such as hepatic, cardiac and neurological disease, which were not directly indicating 3-MCC. Another interesting feature of our cases was the lack of laboratory findings of metabolic decompensation.

P-157

A novel missense homozygous mutation in *SLC1A4* gene in two cousins with hydrocephalus and polyneuropathy

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Background:Neutral amino acid transporter encodes a sodium-dependent transporter for neutral aminoacids. Defects in this transportation cause a disorder called as glutamate/neutral aminoacid transporter disease. Clinical findings are severe developmental delay, microcephaly, seizures, corpus callosum(CC) defects, and spasticity which are treatable by L-serine.
Case Report: Here we report 2 cousins with *SLC1A4* mutations. First patient was a 13 year old girl, born at term after an uneventful pregnancy and delivery from consanguineous parents. 3 children of the family died without a specific diagnosis were suffered from hydrops foetalis and hydrocephalus. She didn't have any dysmorphic features. Spasticity and global developmental delay first noted at the age of 6 months. She needed respiratory support after the age of 12 months. She died at 13 years of age without a specific diagnosis. The second patient is a 2-year-old male, born at term with IUGR from consanguineous parents. His mental-motor deterioration began at the end of first year. Spasticity and deafness were the other prominent features.

Although he didn't need respiratory support, died at the age of 2 years after a PEG operation.
Results: Routine laboratory screening, comprehensive metabolic evaluation, ECHO, and ocular examination were normal in both patients. Cerebellar hypoplasia, hydrocephalus, polyneuropathy, and spasticity were the common features of 2 cousins. Due to the heavy family history and confusing clinical picture, whole exome sequencing was done and a homozygous novel missense c.98G>A mutation in the *SLC1A4* gene was detected.
Discussion:We present these cases in order to report a novel mutation in *SLC1A4* gene and emphasize the different clinical course even in the same family. The common symptoms of cerebellar hypoplasia, hydrocephalus, polyneuropathy, and deafness detected in our patients have not been frequently reported. Absence of CC defects is another striking feature of our cases.

P-158

Clinical presentation of lysinuric protein intolerance (LPI): cases in Spain.

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Background: LPI (OMIM # 222700) is a rare recessive-inherited disease: mutations of SLC747 gene (encoding the cathionic amino acids transport subunit y+ LAT1) affect intestinal absorption and renal reabsorption of arginine, lysine and ornithine, with a leakage in urine and low to normal plasma levels. The clinical spectrum is wide, making diagnosis difficult.
Methods: This is a retrospective, multicentric and observational study of LPI patients identified in Spain. Information was recovered by sending a questionnaire to all metabolic clinical units nationwide.
Results: We identified 17 patients from 11 hospitals (2 patients were lost in the follow-up). We present 15 cases (male: 8). They came from 6 different countries: Pakistan (n=1), Morocco (n=3), Argentina (n=1), Lithuania (n=1), and Spain (n= 9). Consanguinity was known in 4 cases. 7 patients belonged to the same 3 families. 3 cases were diagnosed after neonatal study for having affected siblings. The median age at diagnosis was 5 years old (mean 7.2 years old). Frequent initial symptoms were: failure to thrive (8/12); aversion to proteins (5/12); hepatosplenomegaly (9/12); nausea and vomiting (3/12); pulmonary involvement (3/12); renal disease (9/12); neurologic involvement (10/12): acute hyperammonemia encephalopathy (7/12), developmental delay (4/12). Laboratory findings: increased urinary dibasic amino acids (9/12) with 6 patients having low serum levels; hyperferritinemia (9/12); hypertriglyceridemia (8/12); hyperammonemia (7/12), hypertransaminasemia (5/12), increased LDH (7/12) and anemia (4/12). Mutation analysis showed different variants affecting gen SLC7A7 in every family with patients affected.
Discussion: Although the clinical manifestations of LPI patients appeared in the first years of life, most them suffered a delay in the diagnosis of even some years after the onset of symptoms, highlighting the difficulty in the diagnosis of LPI.

09. Urea cycle disorders

P-159

In depth analysis of *spf^{ash}* mouse model for human X-linked ornithine transcarbamylase deficiency

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Background: The most common ureagenesis defect is X-linked ornithine transcarbamylase (OTC) deficiency, that is also a main target for novel therapeutic interventions. To study these therapies, the *spf^{ash}* mouse that carries the same mutation (c.386G>A, p.R129H) as human patients, is widely used. Male *spf^{ash}* mice have a mild biochemical phenotype with low OTC activity (5-10% of wild-type), resulting in high urinary orotic acid (OA) but no hyperammonemia. We describe here an in depth characterization of this model and new readouts to more precisely assess novel treatment options.
Methods: We measured amino acid in dried blood spots (DBS), urinary OA, blood ammonia levels, liver OTC activity and also measured *in vivo* ureagenesis from DBS using $^{15}NH_4Cl$ as tracer in mice at different ages.
Results: Stable isotopes in *spf^{ash}* mice revealed unexpectedly high inter- and intraindividual variations in ureagenesis; this was mainly age-dependent with young animals showing a significant higher ureagenesis capacity: mice younger than six week of age % of [^{15}N]urea enrichment of 12.43±5.08 % and older than 6 weeks-old 6.52±2.76%. Ureagenesis was stable once mice reached adulthood, showing a clear difference between wild-type and *spf^{ash}* mice. Moreover citrulline and OA levels in the mutant displayed large variation not always reflecting OTC activity. We observed a stress and day time dependence in ammonia values, but even minimizing these, levels fluctuated independent of genotype (29 to >180μM).
Discussion: Despite being used for many years for studies of new therapies, the *spf^{ash}* mouse shows a highly variable biochemical phenotype. As in humans, amino acids, ammonia levels and urinary OA are unreliable outcome parameters. These variations could be a result of the mutation leading to a splicing defect, which can be strongly influenced inter-individually. Our study provides insights in the *spf^{ash}* mouse and helps to determine more precise standards to evaluate potential new therapies.

P-160

Generation of an ornithine transcarbamylase (OTC) deficient pig as a large animal model of metabolic liver diseases

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Background: Mice have become the standard animal model in health research. However, limitations of murine models for clinical translation are the differences in e.g. size, anatomy, immune response etc. compared to human, and therefore, there is a substantial interest to create larger

animal models. The aim of this study was to generate a (domestic) pig with X-linked OTC deficiency to study a classical metabolic defect and novel treatment options.
Methods: By using CRISPR/Cas9, we have targeted the *OTC* locus in porcine kidney cells for genome engineering and generated by homologous recombination with a BAC-fragment harboring a neomycin marker gene the desired *OTC*-c.386G>A/p.R129H knock-in, corresponding to the mouse spf-ash mutation and to a common human mutation resulting in OTC deficiency. In order to conserve the human situation and for potential future treatment, we humanized at the same time the OTC-R129H locus by replacing a 1.3 kb intronic fragment downstream of exon 4 by a human fragment.
Results: Following somatic cell nuclear transfer and embryo transfer, 19 male fetuses from 3 pregnancies were stillborn at term. We observed massive hyperammonemia, high urinary levels of orotic acid and dysmorphic appearance in the dead male offspring, and also in genetically heterozygous females. Liver analysis confirmed the presence of the genomic exon 4 variant c.386G>A. Transcript analysis revealed the expected exon 4 skipping or retention of the exon 4 variant c.386G>A as a result of use of the cryptic splice site at c.386+4. However, exons 6–7 were completely missing on the *OTC*-mRNA and OTC enzyme activity was below detectable level.
Discussion: We hypothesize that lethality and loss of function of the targeted gene in this large animal model were due to two independent phenomena, i.e. non-random X-inactivation and expression interference, the latter most probably induced by the active neomycin gene in the humanized OTC-R129H expression cassette.

P-161

A phase 1/2 clinical trial for AAV8-mediated liver-directed gene therapy in adults with late onset OTC deficiency

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Background: Current therapy for ornithine transcarbamylase (OTC) deficiency is based upon restriction of dietary protein intake and pharmacologic nitrogen scavenging agents. Although this approach lowers the incidence of hyperammonemic episodes, a risk of life-threatening hyperammonemic crises remains and neurocognitive outcomes are often suboptimal. Gene therapy aimed at restoring liver OTC activity represents a potential novel therapeutic strategy.
Methods / case report: DTX301 is a self-complementary recombinant AAV8 vector expressing a codon-optimized human OTC cDNA under the transcriptional control of a liver specific promoter. CAPtivate is a global multi-center open label Phase1/2 dose escalation trial evaluating the safety and preliminary efficacy of a single DTX301 IV infusion in adults with symptomatic late onset OTC deficiency; first patient was enrolled in August 2017. After this initial 52-week study period, subjects will be followed for a total of 5 years after dosing. Efficacy objectives include change in total ureagenesis capacity measured using an *in vivo* stable isotope dilution assay and 24-hr NH3 AUC. Evaluation of candidate DTX301 doses in cohorts of 3 subjects using a Bayesian adaptive design approach is employed for dose finding.
Results: Dosing of the first 3 patients at 2e12 GC/kg level was completed in November 2017. No infusion-related or serious adverse events have been observed to date. One patient achieved normalized ureagenesis rate sustained past 24-weeks post-dose and has discontinued ammonia scavenger medications. The DMC review in March 2018 resulted in recommendation to continue with the 2nd dosing cohort at 6e12 GC/kg. Screening and enrollment of subsequent dose cohorts is on-going.
Discussion: Early preliminary data from CAPtivate, an on-going Ph1/2 AAV gene transfer clinical trial, indicate that DTX301 has an initial benefit/risk profile that is favorable. DTX301 is a promising new therapeutic approach for OTC deficiency.
Conflict of Interest declared.

P-162

Optimizing the Hemodialysis Prescription for Hyperammonemic Patients with Ornithine Transcarbamylase Deficiency.

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Background: An early liver transplant is the key to the survival of infants born with ornithine transcarbamylase deficiency (OTCD), a rare urea cycle disorder. If delayed, their hyperammonemia may cause permanent neurological damage or death. Hemodialysis (HD) is a lifesaving treatment that has been shown to reduce ammonia levels until a transplant is available. Given the rarity of this condition, the optimal dialysis settings that efficiently decrease ammonia levels and the effects of the duration and severity of hyperammonemia have not been identified.
Methods: This is a retrospective study of 7 male children with OTCD who received HD from 2010–2014. Ammonia levels and dialysis session sheets were collected from each dialytic session along with magnetic resonance imaging (MRI) scans. We determined blood flow rate (Qb) in mL/kg/min, dialysate flow rate (Qd) as a factor of Qb, dialyzer size (m^2) as a percentage of body surface area, central venous access size and location, and session duration. Ammonia reduction half-life was calculated for each session and a regression tree by recursive partitioning was implemented.
Results: Data from 172 dialysis sessions involving all 7 patients were collected. When blood flow rate (Qb) ranged from 7.0-9.0 mL/kg/min or when the dialyzer size was at least 1.2 times the body surface area (BSA), a half-life ranging from 0 to 60min was observed. When the Qd to Qb ratio was less than 3.7 and when Qb was greater than or equal to 8.4mL/kg/min, the shortest average half-life was under an hour. The MRI data show minimal cytotoxic cerebral edema and atrophy when HD maintained ammonia levels less than 400umol/L.
Discussion: Using our HD data, the main determinants of ammonia half-life were the Qb and the dialyzer size to BSA ratio, both of which are modifiable and controllable factors during HD. Both the duration and severity of hyperammonemia may contribute to neurological damage if it is not reduced and maintained below 400umol/L.

P-163

Levels of GAA and Creatine in patients with UCDs

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Background: Creatine metabolism is of importance to patients with UCD because it is thought that decreased rates of creatine synthesis may result in secondary creatine deficiency.
Methods / case report: We analyzed levels of GAA and Creatine in 28 patients with UCD (11female, 17 males). We included OTCD (n=10), ASSD (n=6), ASLD (n=8) and ARGD (n=4).Screening for creatine metabolites (creatine, GAA, and arginine) with LC/MS/MS was performed in plasma samples.
Results: In our cohort patients with OTCD showed significantly reduced levels of both GAA (1.73±0.85 umol/L, NR: 2.42±1.10)) and Creatine (42.24±22.67 umol/l, NR: 72.2±33.4). Untreated patients with ASSD (GAA=7.65±2.37 and Creat= 394.4±l30.5) and ASLD (GAA=5.27±9.88 and Creat= 186.6±176.65) showed high levels of GAA and Creatine, whereas in ARGD, GAA and Creatine levels were significantly increased (GAA=7.4±1.8 and Creat= 170±73). Treated patients with ASSD showed low levels of both GAA (2.3±0.62 umol/l) and Creatine (34.36±19.72 umol/l). In patients with ARGD, markedly elevated arginine levels may result in higher concentrations of GAA and creatine. In the case of OTCD and treated ASSD patients, arginine levels are markedly decreased. Three of our patients with ASSD on low protein diets showed significantly reduced levels of GAA (2.3±0.62) and Creatine (34.3±19.7) on subsequent follow ups. In the case of OTCD and treated ASSD patients, arginine levels are markedly decreased unless supplemented. Decreased levels of arginine and low protein diet may result in decreased creatine synthesis. In patients with ARGD markedly elevated arginine levels may result in higher concentrations of GAA and higher rates of creatine synthesis
Discussion: Our studies show that Creatine was low in OTCD and treated ASSD and high in ARGD, and GAA was high in ARGD and untreated ASSD. Decrease in brain creatine levels may contribute to the neurological symptoms exhibited by these patients and/ or an increase in GAA may be neuro- toxic

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Improvements in arginase 1 deficiency-related disease manifestations following plasma arginine reduction with pegzilarginase

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Background: Arginase 1 Deficiency (ARG1-D) is a rare genetic disease that typically presents in early childhood with spasticity, impaired mobility, and developmental delay. The enzyme-deficiency-related hyperargininemia is thought to play a key role in disease pathogenesis and resultant complications.
Methods: This is an ongoing Phase 1/2 study of intravenously (IV) administered pegzilarginase in patients with ARG1-D. In addition to evaluations of safety, pharmacokinetics and effects on plasma arginine and guanidino compounds (GCs: α-keto-δ-guanidinovaleric acid, argininic acid, and N-acetyl-arginine), clinical outcomes assessments were incorporated at baseline and follow-up, including 6-minute walk test (6MWT), Berg Balance Scale (BBS), and Gross Motor Function Measure (GMFM-66). Safety assessments include physical examinations, laboratory tests, anti-drug antibody evaluations and ECGs.
Results: Enrolled patients had disease manifestations including hyperargininemia, neuromotor and neurocognitive deficits. IV pegzilarginase, added to current disease management, rapidly and sustainably lowered plasma arginine and GCs in all patients and was well tolerated except for a single infusion associated reaction in one patient. Clinical improvements were observed in neuromotor test(s) in the first 2 patients who completed repeat dosing, and further improvement was observed during treatment in the open label extension. Dietary protein intake (relative to prescribed) also improved.
Discussion: Pegzilarginase treatment of patients with ARG1-D resulted in marked and sustained reduction of plasma arginine and GCs and was accompanied by clinically relevant treatment effects, including tests of neuromotor function. These data provide the first evidence of clinical benefit from reduction in plasma arginine and GCs beyond what can be achieved with current disease management. Further insights are expected from additional patients and with longer term dosing in the extension study.
Conflict of Interest declared.

P-165

Clinical perspectives on the diagnosis and treatment of patients with n-acetylglutamate synthase deficiency

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Background: Early diagnosis and treatment of N-acetylglutamate synthase deficiency (NAGSd) is crucial to prevent high levels of neurotoxicity that cause irreversible brain damage or death. Targeted treatment with N-Carbamylglutamate (NCG) can prevent complications under good adherence conditions but no previous studies have documented current diagnostic and treatment procedures.
Methods: Interviews were conducted with treating NAGSd physicians from the United Kingdom, France, Belgium, Spain, Germany and Italy. Qualitative interviewers explored physician experiences and perspectives on NAGSd diagnostic procedures, treatment aspects, and disease and treatment impacts on health-related quality of life (HRQoL). Interviews were audio-recorded, transcribed and analyzed using grounded theory.
Results: Data were available from 7 physicians who treat a total of 18 NAGSd patients. Most physicians stated that protocols were well-established in their hospitals and that NCG is administered prior to diagnosis. Patients (n=14) diagnosed and treated with NCG as neonates showed no cognitive or behavioral deficits, and physicians reported no major disease impacts on patient lives. In contrast, late diagnosis and delayed treatment initiation with NCG was related to complications and HRQoL impacts. Delayed diagnoses (n=4) were associated with slow test results, undocumented family history of disease, and lack of awareness of rare diseases. Late diagnosis and cost of treatment impacted treatment choice and initiation. Contrary to recent guidelines (Haberle J, 2012), 3 patients were on a protein restrictive diet. Treatment adherence to NCG was not a concern.
Discussion: Physicians from 7 countries unanimously confirmed the importance of early diagnosis and treatment to avoid complications in NAGSd patients and impacts on patients' HRQoL. Although adherence to treatment with NCG was not a concern, frequent patient follow-up visits are needed to ensure treatment adherence over time.
Conflict of Interest declared.

P-166

The impact of liver transplantation on plasma and CSF amino acids in patients with argininosuccinic aciduria

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Background: The positive effect of liver transplantation (LT) has been reported in few cases in ASLD patients, allowing termination of protein restriction, of arginine supplementation and ammonia scavengers therapy. However, the biochemical impact of LT has been so far only poorly investigated.
Methods / case report: As part of our LT assessment protocol in ASLD, amino acids were determined in plasma (71 samples) and CSF (9 samples) in 4 patients transplanted and 1 listed for LT. Values are expressed as μmol/L. To compare data before and after LT we used unpaired t-test.
Results: After 51 months (range 2–77) follow-up from LT, patient and graft survival were 100%. All children reached normal protein intake, withdrawing arginine and ammonia scavengers. Patients did not experience episodes of hyperammonemia, with a mean plasma ammonia at follow-up of 22 μmol/L (range 7–32, normal < 40). Quality of life significantly improved and intellectual disability parameters remained unchanged. Mean ASA levels in plasma were significantly decreased by LT (445+/−45 vs 112+/−7; p:< 0.0001) and correlated directly with ammonia (r: 0.66, p< 00.1). Remarkably, in contrast to what found in plasma, CSF concentration of ASA before and after LT did not differ (476+/−42 vs 407+/−84). Glutamine (848+/−30 vs 711+/−13) and citrulline (205+/−9 vs 163+/−6) concentrations in plasma also significantly decreased after LT (p:< 0.0001), with persistence of mildly elevated levels in CSF (glutamine 680+/−24 vs 701+/−49, nv< 590; citrulline 22+/−2 vs 16+/−3; nv< 6). CSF arginine fells below normal (13+/−2, nv 15–35).
Discussion: This study demonstrates that LT has no impact on ASA levels in CSF, implying that the metabolic alteration still exist in the CNS compartment beyond hepatic transplant. Indeed, it is not known the impact of persistent metabolic abnormalities on the long-term neurologic outcome.

P-167

Upregulation of hepatic glutamine synthetase enhances ammonia detoxification.

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Background: The urea cycle and the glutamine synthetase (GS) are the two main pathways for waste nitrogen removal and their deficiency results in hyperammonemia. Recent findings suggested that enhancement of GS expression in muscle has therapeutic potential for treatment of hyperammonemia. Whether increased hepatic GS activity is effective at reducing hyperammonemia has not been investigated so far.
Methods: We evaluated the efficacy of hepatic GS overexpression during hyperammonemia in C57BL/6 wild-type (WT) mice and in a mouse model with conditional hepatic deficiency of Carbamoyl Phosphate Synthetase 1 (Cps1), the initial and rate-limiting step of ureagenesis. Deletion of the *Cps1* locus was achieved in adult transgenic animals using a Cre recombinase-expressing adeno-associated viral vector. To increase GS expression, mice were injected with a helper-dependent adenoviral (HDAd) vector expressing the murine GS under the control of a liver-specific expression cassette (HDAd-GS). Control mice received a HDAd vector encoding an unrelated reporter gene (HDAd-AFP). Ammonia clearance was evaluated after intraperitoneal (i.p.) injections of ammonium chloride that induced hyperammonemia. GS protein levels were evaluated by Western blot analysis in liver homogenates.
Results: Compared to controls, WT mice with increased hepatic GS showed reduced blood ammonia levels at 0.5 h after i.p. injections of ammonium chloride and a concomitant increase of blood glutamine whereas blood urea was unaffected. In *Cps1*-deficient mice, the administration of HDAd-GS reduced blood ammonia levels at baseline and protected against acute hyperammonemia following the ammonia challenge.
Discussion: Liver-specific GS overexpression improved ammonia detoxification in WT and in *Cps1*-deficient mice thus confirming a key role of GS in ammonia homeostasis. Hepatic GS augmentation therapy has potential for treatment of both primary and secondary forms of hyperammonemia.

P-168

Urinary Derivatives of Phenylbutyric and Benzoic Acids by LC-MS/MS as Treatment Compliance Biomarkers in Urea Cycle Disorders

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Background: Salts of benzoic acid (BA), phenylacetic acid (PAA) and phenylbutyric acid (PBA) have been used for nitrogen elimination as a treatment for hyperammonemia caused by hereditary urea cycle disorders (UCD). It is necessary to develop a new analytical method for PBA measurement in urine that evaluates the drug dosage, checks the adherence and correlates with clinical parameters.
Methods: Urine specimens from UCD patients receiving PBA or PBA and BA were analysed by liquid chromatography coupled to tandem mass spectrometry to measure phenylacetylglutamine (PAGln) and hippuric acid. Collected data for each patient were age, gender, height, weight, UCD subtype, PBA dosage, protein intake, ammonium and glutamine (Gln) levels. The stability for these metabolites was also determined at different storage temperatures for one month.
Results: Our study included 25 patients suffering from UCD: 17 ornithine transcarbamylase deficiency, 5 Citrullinemia (argininosuccinate synthetase), 2 carbamyl phosphate synthetase deficiency and 1 argininosuccinate lyase deficiency. The PAGln levels did not correlate with height, weight or age. However, the PAGln values shown correlation with PBA dose (r=0.554, P=0.009). Gln and ammonia were related by positive correlation (r=0.644, P=0.002).
Discussion: We have developed a simple method for the determination of PBA, BA and their metabolites in urine, showing them as useful biomarkers of effective drug delivery. PAGln in urine is stable at room temperature at least for 15 days and frozen at −20°C for several months. This procedure is useful for the optimization and monitorization of the drug dose allowing the use of spot urines.

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Lessons learned from combined and comparative data analysis of over 1,000 patients with urea cycle disorders

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Background: Patients with urea cycle disorders (UCDs) are followed by two international registries managed by the North American Urea Cycle Disorders Consortium (UCDC) and the European registry and network for intoxication type metabolic disease (E-IMD) aiming to study the natural history of these rare metabolic disorders.
Methods: Data from 1095 individuals, including 115 patients with liver transplantation were retrieved from the respective data bases. Both registries use remote data entry via electronic forms. Patients are prospectively followed by protocols based on regular follow-up and emergency visits. The UCDC data model comprises about 570 and the E-IMD model about 490 variables with an overlap of approximately 250 variables describing the medical history.
Results: Specific metabolic defects, sex, age at last visit, observation period, survival, mortality, mode of diagnosis (prenatal testing, newborn screening, high-risk family screening, metabolic investigation after onset of symptoms), age at diagnosis, and diagnostic delay were investigated systematically and for the first time compared between both continents.
Discussion: It is demonstrated that combining data from different databases is manageable and can enhance future research of rare diseases. Combined analysis of different databases allows increased sample sizes for natural history research, while comparative analysis can inform on therapeutic options and enhance long-term outcome studies. As the predominant rationale of collaborative databases for rare diseases is to increase overall sample size, combined and therefore larger data sets allow analyses of well-defined subsamples with circumscribed characteristics, e.g. early and presymptomatically treated cases. This approach can also contribute to the development of minimal core data sets ("common data elements") for further registry studies.

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Human liver stem cells in inherited neonatal-onset hyperammonemic encephalopathy

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Background: Human liver stem cells (HLSCs) are a novel progenitor population isolated from adult liver, which show self-renewing capacity, hepatocyte differentiation, and liver restoring in animal models of hepatic injury. Severe urea cycle defects and organic acidurias are life-threatening disorders causing neonatal hyperammonemia, which can benefit from liver transplantation.
Methods / Case report: In a Phase I study, the safety of HLSCs in 3 newborns with neonatal-onset hyperammonemic encephalopathy was evaluated. Formal indication for early liver transplantation was required for patients' inclusion in the study. HLSCs were administered by 2 subsequent ultrasound-guided intrahepatic injections under local anaesthesia within the first months of life, after correction of acute neonatal metabolic decompensation and stabilization of the patients. At each administration, $22x10^6$ HLSCs (first patient) or $44x10^6$ HLSCs (subsequent patients) were provided. The study was approved by Ethical Committee and Agenzia Italiana del Farmaco on favourable opinion of Istituto Superiore di Sanità.
Results: One patient with argininosuccinic aciduria and 2 with methylmalonic acidemia underwent HLSCs injections. In all patients, no short- or long-term complications were registered and all the protocol safety items were accomplished. After the HLSCs administrations, all patients showed transitory improvements of specific disease-related biochemical markers.
Discussion: Intrahepatic injection of HLSCs in newborns with neonatal-onset inherited hyperammonemia due to urea cycle disorders or organic acidemias is safe and well tolerated. The potential clinical effectiveness of this approach will be investigated in subsequent studies.

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Human arginase 1 (*ARG1*): mutational update, patients and protein structure considerations

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Background: Argininemia (ARGD) is a urea cycle disorder (UCD) caused by defects in the enzyme arginase 1 (ARG1). Clinically, ARGD is associated with hyperargininemia, spastic paraparesis, progressive neurological and intellectual impairment and persistent growth retardation. Remarkably, in contrast to other UCDs, hyperammonemia is rare.
Methods: Clinical and biochemical information of 112 patients, including onset, genetic background and ethnic origin was collected. We compiled as well all known missense variants with unknown relevance. For the most common mutations a structural rationalization of their effect was performed.
Results: This study reports a total of 66 mutations (of which 12 are novel), including 30 missense mutations, 7 nonsense, 10 splicing, 15 deletions, two duplications, one small insertion and one translation initiation codon mutation. Only six mutations (p.Ile8Lys; p.Gly106Arg; c.466-2A>G; c.77delA; c.262_265delAAGA; and c.647_648ins32) could be associated with a neonatal onset. The most common mutations (p.Thr134Ile; p.Gly235Arg; and p.Arg21*) clustered in Brazil, China or Turkey. Furthermore, early diagnosis, either by newborn screening (NBS) or prenatal testing enabling treatment from birth, had a positive impact on the development of ARGD patients.
Discussion: Unlike other UCDs, ammonia was not generally elevated in ARGD patients, highlighting that even in absence of ARG1, the urea cycle is not fully interrupted. Thus, the observed symptoms should result from the elevated arginine. Although arginine levels did not clearly correlate with the disease severity, the combination of arginine levels with other amino acid ratios (arginine/ornithine and arginine/leucine x phenylalanine) proved reliable and thus, could be used in NBS.

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Cognitive outcomes post-liver transplant in urea cycle and organic acid disorders: a case series.

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Background: Urea cycle (UCD) and organic acid disorders classically present in the neonatal period with encephalopathy progressing to coma and death. In those who survive, global developmental delay is common with high risk of regression due to recurrent crises. Liver transplant improves the biochemical abnormality and the literature shows excellent patient survival. It remains unclear if stabilization of the delays is achievable in classical forms. We report the neurocognitive and functional outcomes post-transplant of 9 UCD, 3 maple syrup urine disease (MSUD) and one propionic aciduria (PA) patient.
Methods: 13 metabolic patients were matched to 26 non-metabolic patients. All 39 patients received liver transplant at one center. Wilcoxon rank sum test was used to compare full scale IQ (FSIQ) and Adaptive Behavior Assessment System II– General Adaptive Composite (ABASII-GAC) at age 4.5 years between the two groups. Dichotomous outcomes were reported as percentages.
Results: All metabolic patients survived post-transplant. FSIQ median [IQR] was 75 [54, 82.5] compared to 94.5 [79.75, 103.5] for metabolic and control cases, respectively (p-value < 0.001). GAC median [IQR] was 62.0 [47.5, 83] compared to 88.0 [74.25, 97.50] for metabolic and control cases, respectively (p-value < 0.003). 54% of the metabolic cases were in the delayed (FSIQ < 70) category compared to 4% of control cases. In the metabolic cases, 23% had autism and 8% had cerebral palsy compared to zero in controls.
Discussion: To the best of our knowledge, this is the first study evaluating FSIQ and GAC at age 4.5 years through a case–control comparison between metabolic and non-metabolic patients post liver transplantation. While transplant improves the survival in classical forms of UCD, MSUD and PA, the neurocognitive and functional outcomes remain poor with over half of the patients in the delayed category. This information should be included when counseling parents regarding post-transplant outcome.

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Biomarkers of neuronal dysfunction in Urea cycle disorders and Maple Syrup Urine disease.

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Background: Patients with inborn errors of metabolism (IEM) may show a spectrum of neurocognitive dysfunctions and behavioral problems despite accurate metabolic control. In order to identify novel mechanisms and possible peripheral biomarkers involved in the cognitive and behavioral difficulties observed, we analyzed in blood the mRNA expression of a group of key genes for the correct brain functioning and the protein levels of Brain-derived neurotrophic factor (BDNF) in plasma. We focused this study in two different IEMs: Urea Cycle Disorders (UCD) and Maple Syrup Urine Disease (MSUD).
Methods: We analyzed gene expression by Real-Time PCR and BDNF protein expression by ELISA in 19 patients with UCDs, 9 patients with MSUD and 44 age-matched healthy subjects, and the results were correlated with clinical, biochemical and outcome data.
Results: *LIN28A* expression increased in UCD patients compared to controls while *CACNA2D2, MECP2E1 and E2* were downregulated and *THBS1* was upregulated in MSUD patients in respect to controls. When correlating the results obtained from both IEMs as a whole, we observed a correlation between low levels of BDNF with impaired development and elevated *LIN28A* levels with executive function and attention deficits.
Discussion: To our knowledge, this is the first study that investigates genes involved in brain development and synaptic maturation as well as BDNF as possible neuronal dysfunction biomarkers in two different IEM patients. Our results suggest that *LIN28A* may represent a candidate gene contributing to the pathophysiology of neuronal damage in UCD while *CACNA2D2, MECP2* and *THBS1* in MSUD. At the same time, we postulate BDNF, *LIN28A* and their loop of regulation as a possible altered mechanism that could be responsible, in part, for the neuropsychiatric deficits observed in these IEMs. Further studies in larger series in order to support these findings are necessary.

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A frequent mutation in *ASS1* in French Guiana patients

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Background: Citrullinemia type I is a rare autosomal recessive inborn error of distal urea cycle caused by mutation in the *ASS1* gene and biochemically characterised by accumulation of citrulline and ammonia. Clinical phenotype ranges from acute neonatal coma to a late onset presentation.
Case report: We describe here 3 unrelated cases of acute neonatal citrullinemia type I diagnosed at Saint Laurent du Maroni, in a French overseas department.Case 1: After an uneventful pregnancy, this girl presented at day one with poor suckling, vomiting, hypotonia, poor contact and respiratory distress. An hyperammoniemia at 748 μM was found and despite acute management, ammonia rose above 2000 μM. The patient died at 4 days of life of multiple organ failure.Case 2: After a normal pregnancy, this boy presented at 16 hours of life with neurological distress, respiratory acidosis and hyperlactacidemia. He developed hepatic cytolysis, hyperammoniemia (900 μM), renal insufficiency, respiratory distress, coma and hyperammoniemia rose at 2000 μM. He died at 3 days of life.Case 3: This boy was referred at the local hospital at 7 days of life for hypotonia, and poor feeding. Biological investigations revealed hyperammoniemia (377 μM). He was therefore treated according to the recommendations.
Results: Biochemical investigations in the 3 patients revealed highly increased citrulline and orotic acid leading to the diagnosis of citrullinemia type I.The mutation c.892G>A (p.Glu290Lys) was found at the homozygous state in case 1 and 3 and at the heterozygous state in case 2 in *ASS1* gene. The second mutation in case 2 was not identified suggesting a deep intronic mutation.

Discussion: In one year, we identified 3 cases of citrullinemia type I carrying the same mutation in French Guiana suggesting a founder effect. These results raise the question of targeted neonatal screening for this pathology among the French Guyanese population, which seems to be at risk.

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Induction of Aquaporin 9 in patient-derived iPSC-hepatocytes enables modeling and drug screening for urea cycle disorders

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Background: Reprogramming of patient-derived skin fibroblasts into induced pluripotent stem cells (iPSCs) followed by directed differentiation into hepatocytes (iPSC-Heps) allows modeling of genetic liver diseases. We aim to develop an iPSC-Hep-based model of the urea cycle disorder ornithine transcarbamoylase (OTC) deficiency (OTCD) that can be used to screen for pharmacological chaperones (PCs) to treat OTCD.
Methods: We generated iPSCs from fibroblasts of OTCD patients and controls and differentiated them into iPSC-Heps. We compared urea cycle enzyme expression and ammonia metabolism between patient-derived and normal iPSC-Heps and primary human hepatocytes (PHH). We included human fetal and adult liver tissue as additional controls.
Results: Differentiation of OTCD patient-derived iPSCs into iPSC-Heps revealed a disease-specific phenotype with reduced OTC activity and urea secretion. However, urea secretion was also low in control iPSC-Heps, which expressed all urea cycle enzymes at levels comparable to PHH, even after ammonia challenge. iPSC-Heps are known to correspond to immature fetal hepatocytes rather than mature adult hepatocytes. Therefore, we compared gene expression profiles of human fetal and adult liver tissue to identify differentially expressed genes that are responsible for the low urea secretion in iPSC-Heps. We found that fetal liver tissue was lacking expression of aquaporin 9 (AQP9), which is required for urea secretion. Consistent with their fetal state of differentiation, AQP9 was absent in all of our iPSC-Hep lines and its induction led to a significant increase in urea secretion.
Discussion: These findings show that patient-derived iPSC-Heps replicate characteristic features of OTCD. A limitation of this model is low urea secretion by iPSC-Heps because of their fetal state of differentiation. We identify AQP9 as a target for overcoming this deficiency and realize the potential to screen for PCs in our patient-derived iPSC-Heps to treat OTCD.

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Longitudinal electrophysiological assessment of motor and sensory tract in a cohort of patients with HHH syndrome

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Background: Hyperornithinemia–hyperammonemia–homocitrullinuria (HHH) syndrome is a rare disorder of the urea cycle (UCD). Regardless of the good metabolic control, the clinical course of HHH is characterized by a progressive pyramidal and cerebellar dysfunction, whose pathophysiology still remains to be elucidated.
Aim of the study: To investigate long motor and sensitive fiber affection in a cohort of HHH pts searching possible correlation between clinical, electrophysiological and neuroradiological data.
Methods: We report on the long-term follow-up (18.0±13.0 yrs) in 9 pts aged 7–52.6 yrs with HHH syndrome. Patients assessment included periodical clinical evaluations, motor evoked potentials (MEP) and somatosensory evoked potentials (SSEP) at lower limbs (LL) focused on central motor and sensory conduction times, nerve conduction velocity (NCV) at LL, and brain (7 pts) and spinal cord (5 pts) MRI imaging.
Results: Brisk tendon reflexes were detectable in 9/9 pts, spastic paraparesis in 5/9 pts and spastic ataxia in 6/9; one pt had sensorial deficit and one peripheral neuropathy. MEP were abnormal (20.7±3; nv 15.6 ±1.3 m/s), while SSEP (19±4; nv +3DS=20.6) were borderline, and frankly abnormal only in the eldest pt. NCV was normal in all but one pt. MRI showed variable brain atrophy [mild (4/6) and moderate (2/6) supratentorial; mild (5/7) cerebellar] and spinal cord atrophy (1/5 mild, 1/5 moderate, 1/5 severe).
Discussion: Our study highlights the predominant affection of corticospinal tract in HHH syndrome. The abnormal SSEP in the eldest pt suggests a possible delayed involvement of sensitive long fibers tract. Rarely, peripheral neuropathy can be an associated finding. Clinical electrophysiological and neuroradiological data show a more severe involvement of long fibers tracts compared to cerebellum. The clinical similarities of neurological involvement in HHH and argininemia suggests the possibility of common mechanisms affecting the distal part of urea cycle.

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The Phenotypic Spectrum of *ASS1* A118T Mutants: Case Report and Review of the Literature

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Background: Citrullinemia type 1 is caused by mutations in the *ASS1*, resulting in decreased activity of the urea cycle enzyme, argininocsuccinate synthetase. The *ASS1* A118T mutation has been shown to result in high residual ASS activity, but a markedly decreased affinity for its substrates. We report the outcome of our patient who is a compound heterozygote for A118T and a null mutation, and also review the published cases of A118T mutants.
Case report/results: We report the case of a 4 day-old female ascertained by DC newborn screening with an elevated citrulline of 12.59 mg/dL (N < 1.34). At initial evaluation, plasma ammonia was 70 μM, glutamine 1066 μM, citrulline 1701 μM, and arginine 24 μM. These results were thought to be consistent with those observed in symptomatic patients with citrullinemia type 1. However, after initiation of metabolic formula and arginine, plasma ammonia and glutamine surprisingly normalized without administration of ammonia scavengers. DNA sequencing of *ASS1* revealed two known pathogenic changes: c.1168G>A (p.G390R) and c.352G>A (p.A118T). G390R results in null enzyme activity, whereas A118T increases the Km of ASS. Our patient, now age 29 months, has been clinically stable on an essentially unrestricted diet with only oral

arginine supplementation. She has maintained normal ammonia and glutamine levels, even during intercurrent childhood illnesses. Neurodevelopmental evaluations at 6 and 19.5 months revealed normal psychomotor development.
Discussion: In our patient, limited medical or dietary intervention has been required. We suggest that A118T heterozygotes may have sufficient residual enzyme activity to result in near normal urea cycle flux during baseline health or even during mild intercurrent illness. However, this capacity may be overwhelmed during severe illness or catabolism, and require urgent medical intervention, as described in published case reports.

10. Organic acidurias: branched-chain

P-178 Movement disorder associated with 3-Hydroxyisobutyryl-Coa hydrolase (*HIBCH*) deficiency

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Background: HIBCH deficiency is a rare inborn error of valine metabolism identified in 1982. However, most cases so far reported were diagnosed in the last few years by (next generation sequencing (NGS). We report a new case with a prolonged follow-up, diagnosed on the basis of a chemical biomarker.
Methods / case report: This 34-year-old man showed motor skill developmental delay and recurrent attacks of chorea with febrile illnesses since the age of 8 months. He walked unsupported with an ataxic gait at the age of 2. When he was 6, he experienced neurological regression and epileptic seizures during a febrile disease, with severe hypotonia and hypokinesia. Partial neurological recovery was then observed. At the age of 9 he showed an ataxic-dyskinetic-spastic gait and upper limbs dystonia. At the age of 34, he was wheelchair bound and showed severe intellectual disability, spastic-dystonic tetraparesis, hypo-bradykinesia, head titubation, ophthalmoplegia, optic atrophy and retinopathy, lower limb axonal peripheral neuropathy.
Results: Serial MRI disclosed bilateral pallidum alteration on T2 sequences and cerebral atrophy. 123I-ioflupane brain SPECT and H-MRS were normal. Mitochondrial respiratory chain on a muscle biopsy was normal. A dosage of acylcarnitine on dried blood spot (DBS) showed high levels of 3-hydroxybutyrylcarnitine (C4OH) and Sanger sequencing of the *HIBCH* gene revealed a novel homozygous variant (c.777T>A, p.Phe259Leu).
Discussion: HIBCH defect is an underestimated cause of early onset and slowly progressive Leigh syndrome due to a secondary damage of mitochondrial function. The diagnosis may be detected by acylcarnitine on DBS, possibly as part of neonatal screening program. If diagnosed early the disorder is potentially treatable by handling valine intake with the diet and by contrasting oxidative damage. With respect to the few reported cases our patient developed retinopathy. Finally, isolated pallidal lesions may be suggested as a neuroimaging diagnostic marker of this condition.

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High prevalence of 3-hydroxy-3-methylglutaric aciduria in a high-risk Brazilian population

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Background: 3-Hydroxy-3-methylglutaric aciduria (3-HMG) is an inborn error of metabolism caused by deficiency of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) lyase, that catalyzes the cleavage of HMG-CoA to acetoacetic acid and acetyl-CoA, the last step of both ketogenesis and leucine catabolism. The disease is very rare, with approximately 150 cases described in literature. Patients usually develop severe metabolic decompensations with hypoketotic hypoglycemia, metabolic acidosis and encephalopathy that may have a fatal outcome if not treated immediately. Variable CNS damage is commonly observed in untreated patients.
Methods / case report: The Medical Genetics Service of Clinical Hospital of Porto Alegre carries out diagnosis of organic acidurias by GC/MS in symptomatic (high-risk) patients since 1993 and it is a reference center for diagnosis of these diseases in Latin America. In this work we report 40 patients from different Brazilian states, with biochemical diagnosis of 3-HMG.
Results: Urine from all 40 diagnosed patients contained large amounts of 3-hydroxy-3-methylglutaric, 3-methylglutaconic, 3-hydroxyisovaleric and 3-methylglutaric acids. The prevalence of 3-HMG in our selective screening corresponded to 8.7% of total organic acidurias detected (40/461) in our laboratory (1993–2016) and the fourth more frequent in studied population indicating a high incidence of this disorder in Brazil. The major symptoms presented were hypoglycemia, seizures, metabolic acidosis, vomiting and hepatomegaly. It is important to emphasize that most patients were of Portuguese ancestry and that molecular characterization (although limited - 11/40) revealed similar mutations of individuals of Portuguese/Spanish ancestry (P.Glu37*and c.504_505delCT), indicating a founder effect
Discussion: Our results expand significantly the number of 3-HMG reported in the literature and demonstrate a relatively high prevalence of the disease in Brazilian population.
Support: FIPE/HCPA, CNPq, FAPERGS

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Two siblings with type VII 3-methylglutaconic aciduria due to mutations in *CLPB* gene

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Background: 3-methylglutaconic acidurias are a group of conditions unified by the presence of significantly elevated levels of 3-methylglutaconic aciduria (3-MGA). These rare conditions are diversified by variable clinical presentations, thus we are expanding the phenotype in reporting a case of two siblings.
Case report: We report the case of 2 siblings of African descent with caseinolytic peptidase B (*CLPB*) defect-associated 3-methylglutaconic aciduria with clinical signs and symptoms of neutropenia and brain atrophy.
Results: The index case presented at 14 months with bilateral cervical lymphadenitis, fever, and neutropenia (0.1 x 109/L, 1.5-8.5 x 109/L) followed by recurrent infections. The neutropenia and clinical picture normalized with G-CSF. He was developing normally until 2 years of age, at which point a gait abnormality became prominent. By 3.5 years, he had progressive dysarthria, ataxia, delayed speech, and mild global developmental delay. Subqequent work up revealed plasma 3-MGA to be 2892 (103–384) and urine 3-MGA to be 43.2 (1.9-9.1). Molecular testing confirmed the diagnosis with compound heterozygous mutations in the

CLPB gene. A review of his family history revealed an older sibling with severe neutropenia and generalized atrophy who had died at 16 months of age following adenovirus pneumonitis.
Discussion: This case represents a pair of two siblings with neutropenia, ataxia, and developmental delay with a confirmed *CLPB* defect and associated 3-methylglutaconic aciduria in the index patient. It is a rare autosomal recessive condition associated with two mutations in the *CLPB* gene reported in only about 35 cases. It highlights the importance of timely genetic diagnosis to ensure appropriate follow-up for sequelae based on the genotype. In this case, follow-up is required for increased likelihood of seizures, intellectual disability, cataracts, cardiac anomalies, hematologic malignancy, renal cysts, and nephrocalcinosis.

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Exploring branched chain amino acid ratios to guide nutrition therapy in propionic and methyl malonic acidemias (PPA and MMA)

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Background: Recent published findings demonstrate that MMA and PPA patients thrive better on a prescribed diet where intact protein is dominant over medical food (MF) protein. A current theory states the high leucine content and diminished amount of other branched chain amino acids (BCAAs) in MMA and PPA specific MF lead to a deleterious effect of high leucine intake on hormone regulation and brain metabolism.
Methods / Case report: Review and present current MMA and PPA medical food composition for BCAA relative ratios. In addition, medical history, anthropometrics, diet analysis completed with Metabolic Pro, and plasma amino acid concentrations over time were extracted from a retrospective chart review. For selected cases, impact of MF and intact protein ratios and their relationship with plasma amino acids were evaluated. Additionally, traditional treatment protocols for MMA and PPA were compared with recent updates and recommendations.
Results: Ratio of leucine to other BCAAs in current medical foods varies broadly. Ratio of leucine to isoleucine ranged from 1.7 in breast milk to 160 in a current medical food. Leucine to valine was 1.5 in breast milk and up to 1600 ratio in commonly used medical foods. Ratio of leucine to other plasma BCAAs approached normal concentrations with higher intact protein introduced to the diet and decreases to medical food amount. Free amino acid supplementation was not required to normalize offending plasma amino acids with observed improvements in appetite.
Discussion: Modification of current nutritional therapy is crucial to optimize outcomes in MMA and PPA patients. This relies on discovering an ideal balance of essential amino acids while meeting other nutritional needs. Dietary ratio of leucine to valine and isoleucine may offer an approach to balance the medical food and intact protein content of MMA and PPA patient diets for improved long term outcomes.

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Evaluation of dietary treatment in classic OAD and UCD. *Information from a European multicenter database (E-IMD)*

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Background: Organic acidurias (OAD) and urea cycle disorders (UCD) are a group of rare inherited disorders affecting amino acid and protein metabolism, with an estimated incidence of 1 in 50,000-1,000,000 newborns per individual disease (Kölker et al. 2007; Summar et al. 2013). Current dietary practice in organic acidurias (OAD) and urea cycle disorders (UCD) may vary widely (Zwickler et al. 2008; Manoli et al. 2016). We assess the long-term dietary treatment.
Methods: Cross-sectional baseline visits of patients from the European registry and network for intoxication type metabolic diseases (E-IMD, Chafea no. 2010 12 01) were analyzed. Protein intake was compared to recommended daily allowance (RDA) by WHO 2007.
Results: Totals of 418 classic OAD and 460 UCD patients were included. In MMA and PA, plasma L-valine and L-isoleucine levels were below the lower level of reference ranges in 56% and 55% of the patients respectively. Levels were mainly low in patients receiving amino acid mixture (AAM), despite a median natural protein intake according to current RDA. In UCD natural protein intake was according to RDA, except in symptomatic ASS-D, ASL-D, CPS1-D, OTC-D male and HHH syndrome patients receiving AAM. In UCD plasma BCAA levels in patients receiving AAM were similar to those who did not receive AAM, despite a lower natural protein intake %RDA in patients receiving AAM. In CPS1-D, OTC-D and HHH syndrome, those receiving selective supplementation with L-citrulline had higher plasma L-arginine levels compared to those receiving selective L-arginine.
Discussion: In MMA and PA patients receiving AAM supplements had very low BCAA levels. In UCD AAM products seem beneficial in patients with a low natural protein intake. In OTC-D, CPS1-D and HHH syndrome patients, selective L-citrulline supplementation seems preferable. The above findings are important to further improve chronic dietary treatment. and adverse effects of decreased plasma BCAA levels needs to be determined.

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Status of Glutathione in Indian children with Branched Chain Amino acid Metabolism Defects- MMA, PA, IVA and MSUD

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Background: Oxidative stress is a major factor affecting pathogenicity in various inborn errors of metabolism. Increasing literature has emerged suggesting a possible role of free radical generation in the pathophysiology of neurodegenerative disorders including some branched chain amino acid metabolism defects like MMA, PA, IVA and MSUD. Objective was to analyze the levels of glutathione as a measure of oxidative stress in patients with MMA, PA, IVA and MSUD
Methods / case report: We analyzed glutathione levels in 600 patients with suspected IEMs. Out of these 23 patients were diagnosed to have MMA, 4 with PA, 4 with IVA and 6 with MSUD. Estimation of Glutathione was done along with other biochemical parameters. Blood samples of 54 healthy individuals were also analyzed for reference ranges of glutathione in normal population. Quantification of GSH was performed by enzymatic recycling method, using Glutathione reductase and Ellman's reagent
Results:We observed considerably low levels of glutathione in patients with MMA (24.72 ± 9.37nmol/mg Hb, n=23), PA (14.13 ± 5.97 nmol/mg

Hb, n=4), IVA (18.32 ± 9.84 nmol/mg Hb, n=4) and MSUD (18.44 ± 10.52 nmol/mg Hb, n=6) as compared to healthy individuals (46.64 ± 17.51 nmol/mg Hb, n=54). Glutathione levels were found to improve upon treatment in one patient with MMA and two patients with MSUD
Discussion: Low levels of glutathione were observed in patients with branched chain amino acid metabolism defects like MMA, PA, IVA and MSUD. Improvement in levels of glutathione was seen upon initiation of treatment in these patients. We hypothesize that supplementation with anti- oxidants (Vitamin C, Vitamin E and NAC) during intercurrent illness may help to prevent brain damage due to excessive accumulation of oxidative metabolites.

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Efficacy of Riboflavin supplementation in carnitine-induced fish odor syndrome

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Background: Fishy odor syndrome (FOS) is an infrequent side effect of carnitine supplementation. L-carnitine supplementation is recommended for long-term treatment of methylmalonic acidemia (MMA). FOS has also been reported as side effect of betaine treatment with Riboflavin (B2) supplementation being effective on odor relief. Interestingly betaine and carnitine share the same catabolic pathway. We report on a boy with MMA and FOS who was successfully treated with B2 supplementation.
Case report: A 15-year-old male with B12-responsive MMA (*mut* c.682C>T/c.765C>T), treated with 5200 mg of carnitine per day (105 mg/Kg/day) developed a strong body odor, described as fish-like. Significant impact of body odor on his daytime activities was reported. Tracing back the patient's history the odor had been noted since he was 5 years old disrespectfully of a number of cosmetic products. Basing on 6-point intensity scale suggested by The German standard Olfactometry Determination of Odour Intensity Guideline 3882 the odor was classified as Strong (4/6) by both the patient and his parents and Very strong (5/6) by the physicians.
Results: A trial with B2 supplementation (100 mg/day) was started. Immediate improvement on his body odor was noticed. One week after the onset of the B2 treatment the odor was classified as Not perceptible (0/6) by the patient and his family and Very weak (1/6) by the physicians without withdrawing carnitine therapy.
Discussion: FOS can be caused by lack of trimethylamine (TMA) N-oxidation by the hepatic enzyme Flavin-containing mono-oxygenase type 3. It has been reported as an uncommon side effect of oral L-carnitine supplementation. In humans oral L-carnitine is converted to TMA. Increased substrate intake (e.g. high carnitine) might cause increased TMA excretion in sweat and breath resulting in fishy odor. Benefits of the therapy in the patient herein reported suggest to consider B2 supplementation in patients on high dose carnitine who develop FOS.

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Liver transplantation in Propionic Acidaemia: a single centre experience in the UK

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Background: Liver transplantation for patients with Propionic Acidaemia (PA) remains a therapeutic option.
Methods: Retrospective review of patients with PA who underwent liver transplantation at a tertiary liver centre between 1995 and 2015.
Results: Fourteen children identified; 8 male; median age at presentation 3 days (0–77). Pre-transplant median protein restriction 1g/kg/day (0.63-1.75), 86% had developmental delay, 71% required supportive feeding. Frequent decompensations was the main indication. Median age at transplantation 2 years (0.8–8). Thirteen received cadaveric grafts (4 auxiliary) and 1 live-related donor. Patient and graft survival was 79% and 69% respectively. Three died; 43 days (biliary peritonitis), 225 days (acute on chronic rejection) and 13.5 years (post-transplant lymphoproliferative disease). Of 11 survivors 5 had at least one episode of acute cellular rejection, 2 sustained metabolic stroke and 3 developed cardiomyopathy. Median follow-up duration was 4 years (2–22). All liberalised protein intake and 9 had no further metabolic decompensations (median episodes pre-transplant 4 (1–30) and post-transplant 0 (0–5)). All survivors made developmental progress but were behind compared with unaffected children. Plasma glycine and propionylcarnitine remained elevated but reduced post-transplant.
Discussion: Liver transplantation in PA reduces frequency of metabolic decompensations and liberalises protein intake and can be considered in selected cases.

P-186

The molecular landscape of isolated Methylmalonic Acidemia in Malaysian patients

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Background: Isolated MMA is caused by one of the following: deficiency of the enzyme methylmalonyl-CoA mutase (Mut), a defect in the biosynthesis of its cofactor adenosyl-cobalamin (CblA, CblB and CblD-variant2) or deficiency of the enzyme methylmalonyl-CoA epimerase. All are recessively inherited disorders. In this study, we explored the molecular etiology of Malaysian patients with isolated MMA by analyzing the 5 genes implicated: *MUT, MMAA, MMAB, MMADHC* and *MCEE.*
Methods: 15 patients diagnosed with isolated MMA based on their clinical and biochemical profile were included. Their blood DNA were analyzed by PCR-Sanger sequencing using in-house designed primers that targeted coding exons and intron-exon boundaries of the genes studied. New variants identified were evaluated using *in silico* programs such as MutationTaster2, FATHMM-XF, NNSplice, NetGene2 and Human Splicing Finder. Primer walking was used to investigate a possible homozygous large deletion in *MMAA*.
Results: We identified 11 patients with Mut, 1 with CblA, 2 with CblB and 1 with CblD-variant2 MMA. All Mut and CblB patients had severe phenotype with metabolic decompensation before 2½ months old whilst CblA and CblD-variant2 patients presented later with variable signs. *MUT* analysis yielded 7 pathogenic variants; the commonest c.982C>T was found in 14/22 alleles (64%). Of the 8 patients who harbor c.982C>T, 5 were ethnic Iban, an indigenous inhabitant of Borneo. Analysis of *MMAA*, *MMAB* and *MMADHC* showed 1, 2 and 1 mutations respectively. 5 novel mutations were detected in this study: c.300T>A & c.1444+3A>T in the *MUT*, a large deletion (≈17.3kb) spanning introns 1→3 in the *MMAA*, c.644+1G>A in the *MMAB* and c.2T>G in the *MMADHC*.

Discussion: Molecular study is crucial for diagnostic confirmation where cellular biochemical tests are not available. The spectrum of mutations ranged from single nucleotide variant to large deletion, it is hence important that accurate molecular techniques are used to characterize them.

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Clinical, molecular data and outcome of 11 Iranian patients affected of Propionic Acidemia: A case series

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Background: Propionic Acidemia, an autosomal recessive disorder caused by defect of Propionyl-CoA Carboxylase, a dodecamer composed of alpha and beta subunits encoded by genes: PCCA and PCCB respectively is a disease of wide clinical spectrum which even with optimal treatment there is still some neurologic impairment with limited published information on patients' developmental outcome.In this study we aim to report a case series of propionic acidemia to explore clinical, molecular characteristics and their outcome.
Methods: Propionic acidemia patients, treated in Research Unit of Iranian National Society for Study on Inborn Errors of Metabolism 2010–2018 entered the study. Diagnosis confirmed by dried blood spot acyl carnitine and urine organic acid profiles, done with MS/MS and GC/MS respectively. A retrospective descriptive analysis was done on the patients' data.
Results: 11 patients:7male,4female;9/11 consanguineous; mean birth body weight:2907gram(2700–3700);mean birth head circumference:34.3cm(33–37);5/11 early onset(≤3 months) ;mean onset age:4.2 months(0.1-14);mean diagnosis age:9.35 months(0.1-24);mean follow up duration:2.9years (0.3-8.5);first presentation:2/11Neonatal cholestasis;4/11seizure;4/11neurodevelopmental delay;3/11vomitting; 1/11hypoglycemia;3/11 metabolic acidosis; mean age of complication:13.1months(2.5-26);complications: 10/11neurodevelopmental delay;3/11optic atrophy; 8/11lactic acidosis;6/11 permanent metabolic acidosis;7/11seizure;6/11death;mean plasma C3 level: 16.8μmol/L (10.2-30.7); mean C3/C2 ratio:1.2(0.82-1.88);mean urine 3-hydroxy propionic acid:948.2mmol/molcr(72–3860) ;mean2-methylcitric acid:345.4mmol/molcr(34–1007);Treatment response:1/11good;4/11fair;6/11poor(70%early onset); two patients showed unreported homozygote mutations:delc.740-742PCCA; del exon 10 PCCB.
Discussion: Propionic acidemia in our Iranian cohort is a disorder with a fairly poor treatment response, especially if presented early in life or diagnosed late.

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Evaluation of the effect of sodium phenylbutyrate therapy on plasma leucine levels as an emergency treatment for maple syrup urine disease

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Background: Maple syrup urine disease (MSUD) is an autosomal recessive metabolic disorder caused by deficiency of the branched-chain α-keto acid dehydrogenase complex. Clinical manifestations are irritability, poor feeding, encephalopathy, apnea attacks, opisthotonus and stereotyped movements that can lead to coma and central respiratory failure. Standard treatment of MSUD comprises a protein restricted diet in addition to a specific formula including essential amino acids and micronutrients. Emergency treatment includes intravenous lipid and glucose perfusions with a high ratio of perfusion rate, insulin perfusions and hemodialysis. The aim of this study is to evaluate the effect of sodium phenylbutyrate therapy on the decrease of plasma leucine levels in a MSUD attack.
Methods: Ten patients with the classical phenotype of MSUD were included in the study. All 10 MSUD patients had been regularly following up and were treated with a protein restricted diet supplemented with specific formulae. Patient data including plasma amino acid analysis at the time of metabolic decompensation and therapy responses were collected retrospectively.
Results: The difference in therapy response between standard emergency treatments and 250 mg/kg/day sodium phenylbutyrate accompanied by standard treatment were compared. A significant difference was observed in the daily decrease rate in plasma leucine levels under phenylbutyrate therapy. In this group normalization of plasma leucine levels could be reached in a shorter time. Hemodialysis was not required even in patients with plasma leucine levels of 1000 μmol/L.
Discussion: Metabolic decompensation in MSUD can be life threatening if it is not managed early and appropriately. For treatment of higher plasma leucine levels above 750 μmol/L associated with encephalopathy, hemodialysis stands out as the most effective method. In conditions where hemodialysis cannot be performed, phenylbutyrate therapy could be an optional and effective alternative therapy.

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Cardiomyocytes derived from induced pluripotent stem cells as a model for propionic acidemia

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Background: Propionic acidemia (PA), one of the most frequent life-threatening organic acidemias, is caused by defects in either the *PCCA* or *PCCB* genes, encoding both subunits of the mitochondrial enzyme propionyl-CoA carboxylase. PA leads to a multisystemic disorder that mainly affects the cardiovascular and nervous systems. Cardiomyopathy features are associated with high morbidity and mortality and have been attributed to bioenergetics failure and intracellular accumulation of toxic metabolites.
Methods / case report: To overcome limitations of current cellular models of PA and investigate the cardiac pathology of the disease, we aimed to generate induced pluripotent stem cells (iPSCs)-derived cardiomyocytes from a PCCA-deficient patient using well-established protocols.
Results: We derived iPSCs from PA patient-derived fibroblasts and along with a control iPSC line successfully differentiated them into cardiomyocytes. The presence of cardiomyocytes was easily established by visual observation of spontaneously contracting regions. Immunofluorescence assay showed high expression of cardiac-specific proteins such as cardiac troponin T (cTnT), smooth muscle actin, alpha-actinin and GATA4. cTnT was expressed in more than 95% of control and patient differentiated cells showing high purity of iPSC-derived cardiomyocytes. Interestingly, patient cardiomyocytes exhibited a highly reduced beat frequency (51 bpm) compared to control cardiomyocytes

(86 bpm). In addition, PA iPSC-derived cardiomyocytes expressed biomarkers associated with altered cardiac function/development, with deregulated expression of ANP, alpha/beta MHC and heart-enriched miRNAs previously associated with cardiac hypertrophy.
Discussion: Our data show for the first time that PA iPSC and its cardiac derivative represent a promising model for the study of PA related cardiomyopathy.

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Persistent CSF biochemical abnormalities in transplanted patients with methylmalonic aciduria: a longitudinal study

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Background: Liver transplantation (LT), with or without kidney transplantation (KT), has been proposed as a potentially curative treatment for methylmalonic acidemia due to *MUT* mutations as it reduces the risk of metabolic decompensations and allows less restricted protein intake. Although transplanted organs are an enzyme source, they only partially correct the biochemical defect. De novo methylmalonic acid (MMA) production in CNS may contribute to neurological dysfunction after transplantation. Only one study described longitudinal determination of CSF and plasma MMA after LT.
Methods: To study the effect of LT/KT on biochemical abnormalities in methylmalonic acidemia, we did serial MMA measurements in plasma (118 samples) and CSF (9 samples) in 6 patients, all on free diet after transplantation. Patients were divided into 2 groups. Group 1 (age 3-4y) included 2 LT and 1 combined LT/KT patient; Group 2 (age 19-43y) included 3 KT patients. Unpaired t-test with SPSS Statistics 21.0 package was used to compare data before and after transplantation.
Results: Mean MMA levels in plasma were decreased either by LT or by combined LT/KT (620±390 vs 207±121 μmol/L; $p< 0.0001$) or by KT alone (2030±1793 vs 711±395 μmol/L; $p=0.0112$). Differences of MMA before transplantation in KT reflect renal failure, whereas higher levels after transplantation could be due to older age in KT patients or to a lower enzymatic supply provided by KT. In contrast to plasma, CSF MMA concentration was unchanged by transplantation, with persistently high levels (476±215 vs 420±258 μmol/L) in patients receiving LT alone or LT/KT and in a single determination in a KT patient (250 μmol/L).
Discussion: Our study shows that organ transplantation does not affect the concentration of MMA in CSF. The persistent elevation of offending metabolites may possibly contribute to long-term neurological damage. Novel strategies targeting CNS should be investigated to improve neurological outcome in methylmalonic acidemia.

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Identification and functional characterization of novel mutations in propionic acidemia

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Background: Propionic acidemia (PA) is characterized by the toxic accumulation of propionyl-CoA and derived metabolites due to propionyl-CoA carboxylase (PCC) deficiency. PA is caused by mutations in the *PCCA* and *PCCB* genes, encoding the α and β subunits of the PCC enzyme, respectively. Up to date, more than 200 pathogenic mutations have been identified, mostly missense defects.
Methods:Genetic analysis in PA patients was performed by Sanger or next generation sequencing. Structural analysis in the 3D model of the PCC enzyme was performed using Pymol. Expression analysis of missense variants was performed in a eukaryotic system, measuring PCC activity and protein levels. Available patients-derived fibroblasts were grown at 28°C or 37°C and PCC activity and protein measured to examine the potential effect of missense variants on folding and/or protein stability.
Results: We report 20 novel variants in the *PCCA* and 14 in the *PCCB* genes. Most potentially disease-causing variants were predicted to disturb protein structure. Functional analysis revealed pathogenic effect of 21 missense variants as they exhibited reduced or null PCC activity and protein levels, compared to wild-type constructs. *PCCB* variants p.E168del, p.Q58P and p.I460T resulted in medium-high protein levels and no activity. Only p.R230C and p.C712S in *PCCA,* and p.G188A, p.R272W and p.H534R in *PCCB* retained both partial PCC activity and medium-high protein levels. Moreover, we could detect a slight increase in PCC activity or protein in patient-derived fibroblasts carrying some of these mutations. Examination of available clinical data allowed to establish genotype-phenotype correlations in most cases, with some notable exceptions.
Discussion: Functional analysis confirmed the pathogenicity of novel missense variants in the *PCCA* and *PCCB* genes. Most of them affect protein structure and stability and could be targeted by therapies improving folding.

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The use of carglumic acid in the treatment of acute hyperammonemia associated with organic aciduria: A single center experience

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Background: Carglumic acid (CA) acts as a cofactor for the enzyme carbomylphosphate synthase and therefore bypassing the effect of inhibition of the enzyme N acetylglutamate synthase (NAGS)by organic acid. Therefore, CA emerged as a treatment modality for acute hyperammonemia in patients with organic aciduria in addition to scavengers. The aim of this study is to review our experience with treatment of acute hyperammonemia using CA with or without other scavengers in patients with organic aciduria.
Methods: This retrospective study recruited all children < 16 years of age with organic aciduria who developed acute hyperammonemia and treated with CA with or without other ammonia scavenger drugs between December 2015 and April 2018. Data were collected include demographic characteristics, clinical diagnosis, duration of CA administration, level of ammonia before and after treatment and possible side effects secondary to CA.
Results: Ten patients with organic aciduria presented with acute hyperammonemia and received CA. during the study period. Four were males. The mean age was 38 month (9–86). Underline diagnosis was propionic aciduria in 7 and methylmalonic aciduria in 3. A founder mutation was observed in *PCCA* gene in 6 patients. One patient had mutation in *PCCB* gene and 3 patients had variant in *MUT* gene. These patients were admitted 32 times with acute hyperammonemia during the study period. CA was given for 125 days during these admissions with a mean of 3.9 days per admission. The mean ammonia encountered at the start of CA was 227

(134–348) with mean level of 45 (33–61) at discontinuation of CA. None of the patients needed dialysis during the study period. No side effects were observed in any of the patients.
Discussion: CA seems to be safe and well tolerated by patients in this cohort with no side effects observed. Acute hyperammonemia responded to treatment with CA with or without other ammonia scavenger drugs.

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Acylcarnitine profile of 3-hydroxy-3-methylglutaryl CoA lyase deficiency patients

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Background: 3-hydroxy-3-methylglutaryl-coenzyme A lyase deficiency (HMGCLD, OMIM 246450) is a rare autosomal recessively inherited metabolic disorder caused by mutations in *HMGCL* gene. The mitochondrial enzyme catalyzes the cleavage of HMG-CoA to acetyl-CoA and acetoacetic acid. This conversion is a common last step in leucine catabolism and ketogenesis from fatty acids. Diagnosis is established by tandem mass spectrometry based newborn screening that contains SRM transition for non-butylated C5-OH carnitine (262 à 85) representing four different compounds (methylmalonylcarnitine, 3-hydroxyisovalerylcarnitine, succinylcarnitine and 2-methyl-3-hydroxybutyrylcarnitine), pointing to different inborn errors of metabolism.
Methods / Case report:We analysed plasma samples of two HMGCLD patients with a liquid chromatography coupled with high-resolution mass spectrometry.
Results:Apart from 3-hydroxyisovalerylcarnitine and 3-methyl glutarylcarnitine, other acylcarnitine species derived from intermediates in the leucine degradation pathway were observed. These acylcarnitines were annotated based on accurate mass of precursor ions and predicted fragmentation behavior.
Discussion: Newly found elevated acylcarnitines could hypothetically be also expected in other condition, 3-methylglutaconyl-CoA hydratase deficiency (MGCA) of which defective enzyme is located one step upstream of the leucine degradation pathway. HMGCLD could be distinguished from MGCA by measuring of 3-hydroxy-3-methylglutarylcarnitine.The result suggests that 3-hydroxy-3-methylglutarylcarnitine could be specific marker in dried blood spots by FIA-MS screening (based on SRM transition 306 à 85) and thus speed up differential diagnoses among diseases characterized/ screened by C5-OH transition (262 à 85). That would allow for earlier introducing dietary intervention directly from first screening sample without necessity of re-sampling.
This work was supported by NPU I (LO1304) and IGA_LF_2018_010.

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Methylmalonic acid compromises energy metabolism in rat C6 astroglial cells

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Background: Methylmalonic acidemia is an inherited metabolic disorder biochemically characterized by tissue accumulation of methylmalonic acid (MMA). Clinical manifestations are mainly neurological, including encephalopathy, cerebral atrophy, coma and seizures during metabolic decompensation. Considering that the mechanisms underlying neurological alterations of this disorder are not fully understood, the aim of this study was to investigate the toxic effects of MMA in an astroglial cell line presenting astrocytic features.
Methods: Astroglial C6 cells were exposed to MMA (0.1-10mM) for 24 or 48 hours and afterwards viability, glucose consumption, oxidative stress and cell respiration were analyzed. Cell viability and glucose uptake were determined by MTT reduction and glucose oxidase assays, respectively. Basal and maximal cell respiration rates were assessed by measuring oxygen consumption in an Oroboros O2K high-resolution respirometer. Redox homeostasis was evaluated by the lipid peroxidation (TBA-RS) and by non-enzymatic antioxidants content (GSH).
Results: Cell viability was reduced after 48 hours exposition to MMA, whereas glucose consumption was increased at the same time period. Basal and maximal respiration rates were also reduced when cells were exposed to MMA. No differences in GSH levels were found, whereas an increase in cell malondialdehyde content was observed both 24 and 48 hours after MMA exposition.
Discussion: Our results demonstrated that MMA reduces cell viability, stimulate anaerobic glycolysis while induces dysfunction in mitochondrial respiration and causes lipid peroxidation in rat C6 cell line, in concentrations similar to those found in patients affected by this disease, suggesting that these effects could be involved in the pathophysiology of neurological dysfunction of this disease.
Financial support: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP #2015/25541-0) and Universidade Federal do ABC (UFABC).

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Long-term use of carglumic acid in the management of hyperammonemia in patients with methylmalonic and propionic acidemias

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Background: Hyperammonemia in patients with branched chain organic acidemias appears to be caused by reduced N-acetyl glutamate synthesis secondary to lowered acetyl-CoA and/or glutamate levels and to the accumulation of inhibitory metabolites. Based on the currently recognized pathophysiology, the application of carglumic acid has emerged as a therapeutic option for the secondary hyperammonemia caused by organic acidemias. However, long-term management with carglumic acid has never been reported.
Methods: We reviewed all patients with branched chain organic acidemias diagnosed in the Istanbul University Cerrahpasa Children's Hospital. Patients that had undergone long-termcarglumic acid treatment were included in the study. Patients' ammonia levels, plasma amino acid levels, carglumic acid treatment schedule, dosage, treatment response and adverse events were recorded. Additional information on the nutritional and medical therapies utilisd were also recorded.
Results: Twenty two patients with organic acidemia aged between 6 days and 26 months at the time of diagnosis were included in the study. The median period of carglumic acid treatment was 18.8 months (Range: 1–53 months), the median dose of carglumic acid was 89.46 mg/kg (R: 15–177 mg/kg). The lowest dose was 12.5 mg/kg while maximum dose was 250 mg/kg in patients with MMA and PA. A total of 81 episodes of hyperammonemia were treated with dosage adjustment of carglumic acid, only 15 episodes of hyperammonemia required hospitalization.

Discussion: N-carbamylglutamate, a stable structural analogue of N-acetylglutamate, has been shown to be effective in reducing the ammonia concentration in neonates with PA and MMA. In this study we have shown that carglumic acid can be used in the long-term management of hyperammonemia in patients with propionic and methylmalonic acidemias without severe side effects.

P-196

Improvement in neuropsychological outcomes of a child with methylmalonic acidemia after liver transplantation

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Background: Clinical signs and symptoms of Methylmalonic acidemia (MMA) usually appear in early infancy and include, among others, developmental delay and intellectual deficit. Long-term complications include neurological damage due to metabolic stroke affecting the brain stem, intellectual disability, chronic kidney disease and pancreatitis. Some studies aimed to analyze the longitudinally neurocognitive outcomes, but few detailed reports of the changes in neuropsychological function in MMA individuals after organ transplantation.
Methods: 10-years-old boy presented in the first days of life with metabolic acidosis and coma, leading to the diagnosis of MMA. In spite of adequate treatment, disease progressed and a liver transplantation (LT) was performed. We describe his neurocognitive functioning prior and one year post LT. Up to 6 years of age, cognitive evaluation was performed using the Griffith's scales. After this age, WISC-III was used. Mean cognitive measures is 100 (SD=15).
Results: Pre-LT language, performance and global Developmental Quotient obtained in Griffith's scales scored borderline to normal range. Three years before LT, full scale Intellectual Quotient (FSIQ) was 82. Verbal IQ (VIQ) and performance IQ (PIQ) were 96 and 76, respectively and their difference had statistical significance. Fifteen days prior LT, we observed a decrease in VIQ score (75) and no changes in PIQ score (77). One year post-LT VIQ, PIQ and FSIQ scores displayed a positive change (VIQ=91; PIQ=84; FSIQ=84).
Discussion: Despite being limited to a patient, a recovery of neuropsychological function of patients with MMA seems to occur following LT. Although the metabolic deficit is not totally corrected by LT, there is a significant reduction of neurotoxic metabolites, methylmalonic acid included, which might improve neurological outcomes. A neurocognitive surveillance protocol should be defined and applied to a large cohort of patients, in order to clarify these results.

11. Organic acidurias: others

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Clinical and genetic findings in Chinese patients with methylmalonic aciduria: from heterogeneity to clinical strategy

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Background: To analyze the heterogeneity of frequent symptoms among Chinese children with methylmalonic aciduria (MMA) originating from a mixed genetical background.
Methods / case report: We investigated 26 Chinese patients (17 males and 9 females) diagnosed by elevated urinary MMA, elevated serum C3, C3/C2 ratio and decreased serum free carnitine. Genetic diagnosis of these children was made by sequencing the MUT, MMAA, MMAB, MMACHC, MMADHC, LMBRD1, MCEE, SUCLG1, SUCLG2 and ABCD4 genes. The clinical and biochemical features were analyzed.
Results: We identified a considerable variation between clinical manifestations of these children. Symptoms of MMA varied from mild vomiting to severe encephalopathy and the age of onset from 3 days to 13 years. Less frequent symptoms (< 10%) such as hydrocephalus, isolated pulmonary hypertension, peripheral neuropathy and diabetic ketoacidosis were found as the first signs in MMA patients. Mutations in MUT, MMACHC and MMADHC gene were identified in 22 patients. Among these mutations, two novel missense mutations in MUT gene (c.1540C>A and c.505G>T) were identified. In 2 patients with isolated pulmonary hypertension, we found the same mutation sites as that in 2 patients with hydrocephalus.
Discussion: The heterogeneous clinical manifestations identified among our Chinese patients with MMA indicate that the genetic profiles of MMA patients vary with ethnicity and there were phenotype-genotype correlations according to our genetic sequencing results.

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Human D-glycerate kinase is a mitochondrial protein

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Background: D-glyceric aciduria is a rare inborn error of serine and fructose metabolism which is caused by D-glycerate kinase (GLYCTK) deficiency. Patients present with a wide range of clinical phenotypes. Also, asymptomatic cases have been described (Sass et al. Hum Mutat 2010; 31: 1280–5). Given the highly variable clinical outcomes it has been suggested that D-glycerate deficiency is a mere biochemical variant rather than a disease (Kalim et al. Brain Dev 2017; 39: 536–8). Previously, it was claimed that two GLYCTK isoforms, encoded by *GLYCTK1* and *GLYCTK2*, which may result from alternative splicing, are ubiquitously expressed in human (Guo et al. DNA Seq 2006; 17: 1–7). The authors further proposed that the isoforms exhibit differential subcellular localizations and that they may fulfill different functions, but comprehensive analysis is lacking. So far, endogenous GLYCTK protein expression has only been reported in the liver.
Methods: Here, a characterization of GLYCTK1 and GLYCTK2 was carried out on protein and nucleic acid levels in cultured human-derived cell lines overexpressing GLYCTK.
Results: Immunoblotting revealed that GLYCTK1 was efficiently overexpressed while GLYCTK2 expression could not be established. *In silico* analyses revealed the presence of mitochondrial presequences in both GLYCTK1 and GLYCTK2. In contrast to published results, immunofluorescence analysis and differential centrifugation verified that overexpressed GLYCTK1 exclusively localizes to mitochondria while GLYCTK2 could not be detected. However, quantitative PCR analysis confirmed that both transcripts are present in various human tissues.
Discussion: We propose that mitochondrial GLYCTK1 represents the functional enzyme and that GLYCTK2 is non-functional and being degraded. This notion is further supported by the fact that all published GLYCTK mutations which affect enzyme function are located in exon 5 which is not present in GLYCTK2.

P-199

Effects on redox homeostasis and histopathology provoked by intracerebral administration of D-2-HG in neonatal rats

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Background: D-2-hydroxyglutaric aciduria (DHGA) is an inborn error of metabolism biochemically characterized by tissue accumulation and elevated urinary excretion of D-2-hydroxyglutaric acid (D-2-HG). Affected patients usually present neurological symptoms, such as epilepsy, hypotonia and psychomotor/mental retardation whose pathomechanisms are poorly known.
Methods: D-2-HG was intracerebrally administered to rat pups at postnatal day 1 to induce a rise of D-2-HG levels in the central nervous system. Thereafter, we investigated whether D-2-HG in vivo administration could disturb redox homeostasis and induce brain histopathological alterations in the cerebral cortex of the neonatal rats.
Results: D-2-HG markedly induced the generation of oxygen (increase of 2',7'-dichloroflurescein-DCFH-oxidation) and nitrogen reactive species (increase of nitrate and nitrite concentrations), lipid peroxidation (increase of malondialdehyde concentrations), and protein oxidation (increase of carbonyl formation),besides decreasing the tissue antioxidant defenses (reduced glutathione-GSH). Increase of the activities of the antioxidant enzymes catalase and superoxide dismutase were also observed in the cerebral cortex following D-2-HG administration. Moreover, D-2-HG-induced increases ofmalondialdehyde concentrations and 2',7'-dichloroflurescein-DCFH-oxidation were fully prevented by the NMDA-receptor antagonist MK-801 and the antioxidant melatonin. It was also observed that D-2-HG provoked significant vacuolation and edema in the cerebral cortex as determined by hematoxylin and eosin staining.
Discussion: The data indicate pro-oxidant effects of D-2-HG disturbing brain redox cell status that probably induced the histopathological alterations observed.
Financial support: CNPq, PROPESq/UFRGS, FAPERGS, CAPES

P-200

Neural cell damage caused by acute intrastriatal administration of lysine in the model of glutaric acidemia type I

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Background: Glutaric acidemia type I is an inherited neurometabolic disorder caused by deficiency of glutaryl-CoA dehydrogenase (GCDH) activity. Patients usually present progressive cortical leukodystrophy and develop acute striatal degeneration mainly during crises of metabolic descompensation.
Methods: We investigated the effects of an acute intrastriatal administration of lysine (Lys, 1.5 or 4 μmol) or NaCl to 30-day-old wild type (WT) and GCDH deficient ($Gcdh^{-/-}$) mice. We evaluated synaptophysin expression by western blotting and GFAP, S100B, NeuN and myelin basic protein (MBP) by immunohistochemistry after Lys injection.
Results: Sinaptophysin expression was significantly reduced in striatum from $Gcdh^{-/-}$ as compared to WT. Furthermore, Lys administration at the lowest dose (1.5 μmol) provoked increased GFAP protein expression only in $Gcdh^{-/-}$ mice, suggesting astrocytic reactivity in the $Gcdh^{-/-}$ animals. In contrast, S100B was not altered, despite GFAP increased. NeuN expression was reduced in striatum of $Gcdh^{-/-}$ but not in WT mice submitted to 1.5 μmol Lys or NaCl injection, indicating neuronal loss. Lys also provoked a reduction of neuronal fiber size and overall myelination in striatum of both WT and $Gcdh^{-/-}$, as determined by MBP antibody. In cerebral cortex, myelination was decreased in the $Gcdh^{-/-}$ mice only when the highest Lys dose (4 μmol) was injected, with no change in the other parameters.
Discussion: Our results showed that Lys provoked increased astrocytic reactivity, neuronal loss and reduction of both neuronal size and myelination in $Gcdh^{-/-}$ mice striatum, whereas in cerebral cortex only myelination was decreased in $Gcdh^{-/-}$ mice injected with Lys at 4 μmol. The data indicate a greater susceptibility of the striatum to Lys overload in $Gcdh^{-/-}$ mice, which may be due to increased GA production ultimately leading to the imunohistochemistry alterations observed in these animals. Financial support: PROPESq/UFRGS, FAPERGS, CNPq, CAPES

P-201

Impairment of mitochondrial respiration caused by metabolites accumulating in propionic acidemia in rat heart and brain

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Background: Propionic (PA) and 3-hydroxypropionic (3OHPA) acids are predominantly accumulated in biological fluids and tissues of patients affected by propionic acidemia that usually manifest severe neurological symptoms and cardiomyopathy. Maleic acid (MA) probably derived from PA and 3OHPA is also excreted in the urine of some patients.Considering that the underlying mechanisms of brain and heart dysfunction in this disorder are not yet fully established, we investigated the effects of PA, 3OHPA and MA (0.05 - 5 mM) on brain and heart mitochondrial respiratory parameters.
Methods: Resting (state 4), ADP-stimulated (state 3) and CCCP-stimulated (uncoupled) respiration measured by oxygen consumption were evaluated using mitochondrial preparations from brain and heart of developing rats supported by glutamate/malate, pyruvate/malate, α-ketoglutarate or succinate.
Results: PA and 3OHPA significantly decreased state 3 and uncoupled respiration in pyruvate/malate and α-ketoglutarate-supported heart mitochondria. The effects caused by 3OHPA were less pronounced using glutamate/malate and succinate. In contrast, these organic acids caused no significant alterations on brain mitochondria. Furthermore, MA strongly decreased state 3 and uncoupled respiration in both brain and heart mitochondria supported by glutamate/malate and α-ketoglutarate, with less intense inhibition verified when using succinate. Interestingly, MA also provoked a strong inhibition of these respiratory states supported by pyruvate/malate in heart but not in brain.
Discussion: Taken together, the data indicate that PA and 3OHPA disturb mitochondrial bioenergetics in heart, whereas MA behaves as a much stronger metabolic inhibitor in both tissues. It is presumed that these deleterious effects reflecting disturbance of oxidative metabolism may be involved in the cardiomyopathy and brain damage occurring in propionic acidemic patients.
Financial support: PROPESQ/UFRGS, FAPERGS and CNPq.

P-202

A knock-in rat model for glutaric aciduria type I confirms cerebral ammonium accumulation

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Background: Glutaric aciduria type I (GA-I) is caused by deficiency of glutaryl-CoA dehydrogenase (GCDH). Most untreated patients are asymptomatic at birth and then develop encephalopathic crises most often triggered by a catabolic stress, which lead to irreversible neurological impairment. Despite numerous *in vitro* and *in vivo* studies, the pathogenesis of neurological damage in GA-I remains poorly understood.
Methods / case report: R411W, the rat homologue of the frequent human mutation R402W, was introduced into the *Gcdh* gene of Sprague–Dawley rats by CRISPR/Casp9-mediated genome engineering.
Results: Homozygous $Gcdh^{ki/ki}$ rats showed normal growth and were fertile. They revealed a biochemical phenotype typical for GA-I including elevations of 3-OH-glutaric acid (3-OHGA), glutaric acid (GA) and glutarylcarnitine in tissues and body fluids. Furthermore, a significant increase of ammonium (NH_4^+) was found in plasma accompanied by glutamine decrease and glutamate increase, suggesting that NH_4^+ was produced by the enzyme glutaminase (Gls). Histologically, $Gcdh^{ki/ki}$ rats developed the typical diffuse spongiform myelinopathy as known from autopsies of GA-I patients. However, $Gcdh^{ki/ki}$ rats did not present any signs of an encephalopathic crisis. Proteomic analyses on brain tissues revealed dysregulated genes in $Gcdh^{ki/ki}$ rats that are implicated in synaptic signal transmission and synapsis organization. Furthermore, Gls was found 12-fold up-regulated in brain tissues of $Gcdh^{ki/ki}$ rats.
Discussion: We successfully created the first transgenic rat model for GA-I. The characterization of this new model showed further evidence for a role of cerebral NH_4^+ production by Gls in the neuropathogenesis of GA-I. New therapeutic strategies targeting cerebral NH_4^+ accumulation have to be developed and can be tested in the $Gcdh^{ki/ki}$ rat.

P-203

Report of clinical, neuroradiological and genotypic characteristics of 24 L-2-hydroxyglutaric aciduria patients from a single center in Turkey

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Background: L-2-hydroxyglutaric aciduria (L2HGA) is an autosomal recessive neurometabolic disorder that is caused by deficiency of 2-hydroxyglutarate dehydrogenase enzyme. Developmental delay, seizures, progressive ataxia, pyramidal tract signs, extrapyramidal signs and progressive mental retardation are characteristic clinical features. Different types of malignant brain tumors have been reported. Although neurological symptoms are well defined, the pathophysiology of brain damage and genotype-phenotype relations are poorly understood. In this study, clinical, neuroradiological and genotypic characteristics and therapy responses of 24 L2HGA patients were summarized.
Methods: Patients in whom the diagnosis was made by molecular analysis of the *L2HGDH* gene and/or by showing elevated L-2-hydroxyglutaric acid in urinary organic acid analysis, were enrolled in the study. Data of patients including demographic features, detailed physical examination and abnormal MRI findings and therapy responses were collected retrospectively.
Results: 24 L2HGA patients from 16 unrelated families were enrolled in the study. 11 patients were male and 13 were female. Consanguineous marriage was reported in 22 patients. 22 patients exhibited neurological symptoms. Two unaffected patients were diagnosed at 7 months and 12 months respectively. Brain tumors were detected in three patients. Subcortical white matter and dentate nuclei alterations were found to be the most frequent MRI abnormality. Brain MRI revealed isolated basal ganglia involvement in two patients. Brain MRI of all patients was abnormal even in two patients with normal physical examination.
Discussion: L2HGA is a rare progressive neurometabolic disorder. Recognition of early clinical findings and different brain MRI features should help to overcome the diagnostic difficulties.

P-204

Bezafibrate prevents impairment of redox homeostasis and mitochondrial biogenesis induced by 3-methylglutaric acid.

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Background: 3-Methylglutaric acid (MGA) is an organic acid that accumulates in 3-hydroxy-3-methyglutaric (HMGA) and 3-methylglutaconic (MGTA) acidurias whose patients present neurological dysfunction. Aiming to elucidate the pathomechanisms underlying the brain injury observed in these disorders, we evaluated the effects of MGA administration on redox homeostasis, signaling pathways involved in mitochondrial biogenesis and neuronal damage markers in rat cerebral cortex. The effects of bezafibrate (BEZ), a compound that induces mitochondrial biogenesis, were also examined on the same parameters.
Methods: Thirty-day-old rats were intraperitoneally administered with MGA (one injection of 10μmol/g followed by two injections of 5 μmol/g) and euthanized 1h after the last injection for evaluation of biochemical parameters and content of proteins. Treatment with BEZ (100 or 30mg/kg/day) was performed by gavage for 7 days (1 daily injection) before the first administration of MGA.
Results: We verified that MGA induced lipid peroxidation, increased heme oxygenase-1 content and altered the activities of antioxidant enzymes, as well as of glutathione S-transferase and glucose-6-phosphate dehydrogenase. Furthermore, MGA diminished the levels of sirtuin 1 and nuclear PGC-1α, indicating impairment in mitochondrial biogenesis. MGA also induced neuronal damage, as reflected by decreased synaptophysin content and increased Tau phosphorylation. BEZ pretreatment prevented all effects elicited by MGA.
Discussion: Our findings provide strong evidence that MGA impairs redox homeostasis and mitochondrial biogenesis that cause neuronal damage in rat

brain. Moreover, considering that BEZ induced neuroprotective effects, it is suggested that therapeutic activation of mitochondrial biogenesis may ameliorate the neurological symptoms in patients with HMGA and MGTA. Financial support: CNPq, CAPES, Propesq-UFRGS, FAPERGS, INCT-EN.

P-205

Increased lipid damage and reactive oxygen species production in the cortex of glutaryl-CoA dehydrogenase deficient mice

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Background: Deficiency of the enzyme glutaryl-CoA dehydrogenase (GCDH), known as glutaric acidemia type I (GA-I), leads to the accumulation of glutaric acid (GA). Treatment is based on protein restriction and L-carnitine (L-car) supplementation, which also has an important role as an antioxidant, inducing the excretion of accumulated metabolites. The mechanism of brain injury seen in GA-I patients is poorly understood. To help understand the pathogenesis of GA-I, a knockout mouse model (*Gcdh*$^{-/-}$) was developed and subjected to a dietary overload of lysine (Lys).
Methods: We aimed to evaluate the lipid damage and reactive oxygen species (ROS) production in the cortex of *Gcdh*$^{-/-}$ and Wild-type (WT) mice, supplemented or unsupplemented with Lys, and to investigate if treatment with L-car can protect against these processes. Thirty-day-old *Gcdh*$^{-/-}$ and WT mice were submitted to a normal diet (0.9% Lys) or to a diet with 4.7% Lys for 72 hours. In addition, these animals underwent three intraperitoneal injections, each at a different time, of saline or L-car (two hours prior, after 24 and 48 hours of the diet introduction). Euthanasia was performed 24 hours after the final injection. *Gcdh*$^{-/-}$ and WT mice were separated into Group A (0.9% Lys + saline), Group B (4.7% Lys + saline) and Group C (4.7% Lys + L-car). The cortices of these mice were dissected and the homogenate used to evaluate TBARS and DCFH oxidation.
Results: Knockout animals had higher levels of TBARS and DCFH than WT mice, indicating lipid peroxidation and increased production of ROS, respectively, in the cortex of *Gcdh*$^{-/-}$ mice. Separately, *Gcdh*$^{-/-}$ mice exposed to an overload of Lys had TBARS and DCFH levels higher than those receiving a normal diet. These levels were significantly decreased when this group received L-car.
Discussion: In conclusion, we demonstrate a novel beneficial effect of L-car treatment by preventing lipid peroxidation and ROS formation in the cerebral tissue of *Gcdh*$^{-/-}$ mice.

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Variants of mevalonic aciduria – our experience

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Background: Mevalonic aciduria (OMIM# 610377) is at the severe end of the clinical spectrum of diseases caused by mevalonate kinase deficiency, with considerably heterogeneous, multisystem symptomatology. Biochemically it is characterised by high urinary excretion of mevalonic acid. We present clinical and key laboratory findings of two distant relatives with mevalonic aciduria with identical causal mutation in the *MVK* gene.
Case reports:A girl was repeatedly hospitalized since the 2nd week of life for failure to thrive and recurrent febrile attacks with increased inflammatory markers, accompanied by lymphadenopathy, hepatosplenomegaly, arthralgia and exanthema. At the 3rd month of life, the hypotrophy was −2.7 SD. An overall infection, congenital immunodeficiency or malignant process could not be identified; targeted OA analysis in the urine showed a high excretion of mevalonolactone at 1990 mmol/mol creat. The girl died at 2.5 years of age. A 19-day old eutrophic boy was hospitalized due to conjugated hyperbilirubinemia. Icterus, mild lymphadenopathy, mildly increased liver enzymes and inflammatory markers were found after admission to the hospital. Hepatotropic infection was not detected. Investigations of galactosemia, tyrosinemia and α1-AT deficiency were negative. Biliary atresia/hypoplasia was assumed, a liver biopsy was planned. Due to the gradual increase of inflammatory markers, cholangitis was suspected. In further metabolic examinations, urinary OA analysis was indicated and showed a high excretion of mevalonic acid at 40.5 and mevalonolactone at 393 mmol/mol crea. The boy died aged 4 months.
Results:Our patients demonstrate the clinical variability of mevalonic aciduria even within the same genotype.
Discussion: The clinical manifestation of the 2nd patient has been described so far only in very few neonates. The cause of "non-rare" neonatal cholestasis could be a large variety of diseases, a wider spectrum of IMD should be considered in differential diagnostics.

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Evaluation of dynamic thiol/disulfide homeostasis as an indicator of oxidative stress in L-2-hydroxyglutaric aciduria disease patients

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Background: L-2-hydroxyglutaric aciduria (L2HGA) is an autosomal recessive disorder that is caused by deficiency of 2-hydroxyglutarate dehydrogenase. Although neurological symptoms are well defined, the pathophysiology of brain damage is poorly understood. Accumulation of L-2-hydroxyglutaric acid, impairment of energy metabolism or alteration of neurotransmission are thought to be responsible for neurotoxicity. In recent years, elevated oxidative stress was proposed to be found and lead to brain injury in L2HGA. The aim of this study is to evaluate thiol/disulphide homeostasis as an indicator of oxidative stress in L2HGA patients who have been receiving antioxidant treatment.
Methods: Sixteen patients with L2HGA and 16 healthy individuals were included in the study. All 16 L2HGA patients had been regularly followed and were receiving antioxidant treatment. They presented neurological dysfunction at different grades at the time of testing. Serum native thiol (−SH), total thiol (−SH+−S-S-) and disulphide (−S-S) levels were measured in all subjects. Disulphide/native thiol, disulphide/total thiol and native thiol/total thiol ratios were calculated from these values.
Results: No significant difference was observed in -SH, −SH+−S-S-, −S-S levels between the two groups. In addition no increase of disulphide/native thiol and disulphide/total thiol ratios was detected.

Discussion: This study is the first to evaluate dynamic thiol-disulphide homeostasis as an indicator of oxidative stress in L2HGA patients. In recent studies, it was claimed that oxidative stress could be responsible for the neurotoxicity and antioxidant therapy was offered to prevent oxidative damage. Reduction in thiol levels and an increase in dynamic disulphide bonds have been found to be associated with oxidative stress in several diseases. In our study, the dynamic thiol/disulfide homeostasis status in our patient group showed that antioxidant therapy could prevent oxidative stress in L2HGA patients.

P-208

Improving qualitative interpretation of urinary organic acids profile

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Background: Analysis of urinary organic acids (OA) profiles constitutes the biochemical confirmation of organic acidurias. Interpretation of such profiles includes the identification of increased amounts of metabolites as well as the appearance of abnormal ones. Although the classical metabolic profiles of most organic acidurias have been well established, there is limited information regarding the variations of organic acids profile in young infants. Since, clinical onset of most organic acidurias occurs during the first 3 years of age, it is important to know normal variation in order to diminish false positive results and improve organic acidurias diagnosis using either qualitative or quantitative OA interpretation. This work shows our findings regarding the OA profiles of healthy children
Methods: 160 samples were collected from healthy children (0–3 years old) without familial history of neonatal death. OA profiles were obtained by GC-MS. After manual interpretation, relative quantification of the metabolites was performed using a double normalization with internal standard and urinary creatinine.
Results: OA profile included 45 metabolites approximately. RESULTS showed variations in the metabolites and their quantity associated to diet and age, respectively. Variations involved mainly metabolic products of gut microbiota, Krebs cycle and amino acid metabolism intermediaries. In addition, metabolites associated to organic acidurias were observed as part of the normal profile either constantly or associated to age or diet (e.g. glutaric, 3-OH-3-methyl glutaric and methylmalonic acids).
Discussion: Our results point out the importance of age and diet as important factors to take into account for OA profile interpretation. The implementation of semiquantitative interpretation contributes to avoiding overestimation of some findings and variations that could be normal in this period of life and that may lead to unnecessary metabolic studies and family stress.

P-209

Oxysterol levels as oxidative stress biomarkers in organic acidemia patients

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Background & AIM: Limited studies showed a relation between oxidative stress and toxic metabolite accumulation in the pathophysiology of organic acidemias (OA). Restricted diet treatment may also lead to a decrease in the antioxidant defense systems due to the lack of essential substances. This study evaluated markers of oxidative stress in OA patients.
Methods: 111 OA patients in routine clinic visits (RCV) or referred to emergency department (ED) and 100 healthy children were enrolled. Plasma levels of oxidation biomarkers, 7-keto cholesterol (7-KC) and cholestane-3β,5α,6β-triol (C-triol) that result from non-enzymatic oxidation of cholesterol, were determined by LC-MS/MS method.
Results: OA patient group consisted of 43 maple syrup urine disease, 26 methylmalonic acidemia, 13 propionic acidemia, 13 isovaleric acidemia and 16 glutaric acidemia type 1. There were 118 episodes: 106 during RCV and 12 when they were referred to ED. 7-KC levels were significantly higher in OA patient group [mean 36.85±6.30 ng/mL, 21.53 ±3.20ng/mL] ($p< 0.001$). Statistically significant increase in C-triol levels in OA patients [mean 21.69±1.77 ng/mL, 9.39±1.43 ng/mL] ($p< 0.001$) was also observed. Plasma 7-KC and C-triol levels didn't significantly differ between OA subgroups ($p=0.537$ and $p=0.347$ respectively) and between RCV and ED group ($p=0.170$ and $p=0.890$ respectively). No strong correlations between oxysterol levels and total cholesterol and LDL levels in OA patients were observed.
Discussion: To our knowledge, this is the first study evaluating oxysterol levels in OA patients. Oxysterols, seemed to be caused by increased oxidative stress with toxic metabolite stimulation independent of serum total cholesterol and LDL levels in these patients. Increased oxidative stress may be a chronic process and play a role in the long-term pathophysiology and prognosis. Oxysterols can be used in the long-term follow-up of these patients and the use of antioxidants as adjuvant therapy may be considered.

P-210

Intracerebroventricular octanoic acid administration decreases Na^+,K^+-ATPase activity in rats

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Background: Medium-chain acyl-CoA dehydrogenase deficiency (MCADD) causes octanoic acid (OA) accumulation in tissues and biological fluids of patients. During crises, affected individuals may experience hypotonia, lethargy and convulsions, that may progress to coma and death. Patients also present delayed psychomotor development, cerebral palsy and behavioral changes. In the present study, the effect of intracerebroventricular (ICV) OA administration on Na^+,K^+-ATPase activity and expression, thiobarbituric acid-reactive substances (TBA-RS) levels and sulfhydryl and water contents in rat brain was investigated.
Methods: Sixty-day-old male Wistar rats received a single ICV administration of OA (0,83 μmol/μL; OA group) or artificial cerebrospinal fluid (control group). The animals were euthanized one hour after administration, the brain was removed and the cerebral cortex, striatum, hippocampus and cerebellum were dissected and used for the analyses.
Results: OA administration significantly decreased Na^+,K^+-ATPase activity in cerebral cortex and hippocampus, without affecting its expression. Furthermore, increased TBA-RS levels, a marker of lipid peroxidation, were observed in all brain structures analyzed from the OA group animals compared to the controls. In addition, sulfhydryl group content was significantly decreased in cerebral cortex, striatum and cerebellum of OA group animals.

On the other hand, no difference in brain water content between groups was observed, suggesting that OA administration does not induce cerebral edema.
Discussion: Taken together, the present results suggest that OA decreases Na^+,K^+-ATPase activity, an important enzyme for the maintenance of cerebral homeostasis, possibly by inducing oxidation of sulfhydryl groups in its catalytic site and/or plasma membrane lipids. These findings may contribute to the understanding of the pathophysiological mechanisms underlying the neurological findings observed in patients affected by MCADD.

P-211

Methylmalonic acid induces changes in metabolic and redox homeostasis in C6 astroglial cells

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Background: Methylmalonic acidemia is one of the most prevalent inherited metabolic disorders involving neurological deficits with significant progressive deterioration. Although the consequences of methylmalonic acidemia are closely related to the central nervous system, the role of methylmalonic acid (MMA), which accumulates in this disorder, in glial cells remains unclear. Therefore, we investigated the effects of MMA on metabolic and oxidative stress parameters, and glial reactivity in C6 astroglial cells.
Methods: C6 cells were cultured in DMEM containing 5% fetal bovine serum and, at confluence, were exposed to MMA (0.5 and 5.0 mM) for 24 h. After exposure, we determined the levels of reduced glutathione (GSH) and malondialdehyde (MDA), and the activities of superoxide dismutase (SOD), glutathione S-transferase (GST), glutathione reductase (GR) and glutathione peroxidase (GPx). Amino acid content, lactate release and S100B levels were also determined.
Results: Our results demonstrated that MMA significantly decreased the concentrations of GSH, and the activities of SOD and GPx. However, MMA did not change GST and GR activities as well as MDA levels. MMA also decreased lactate release, but did not alter amino acid profile and S100B secretion in the extracellular medium.
Discussion: These data show that MMA induces glial response with metabolic changes and impairment of enzymatic and non-enzymatic antioxidant defenses leading to oxidative stress. We suggest that these pathomechanisms are involved in the neurological dysfunction and brain abnormalities observed in methylmalonic acidemia.

P-212

A single center experience of thirty five Glutaric Aciduria type 1 patients

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Background: Glutaric aciduria type 1 (GA1) is an inherited metabolic disorder caused by glutaryl-CoA dehydrogenase deficiency. Severity of the disease is markedly variable. Children with GA1 present with severe dystonia, speech and motor impairment after a catabolic state (e.g., infections, vaccination, trauma or surgery) in the first six years of life.
Methods: Records of 35 (18M, 17F) children (median current age:53 months) with GA1 were retrospectively reviewed.
Results: First complaints were neuromotor regression (88,5%), seizures (39,2%), macrocephaly (15,2%), hypotonia (9,1%), and family history of GA1 (3%).The median age of diagnosis was 10 months. On physical examination; axial hypotonia (85,7%), macrocephaly (57,1%), peripheral spacticity (57,1%), dystonia (31,4%), and nystagmus (5,7%) were detected. Glutaric acid, 3-hydroxyglutaric acid (3-OH-GA), glutarylcarnitine (C5DC) levels were high, and free carnitine and lysine levels were low in all patients. The first MRI findings were frontotemporal atrophy (80%), white matter changes (51,5%), hydrocephaly (%48,5), cerebral cysts (39,4%), delayed myelination (36,4%), subdural hematoma (33,3%), and globus pallidus abnormality (30,1%). Patients were treated with a low lysine diet, carnitine supplementation and high energy intake during catabolic episodes. Twenty of 35 patients had epileptic seizures. Multiple antiepileptic drugs were used to control seizures in 7 of 20 patients. The most common mutations in *GCDH* gene were p.P248L (c.743C>T), p.L340F (c.1018C>T) and p.E365K (c.1093G>A). Four novel mutations were detected in this study.
Discussion: Here, we report a large series of Turkish GA1 patients. Absence of GA1 in the Turkish newborn screening programme (NBS) and lack of p\awareness about inherited metabolic diseases cause mild to severe neurologic sequelae in all inedex cases due to the delay in diagnosis. Early diagnosis with NBS and good adherence to therapy are essential to avoid undesirable neurological damages.

12. Carbohydrate disorders

P-213

Adherence to treatment of hepatic glycogen storage disease type I patients followed by a Referral Center of Metabolic Diseases in Brazil

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Background: Glycogen Storage Disease Type I (GSDI) is an Inborn Error of Metabolism (IEM) caused by pathological mutations on genes that code enzymes involved in glycogen metabolism, leading to the accumulation of substrate in the liver, kidneys and intestinal mucosa, resulting in important metabolic alterations that compromise the individual's quality of life. Treatment for GSDI is mainly dietetic and aims to avoid hypoglycemia and to prevent secondary metabolic disorders through the frequent administration of uncooked cornstarch and/or continuous nocturnal nasogastric feeding or gastrostomy, besides dietary restrictions. Adherence to treatment is a world-wide public health issue and it is known that low adherence to prescribed interventions is a complex matter present in patients with chronic diseases. To date, no studies were found on adherence to

treatment of hepatic GSD. This study aims to characterize adherence to treatment of GSDI patients and identify its contributing factors.
Methods: Convenience sampled cross-sectional study, including GSDI individuals followed by the Ambulatory Clinic in a referral Center of Metabolic Disorders. Data was collected through medical records review and interviews with patients and/or family members. Adherence was evaluated by biochemical biomarkers and the variables were statistically analyzed.
Results: 18 patients were included. Eleven patients were classified as adherent to treatment. All the GSDIb patients were adherent. Three patients had an optimal level of adherence.
Discussion: The study had a higher index of adherence than referrenced by the literature. Important data was obtained that provides better comprehension of the possible factors that influence adherence for the treatment of GSDI. Strategies must be considered and incorporated into standards of health by the Health Care System to insure that patients have access to the requirements needed to improve their quality of life and to minimize difficulties associated with treatment.

P-214

Congenital disorders of glycosylation: clinical, biochemical and genetics studies and potential pitfalls in the screening

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Background: Congenital Disorders of Glycosylation (CDG) are human genetic diseases caused by defects in the synthesis or remodeling of N, O-glycoproteins and even in the biosynthesis of glycolipids. Glycans are involved in many processes necessary for normal functioning of biological systems. Alterations in this pathway cause a very variable range of phenotypes ranging from clinical multisystem presentation with severe neurological impairment to only one organ or tissue affected.
Methods / case report: In patients with clinical phenotype, transferrin from serum samples was analyzed by isoelectric focusing (IEF), High Performance Liquid Chromatography (HPLC) and/or Capillary Electrophoresis and Mass Spectrometry (MS). The search of mutations on the most frequent affected gene, was performed using Sanger sequencing or Whole Exome Sequencing (WES).
Results: Altered transferrin sialylation was observed and six of ten had a CDG type I IEF pattern with increased di- and asialotransferrin. Four patients showed a CDG type II pattern with more than two increased glycoforms. Genetic tests detected the affected genes. Mostly, CDG-I patterns were diagnosed as *PMM2-CDG* and 100% of these patients carried the most frequent variant (R141H). We also detected *ALG2-CDG* patients; a less frequent CDG subtype. To date, only a single *ALG2-CDG* patient has been described. Secondary defects in protein glycosylation could result in potential pitfalls in CDG screening. For the first time a patient with *COL6A2* variants (myopathy), *GALT* variants and atypical clinical presentation of galactosemia, and the third, a protein variant causing changes in transferrin isoelectrofocusing, were diagnosed as genetic metabolic disorders different from CDG.
Discussion: A broad spectrum of new CDG subtypes have been described, most of them presenting normal transferrin analysis. Together new defects in protein glycosylation, different from CDG, were detected leading the CDG screening a big challenge.

P-215

The hepatic clinicopathological features of glycogen storage disease type III

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Background: Glycogen storage disease type III (GSDIII) is an inherited metabolic disease characterized by deficiency of glycogen debranching enzyme (AGL) that leads to the accumulation glycogen. This clinically appears as variable symptoms in the liver and muscle. The only treatment for GSDIII is symptomatic; a diet including cornstarch and increased protein is used to lessen hypoglycemia. However, the long term hepatic symptoms continue to appear despite these interventions. In preparation for a definitive therapy for GSDIII, the natural history needs further characterization to accurately describe the endpoint of a clinical trial. As Duke serves as a tertiary care center for GSD, a natural history study was conducted using our cohort, to better describe the hepatic manifestations of GSDIII.
Methods: We included pediatric participants (defined as age 0 to 21yo 11mo) with GSD III consented to a natural history study. Electronic /paper charts were reviewed for biochemical, radiological and histological profiles. We also reviewed all available liver biopsies, including adults.
Results: We enrolled 26 pediatric patients (mean age 13.7yrs). We analyzed the labs and most recent radiological studies. The mean alanine aminotransferase (ALT) value was 318U/L (range 27-1441U/L) and the mean aspartate aminotransferase (AST) was 319U/L (range 25-2548U/L). Hex4 values were found to be abnormal in many patients. All had hepatomegaly. We reviewed liver biopsies from 11 participants (range 7mo-39yrs); findings included swollen hepatocytes, with evidence of glycogen accumulation in cytoplasm and various stages of fibrosis/cirrhosis.
Discussion: Dietary interventions have improved the life expectancy of individuals with GSDIII, making long term hepatic findings better recognized, albeit unnoticed until later as muscle symptoms prevail. A definitive therapy, one that would prevent the progressive buildup of glycogen in various organs with time, is needed.

P-216

Profiling of intracellular galactose metabolites: potential for galactosemia research

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Background: Patients with classical galactosemia, even with the same genotype, have a highly variable outcome. Unfortunately, there are no biomarkers at present which predict clinical outcome early in life. In order to fill this gap, we developed a new, quantitative method called galactose

metabolite profiling (GMP) which is based on the quantitative assessment of different metabolites produced from galactose in fibroblasts.
Methods: GMP analysis was performed in fibroblasts of three patients with a classical presentation, three patients with a variant presentation (no illness at diagnosis by newborn screening, erythrocyte galactose-1-phosphate uridyltransferase activity 4-9%) and three healthy controls. The following metabolites were analyzed: [$U^{13}C$]-galactose, [$U^{13}C$]-galactose-1-phosphate (Gal-1-P) and [$^{13}C_6$]- uridine diphosphate (UDP)-galactose. The ratio of [$U^{13}C$]-Gal-1-P/ [$^{13}C_6$]-UDP-galactose was defined as the galactose index (GI).
Results: All patient cell lines could be clearly distinguished from the control cell lines and there was a clear difference between patients with a classical and patients with a variant presentation. Variant patients had lower levels of [$U^{13}C$]-galactose and [$U^{13}C$]-Gal-1-P than classical patients (but higher than healthy controls) and higher levels of [$^{13}C_6$]-UDP-galactose than classical patients (but lower than healthy controls) which resulted in a different GI in all groups (mean GI classical patients vs controls $p< 0.001$, classical patients vs variant patients $p< 0.001$).
Discussion: We conclude that GMP in fibroblasts is a sensitive method to determine the residual capacity to metabolize galactose, and allows discriminating between patients with a classical and variant presentation of galactosemia and healthy controls. After further validation in a larger cohort of patients representing the full phenotypic spectrum of galactosemia, GMP could well turn out to be a useful method for early prognostication.

P-217

Combined liver and renal transplantation in a female with Glycogen Storage Disease type Ia caused by an exon deletion.

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Background: Glycogen Storage Disease Ia (GSDIa) is a rare inherited condition resulting from hepatic glucose-6-phosphatase deficiency. Renal manifestations start in childhood and progress silently. Liver and kidney transplantation (LKT) has been previously described.
Case report: An 18 year old Asian girl diagnosed at 6 months with GSDIa (exon 1–3 deletion) was referred to adult services with fasting tolerance of 90 min; required continuous PEG feeding overnight for 3.5 years. Her metabolic control was poor with total cholesterol (4.7-13.2 mmol/L) and triglycerides (8.6-35 mmol/l). Renal function rapidly declined with proteinuria.There was evidence of distal renal tubular acidosis. Kidney biopsy showed moderate tubular atrophy and interstitial fibrosis, a mixture of globally sclerosed, mildly mesangial expanded and normal glomeruli. The proximal convoluted tubules showed extensive vacuolation of the lining epithelial cells and glycogenisation of the nucleus. There was no evidence of a second kidney disease. Despite treatment with enalapril, her eGFR fell from 71 (July'14) to 6 ml/min (Nov'17) when she started haemodialysis. 5 months later she had a cadaver LKT.
Results: Simultaneous LKT was uncomplicated. After 3 weeks, liver function was normal (ALT 34U/L, ALP 157U/L), and kidney function was excellent with creatinine 102 μmol/L and eGFR 50ml/min. She no longer experienced nocturnal hypoglycaemia and her PEG tube was removed. Her lipid profile improved. Medications included statin, vitamin D, aspirin, antihypertensive agents and anti-TB prophylaxis, prednisolone, tacrolimus, mycophenolate mofetil.
Discussion: End-stage renal disease (ESRD) in GSDIa developed as a result of the genetic defect. Combined LKT corrected the metabolic defect responsible for impaired glucose homeostasis, and hyperlipidaemia. It allowed a normal diet, cessation of PEG feeding and an improved quality of life. LKT is an option for patients with GSDIa-related ESRD.
Conflict of Interest declared.

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Quality of blood glucose control and complications in glycogen storage disease type I: data from the swiss registry

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Background: Regular carbohydrate intake to avoid hypoglycemia is the mainstay of dietary treatment in glycogen storage disease type 1 (GSDI). The aim of this study was to evaluate the quality of dietary treatment and glucose control in a Swiss cohort of GSDI patients, in relation to the presence of long-term complications.
Methods: Data of 25 patients (22 GSDIa, 3 GSDIb, median age 20y) from the Swiss hepatic glycogen storage disease registry were analyzed cross-sectionally. Frequency and type of hypoglycemia symptoms were assessed prospectively using a structured questionnaire. Continuous glucose monitoring (CGMS) was performed as part of usual clinical care to assess metabolic control in 14 patients.
Results: Although maintenance of euglycemia is the primary goal of dietary treatment, few patients (n=3, 13%) performed capillary blood glucose measurements regularly. Symptoms of hypoglycemia were present in 13 patients (57 %), but CGMS revealed periods of low glucose (< 4mmol/l) in all patients, irrespective of the presence of symptoms. GSDIa patients with liver adenomas showed a higher frequency and area under the curve (AUC) of low blood glucose than patients without adenomas (frequency 2.7±0.8 vs 1.5±0.7 per day, AUC 0.11±0.08 vs 0.03±0.02 mM/d; $p< 0.05$). The presence of microalbuminuria was also related to the frequency of low blood glucose. Z-Scores of bone density correlated negatively with lactate levels.
Discussion: The quality of glucose control is related to the presence of typical long-term complications in GSDI. Many patients experience episodes of asymptomatic low blood glucose. Regular assessment of glucose control is an essential element to evaluate the quality of treatment, and increasing the frequency of glucose self-monitoring remains an important goal of patient education and motivation. CGMS devices may support patients to detect fluctuations of serum glucose in everyday life and to optimize dietary therapy.

P-219

P.G991A variant in *PHKA2* gene may be one major cause of ketotic hypoglycemia in Japanese children.

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Background: Ketotic hypoglycemia(KH) accounts for 30~50% of hypoglycemic cases during childhood. But its pathophysiology has not been demonstrated fully yet.
Methods: A gene panel consisting of 59 genes associated with hypoglycemia were performed in KH patients. For functional evaluaion of *PHKA2* p.G991A, transient expression of wild-type and variant expression vectors were done in HEK293T cells, followed by measuring phosphorylase b kinase(Phk) activity in cell extracts. Thermal stability of the variant PhK activity was examined using blood cells of one KH boy with this variant.
Results: The gene panel analysis was done for 10 KH cases(9 boys, 1 girl) without hepatomegaly and liver dysfunction. p.G991A variant in *PHKA2* was identified in 3 cases. Successively another 10 KH boys were examined and this variant was identified in 3 cases. In transient expression analysis, the activity of wild-type and the variant PhK were 29.6(±4.5) and 31.0(±8.7) nmole/min/mg, respectively. Residual activities of the patient's sample after incubating at 45 °C for 5 to 10 minutes was about 50 to 60% of those of the control samples after 5 to 10 minutes incubation.
Discussion: Considering the frequency of the variant in Japanese population (about 1 in 200 males), a high frequency of this variant in KH patients (6 in 20) is significant. This may explain why KH is more common in boys. p.G991A variant did not reduce the activity of PhK in our expression analysis. However, the variant protein may be unstable under higher temperatures. This temperature-sensitive character of the variant could explain why children with the variant do not present hepatomegaly but sometimes present hypoglycemia, especially in association with febrile infections.

P-220

The F170V mutation in *PHKA2* causes fasting intolerance without other features of Glycogen Storage Disease Type IX.

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Background: GSDIX is the commonest GSD. Typical features seen in most patients include hepatomegaly, elevated liver enzymes & hyperlipidaemia. Fasting intolerance usually indicates a more severe phenotype. We describe 3 Pakistani origin boys with ketotic hypoglycaemia who are hemizyogus for a novel variant in *PHKA2 despite no other features of GSD.*
Case report: Case 1 is a 6 year old boy found to be hypoglycaemic (2.2 mM) prior to endoscopy. A fasting study showed a 7 hour tolerance. Case 2 is an unrelated 3 year old boy found to be hypoglycaemic (1.2 mM) prior to orchidopexy. A fasting study showed a 4 hour tolerance. Case 3, the 3 year old cousin of Case 2, had a morning blood glucose of 1.7 mM. A formal fasting study showed a 5 hour tolerance. No boy had hepatomegaly or abnormal liver enzymes, lipid profiles, acylcarnitines or organic acids. All improved with a glucose polymer emergency regimen and avoidance of fasting longer than their proven tolerance.
Results: Cases 1 & 2 were found through whole exome sequencing to be hemizygous for a novel maternally inherited c.508T>G (F170V) variant in *PHKA2*. Case 2 was also found to have citrin deficiency. Targeted testing in Case 3 showed he was hemizygous for the *PHKA2* variant but did not carry the citrin mutation. A liver biopsy from Case 1 showed no significant glycogen storage. Phosphorylase kinase activity assayed in red blood cells was clearly abnormal in Case 1 & Case 2 [0.6 & 1.1 nmol/min/mg] compared to control samples [range 6–19 nmol/min/mg].
Discussion: These results demonstrate that the F170V variant in *PHKA2* is pathogenic and is the likely explanation for fasting intolerance in these boys. The mechanism for hypoglycaemia may not be as straightforward as an inability to release stored glycogen and requires further investigation. In the meantime, otherwise unexplained fasting intolerance in a male, particularly of Pakistani origin, may justify sequencing of *PHKA2* even in the absence of typical features of GSD.

P-221

Lipid markers in Glycogen storage disease type I (GSDI): towards assessment of metabolic risk

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Background: Higher risk of developing insulin-resistance (IR) and metabolic syndrome (MS) has been shown in GSDIa patients. Hyperlipidemia is a typical feature of GSDIa. Notably, non-HDL-cholesterol (non-HDL-C), triglycerides (TG)/HDL-C ratio, non-HDL-C/HDL-C ratio and plasma leptin are reliable markers of IR and MS. The aim of the current study was to assess lipid markers in GSDI patients.
Methods: 12 GSDIa patients, 6 GSDIb patients, 26 and 12 age and sex-matched controls were enrolled. Waist circumference (WC), serum glucose and plasma cholesterol (C), TG, HDL-C, LDL-C, leptin were measured. Non-HDL-C, TG/HDL-C ratio and non-HDL-C/HDL-C ratio were also assessed as well as serum insulin and markers of IR (HOMA-IR, QUICKI, ISI).
Results: GSDIa patients showed higher WC, C, TG and insulin levels ($p< 0.001$), non-HDL-C ($p< 0.01$), TG/HDL-C ratio ($p< 0.01$) non-HDL-C/HDL-C ratio ($p< 0.05$) and HOMA ($p< 0.001$) than controls. QUICKI and ISI were lower than controls ($p< 0.001$). Glucose, HDL-C, LDL-C and leptin showed no significant difference. GSDIb patients showed lower C, LDL-C, HDL-C ($p< 0.001$) with no significant difference in WC, glucose, non-HDL-C, leptin, insulin, TG/HDL-C, non-HDL-C/HDL ratio, HOMA, QUICKI, ISI. In GSDIa patients non-HDL-C and TG/HDL-C ratio directly correlated with glucose levels ($p< 0.05$); HDL-C inversely correlated with WC ($p< 0.05$); also, leptin levels directly correlated with insulin and HOMA-IR ($p< 0.05$) and inversely correlated with QUICKI and ISI ($p< 0.05$).
Discussion: Lipid markers are currently employed to assess metabolic risk in the general population. Our findings suggest that non-HDL-C, TG/HDL-C ratio and non-HDL-C/HDL-C ratio might be useful tools for early detection of the risk of IR and MS in GSDIa patients. Correlation data in GSDIa, together with normal lipid markers in GSDIb patients, support the hypothesis that chronic hyperlipidemia and adipose tissue dysfunction might play a role in the development of IR and MS in GSDIa patients.

P-222

Profiling of sugar metabolites for diagnostics of genetic diseases that escape CDG

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Background: The Congenital Disorders of Glycosylation (CDG) have expanded both clinically and in the number of gene defects that are being

classified as CDG. Easy availability of transferrin isofocusing for screening of N-glycosylation disorders has strongly contributed to the discovery of novel CDG defects. Via clinical exome sequencing, an increasing number of genetic defects in sugar metabolism have been discovered, however, with normal transferrin glycosylation, i.e. normal CDG screening. Still, these defects are classified as CDG, such as PGM3-CDG, GFPT1-CDG, GMPPB-CDG, GNE-CDG, etc.
Methods: For research and functional confirmation of gene defects in sugar metabolism, we have established a highly sensitive and specific LC-QqQ mass spectrometry assay via ion-pairing reverse phase LC. The method allows to analyze levels of nucleotide sugars and sugar-phosphates in patient fibroblasts in 6-well plates, blood cells, tissues and model organisms.
Results: As example, low levels of CDP-ribitol were observed in blood cells and fibroblasts of ISPD-CDG patients, which can be used as facile diagnostics. Furthermore, our research focuses on defects in CMP-sialic acid biosynthesis, presenting with contrasting clinical phenotypes, such as myopathy in GNE-CDG, intellectual disability in NANS-CDG and SLC35A1-CDG and skeletal abnormalities in NANS-CDG. By application of our methodology in HAP1 knock-out cells for all steps in sialic acid biosynthesis, we could uncover an unexpected bypass route for NANP, the phosphatase that converts sialic acid-9-P to sialic acid.
Discussion: Application of our methodology to additional cell and animal models will provide improved diagnostics for glycosylation defects that escape routine CDG screening and will allow to elucidate the pathophysiology of the diverse clinical symptoms in patients with a defect in sugar metabolism.

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Skeletal and cardiac muscle involvement in children with glycogen storage disease type III

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Background: Glycogen storage disease type III (GSD III) may present with hepatic disease or may involve both skeletal and cardiac muscles as well. The aim of this work is to assess the prevalence of neuromuscular and cardiac involvement in a group of children with GSD III.
Methods / Case report: 28 children with GSD III, diagnosed by enzyme assay, were enrolled in the study after an informed consent was obtained from their parents/guardians and after the study protocol was approved by our institutional ethical committee.
Results: Their mean age was 6.6+3.1 years. All cases were assessed neurologically by clinical examination, electromyography (EMG), and nerve conduction velocity. The heart was examined clinically by electrocardiogram and echocardiography. Seventeen patients (61 %) had myopathic changes by EMG, three of them had associated neuropathic changes. Creatine kinase (CK) was elevated in all myopathic cases except one. Children with myopathic changes were significantly older (p=0.02), and CK was significantly higher (p< 0.0001). Nine cases had left ventricular (LV) hypertrophy, seven of them had myopathic changes by EMG.
Discussion: Myopathic changes are not uncommon in children with GSD III. Myopathic changes tend to occur in older age and are associated with higher CK level. Cardiac muscle involvement is less common in this age group and may, on occasion, occur alone without skeletal muscle involvement. Despite mild degrees of affection in this age group, it is recommended to perform prospective annual screening using EMG and echocardiography in order to augment dietary therapy regimen to prevent progression to life threatening complications.

P-224

Comparative liver pathology in Glycogen Storage Disease type III

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Background: Glycogen Storage Disease type III (GSD III) is an autosomal, recessive disorder caused by a deficiency of glycogen debrancher enzyme from mutations in the amylo-1,6-glucosidase (*AGL*) gene causing hepatic and muscular disorders in those affected. Progressive hepatic fibrosis is commonly seen in patients with GSD III and liver failure, hepatic adenomas, and hepatocellular carcinoma have been reported in some cases. We had previously characterized the disease progression in curly-coated retrievers (CCR), a large breed canine model that proved difficult to maintain in a laboratory setting.
Methods: We have created a more manageable canine model via crossbreeding the CCR dogs with smaller, mixed-breed dogs and generated a novel *AGL* KO mouse model via deletion of exons 6–10 from the *Agl* gene. Both animal models were followed up to 18 months of age to monitor for disease progression. In addition, they were compared with a previously undescribed cohort of child and adult GSD III patients (n=11) with hepatic fibrosis.
Results: Both animal models developed similar liver pathologic features shown in human patients, such as elevated liver enzymes, increased hepatic glycogen content, and progressive hepatic fibrosis leading to cirrhosis. Hepatocellular adenoma was also described in an 18-month *AGL* KO mouse. These features correspond to findings of similar elevations in ALT and AST, as well as hepatic fibrosis in GSD III patients.
Discussion: Robust animal models, such as these, can prove invaluable in the development of novel therapeutic agents to prevent hepatic fibrosis and other complications of GSD III.
Conflict of Interest declared.

P-225

Genetic analysis of Glucose transporter-1 deficiency syndrome (GLUT1DS)

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Background: Classic patients with glucose transporter-1 deficiency syndrome (GLUT1DS) present hypoglycorrhachia, drug-resistant epilepsy, developmental delay, a complex movement disorder and, in the 50% of the cases, an acquired microcephaly. Furthermore, patients respond to ketogenic diet.
Methods / Case report: In this study, we have included 49 suspected cases of GLUT1DS with a CSF glucose level ranged from 1.1-2.9 mmol/L (glucose CSF /blood *ratio*: 0.35-0.63). We have performed the sequencing of the *SLC2A1* gene by Sanger combined with MLPA and/or next generation sequencing using two NGS panels. PANEL 1 - an in-house customized

exome sequencing panel to capture the exome including the entire sequence of *SLC2A1*. PANEL 2 - an extended panel that includes all the known (in 2013) disease-associated genes described in the OMIM database.
Results: As expected, we have detected 27 patients with variations in *SLC2A1*. The majority were *de novo* mutations, while five mutations were associated to maternal or paternal inheritance (missense variants). The mutational spectrum in *SLC2A1* includes two large deletions, two small deletion, one duplication and 22 nucleotide changes (nineteen likely missense, one nonsense, and two splice site mutations). Seventeen variants are novel, six loss-of-functions and eleven likely missense, four with unknown significance following the ACMG guidelines. In twenty-two cases no pathogenic mutations in *SLC2A1* were detected and were further analysed by PANEL 2, allowing the detection of pathogenic variants in five different genes related to ion channels, transcriptional factor or protein cellular trafficking.
Discussion: Our results show that a response to ketogenic diet, drug-resistant epilepsy and/or hypoglycorrhachia is not a pathognomonic marker for GLUT1DS. Moreover, the results highlight the fact that genetic analysis should be a must-have for GLUT1DS classification.

P-226

Clinical, biochemical and molecular spectrum of glycogen storage disorders in India : A ten year experience from a tertiary center.

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Background: Glycogen storage disorders (GSDs) may affect liver, muscle, kidneys, and heart. Long-term complications constitute the main disease burden over time, especially in developing countries like India.
Methods: Retrospective data of all patients with GSD (n=46) who presented to our Department during the time period 2008–17 were screened. Records of the clinical examination, demographics and relevant investigations including liver biopsy were obtained. Molecular testing data was collected and genetic studies were offered to cases without genetic confirmation.
Results: In our cohort, GSD 1a was present in 15 patients, type Ib, III, IV, V, IX, XI were present in 3, 19, 4, 2,2 and 1 patient, respectively. In patients with GSD-I, mean age at presentation was 4 years. Main presentations included short stature (70%), abdominal distension (82%). Major clinical and laboratory findings: Hepatomegaly (100%), anemia (81%), renal enlargement (65%), osteopenia/fractures (7%), increased ALP (61%), transaminitis (75%), increased triglycerides (100%), hyperuricemia (89%). Liver biopsy confirmed the diagnosis in 88%. One patient was found to have hepatic adenoma. 80% of GSD-Ib patients had recurrent bacterial infections with a mean ANC of 900. In GSD-III, increased CK (78%), cardiac involvement (65%), liver enzyme elevation as well as hepatomegaly was present in all patients. RBC glycogen was raised in 4 cases. Liver enzymes were significantly higher in GSD-III ($p< 0.05$). In the four cases of GSD-IV, all had hepatomegaly, portal hypertension, and high RBC glycogen was present in one case. Molecular diagnosis was performed in 23 cases with a diagnosis in 19 and 5 novel mutations.
Discussion: We report the largest Indian cohort comprising six types of GSD. Type III was the commonest consistent with the previously published Indian reports. RBC glycogen, a known marker of GSD III, could be present in type IV also. Earlier, histological confirmation was the mainstay of diagnosis, now it has largely been replaced by non-invasive and accurate gene testing.

P-227

The impact of classical galactosaemia on daily living – a Swiss observational study

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Background: Despite newborn screening and early treatment with a galactose-restricted diet, many patients with classical galactosaemia (CG) develop long-term complications, such as impaired neurocognitive and neuropsychological development. The aim of the study was to improve our understanding of the impact of CG on daily life.
Methods: Twenty-two adult patients with confirmed CG, i.e. 58% of all known Swiss patients, were assessed with different self- and informant-reported questionnaires, for their executive functioning (BRIEF-A), adaptive behavior (ABAS-3) and level of anxiety and depression (HADS).
Results: Analysis of the BRIEF-A revealed no general deficits but significant problems in the field of planning and organizing while on the other hand, subjects appeared to perform above average in the ability to inhibit and control impulses and actions. ABAS-3 revealed substantial deficits in adaptive behavior in general, and especially in skills needed to autonomously function both at home and outdoors. In the HADS, the subjects did not show elevated levels of anxiety and depression.
Discussion: Interestingly, adaptive behavior appears to be more affected than executive functions in our CG population. Particularly, skills needed to autonomously function at home, and take care of domestic chores, to communicate, and to engage in leisure time tend to be most affected. While others describe a higher prevalence of anxiety and depression in CG patients, this was not the case in our study population. The HADS may not be a sensitive tool to detect these symptoms in CG patients. Due to the yet unclear factors that lead to problems in neurocognitive performance and the considerable heterogeneity among the individuals, it is essential to test every individual for their neurocognitive performance to find the individuals at risk. This would allow the physicians to start interventions sooner, and more adjusted to the specific needs of their patients.

P-228

Glycogen storage disease type I B and amyloidosis: should we look out for this complication?

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Background: Glycogen storage disease type I B (GSD IB) (OMIM#232220) is an inherited metabolic disease caused by functional deficiency of glucose-6-phosphatase due to mutations in the *G6PT1* gene encoding glucose-6-phosphate translocase. Recurrent infections, neutropenia, and chronic inflammatory bowel disease have been recognized as the distinctive features of GSD IB. We present a 17-year-old female patient with GSD IB who was followed since infancy and developed amyloid nephropathy.
Case report: A girl born to related parents had hypoglycaemia (13 mg/dL) on postnatal day one. Hepatomegaly, hyperuricemia,

hypertriglyceridemia, and hyperlactatemia were detected. Her sister had died at the age of 14 months from GSD IB. Deoxyglucose transport in PMN cells (1.7 nmol/min/mg protein, normal 3.5-9.5) was defective. The *SLC37A4* gene analysis revealed homozygous c.1042_1043delCT mutation. She received dietary treatment with good metabolic control after 2 months. Recurrent infections subsided after 2 years. She did not have bowel complaints. She received growth hormone treatment for 3 years because of short stature. She had neutropenia but G-CSF was not recommended. She was referred at 17 years with paleness, diffuse edema, hyperpnea, diarrhea, abdominal distension, and hepatosplenomegaly. Blood urea 118 mg/dL, creatinine 1.1 mg/dL, albumin 1.64 g/dL, uric acid 13.1 mg/dL were abnormal. Urine analysis showed proteinuria (+3). Renal function progressively decreased leading to renal failure, necessitating renal replacement therapy. Biopsies revealed amyloid deposits in the gastrointestinal tract and kidney.
Results-discussion: Renal complications such as focal segmental glomerulosclerosis, nephrocalcinosis, gout nephropathy, cresenteric glomerulonephritis and Fanconi-like syndrome are common in GSD I. Chronic inflammation in GSD IB may potentiate amyloidosis and patients should be evaluated for this complication.

P-229

Missed cases of hereditary fructose intolerance incidentally diagnosed via non-targeted genetic testing

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Background: Hereditary Fructose Intolerance (HFI) is caused by biallelic loss-of-function mutations in the *ALDOB* gene. The gene product, aldolase B, is responsible for the catabolism of fructose-1-phosphate and fructose-1,6-bisphosphate. Although HFI was first reported in an adult with "idiosyncracy to fructose", classical teaching focuses on recognizing infantile acute liver failure following first exposure to fructose, sucrose, or sorbitol containing feeds. Patients with protean manifestations are prone to missed diagnosis.
Case reports / results: We report the incidental diagnosis of HFI in 4 patients via non-targeted genetic testing ordered for alternative indications. Patient 1 is a 5-year-old female who presented at 18 months with short stature and developmental delay. Whole exome sequencing at 4 years revealed compound heterozygosity for the p.A150P and p.A175D mutations in *ALDOB*. Patient 2 is a 3-year-old female who presented at 2 years following the diagnosis of HFI in her sister (*i.e.,* Patient 1). Presymptomatic *ALDOB* sequencing revealed the same mutations. Patient 3 is a 30-year-old female who presented at 30 years with irregular menses and interest in assistive reproductive technologies. Carrier screening revealed compound heterozygosity for the p.A175D and c.113-1_115delGGTA mutations in *ALDOB*. Patient 4 is a 68-year-old male who presented at 67 years with "indeterminate" non-targeted direct-to-consumer testing. Diagnostic *ALDOB* sequencing revealed homozygosity for the p.A150P mutation.
Discussion: The lack of newborn screening and diagnostic clinical and biochemical findings for HFI makes establishing the diagnosis difficult. Improved accessibility of non-targeted comprehensive sequencing has increased the probability of doing so incidentally. HFI should be considered for patients with non-specific findings (*e.g.*, failure to thrive, developmental delay), and for asymptomatic individuals with significant aversion to sweets.

P-230

GALK deficiency – more than cataracts

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Background: Galactokinase deficiency (GALK-D) is a rare autosomal recessive disorder of galactose metabolism. Cataract and *pseudotumor cerebri* have been described due to galactitol accumulation. Treatment consists of a galactose restricted diet, although cataract seems irreversible when diet is introduced beyond four to eight weeks of life.
Case report: Authors present three cases of GALK-D (Caucasians aged 16 months, 6 and 8 years) followed at a Portuguese Metabolic Diseases Unit. All patients presented with bilateral cataracts (ages between 5 months and 4 years); the youngest had marked pendular nystagmus, another developed *pseudotumor cerebri* refractory to acetazolamide. All patients showed elevated values of urinary galactitol and galactose with normal enzymatic activity for GALT (galactose-1-phosphate uridylyltransferase). One patient was diagnosed by enzymatic assay with reduced GALK activity (0.6mU/g Hb; ref.v.:17.3-39.7), the other two underwent molecular analysis that revealed compound heterozygosity in the *GALK1* gene – p.D46N/p.R256W and p.T352K/p.R256W, with two novel mutations identified – p.D46N and p.T352K. All patients initiated a galactose restricted diet and surgical cataract removal, with marked improvement of nystagmus, visual acuity and *pseudotumor cerebri*.
Results / discussion: Apart from cataracts, other symptoms have been rarely described in GALK-D patients. Nystagmus was previously described in only one published case, and its presence may indicate a worse visual outcome. The two novel mutations (p.D46N and p.T352K) appear to be pathogenic according to bioinformatic analysis using the PolyPhen2 program, but analysis of the enzymatic activity of GALK should be performed in these patients for unequivocal determination of pathogenicity.

P-231

Biochemical and molecular characterization of Galactokinase deficiency - possibility of a common mutation in the north Indian population.

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Background: Galactokinase (GALK) catalyses the first committed step of the Leloir pathway and its deficiency causes Galactosemia type II. Screening for congenital cataracts often includes the enzyme. Though its incidence its very low in Asian population, identification is essential as this is treatable and recurrence can be prevented.
Methods: Retrospective data was collected for the period of 2002–18 for all patients with congenital cataract and patients with GALK deficiency were screened. Their clinical records and biochemical investigations (GALK enzymes) were analysed. Molecular studies were obtained and suggested to patients where previously missed. Follow-up of the diagnosed cases was performed.

Results: Amongst 73 patients with congenital cataract presenting to genetic clinic at our hospital, 11 were diagnosed with GALK deficiency. The mean age at presentation was 1.5 years. All patients were from north India. Consanguinity was present in 2 patients. Cataract was the presenting complaint in all with additional complaint of developmental delay in one. GALK enzyme levels were performed in all patients with a mean value of 9.9 nmol/min/gHb. All patients had elevated galactose, normal galactose-1-phosphate. Molecular studies were done in three patients showing a single homozygous truncating mutation in *GALK* gene, c.410delG; p.Glu137Valfs*27. Dietary galactose restriction was not being followed in all. On follow up, children faired very well even while being on normal diet. One child with associated developmental delay at diagnosis improved upon dietary restriction.
Discussion: The mutation identified in our patients has been reported by Yasmeen et al. in 6 members of a consanguineous Pakistani family. The geographical closeness further strengthens the possibility of a common 'Indian' mutation. GALK deficiency is an important cause of cataract and must be evaluated in all patients. The role of dietary galactose/lactose restricted diet is not substantiated.

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Clinical and histopathological phenotyping in a cohort of 17 patients with *GYG1*-related polyglucosan body myopathy

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Background: The aim of this study was to detail the clinical and morphological features in patients with polyglucosan body myopathy 2 associated with *GYG1* gene mutations, a recently described muscle glycogenosis.
Methods / case report: We retrospectively analysed muscle symptoms and muscle biopsies from 17 patients from 12 families with pathogenic *GYG1* gene mutations.
Results: 7 patients were male and 10 patients were female. Age at onset varied from 15 to 79 years. Initial symptom was lower limb girdle weakness (4 patients), distal lower limb weakness (3 patients), upper limb girdle weakness (2 patients), muscle fatigability (2 patients), scapuloperoneal weakness (1 patient), asymmetric hand and lower proximal limb weakness (1 patient), exercise intolerance followed by limb girdle weakness (1 patient), and sports difficulties (1 patient). Clinical course was slowly progressive, with development of weakness to proximal muscles in patients with an initial distal phenotype. Mild axial weakness was found in 3 patients. Strikingly asymmetric weakness was noted in 6 patients. Serum CK were normal or slightly elevated in 8 patients, and highly elevated in 9 patients. None of the patients developed cardiac or respiratory involvement. Muscle biopsy showed vacuoles filled with hyperintense PAS-positive material partially resistant to α-amylase in 5-50% of fibers (14 patients). All patients presented one homozygous or two compound heterozygous *GYG1* mutations. 9 patients were homozygous and 3 were heterozygous for the common c.143+3G>C mutation.
Discussion: Polyglucosan body myopathy 2 shows an extremely variable clinical phenotype including limb girdle, scapulo-peroneal or distal, often-asymmetric weakness. Muscle fatigability and exercise intolerance are rare manifesting symptoms. On the other hand, the histopathologic phenotype is homogenous and easily recognisable. A multicentre international natural history study will be useful in view of a possible therapeutic approach.

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Motor function evaluation of 17 Turkish infantile Pompe patients: Çukurova University experience

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Background: Infantile Pompe disease(IPD) is a rare lysosomal storage disorder caused by deficiency of acid maltase. It presents usually during the first weeks of life with hypertrophic cardiomyopathy, hypotonia, macroglossia, and progressive muscle weakness. Although enzyme replacement treatment (ERT) is a life-saving therapy, effectiveness of muscle function on mobility is not evaluated in detail.
Methods: The following tests were performed on 17 patients from a group of 19 mobile patients and also on healthy children and patients with Duchenne muscular distrophy (DMD): the 6-minute walk test (SMWT), timed 10-meter walk test (10mWT), quick motor function test (QMFT, elasticity, tonus and stiffness of biceps, vastus medialis, gastrocnemius and tibialis anterior muscles by myotonometry & MicroFET3), 3 minute stair climb test (3MSCT), and muscle strength (hand-held dynamometry).
Results: Out of 39 patients, 24 were alive, 2 were on mechanical ventilation and 3 were younger than 18 months of age. Ten girls and 7 boys were started on ERT at 3,5 ± 3,1 months of age. Their current ages were 57,6 ±25,9 months, and the mean duration of ERT was 53,2±27,8 months. The p.L299P (c.896T>C) was the most common mutation.There was a significant difference in SMWT and 10mWT, between healthy children and IPD ($p< 0,005$). SMWT and 10mWT were nearly similar for DMD and male IPD patients. The tonus of *vastus medialis*, elasticity of *vastus medialis* and *gastrocnemius* muscles, strength of hand-held were significantly higher in healthy children compared to IPD patients ($p< 0,005$). Distal muscles of lower extremities and *biceps brachii* muscles in IPD were less affected.
Discussion: ERT has increased the survival in IPD with respect to cardiomyopathy; but there are sparse data on ambulatory patients. QMFT is valid for non-classic Pompe, however, there are a few reports with limited number of IPD patients. We report motor function evaluation results of a large group of IPD patients.

P-234

Infectious and gastrointestinal complications in glycogenosis of type Ib : study of a french cohort

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Background: Glycogenosis type Ib (GSD Ib), an inherited disease of glycogen metabolism, causes severe hypoglycaemia, but also infections and « Crohn-like » inflammatory bowel disease (IBD) that sometimes significantly impair patient's quality of life.

Methods: The aim of this retrospective study is to describe these infectious and gastrointestinal complications in patients followed in two French centers. Data from 9 patients (3 girls, 6 boys) were collected, during an average follow-up time of 19.1 years.
Results: Patients were diagnosed at 0.8 years on average. Infections began earlier than IBD (at 1.7 years against 3.8 years on average). The number of acute hospitalizations was 0.7/year, with an average of 0.4/year for infectious reasons and 0.4/year for digestive reasons. Harvey Bradshaw's score, originally used to measure clinical activity of Crohn's disease, was able to distinguish two significantly different groups of severity for gastrointestinal complications in our cohort : «severe» and «less severe». As expected, patients in the «severe» group presented more serious gastrointestinal features but also worse overall clinical and biological characteristics than other patients: earlier neutropenia (median 0.3 years vs. 1.5 years, p=0.046) and higher number of acute hospitalizations (median 1.3/year vs. 0.2/year, p=0.014), including for infectious reasons (median 0.8/year vs 0.2/year, p=0.014). Seven patients were treated with G-CSF and cotrimoxazole, 2 with 5- aminosalicylic acid, and 4 (all in the «severe» group) with a polymeric solution enriched in anti-inflammatory cytokines TGF-β. One patient required immunomodulatory treatments.
Discussion: Infections and IBD are therefore major complications in GSDIb but their severity is inconsistent. Dietetic treatment with specific anti-inflammatory agents seems particularly appropriate in these patients.

13. Disorders of fatty acid oxidation and ketone body metabolism

P-235

X-linked adrenoleukodystrophy (X-ALD) in Argentina: Ten years of follow-up in a cohort of patients

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Background: X-ALD is an inherited metabolic disease characterized by increased concentrations of very long-chain fatty acids (VLCFAs) in plasma and tissues, due to a defect in peroxisomal VLCFA β-oxidation, associated with mutations in the *ABCD1* gene.
Case report: Our aim was to report outcomes as measured by health status, disease progress and damage in a group of Argentinean patients with X-ALD, monitored prospectively for 10 years. The diagnosis of X-ALD patients was established by measuring VLCFA concentrations in plasma. Genomic variants were analyzed by PCR/sequencing of the *ABCD1* gene. The validation of identified variants was accomplished through a combination of two methods: a) bioinformatic tools b) functional analysis. Disease progress was assessed by clinical and laboratory measures of disease progress and evaluation of disease outcome.
Results: Throughout 10 years we made clinical, biochemical and genetic diagnoses in a total of 14 Argentine families. We identified 16 genomic changes, 10 mutations, (8 of which are novel, and two known mutations) and 6 polymorphisms (3 novel and 3 known). The mutations were widely distributed along the *ABCD1* gene and included missense, frameshift and splice site mutations. The majority of X-ALD patients in our study group had non-recurrent (89%) mutations, apart from two patients that had the same mutation. We could confirm the carrier status of 20 women under study, of whom 4 were symptomatic.
Discussion: This study represents the clinical, molecular and functional characterization of the largest cohort of Argentinean X-ALD patients studied to date. In addition, it increases the mutational spectrum of *ABCD1* gene allelic variants and facilitates genetic counselling and prenatal diagnosis in affected families. Our results, in accordance with the published data, confirms that there is no genotype-phenotype correlation.

P-236

A novel loss of function *FLAD1* variant, causing riboflavin-responsive multiple acyl-CoA dehydrogenase deficiency (MADD)

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Background: MADD is a heterogeneous disorder affecting multiple flavoproteins involved in energy metabolism. Presentations range from severe to mild forms, with response to riboflavin. Genetic testing for MADD comprises analysis of *ETFA*, *ETFB*, *ETFDH* and genes involved in riboflavin transport and flavoprotein biosynthesis. Deficiency of FAD synthase (FADS) caused by *FLAD1* variations was identified as a cause of MADD with potential effect of riboflavin treatment. We describe a novel, *FLAD1* loss-of-function (LOF) variant causing riboflavin-responsive MADD myopathy in an 8-year-old boy.
Methods / case report: The patient referred to the metabolic service with a positive newborn screen for medium-chain acyl-CoA dehydrogenase deficiency. Confirmatory biochemical analysis was diagnostic for MADD, but with negative findings in *ETFA*, *ETFB* and *ETFDH* genes. Riboflavin was initiated with biochemical and clinical response. By 8 years of age, the child had developed myopathy, and an expanded gene analysis revealed homozygosity for a *FLAD1 variant,* c.745C>T (p.Arg249*). Functional assays were performed to better characterize the variant.
Results: Immunoblotting showed almost no detectable full-length FADS protein in patient fibroblasts, but revealed a 26 kDa band, corresponding to the recently characterized FADS isoform, which lacks the N-terminal region of the protein containing the c.745C>T variant, but with a functional FADS domain in the C-terminal. While there was almost total lack (< 10%) of FAD synthesis rate, cellular FAD content was 54% that of controls.
Discussion: Compared to the previously reported cases with biallelic LOF *FLAD1* variants, the present case shows a better response to riboflavin treatment, stressing the importance of early recognition and treatment. Our data support earlier speculations that FADS is not the rate-limiting step in FAD synthesis and that residual synthesis activity, possibly complemented by the 26 kDa truncated FADS protein, determine cellular FAD content.

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Mitochondrial bioenergetics dysfunction caused by fatty acids accumulated in VLCAD deficiency in rat skeletal muscle

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Background: Very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency is the most frequent disorder of long-chain fatty acid β-oxidation with an incidence of 1:50,000-1:100,000 newborns. It is

biochemically characterized by accumulation of long-chain fatty acids (LCFA) and carnitine derivatives, especially cis-5-tetradecenoic (Cis-5) and myristic (Myr) acids. The disease is clinically heterogeneous, although patients usually present cardiomyopathy, hepatopathy with recurrent hypoketotic hypoglycemia, as well as muscle weakness, myalgia, episodic myoglobinuria and rhabdomyolysis. The manifestations are mainly induced by catabolic situations such as fasting, exercise, illness and fever that are also associated with marked rise of the accumulating metabolites concentrations.
Methods: We investigated the effects of Cis-5 and Myr on important parameters of bioenergetics (high-resolution respirometry, ATP production and anisotropy) in skeletal muscle mitochondrial preparations.
Results: Myr and Cis-5 significantly increased state 4 respiration in skeletal muscle mitochondria using glutamate plus malate (GM), or succinate as substrates, indicating an uncoupling behavior. In addition, these fatty acids markedly decreased state 3 and uncoupled respiration, suggesting a metabolic inhibition. In this regard, we observed that the inhibitory effects were more pronounced with GM and Myr rather than with succinate and Cis-5. We also observed that Myr and Cis-5 markedly decreased ATP production without altering membrane fluidity, making unlikely a destabilization of mitochondrial membranes by interaction of these fatty acids with membrane phospholipids.
Discussion: We propose that the mitotoxicity caused by the fatty acids that most accumulate in VLCAD deficiency disrupting mitochondrial respiration may be possibly associated with the muscular symptoms and skeletal abnormalities frequently observed in patients affected by this disease.
Financial support: PROPESQ/UFRGS, FAPERGS and CNPq.

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Reduction of acylcarnitines restores electrophysiological abnormalities in VLCAD deficient hiPSC-cardiomyocytes

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Background: Patients with very long-chain acyl-CoA dehydrogenase deficiency (VLCADD) are at risk of developing arrhythmias. Treatment options are scarce, partly because the underlying mechanism leading to arrhythmias in these patients is unknown. Electrophysiological derangements may be related to (1) energy shortage because of defective fat use, or (2) accumulation of long-chain acyl-CoAs and/or long-chain acylcarnitines. We used a pharmacological approach involving either enhanced mitochondrial biogenesis or substrate reduction in human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) derived from VLCADD patients.
Methods: We measured electrophysiological and biochemical parameters in CM from one control and two VLCADD patient-derived hiPSC lines: VLCAD1 (p.V283A/p.E381del), and VLCAD2 (homozygous c.104delC). We used two strategies to reduce accumulation of intermediates by pre-incubation with (1) 50 μM resveratrol or (2) 100 μM etomoxir.
Results: Both VLCADD hiPSC-CM lines accumulated long-chain acylcarnitines. Action potentials, measured with patch clamp and Kir2.1 injection via dynamic clamp, were shorter and displayed lower amplitudes in VLCADD. The susceptibility to delayed afterdepolarizations (DADs) was increased in both VLCADD lines. In VLCAD1, but not in VLCAD2, long-chain acylcarnitine accumulation was decreased by resveratrol. Accordingly, action potentials were normalized and amount of DADs was reduced. Etomoxir led to reduction of acylcarnitines and reduced amount of DADs in both lines.
Discussion: hiPSC-CM of VLCADD patients show accumulation of long-chain acylcarnitines, shorter action potentials, and a higher susceptibility to DAD generation, an important cellular mechanism for arrhythmias. Reduction of acylcarnitine accumulation by etomoxir reduced DADs in two patients cell lines, indicating that DADs in VLCADD hiPSC-CM are caused by acylcarnitine accumulation rather than energy shortage.

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Effects of GSK773, an AMPK activator, on metabolism and differentiation of Carnitine Palmitoyl Transferase 2 (CPT2) deficient myotubes

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Background: Carnitine Palmitoyl Transferase 2 (CPT2) deficiency is among the most common inherited defects of mitochondrial fatty acid oxidation (FAO). A frequent phenotype is an early adult-onset myopathy characterized by myalgia and recurrent episodes of rhabdomyolysis usually triggered by prolonged exercise or fasting. To date, there is no treatment of this disorder other than dietary management. AMPK is considered as a potential therapeutic target in many common metabolic disorders associated with mitochondrial dysfunctions.
Methods/results: We tested the therapeutic potential of a new direct AMPK activator GSK773 provided by GlaxoSmithKline (GSK) in myotubes from 4 CPT2-deficient patients. We show that GSK773 is able to stimulate residual FAO capacities in a dose- and time-dependent manner. Correction of CPT2 defect is achieved after treatment with GSK773 at 30 μM for 48h. Western-blots analysis shows that GSK773 increases the amount of CPT2 mutant protein. Analysis of acylcarnitine intermediates in the culture media shows that CPT2-deficient myotubes exhibit an accumulation of C16-acylcarnitines that is significantly decreased after GSK773 treatment. Surprisingly, immunofluorescence and Xcellligence (real-time monitoring of cell culture) show an impaired differentiation process in CPT2-deficient myotubes that is corrected by GSK773. We also show that GSK773 induces a shift in myosin-heavy-chain isoforms toward slow oxidative fiber types, improves the quality of mitochondrial network and increases ROS levels, suggesting that GSK773 might represent an exercise mimetic. From a mechanistic point of view, siRNAs experiments showed the crucial roles of AMPK and PGC-1alpha in the signalling cascade triggered by GSK773.
Discussion: Altogether these results suggest that GSK773 improves metabolic and structural parameters in CPT2-deficient myotubes and that AMPK might represent a highly relevant therapeutic target for pharmacological correction of inborn CPT2 deficiency.

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Investigation of the beta-oxidation process in MCAD-deficient patients with normal enzyme activity

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Background: We have recently identified individuals with consistent elevation of serum octanoylcarnitine (C8) levels but normal medium-chain acyl-CoA dehydrogenase (MCAD) activity, measured using octanoyl-CoA as a substrate. In order to verify whether these individuals are MCAD deficient, beta-oxidation activity was measured in their peripheral blood mononuclear cells (PBMCs) using a stable isotope-labeled fatty acid, in addition to *ACADM* gene analysis.
Methods: Blood samples were collected from 8 patients with normal MCAD activity but increased serum C8, 2 patients with decreased MCAD activities, and 24 healthy adults. PBMCs were fractionated and cultured in a medium containing [$^2H_{31}$] (d_{31})-palmitic acid, and acylcarnitines in PBMCs were quantified using tandem mass spectrometry.
Results: In controls, the ratio of d_7-butyrylcarnitine to d_{11}-hexanoylcarnitine ($d_7C4/d_{11}C6$) ranged from 1.82 to 4.12, $d_{11}C6/d_{15}C8$ from 1.74 to 5.38, and $d_7C4/d_{15}C8$ from 4.69 to 13.58. In all 10 patients, $d_7C4/d_{11}C6$ (0.02-0.47) and $d_7C4/d_{15}C8$ (0.00-2.46) ratios were decreased as compared to controls, while $d_{11}C6/d_{15}C8$ ratios in the 8 patients with normal MCAD activity ranged from 1.57 to 5.03.
Discussion: The decreased $d_7C4/d_{11}C6$ and $d_7C4/d_{15}C8$ in the present patients indicated the metabolic block in beta-oxidation, and all patients were diagnosed with MCAD deficiency in combination with the confirmation of pathogenic variants in the *ACADM* gene. Acyl-CoA dehydrogenases catalyzes a series of substrates and it might be difficult to evaluate the enzyme activity using a single substrate, especially in patients with a milder form of MCAD deficiency. Our assay system is useful to identify the specific enzymatic dysfunction in fatty acid oxidation disorders.

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Induction of fatty acid omega-oxidation pathway in 2-hydroxyacyl-CoA lyase knockout mice

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Background: Peroxisomal fatty acid (FA) alpha-oxidation is an essential pathway for the degradation of beta-carbon methylated FAs such as phytanic acid. One enzyme in this pathway is 2-hydroxyacyl-CoA lyase (HACL1) which is responsible for the cleavage of 2-hydroxyphytanoyl-CoA into pristanal and formyl-CoA. Hacl1 deficient mice do not present with a phenotype, unlike other deficiencies in alpha-oxidation enzymes such as phytanoyl-CoA hydroxylase deficiency in Refsum's disease mice in which neuropathy and ataxia are present.
Methods: Liver from wild-type (WT) and Hacl1 knockout (KO) mice fed a high phytol diet were obtained for lipidomic and proteomic analysis. Tissues were homogenized followed by protein precipitation and trypsin digestion for untargeted proteomic liquid chromatography-mass spectrometry analysis and FA hydrolysis, extraction, and derivatization for FA gas chromatography–mass spectrometry analysis.
Results: The liver FA profile in the WT and KO did not show a significant difference with the exception of 2-hydroxyphytanic acid detection in the KO liver. Proteomic analysis showed a 40 fold decrease in the abundance of Hacl1 protein ($P< 0.05$) and increase of 2 fold and above in the abundance of proteins involved in PPAR signalling, peroxisome proliferation and omega-oxidation, particularly Cyp4a10 and Cyp4a14. These proteins were not upregulated in KO mice fed a normal diet.
Discussion: A phytol diet in Hacl1 KO mice induces the omega-oxidation pathway for phytanic acid degradation. This alternate pathway appears sufficient to degrade FAs without manifesting the typical phenotype in alpha-oxidation disorders.

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Functional analysis of mutant recombinant HSD17B10 proteins using an *E. Coli* expression system

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Background: HSD10 disease is a rare X-linked recessive disorder caused by a mutation in the *HSD17B10* gene. HSD10 is a multifunctional protein which has three functions in: (1) Isoleucine metabolism, as 2-methyl-3-hydroxybutyryl-CoA dehydrogenase (2M3HBD), (2) the metabolism of neuroactive steroids, as 17β-hydroxysteroid dehydrogenase and (3) the processing of mitochondrial tRNA transcripts, as mitochondrial RNaseP. The clinical severity of this disorder ranges from a severe neonatal form to an atypical form. In Japan, we identified two cases of the atypical form in which patients had p.A154T or p.A157V mutations and one case of the severe form in which the patient had the p.R226Q mutation.
Methods: The *HSD17B10* gene was incorporated into pET28a and the vectors containing wild-type or mutant cDNA (p.A154T, p.A157V, p.R226Q) were used to produce high-purity recombinant protein in an *E. coli* expression system. The activity of 2M3HBD, an enzyme in the isoleucine metabolic pathway, was measured by monitoring the enzymatic reduction of NAD^+ to NADH using a spectrophotometric coupling assay.
Results: The activity of mutant 2M3HBD enzyme was significantly decreased compared to wild-type. Also, enzyme activity was lower for the p.R226Q mutation compared to the p.A154T and p.A157V mutations.
Discussion: In this experiment, a correlation was identified between the activity of 2M3HBD, an isoleucine metabolic enzyme, and the clinical severity of HSD10 disease. Recently, several reports have suggested that 17β-hydroxysteroid dehydrogenase activity modulates the neurological severity of HSD10 disease and another report has suggested that dysfunction of RNaseP is also correlated with clinical severity. It is important to know which of these enzymatic functions is more important in this regard. Accumulation of additional cases and further studies on the molecular mechanism are necessary for better understanding of this disorder. We are planning to proceed with further functional analysis.

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Phenotypic variability in 30 children and young adults with Carnitine Palmitoyl Transferase (CPTII) deficiency in Greece

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Background: CPT II defidiency is an Inborn Error of Metabolism and belongs to the β-oxidation defects. It presents with muscle and liver disease, cardiomyopathy, very high CK levels, muscle weakness, cramps after exercise, myoglobinuria, rhabdomyolysis, acute renal failure, and abnormal acylcarnitine species (C16-C18). Usually the risk factors for the onset of symptoms are infections and exercise.
Methods / case report: Our study describes the phenotypic variability of our patients and the correlation with their genotype (21 children and 9 adults). Diagnosis was confirmed by sequencing of *CPT2* gene.
Results: Children: Age of first onset ranged from 2–8 years. All presented with high CK levels (40.000-300.000 mg/dl) and liver disease (16 after infection and 5 after exercise intolerance). Six mutations were found in exons 2, 3 and 4. Eleven patients were homozygotes for the common c.338C>T mutation, and 10 were compound heterozygotes. Adults: Age of first onset of symptoms ranged from 24–40 years. All presented with high CK levels (90.000-170.000 mg/dl) and rabdomyolysis. Five of them had infections. Extremely interesting is that 4 patients are sport teachers with University Degrees in Sports Medicine. Their first onset was with acute renal failure after exercise at the age of 24–26 years. Protective factors for the late onset are unknown. All adults were homozygotes for the c.338C>CT (p.Ser113Leu) mutation.
Discussion: There is no good correlation between phenotype/genotype in our patients. The disease is probably underdiagnosed.

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Beta-Ketothiolase deficiency: unusual clinical presentation of non-ketotic hypoglycemic episodes due to secondary carnitine deficiency.

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Background: β-ketothiolase (T2) deficiency is an autosomal recessive disorder of isoleucine catabolism and ketone body metabolism. Most patients with T2 deficiency develop severe ketoacidotic events. We encountered a case of T2 deficiency who developed hypoglycemic crises without ketosis during her infancy and early childhood. This is a very atypical clinical phenotype in T2 deficiency.
Case report: A girl was born to healthy related Iranian parents at 34 weeks of gestation with a birth weight of 2kg. On the 17thday of life, she had an episode of tachypnea with hypoglycemia and elevated lactate. She was treated with dextrose intravenous fluids. At the age of 53 days, she had an episode of poor feeding, lethargy and hypotonia. At the age of 21.5 months, she again developed respiratory distress and lethargy with severe metabolic acidosis (pH: 7.11, PCO_2: 16.1 mmHg, HCO_3: 5.1 mmol/L) and normal blood glucose (3.9 mmol/L). Urinary ketones were negative. Urinary organic acid analysis showed elevation of 2-methyl-3-hydroxybutyric acid and tiglyglycine without ketone bodies. At 24, 28 and 41 months of age she had similar episodes with mild metabolic acidosis, hypoglycemia, and negative urinary ketone.
Results: The diagnosis of T2 deficiency was confirmed by demonstrating a homozygous c.1035-1037delAGA (p.E345del) mutation in *ACAT1*-gene. This mutation did not retain residual activity.
Discussion: We could not explain why this patient had no ketosis even during serious crisis. We performed DNA panel analysis for defects in beta-oxidation, carnitine cycle disorders and glycogenolysis. However, no significant variants were identified. At this moment, we re-evaluated previous metabolic analyses and found that she had very low plasma free carnitine levels. After L-carnitine supplementation (50 mg/kg/day) she had two mild episodes with positive urinary ketone. Hence we concluded that she was affected with secondary carnitine deficiency which resulted in hypoketotic hypoglycemia.

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Urinary organic acid profiles in mitochondrial HMG-CoA synthase deficiency

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Background: Mitochondrial 3-Hydroxy-3-Methylglutaryl-CoA Synthase (HMGC2) is one of the enzymes involved in ketogenesis. Patients with HMGC2 deficiency (HMGC2D) are asymptomatic in a non-catabolic state and develop hypoketotic hypoglycemia, encephalopathy, and hepatomegaly in a catabolic state, often triggered by an intercurrent infection or prolonged fasting. Organic acid analysis in the urine obtained during episodes of metabolic decompensation, shows non-specific findings except for several specific metabolites. 4-Hydroxy-6-methyl-2-pyrone (4-HMP) is considered as the most specific for HMGC2D. Diagnostic value of urine 4HMP in HMGC2D was studied.
Methods: A 7-month-old Japanese female with history of failure to thrive, developmental delay, hypoglycemia and a high acetylcarnitine level was diagnosed with HMGC2D confirmed by the *HMGC2* gene mutation study (p.Met235Thr/p.Val253Ala). Organic acid analysis was performed in 12 urine specimens from the patient: 2 from onset, 1 from sick day, 4 from hospitalization/recovering stage, and 5 from healthy state. Control urine specimens (n=1053), which were sent to our laboratory for organic acid analysis for diagnosis, were analyzed and compared to the patients' specimens.
Results: 4-HMP and 4-HMP/3-Hydroxybutyrate (3OHB) were measured/calculated. Forty-one and 34 out of 1053 control urine specimens were positive for 4-HMP (all specimens: < 20 mmol/molCr) and ketones, respectively. All control specimens showed 4-HMP/3OHB ratios < 1. In the patient's specimens, 9 out of 12 showed positive 4-HMP (6 specimens: > 20 mmol/molCr). Ten out of 12 specimens showed 4-HMP/3OHB ratios > 1. The other two showed the ratio < 1 due to a significant increase of 3OHB although 4HMP levels were increased as well (> 20 mmol/molCr).
Discussion: Although 4-HMP can be increased in non-specific ketosis, 4-HMP/3OHB ratio may have diagnostic value in distinguishing HMGC2D from non-specific ketosis.

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Neonatal carnitine acylcarnitine translocase deficiency: 8 new cases with survival in 6.

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Background: Carnitine-acylcarnitine translocase (CACT) deficiency (OMIM#212138) is a rare disorder of fatty acid oxidation (FAOD). Patients typically present under 48 hours of age with cardiomyopathy or arrhythmias, hypoketotic hypoglycaemia, hyperammonemia, hepatomegaly with raised liver transaminases and elevated CK. Mortality is the highest amongst FAODs and sudden unexpected death is common in infancy. Outcomes remain poor despite adherence to a long-chain fat restricted, MCT supplemented, high carbohydrate diet. Reported longer-term survivors are few and largely limited to rare milder cases, with residual enzyme activity in fibroblasts 5% or more of control levels (method as described by Pande et al. 1993).
Methods: This is a retrospective review of 8 cases of CACT deficiency from metabolic centres in Australia, Canada, France and New Zealand. All patients with a diagnosis of CACT deficiency confirmed by CACT enzyme activity and/or molecular analysis were included. Previously published cases were excluded.
Results: Seven cases presented within 48 hours of birth (12–48 hrs), with hypoglycaemia noted in six. One case was diagnosed pre-symptomatically by newborn screening. Of seven cases with documented serum ammonia, five were elevated (190 – 1142 μmol/L). Aspartate transaminase was elevated in four of five documented cases (163 – 444 U/L) with the remainder of the liver function typically normal. CK ranged from 96 to >25,000 U/L in six cases. Cardiomyopathy was demonstrated in four cases and arrhythmia in five. Free carnitine was low in four cases (1–7.1 μmol/L) with total carnitine typically normal. Two died in the neonatal period, with six surviving to 4–10 years.
Discussion: Neonatal CACT causes acute metabolic decompensation predominantly with hyperammonaemia, hypoglycaemia, cardiac dysfunction and encephalopathy. Energy provision in critically ill babies is challenging but survival is possible with prompt identification and treatment.

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A double homozygous mutation in *ACADVL* gene presenting with a mild phenotype

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Background: Very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency is an autosomal recessive disorder of fatty acid oxidation.
Case report: The proband is a 5 year old boy. His parents are first cousins. He presented at the age of four years with episodes of infrequent hypoglycaemia. The first attack occurred when he was 2 years old following acute gastroenteritis and the second attack at three years following prolonged sleep. Both episodes were associated with seizures but no symptoms or signs suggestive of central nervous system infection were noted. His development was age appropriate and his growth parameters within normal centiles. Systemic examination was unremarkable apart from hepatomegaly.
Results: Ammonia, lactate, hepatic and renal profiles were normal. Free carnitine (C0) was 6.8 μmol/l (N >8.8) and acetylcarnitine (C2) = 3.7 μmol/l (N > 10). He was started on carnitine. Repeated acylcarnitine profiling 6 months later revealed high C14 level of 1.2 μmol/l (N < 0.8), C14:1 was 2.7 μmol/l (N< 0.6) and C2 level increased to 8 μmol/l (> 8) which is diagnostic of VLCAD. His urine organic acid persistently showed increased dicarboxylic acids. Echocardiography and creatinine phosphokinase were normal. Molecular study revealed two different homozygous variants in the *ACADVL* gene. Segregation studies in both parents and a sister, who were asymptomatic, showed them to have the two variants in heterozygous state in the *ACADVL* gene in cis form.
Discussion: We here report a patient with double homozygous mutations in the *ACADVL* gene who presented with a mild phenotype. However, it is extremely rare to find a double homozygous mutation in one patient. Having a double homozygous variant does not seem to increase the severity of the disease in our patient. This family also reminds physicians that a compound heterozygous mutation does not cause disease if the variants are inherited in cis form as the two variants are in the same allele and the other allele is spared.

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Molecular and clinical characteristics of Very Long-Chain Acyl-CoA Dehydrogenase deficiency in 13 patients attending a single center

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Background: Very Long-Chain Acyl-CoA Dehydrogenase deficiency (VLCADD) is an autosomal recessive disorder of fatty acid oxidation. The presentation of VLCADD varies from asymptomatic babies diagnosed on newborn screening, the mild form presenting with hypoglycemia and rhabdomyolysis to a severe form with cardiomyopathy. Early diagnosis is likely to improve the outcome.
Methods: This is a retrospective review of all patients with VLCADD who presented to our tertiary metabolic center between 2000 and 2018. Demographic, clinical and laboratory data were abstracted from the hospital electronic records using a case report form.
Results: A total of 13 children were included, 7 were males. Mean age was 4.5 years (0.2-18). Consanguinity was reported in 9 parents. One patient was diagnosed antenatally, 4 at birth by newborn screening, 8 presented clinically with hypoglycemia (2), cardiomyopathy (1), and 2 with lethargy and vomiting. C14:1 was high and urine organic acid analysis showed dicarboxylic aciduria in all patient. Molecular study revealed homozygous mutations in *ACADVL* gene in all patients. All patients were treated with medium-chain triglyceride and carnitine supplementation. All patients are alive and have a normal development except one who presented with cardiomyopathy and died at 2 months of age.
Discussion: Most patients in this cohort presented in the neonatal period either by newborn screening or clinically with hypoglycemia, lethargy and vomiting. Only one patient presented with cardiomyopathy and later died at 2 months of age.

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Sex specific metabolic phenotype in very-long chain acyl-CoA dehydrogenase deficient (VLCAD$^{-/-}$) mice

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Background: VLCAD deficiency is the most common disorder of mitochondrial long-chain fatty acid β-oxidation with an incidence of 1:50,000 in newborns. The clinical phenotype is very heterogeneous involving organs and tissues that mostly rely on fatty acid β-oxidation for energy production. The official treatment recommendations include a fat-restricted and -modified diet in which long-chain fatty acids are fully or in part replaced by medium-chain triglycerides (MCT).
Methods / case report: Our studies on the VLCAD$^{-/-}$ mouse have shown that a long-term application of MCT results in a severe metabolic syndrome in female mice whereas males are protected. Our aim is to explore the sexually dimorphic response to defective mitochondrial β-oxidation as well as the effects of MCT on signalling pathways downstream mTOR which are involved in lipogenesis and adipogenesis.
Results: We performed *in vivo* metabolic phenotyping of untreated and with MCT treated fibroblasts by measuring cellular energetics, oxygen consumption, ATP production and proton efflux using Seahorse Technology. Regulation of mTOR pathway was investigated by WB. SILAC-based proteomic analysis was performed to quantify protein expression under normal conditions and under MCT diet.
Discussion: Cellular respiration was significantly reduced in both sexes, however, male showed a better compensatory ability for defective β-oxidation than females. We detected a clear difference in the phosphorylation degree expressed as the ratio mTOR-P/ mTOR between male and female VLCAD$^{-/-}$ mice. Permanent incubation with MCT resulted to a significant reduction of mTOR phosphorylation degree in fibroblasts from male VLCAD$^{-/-}$ mice. In contrast, we observed a marked increase in the ratio mTOR-P/ mTOR in fibroblasts from female VLCAD$^{-/-}$ mice on MCT.
Our results showed for the first time the mechanisms of sex-specific difference in the metabolic regulation of cells with a β-oxidation defect in response to MCT treatment.
Conflict of Interest declared.

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Clinical features and treatment responses of multiple acyl-CoA dehydrogenase deficiency: results of a single center from Turkey

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Background: Multiple acyl-coA dehydrogenase deficiency (MADD) is an autosomal recessively inherited metabolic disorder caused by either electron-transfer flavoprotein (ETF) or ETF dehydrogenase (ETFDH) deficiency. Classical clinical manifestations include hypoketotic hypoglycemia, hypotonia and cardiomyopathy in infants and rhabdomyolysis attacks in adolescents. Fat restricted diet with frequent meals, avoiding from fasting and riboflavin are principles of treatment. With early diagnosis and appropriate therapy, prognosis is expected to be better. In this study, clinical features and treatment responses of 27 MADD patients from a single center are reported.
Methods: Patients in whom the diagnosis was made by molecular analysis of *ETFA, ETFB, and ETFDH* genes and/or by showing chracteristic acylcarnitine profile with organic acid excretion, were enrolled in study. Demographic features, detailed physical examination, complaints in admission, biochemical investigations and plasma acylcarnitine profile, ultrasonographic and echocardiographic findings and therapy responses were collected retrospectively.
Results: 27 MADD patients from 23 unrelated families were enrolled to study. Eighteen patients were male and 9 were female. Consanguineous marriage was reported in 18 patients. Seven patients were detected in the newborn period, 4 were < 3 years, 5 between 3–11 years and 11 ≥11 years of age. All but one were detected due to clinical findings. Eight patients exhibited exercise intolerance and rhabdomyolysis. Four patients were detected due to a sibling history. Hypertrophic cardiomyopathy was detected in two patients. Seventeen patients were treated with dietary treatment and riboflavin, 5 with riboflavin alone. Five patients died during the follow up.
Discussion: MADD is a rare and life threatening metabolic disorder. Especially in countries where expanded newborn screening is not a part of national screening programme, early recognition of clinical findings and initiation of appropriate therapy is needed.

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Fractional excretion of free carnitine in urine as an important marker for carnitine uptake defect

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Background: Carnitine uptake defect (CUD) is a rare autosomal recessive disorder, resulting in urinary carnitine wasting and low serum carnitine. Within the differential diagnosis it is important to distinguish CUD from secondary causes of carnitine deficiency. During prospective pilot study we identified a newborn girl and her asymptomatic mother with very low plasma free carnitine < 5 μmol/l. easurement of free carnitine in urine was performed by direct injection triple quadrupole MS.
Methods: Free carnitine (FC) and D9-FC used as an internal standard were determined as their butylesters and detected in SRM mode. The method was tested using selected real urine samples with low, medium and high concentrations of FC and external quality control urine samples, obtaining intra-day and inter day CV 1.2 to 11.5% and 5.5 to 16%, and bias up to −10 and −6%, respectively. We examined 165 urine samples of healthy subjects aged 2 days to 64 years, 70 urine samples of newborns and their relatives suspected to CUD and of patients with confirmed inborn metabolic disorders and of other diseases.
Results: Analysis of urinary free carnitine showed differences between the newborn girl and her mother (before carnitine substitution): 0.69 μmol/mmol creat and 9.78 μmol/mmol creat, (controls: 1.4 – 27.0) respectively. Fractional excretion of free carnitine in urine in the newborn was 0.17% and in her mother 5.63% (controls: 1.6 – 4.0), so the mother was suspected of having CUD. We confirmed CUD in the mother by DNA analysis, which revealed compound heterozygosity with c.136C>T in exon 1 and a novel mutation c.824G>A in exon 4. Her newborn girl was heterozygote for the c.136C>T mutation. After confirmation of maternal CUD, prompt treatment with L-carnitine was initiated.
Discussion: Fractional excretion of free carnitine in urine allows to differentiate CUD from secondary causes of carnitine deficiency.
The work was supported by R&I ERDF project [ITMS 2014+ 313021D075] and by Research and Development Agency [APVV-0840-11].

P-252

It doesn't always take two to TANGO: co-existence of 22q deletion syndrome and *TANGO2* mutations

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Background: Mutations in *TANGO2* have been recognized as a cause of cardiac arrhythmias and recurrent rhabdomyolysis. The TANGO2 (Transport ANd Golgi Organisation) protein is mitochondrial in humans and deficiency is believed to impair fatty acid oxidation. The gene locus lies at 22q11.2 and therefore patients with 22q deletion syndrome (22qDS) are at an increased risk of disease. However, to our knowledge no such cases have been reported.
Case report: Case 1 was diagnosed with 22qDS in infancy and presented at 5 years of age with rhabdomyolysis, hypoglycaemia and a prolonged QT interval. She recovered and returned to her premorbid state. She was commenced on a medium-chain triglyceride (MCT) formula and remains well. Case 2 presented with a phenotype consistent with a long chain fatty acid oxidation (LCFAO) defect along with developmental delay and dysmorphism and was subsequently found to have 22qDS on a chromosomal microarray. He has had several admissions with rhabdomyolysis complicated by a prolonged QT interval. He required a sympathectomy and the insertion of a cardiac monitor after episodes of torsade de pointes.
Results: Both cases have had abnormal acylcarnitine profiles suggestive of a LCFAO defect. Interrogation of case 1's whole exome sequencing data concluded that she carried the common European deletion of exons 3–9 in the *TANGO2* gene on the opposite allele to her 22q deletion. Case 2 was found to have a nonsense mutation in *TANGO2* co-existent with the 22q deletion on the second allele.
Discussion: The locus for *TANGO2* is in the area involved in 22qDS. We therefore conclude that it should be considered as a cause of prolonged QT and recurrent rhabdomyolysis in patients with 22qDS. Additionally, the use of an MCT formula may help to prevent episodes of metabolic decompensation.

P-253

Carglumic acid decreases ammonia in MADD but not in CACT deficiency

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Background: Carglumic acid (CA) is a structural analogue of N-acetylglutamate (NAG), which activates carbamyl phosphate synthetase I (CPS-I), the first enzyme of the urea cycle. In fatty acid oxidation defects (FAOD), the lack of acetyl-CoA with subsequent NAG underproduction may be one of the pathogenic factors for elevated ammonia.
Methods: We describe two hyperammonemic FAOD patients treated with CA with opposite responses.
Results: The first patient was diagnosed with multiple acyl-CoA dehydrogenase deficiency (MADD, ETF mutations), who presented in neonatal period with hypoglycaemia, hypotonia and metabolic acidosis. Despite dietary management, carnitine and riboflavine, the evolution was marked by numerous decompensations, characterised by hypoglycemia, hyperammonaemia and liver or cardiac failure. From age 13, he developed chronic hyperammonaemia, maximal in the morning before feeding, treated with normoprotidic diet (1 g/kg/d) and Sodium benzoate during one year without benefit. After introduction of CA with higher evening dosages (total daily dosage of 4000 mg/ d, weight: 88kg), ammonia was steadily reduced by 50% in the morning (63 versus 128 μmol/L). The second patient was diagnosed with Carnitine-acylcarnitine translocase deficiency (CACTD). He presented with progressive degradation from around H12 with hypotonia, coma, hyperammonaemia up to 1142 μmol/L and liver failure, without hypoglycaemia. Despite appropriate management, the first year of life was marked by many decompensations with hyperammonaemia, hypoglycaemia, liver failure, cardiac arrest. He also presented chronic hyperammonaemia that did not respond to sodium benzoate and sodium phenylbutyrate. CA was tested at 100 mg/kg/d for 2 weeks without success.
Discussion: Carglumic acid is used for NAGS deficiency and organic acidurias. The efficacy in FAOD seems variable, and might depend on the underlying pathophysiology, with striking efficacy in one MADD adult patient.

14. Mitochondrial disorders: nuclear encoded, disorders of pyruvate metabolism and the Krebs cycle

P-254

Susceptibility to post-traumatic stress disorder is inversely correlated with brain mitochondrial capacity in mice.

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Background: Mitochondrial dysfunction has been increasingly implicated in several psychopathologies. Post-traumatic stress disorder (PTSD) is a debilitating psychiatric disorder induced by exposure to a traumatic event. Disease presentation is highly heterogenous, and the severity of the inducing trauma does not predict the severity of the disorder. This suggests a possible underlying metabolic susceptibility factor. We therefore investigated whether susceptibility to PTSD is associated with mitochondrial dysfunction.
Methods: We exposed 48 wildtype male mice to a PTSD-induction paradigm previously shown to reliably induce a PTSD-like phenotype similar to that of humans. 8 PTSD-vulnerable and 16 PTSD-resilient animals were identified through a series of behavioral tests for the physical symptoms of PTSD. The activities of the mitochondrial electron transport chain (ETC.) complexes I, II, III and IV isolated from brain and muscle of PTSD vulnerable and resilient animals, were measured using a Konelab autoanalyzer.
Results: Significant variation in brain ETC. complex activity was observed among these 48 WT animals. PTSD-vulnerable mice showed a 20% reduction in brain mitochondrial complex activity compared to their resilient littermates, and brain mitochondrial complex activity was inversely correlated with PTSD susceptibility across all 48 animals. Mitochondrial complex activity was not correlated with behavior in any behavioral test for PTSD symptomatology. Muscle ETC. complex activity was not correlated with PTSD susceptibility or symptomatology.
Discussion: Our data indicate that our wildtype mice show significant variation in mitochondrial function, and that reduced

mitochondrial function in the brain of those animals is associated with increased vulnerability to PTSD. These findings have important implications for the prevention, diagnosis and treatment of PTSD in patients with inherited or acquired mitochondrial dysfunction or suboptimal mitochondrial dysfunction.

P-255

Clinical, metabolic and structural characterization of a new patient with *Mitochondrial Fission Factor* (MFF) gene alteration

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Background: MFF is part of a protein complex that promotes mitochondria and peroxisome fission. This far, only 4 patients have been reported showing mutations in MFF, all of them with the clinical features of a very early onset Leigh syndrome.
Methods / case report: This 11-year-old Italian boy was born from consanguineous parents after a pregnancy characterized by intrauterine growth retardation. An older brother with an early onset severe epileptic encephalopathy died at 16 years. The proband showed a normal psychomotor development until the age of 9 months, when neurological regression was observed. Epileptic myoclonic seizures emerged at the age of 18 months. On examination at the age of 5 years he showed: severe intellectual disability, microcephaly, spastic-dystonic tetraparesis, severe optic atrophy, and ophthalmoplegia. Mild hepatic steatosis and cholestasis were also detected. Brain MRI showed an increased T2 signal in the putamen and caudate nuclei. An extensive metabolic work-up showed increased lactate in CSF and in brain (1H-MRS), with normal respiratory chain activities in muscle biopsy and PDH activity in fibroblasts.
Results: NGS panel for mitochondrial disorders revealed a homozygous c. 892C>T (p. Arg298*) in the MFF gene. Immunofluorescence staining detected abnormal morphology of mitochondria and peroxisomes in fibroblasts from the patient, together with a strong reduction in MFF protein levels. No biochemical alterations denoting peroxisomal disorders (VLCFA, phytanic and pristanic acids, DHA, DHCA, THCA) were found.
Discussion: As reported in the other disorders affecting the dynamics of intracellular organelles, such DNM1L deficiency, our patient shows clinical features suggesting both mitochondrial and peroxisomal derangement. Biochemical work-up confirmed in our case a severe involvement of the energetic metabolism, while biomarkers of peroxisomal dysfunction were normal.

P-256

Clinical variability of adenine dinucleotide translocator deficiency due to the homozygous c.368C>A mutation and possible founder effect

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Background: Adenine dinucleotide translocator 1, coded by *SLC25A4* gene, is the main ADP/ATP transporter in inner mitochondrial membrane of skeletal and heart muscle. Dominant mutations are associated with progressive external ophtalmoplegia, recessive mutations cause childhood-onset mitochondrial myopathy and cardiomyopathy, while *de novo* dominant mutations in highly conserved regions cause profound muscle hypotonia with respiratory insufficiency.
Case report: We present four patients from two families homozygous for c.368C>A (p.Ala123Asp) mutation. The mutation was first published in homozygous form in young Slovenian with myopathy and hypertrophic cardiomyopathy.
Results: Patients 1 and 2 were two brothers with hypertrophic cardiomyopathy, muscle weakness and elevated lactate since early childhood. Muscle histology showed mitochondrial myopathy. They had combined deficiency of complexes I, III and IV. On CoQ_{10}, bicarbonates, vitamins B1, B2 and C they are clinically stable for >10 yrs. Patient 3 presented at age of 52 years with respiratory insufficiency. Until then he had fatigue, ptosis, short stature, hyperpigmentation, cachexia, and hypertrophic cardiomyopathy, but was ambulatory. His course was complicated with cerebrovascular accident and he died of complications after several weeks of ventilatory dependency. His sister (patient 4), now at age 54, had muscle weakness since childhood. At the age of 25 years muscle biopsy showed mitochondrial myopathy. Her clinical course was slowly progressive, she developed ptosis, external ophtalmoplegy, hypoventilation and mild heart hypertrophy. Since the age of 45 she uses nocturnal noninvasive ventilation.
Discussion: Homozygous c.368C>A mutation of the *SLC25A4* gene seems to be common in Southeastern Europe, possibly due to a founder effect. It is associated with clinical variability with possible progression to death. It is uncertain if the variability is due to aging or some inherited or acquired modifying factors.

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The genetic and clinical spectrum of *PDHA1*-related PDHc defiency: lessons from nearly 500 patients

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Background: The most frequent cause of PDHc deficiency are mutations in the X-linked *PDHA1*, causing lactic acidosis and various neurological findings. Ketogenic diet and thiamine can positively affect the clinical course.
Methods: The clinical, metabolic and genetic data of PDHA1 deficient patients were collected via literature review or online survey completed by the respective physician.
Results: 496 patients (256 female) from 482 families were included, 211 patients were unpublished. 235 different *PDHA1* variants were found, of which 78 unpublished. Not all data were available for all patients, we therefore report as "n/all available (%)". In 152/211 (72%) patients the mutation had occurred *de novo*. 25 of the 58 *de novo* variants were recurrent. Most frequent findings were lactic acidosis (148/167=89%), intellectual disability (128/149=86%), developmental delay (113/130=87%), muscular hypotonia (134/167=80%), bilateral basal ganglia lesions (104/148=70%), microcephaly (78/124=63%), seizures (102/176=58%), dysphagia (59/103=57%), spasticity (59/129=53%), ataxia (71/140=51%), and dystonia (50/108=46%). Data on activities of daily living: 33/68 (48%) could walk independently, 28/68 (42%) communicated with whole sentences, and 12/68=17% attended a regular school. Impaired vision seen in 28/67 (42%), hearing loss in 18/65 (28%) patients. Most patients were treated with a fat-enriched diet and/or thiamine, this study did not evaluate treatment effects.
Discussion: PDHA1 deficiency is one of the most frequent inborn errors of energy metabolism. Our evaluation of nearly 500 patients proves several aspects that were seen in smaller studies previously (e.g. frequent clinical and neuroradiological findings). The available treatment options, the finding of a high number of (recurrent) *de novo* variants and the equal male:female ratio, warrant early TRIO exome sequencing in all children with a lactic acidosis. This study further adds 78 disease causing *PDHA1* variants.

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Phase II Open Label Single Center Trial Evaluating the Safety & Efficacy of (+)-Epicatechin in Friedreich's Ataxia

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Background: (+)-Epicatechin (Epi) is a flavonoid with similar health benefits to exercise. Observations suggest the existence of a signaling mechanism that may induce mitochondrial biogenesis in mitochondria-depleted muscle fibers & neurons as well as the induction of anti-oxidant enzymes. Our aim was to evaluate the safety & preliminary efficacy of Epi in Friedreich's ataxia (FA).
Methods: We conducted a phase II open label trial evaluating the safety & efficacy of Epi in ten FA subjects for 24 weeks. Participants were 10 to 50 years of age with a confirmed FA diagnosis, disease duration ≤7 y and cardiac/neurological systems affected. Epi 75mg daily was administered orally, with dose escalation to 150mg/day at 3 months for subjects not showing improvement. Primary endpoints assessed safety & efficacy using neurological, cardiac & metabolic evaluations before & after treatment. Secondary endpoints included frataxin up-regulation & urine F2-isoprostane reduction before & after treatment.
Results: Ten patients enrolled & completed all safety & efficacy measurements with the exception of one not completing the FARS & 8 m timed walk at 24 weeks. Epi was safe & tolerable, without clinically significant side effects but with a transient increase in migraine frequency. No subjects discontinued the study. There were FARS improvements in 50% of subjects by a mean 5.5 points (range 2–8) and for the 8 meter walk in 30% of subjects by mean 2.2 seconds (range 2.2-2.3). In the 9 peg hole test an improvement was seen in 50% of subjects by mean 4.2 sec for R hand & 5.4 sec for L hand (range R 1–9, L 3–7) at 24 weeks. A reduction was seen in the MRI-derived left ventricular myocardial mass index (LVMi) by >10% in 6 subjects at 12 weeks and in one additional subject at 24 weeks (70%). LVMi subsequently showed slight worsening at 24 weeks in the 6 subjects. There was no improvement in secondary endpoints.
Discussion: Treatment with Epi was safe & tolerable over 24 weeks & resulted in neurological improvement of total FARS score, 8 m timed walk, 9 peg hole test and a reduction in LVMi in a subset of patients. Further studies of the effects of Epi in FA are warranted.

P-259

A distinct phenotype and mitochondrial OXPHOS disturbances in a second family with novel mutations in the complex I *NDUFA13* gene

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Background: Isolated mitochondrial respiratory chain complex I (CI) deficiency is one of the most frequent OXPHOS disorders in childhood. It is caused by mutations in genes coding for several CI subunits and assembly factors. Clinical features are heterogeneous, being Leigh Syndrome (LS) a common phenotype. Clinical and molecular characterization of a second family with novel mutations in the *NDUFA13* gene, an accessory subunit of CI, is reported.
Case report/methods: A 2-year-old boy presented with psychomotor and language delay, horizontal nystagmus and hyperlactatemia. Brain MRI displayed bilateral necrosis of basal ganglia, characteristic of LS. Muscle biopsy showed lipids drops, and isolated CI enzyme deficiency (50% of control). Next-generation sequencing (NGS) of mitochondrial DNA and of a customized NGS panel including 133 genes coding for OXPHOS subunits and assembly factors were performed. Analysis of mitochondrial functioning were carried out in patient's cultured skin fibroblasts (CF).
Results: Patient's phenotype differs from the first previously reported *NDUFA13* family. NGS studies showed novel biallelic (confirmed by parents' analysis) heterozygous mutations in the *NDUFA13* gene. Growth rate, basal and maximal respiration, and ATP synthesis rate, were all significantly decreased in CF. Both CI enzyme and in-gel activities (IGA) in CF showed a 65% diminution of control. Blue native electrophoresis (BNE-PAGE) studies revealed disturbances in supercomplexes (SC) assembly or stability; particularly, a reduction of CI with respect to controls was observed in SC I+III_2+IV (respirasome) and in SC I+III_2.
Discussion: We identified a second family, harbouring two new mutations in the CI-*NDUFA13* gene by using NGS approaches in a patient with CI deficiency. The proband, who is alive at age of 14, showed LS which were absent in the previously described family. Abnormal functioning of OXPHOS system was demonstrated by enzymatic, respirometry and protein analyses in CF.

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Clinical and genetic evaluation of 35 adult patients suspected for mitochondrial disease in Slovenia

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Background:We describe clinical and genetic evaluation of 35 adult patients suspected for mitochondrial disease in Slovenia.
Methods / case report: Clinical, electrophysiological, histological characteristics and imaging data of 35 adult patients suspected for mitochondrial disease were analyzed retrospectively. In addition 21/35 patients responded to our invitation and genetic analysis with clinical exome sequencing and mtDNA sequencing were performed in these patients. DNA was isolated from blood and in mtDNA analysis cases from bucal swabs.
Results:17/35 patients had a distinct mitochondrial phenotype: 12 patients had chronic progressive external ophthalmoplegia, 3 patients had MERRF, and in 2 patients MELAS was suspected. Muscle biopsy was performed in 28/35 patients and definite abnormalities of mitochondria were found in 14/21 patients on electron microscopy, 12/25 patients had ragged red fibres and 1/25 patient blue ragged fibres. COX negative fibers were present in 12/16 patients. Biochemical studies with OXPHOS activity measurements were decreased in 5 patients. Molecular genetic analysis confirmed mitochondrial disease in 11/35 patients: 4 patients had a large mitochondrial deletion, 3 patients had m.8344A>G point mutation, 1 patient had m.12213G>A point mutation and 3 patients were found to have a nuclear gene defect in genes *TWNK*, *SURF1* or *SLC25A4*.
Discussion: A diagnosis of mitochondrial disease was suspected on clinical findings, but imunohistochemical examination, electron microscopy and molecular genetic analysis mostly contribute for a clasification of definite or probable mitochondrial disease. Analyzing DNA from blood and/or buccal swabs by methods of clinical exome sequencing and mtDNA sequencing provided a genetic diagnosis in only 4/21 patients. It is important to gather more clinical information about adult mitochondrial patients in Slovenia to improve early recognition and diagnosis.

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Physiopathology of TIMM50 defect in a patient with Leigh syndrome and 3-methylglutaconic aciduria

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Background: 3-methylglutaconic aciduria (3-MGA-uria) comprises a heterogeneous group of disorders usually associated to mitochondrial membrane defects. 3-MGA-uria with mutations in *TIMM50* has been described previously in a single report, but physiopathological investigations were very scarce. We present the first detailed characterization of the molecular and physiopathological bases of a patient with mutations in *TIMM50*.
Methods: Genetic analysis was performed by WES. Mitochondrial morphology was analyzed by confocal and electron microscopy. Protein and mRNA expression were determined by western blot and qPCR, respectively. OXPHOS assembly was analyzed by BN-PAGE. Cardiolipin (CL) content was evaluated by UPLC-MS/MS. TIMM50 deficient cell lines were generated using CRISPR/Cas9. Oxygen consumption was evaluated by high-resolution respirometry.
Results: We report on a 17 years old patient with early epilepsy, Leigh syndrome, optic atrophy, transitory *cardiomyopathy* during infancy, persistent 3-MGA-uria and mutations in *TIMM50* (c.[341G>A];[805G>A]). Molecular studies demonstrated that TIMM50 protein was completely absent in patient fibroblasts, regardless of the normal mRNA levels. Confocal and electron microscopy evidenced marked morphological and ultrastructural abnormalities. Biochemical studies showed altered levels of particular CLs, as well as respiratory chain supercomplexes while complexes were normal. High resolution respirometry in TIMM50 fibroblasts demonstrated a significant reduction of the maximal respiratory capacity. HEK293T TIMM50-deficient cell line mimicked this respiratory defect, which was reverted upon transfection with a plasmid encoding for TIMM50 wild type protein.
Discussion: We demonstrated that *TIMM50* mutations cause a mitochondrial disease by targeting key aspects of mitochondrial physiology, including the maintenance of mitochondrial morphology, CL composition and OXPHOS assembly, leading to a reduction of the maximal respiratory capacity.

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Acute liver failure due to *TRMU* defects

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Background: Mitochondrial hepatopathies are usually severe and progressive conditions. When it is accompanied by elevated lactate, often mtDNA depletion syndrome occurs. We report on 5 unrelated infants who presented with acute liver failure and lactic acidemia with normal mtDNA content caused by *TRMU* gene defects; one case promises to be reversible.
Methods: For DNA analysis we conducted target sequencing of 47 nuclear hepatic genes using NGS technology on Ion S5. MtDNA depletion was ruled out by real-time PCR.
Results: We have identified 5 patients with defects in *TRMU* gene. In 3 cases we've found common mutation p.V279M in compound with another previously unknown pathogenic allele. 3 infants carry in-frame insertion (p.355insAVQ) and 2 infants had mis-sense-mutation c.A679G (p.227G), what is more one child had these new variants in compound-heterozygous state.
Discussion: All children were generally healthy during the early neonatal period but were admitted at 2–4 months because of vomiting, poor feeding, ecchymosis, and irritability. On physical examination, all were found to be well-nourished but lethargic, with jaundiced sclerae, distended abdomen, and hepatomegaly. Serology for hepatitis viruses and body fluid cultures failed to detect an infectious etiology. Routine laboratory investigations revealed hyperlactatemia, elevated liver transaminases, hypoglycemia, coagulopathy, and direct hyperbilirubinemia. There was usually no indication of extrahepatic involvement. Despite the

ongoing therapy, four of our *TRMU* infants have not lived through an acute phase of liver failure. However, according to literature, more than 70% of *TRMU* patients survive. Among our cases there is no evident link between proper gene mutations and survival forcast. It could depend on timely and appropriate treatment and any other factors.Newly revealed variants p.355insAVQ and p.227G could be common for Slavic population and should be included in a first line diagnostic.

P-263

Transient infantile liver failure: *TRMU* versus *SCYL1* related disorders

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Background: Mitochondrial diseases are an important cause of pediatric acute liver failure and in some of these disorders (like mutations in nuclear *TRMU* gene, which plays a role in mitochondrial protein translation), the hepatopathy behaves in a transient way with complete recovery after the acute episode. Recently other genetic causes of transient infantile liver failure were recognized, as the mutations in nuclear *SCYL1* gene, which underlies a syndrome characterized by transient episodes of liver failure, peripheral neuropathy and ataxia.
Methods / case report: We present a healthy female child who was admitted at the age of 13 months due to acute hepatic failure. She had normal metabolic evaluation and a positive allopurinol testing. Liver biopsies revealed focal ballooning of hepatocytes and pronounced fibrosis. Muscle mitochondrial chain respiratory analysis showed partial deficiency in complex IV, that normalized in posterior muscle biopsy. From age of 3 years, she developed intention tremor and after the age of 10 presented headache and symptoms of motor-sensory neuropathy. She is now 23 years-old and presents a cerebellar syndrome, without cognitive impairment. There were no further episodes of hepatic failure and serial evaluation showed normal liver function, without evidence of important fibrosis in transient elastography.
Results: Molecular studies revealed two novel variants in heterozygosity in TRMU, probable pathogenic by bioinformatic analysis, and an already known pathogenic variant, in homozygosity, in SCYL1.
Discussion: Although *SCYL1* mutations respond for the main symptomatology of this patient, it is not possible to assure the aetiology of acute liver failure, since they were identified two genetic rare diseases (*SCYL1* or *TRMU*) that could cause transient infantile hepatopathy. The physiopathology of these entities is not yet understood and could be possible the existence of a common underlying mechanism.

P-264

Mutations in Sideroflexin 4 are associated with congenital mitochondrial encephalomyopathy with complex I deficiency

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Background: Prenatal onset of mitochondrial disease with complex I deficiency has been associated with mutations in Sideroflexin 4 (*SFXN4*). We present the third patient known to date with mutations in *SFXN4* and complex I deficiency.
Methods: Investigations included clinical examination, biochemical analysis, skeletal muscle biopsy and genetic analyses by whole-exome sequencing. Functional studies were performed by mRNA expression analysis. Sanger sequencing was used for confirmation and segregation analysis of other family members.
Results: Our patient showed intrauterine growth retardation, neonatal lactic acidosis, failure to thrive, macrocytic anemia, severe visual impairment and psychomotor delay. Brain MRI initially demonstrated delayed myelination followed by white matter hypoplasia/atrophy. Muscle mitochondrial investigations revealed severe complex I deficiency and slight mitochondrial accumulation. Whole-exome sequencing analysis revealed that the patient was bi-allelic for two probably pathogenic mutations in *SFXN4* (NM_213649.1). The first was a 1-base deletion c.969delG leading to frame-shift and a premature stop codon p.(Gln323His*fs**20). The second was a stop-loss mutation in the C-terminal region, c.1012T>C; p.(*388Glnext2) resulting in elongation of the protein by two amino acids. Expression analysis of mRNA from muscle tissue showed loss of SFXN4 transcripts. The mutations segregated with the disease phenotype in the family.
Discussion: Pathogenic mutations in *SFXN4*, encoding mitochondrial sideroflexin 4, are associated with prenatal onset of mitochondrial disease with severe complex I deficiency. We expand the phenotypic spectrum of this disorder including biochemical, neuroimaging and developmental outcomes.

P-265

Natural history of mitochondrial disorders: a systematic review

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Background: Natural history describes the onset, presenting features, clinical phenotype and outcomes of untreated disease. An understanding of the natural history of mitochondrial disorders is essential for early diagnosis, establishing genotype-phenotype-prognosis correlations and planning therapeutic trials.
Methods: A systematic literature review searched for all published natural history studies of mitochondrial disorders containing at least 20 individuals. Data were collected regarding age of onset, presenting and core clinical, biochemical and neuroradiological features. A phenotype was 'common' if it was observed in ≥30% of cases in a study, thereby highlighting common and uncommon phenotypes for each disorder.
Results: Natural history studies for 28 mitochondrial disorders were identified. Fifty-eight percent of disorders had onset < 18m and 80% had onset < 18y. Adult-onset disease predominated in mtDNA deletion syndromes, LHON, MERRF, MIDD, and *TWNK* mutations. Most disorders had multisystemic involvement, most commonly affecting the central nervous system (especially basal ganglia, cortex, white matter), eyes, gastrointestinal system, muscle, auditory system and the heart. High lactate was common in 75% of disorders. Survival was < 1y in 14%, < 5y in 59%, and < 10y in 77% of disorders. Disorders with survival beyond 10y were MERRF, MELAS, *SUCLA2* and *TYMP* mutations. In multiple disorders, poor prognosis was predicted by earlier onset and truncating rather than missense mutations.

Discussion: A thorough knowledge of natural history has helped redefine accepted diagnostic criteria for classical clinical syndromes. As mitochondrial disorders are rare, multicentre collaboration is essential to generate large patient cohorts for high power analyses. Natural history has been utilised in specific circumstances to establish a baseline for comparison in single-arm preliminary clinical trials for novel therapies in disorders that have no existing treatment.

P-266

Different organs have different protein amounts and enzyme activities of the Tricarboxylic Acid Cycle enzymes in mice

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Background: The tricarboxylic acid (TCA) cycle is essential for the production of reducing equivalents used by the electron transport chain. Much of the studies of function have focused on examining these enzymes isolated from the liver due to high enzyme activity, however, enzyme activity differences among organs has not been studied.
Methods: Mitochondria were isolated from wild-type mouse liver, kidney, heart and brain. Activity of the TCA enzymes citrate synthase (CS), isocitrate dehydrogenase 2/3 (IDH2/3), 2-oxoglutarate dehydrogenase (OGDH), succinyl-CoA synthase (SCS), succinate dehydrogenase (SDH), fumarate hydratase (FH), and malate dehydrogenase 2 (MDH2) were measured in these organs. We also measured protein quantity via immunoblot.
Results: TCA enzyme activity was variable across organs. Surprisingly, IDH2/3, SDH, MDH2, and CS activity was highest in heart muscle, whereas OGDH and FH activity was highest in the kidney. Activity broadly correlated with protein amount, except with MDH2.
Discussion: Differing amounts and activities of the TCA cycle enzymes is observed in different organs. The pattern of activity for various enzymes is not the same and may help explain some of the variability seen.

P-267

Two families with isoleucyl-tRNA synthetase (*IARS*) deficiency in Japan and China

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Background: We previously reported the three patients with biallelic mutations in *IARS* that provoke infantile mitochondrial hepatopathy with intrauterine growth retardation, failure to thrive, and zinc deficiency. (Kopajtich et al. AJHG 2016). We herein report other Japanese sisters and Chinese cases with a similar clinical phenotype with biallelic *IARS* mutations.
Case report: Case1: A Japanese girl was born at 35 weeks gestation weighing 1,760 g (−1.7 SD) as the third child of non-consanguineous Japanese parents. At 14 months of age, she developed severe liver failure. A liver biopsy showed steatosis and infiltration of inflammatory cells without fibrosis. She had a mild zinc deficiency. Her elderly sister had been born at 38 weeks gestation, weighing 1,730g (−3.13SD) as the first child. At 3 months of age, she was hospitalized with pneumonia, and developed to meningitis from which she ultimately died. An autopsy was performed and she was diagnosed with subacute liver atrophy, increased of pseudobiliary cells, and fibrosis.
Case2: A Chinese boy was born at 39 weeks gestation weighing 2,500 g (−2.1SD) as the second child of non-consanguineous Chinese parents. At 2 months of age, he developed recurrent pneumonia. At 11 months of age, he manifested severe pneumonia, acute liver failure, and encephalopathy. The patient's zinc level was normal.
Results: A gene analysis of Japanese sisters showed biallelic *IARS* variants with recessive inheritance from her parents. That of Chinese case shows the *IARS* gene homo missense variant with recessive inheritance from his parents. We are now performing validation studies using a yeast system.
Discussion: In the current study, the patients had variants of the *IARS* gene; however, they were all diagnosed with severe infection which led to life-threatening episodes for them. It is therefore important to accumulate and investigate more cases associated with *IARS* mutations.

P-268

***C1QBP* deficiency causes cardiomyopathy associated with combined respiratory chain defects**

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Background: Complement component 1 Q subcomponent-binding protein (C1QBP) is a multi-functional, multi-compartmental mitochondrial matrix protein involved in mitochondrial respiration, inflammation, mitochondrial ribosome biogenesis, apoptosis etc. However, its precise function is unknown. Here we report a neonatal case of intrauterine growth retardation (IUGR), cardiomegaly, and lactic acidosis caused by *C1QBP* mutations.
Case report: A Japanese girl born at 33 weeks 3 days of gestation, the first child of non-consanguineous healthy parents, via an emergency cesarean section owing to oligohydramnios and non-reassuring fetal status had IUGR and weighed 1228 g (−2.6 SD) at birth. Lactic acidosis and cardiomegaly without myocardial hypertrophy developed soon postpartum and deteriorated; she died of cardiorespiratory insufficiency at 4 days of age.
Results: Liver histological analysis revealed marked hepatic steatosis. Liver enzyme analysis revealed complex I, III, and IV deficiency. Exome sequencing revealed compound heterozygous mutations c.739G>T (p.Gly247Trp) and c.824T>C (p.Leu275Pro) in *C1QBP*. Western blot analysis revealed C1QBP level and complex I and IV subunits were decreased in fibroblasts, the latter two analyzed via BN-PAGE. In the complementation assay using *C1qbp*$^{-/-}$ mouse fibroblasts with complex I, III, and IV defects, both variants had significantly lower amounts of C1QBP and mtDNA-encoded proteins (COXI and COXIII).
Discussion: *C1QBP* mutations are associated with combined respiratory chain disorders manifesting as cardiomyopathy. Further studies are required to clarify the mechanism underlying *C1QBP* deficiencies leading to OXPHOS defects.

P-269

Monosymptomatic neurological presentation of *POLG* defect

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Background: Mutation in the DNA polymerase gamma (*POLG*) gene is one of the most common causes of inherited mitochondrial disease, accounting for a heterogeneous spectrum of phenotypes, including childhood Myocerebrohepatopathy Spectrum disorders, Alpers syndrome, several forms of ataxia, neuropathy, and Progressive External Ophthalmoplegia. We report a new, almost monosymptomatic neurological presentation.
Case report: Our patient, a 38-year-old man, was born from non-consanguineous parents. At the age of 5 he was diagnosed with borderline IQ and language delay, and later, when 15 year-old he experienced a severe anxiety disorder. At the age of 19 he developed a postural and action tremor of the upper limbs. In the next years he had the diagnosis of Wolf-Parkinson-White syndrome. On observation, his neurological examination was unremarkable except for asymmetrical postural (right>left) and action tremor of the upper limbs (Fahn Tolosa Marin Tremor Rating Scale: tot score 20; n.v. 0). An inconstant rest tremor was also observed.Osteo-tendineous reflexes were brisk. Liver steatosis with mild elevation of transaminases was evident since his thirties with a BMI of 30 (obesity). Brain MRI showed small T2 hyperintense areas in the subcortical white matter bilaterally. 123I-ioflupane brain SPECT was normal as well as an extended metabolic work-up.
Results: NGS panel for movement disorders showed two homozygous mutations in *Polg1* gene: c.752C>T/p.Thr251Ile in exon 3 and c.1760C>T/p.Pro587Leu in exon10. The two mutations are often found in *cis* with no pathogenic effect suggesting a recessive mode of inheritance. The healthy parents were heterozygotes for the mutations.
Discussion: Movement disorders have been seldom reported as part of the *POLG* spectrum, with two sisters suffering from early onset (20 years) Parkinsonism without PEO. The phenotype of our patients is one of the mildest reported so far. His genotype has been associated with the severe presentation of Alpers syndrome.

P-270

Complex III deficiency due to a homozygous mutation in the nuclear encoded subunit *UQCRQ*

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Background: Complex III deficiency (coenzyme Q : cytochrome c oxidoreductase) is a rare cause of oxidative phosphorylation (OXPHOS) defect. Complex III consists of 11 subunits, one (*MT-CYB*) encoded by mitochondrial DNA, and the remaining ten subunits by nuclear DNA. Until now, reported molecular defects in complex III resided in either the mitochondrial encoded cytochrome *b*, in one of two nuclear encoded subunits (UQCRB, UQCRQ), or in factors involved in complex III assembly.
Methods / case report: The affected subject was a neonate presenting with early encephalopathy, seizures and hypertrophic cardiomyopathy. Brain MRI showed diffuse atrophy of cerebral white matter, cerebral cortex and deep ganglia. A mitochondrial OXPHOS defect was suspected considering the clinical phenotype and an increased lactate concentration in CSF.
Results: A severe complex III deficiency was detected using spectrophotometric analysis in skeletal muscle and cultured skin fibroblasts. Using BN-PAGE followed by in-gel activity staining a clear reduction of both protein amount and activity of complex III was seen, and also a slight decrease of complex I. Western blotting showed reduced signal of complex I and III subunits. Whole exome sequencing (WES) revealed a homozygous c.134C>T mutation (p.Ser45Phe) in *UQCRQ* (complex III, subunit VII of 9.5 kDa).
Discussion: Isolated complex III deficiencies are rare causes of OXPHOS deficiencies. In our patient database containing over 4000 subjects only two siblings were detected with isolated complex III deficiency, both caused by *BCS1L* deficiency. The first paper reporting pathogenic mutations in *UQCRQ* in a consanguineous family was published in 2008 (same mutation as in the proband). The reported members were less severely affected, clearly contrasting with the early and severe course of disease and the severe and global brain atrophy in the proband. WES and selective gene panel analysis was effective in detecting the genetic cause.

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Eight cases of seven families with mitochondrial DNA depletion syndrome due to *MPV17* mutations

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Background: Pathogenic variants in *MPV17* were reported to cause hepatocerebral mitochondrial DNA (mtDNA) depletion syndrome (MTDPS) of infantile onset, which is characterized by hepatopathy and liver failure, developmental delay and other neurological manifestations, lactic acidosis, hypoglycemia, and mtDNA depletion in the liver tissue. Here, we report a summary of eight cases of seven families of MTDPS with *MPV17* gene mutation diagnosed in Japan.
Methods: Mutation analysis was performed on the genomic DNA using primers designed to amplify the coding exons and the exon–intron boundaries of *MPV17*.
Results: Five affected individuals were male, and three were female. *MPV17*-related MTDPS showed an early onset during 1–8 months of age. The universal finding was liver dysfunction, which was described in all affected individuals and includes elevated transaminases, jaundice, and hyperbilirubinemia; six cases resulted in liver failure. Although six patients underwent liver transplantation during infancy, five died before 6 years of age. In addition, three of the five patients died because of pulmonary hypertension. One affected individual who survived after liver transplantation reached adulthood. The c.451dupC mutation represented 7 of 16 mutant alleles.
Discussion: *MPV17*-related MTDPS typically has a poor prognosis because in most affected individuals, liver disease progresses to liver failure typically during infancy or early childhood. Additionally, in this study, affected

individuals died before 6 years of age, which is similar to previously reported cases. Three of the affected individuals died because of pulmonary hypertension, but the association with mitochondrial disorder is unknown. In contrast, we recently discovered one case of MTDPS diagnosed after reaching adulthood. This suggests that cases of MTDPS without diagnosis may be present among patients with acute liver failure whose cause was previously unknown.

P-272

Deletion in *PITRM1* results in juvenile mitochondrial encephalopathy with status epilepticus in dogs

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Background: Canines have emerged as an excellent comparative model for human diseases due to shared genes and because they phenocopy human physiology and dysfunction. Canine complementary models help to uncover new candidate genes, improve understanding of the molecular mechanisms and provide possibilities for therapeutic trials. In this study, we investigated a canine brain disorder with epileptic seizures beginning at 6 to 12 weeks of age. After onset, the disease progresses rapidly to status epilepticus and death.
Methods: Clinical investigations included a detailed neurological examination and serum biochemistry. Post-mortem examination was carried out and tissues were collected from four affected individuals. DNA samples were collected from seven affected dogs and ~400 unaffected dogs. Homozygosity mapping was performed using a high-density SNP array. Whole exome sequencing was done in one affected dog and one obligate carrier and the called variants were filtered using a recessive model against the control data of ~1000 samples. Candidate variants were validated by Sanger sequencing and TaqMan assay.
Results: We discovered a novel rapidly progressing juvenile mitochondrial encephalopathy resulting in lethal status epilepticus. Pathology showed a diffuse necrotizing panpolioencephalopathy with extensive neuronal mitochondrial crowding. Genetic analyses revealed a recessive in-frame 6-bp deletion in *pitrilysin metallopeptidase 1 (PITRM1)*.
Discussion: We have discovered a PITRM1-defective canine model. PITRM1 is a mitochondrial matrix peptidase, that degrades mitochondrial targeting sequences cleaved from imported proteins. Mutations in PITRM1 have been associated with a slowly progressing neurodegeneration in humans. In contrast to the human disease, the canine condition is rapidly progressing after birth leading to status epilepticus and death. Further functional and pathological studies aim to uncover pathophysiological details of the disease and functions of PITRM1.
Conflict of Interest declared.

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Destabilized complex formation as a structure-guided molecular explanation for succinate-CoA ligase (SUCL) deficiency

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Background: Mitochondrial Succinate-CoA ligase (SUCL) catalyses the reversible conversion of succinyl-CoA to succinate, coupled with substrate-level phosphorylation to produce ATP or GTP. SUCL constitutes the first energy extraction step in the Krebs cycle, using metabolites from the catabolism of branched chain amino acids. SUCL is a heterodimeric enzyme composed of the catalytic α-subunit SUCLG1 and one of the two nucleotide-binding β-subunits SUCLA2 (ATP-specific) or SUCLG2 (GTP-specific). In humans SUCL deficiency due to SUCLG1 or SUCLA2 mutations cause encephalomyopathy, methylmalonic acidemia, and mitochondrial DNA depletion.
Methods: The recent article by Carrozzo et al. (JIMD 2016) provided a compendium of clinical and biochemical findings for known SUCLG1 and SUCLA2 genotypes. This feat however could not be paralleled by informative a priori predictions or in vitro characterization of pathogenicity and mutation defect, due in large part to a lack of recombinant reagents, tools and 3D structure. To address this gap, we have generated the recombinant human SUCLG1-SUCLA2 complex for structural and biochemical characterization.
Results: Our SUCLG1-SUCLA2 crystal structure reveals novel insights into the catalytic and disease mechanisms of the complex. The structure reveals the molecular basis for its nucleotide specificity, highlighting residues at the enzyme active site that discriminates binding of ATP in preference over GTP. Structural mapping of missense mutations onto SUCLG1 (n=16) and SUCLA2 (n=9) subunits reveal a large number are located along the heterodimer interface and would interfere with subunit-subunit interactions that lead to destabilisation of the SUCLG1-SUCLA2 complex.
Discussion: Our structural inspection, further characterized by solution studies including size exclusion chromatography and differential scanning fluorimetry, points to protein destabilization and loss of heterosubunit interaction to be molecular causes of SUCL deficiency.

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Two distinct molecular mechanisms for *MRPP2* mutations that cause HSD10 disease

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Background: MRPP2 (also known as HSD17B10) is a moonlighting protein with catalytic and non-catalytic functions. It is involved in the catabolism of isoleucine and steroids. MRPP2 also forms a complex with the MRPP1 (also known as TRMT10C) protein for N1-methylation of purines at position 9 of mitochondrial tRNA, and forms a complex with MRPP1 and MRPP3 (also known as KIAA0391) proteins for 5'-end processing of mitochondrial precursor tRNA. Inherited mutations in the *HSD17B10* gene encoding MRPP2 protein lead to the childhood disorder HSD10 disease, characterised by progressive neurodegeneration, cardiomyopathy or both.
Methods: Using a combination of structural biology, biochemical assays, and mutation characterization, we provide molecular insight into the functions of MRPP2 alone and in complex with MRPP1/MRPP3.
Results: Biochemical characterization of MRPP2 variant proteins constructed from two novel missense mutations in the *HSD17B10* gene (c.34G>C, p.V12L and c.526G>A, p.V176M) identified two distinct molecular mechanisms that explain the underlying biochemical defects associated with HSD10 disease. Both variant proteins showed significant reduction in dehydrogenase, methyltransferase and tRNA processing activities compared to wild-type, associated

with reduced protein stability for p.V12L, whereas p.V176M protein showed impaired kinetics and complex formation. Additionally, the high-resolution structure of MRPP1 shown by crystallography and low-resolution models of MRPP1-MRPP2 and MRPP1-MRPP2-MRPP3 complexes from small-angle X-ray scattering revealed the contribution of MRPP2 towards the overall architecture and assembly of the tRNA modification complexes.
Discussion: This work has characterized novel genotypes for HSD10 disease, and showcased the use of a diverse set of in vitro assays as standards to probe protein functions when evaluating the molecular basis of disease.

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AIF deficiency in five patients: two novel mutations, heart involvement and AIF and CHCHD4 level alterations in autoptic tissues

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Background: AIF (Apoptosis-Inducing Factor) deficiency, caused by *AIFM1* gene mutations, is a rare X-linked disorder with a wide phenotypic spectrum, early-onset encephalomyopathy with rapid progression being the severest form. A novel pathophysiological pathway of AIF deficiency was proposed, involving disruption of the mitochondrial bioenergetics due to a malfunction of AIF and CHCHD4 (Coiled-Coil-Helix-Coiled-Coil-Helix Domain Containing 4), 2 indispensable mitochondrial proteins.
Methods: 5 patients from 2 families were studied on a clinical, biochemical and molecular-genetic level.
Results: 3 male and 2 female patients manifested a neonatal-onset encephalomyopathy with lactic acidosis. Psychomotor retardation was present in all patients, hypertrophic cardiomyopathy developed in 4 and Leigh syndrome in 3 patients, 3 suffered from myoclonic epilepsy, optic atrophy was found in 1. Respiratory chain complexe deficiency was confirmed. Mitochondrial exome sequencing disclosed 2 novel missense *AIFM1* mutations, c.506C>T and c.1391T>G. Interestingly, preferential inactivation of the X-chromosome carrying the mutation was observed regardless to disease manifestation. AIF and CHCHD4 protein levels were severely diminished in various autoptic tissues.
Discussion: Clinical, laboratory and molecular-genetic characterization of 5 patients is presented, with the first female patients and 2 novel *AIFM1* mutations. Heart involvement in our patients expands the clinical phenotype. Autoptic tissues were used for the first time to provide a biochemical evidence of the presumed pathophysiological pathway, studied only in yeast and animal models and human fibroblasts, so far. Further studies are required to clarify the discordance between the X-chromosome inactivation patterns and the clinical and biochemical phenotypes.

P-276

Novel *FDXR* pathogenic variants expand the clinical spectrum related to human ferredoxin reductasedefects

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Background: *FDRX* gene encodes the mitochondrial membrane-associated ferredoxin reductase, the sole enzyme essential for the biosynthesis of iron-sulfur (Fe-S) clusters and heme formation. Fe-S clusters proteins are involved in enzymatic catalysis and gene expression, mitochondrial respiration, DNA replication, DNA repair, and iron homeostasis. Recently, *FDXR* defects have been shown to cause a novel mitochondrial disease with auditory neuropathy and optic atrophy.
Methods / case report: Eight affected individuals from six families were included in the study. Clinical examination, muscle biopsy investigations, biochemical and molecular analyses, including Sanger/whole exome (WES)/WES trios sequencing and Western blot were performed.
Results: Ten *FDXR* pathogenic variants including nine novel ones were revealed. The clinical presentation in these individuals is varied, and includes families with more severe phenotypes than previously described. Early-onset progressive leukoencephalopathy and brain atrophy, Leigh syndrome, and medullary and cerebellar atrophy are reported in three families. Visual and hearing impairment, in keeping with the previously described phenotype in *FDXR* defects, is reported in four of five investigated families. Functional tests performed in two available fibroblast cell lines confirmed reduction in the FDXR protein level on Western blot.
Discussion: Our study contribute to the further delineation of molecular and clinical spectrum related to *FDRX* defects.
This study was partially supported by CMHI grants: S145/16, S148/16, 238/16, mitoNET (German Network for Mitochondrial Diseases) and GENOMIT (European Network for Mitochondrial Diseases).

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A novel *FASTKD2* gene mutation presenting with mitochondrial cytochrome c oxidase deficiency

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Background: The protein encoded by FASTKD2 localizes to the inner mitochondrial membrane and is expressed at its highest levels in the greatly energy-dependent tissues of the brain. Mutations in FASTKD2 is known to underlie respiratory chain complex IV (cytochrome c oxidase) deficiency, a rare and typically fatal disorder that presents in infancy with developmental delay, myopathy and encephalopathy, including demyelinating brain lesions and epilepsy.
Case report: A 14 years old male patient was born at term after an uneventful pregnancy. Mental and motor development was appropriate for his age. When he was 8 years old he had two short absence seizures. He admitted with status epilepticus and treated in the pediatric intensive care

unit. Blood count, liver and renal functions, organic acids, acylcarnitine profile and lactate levels were normal. Echocardiography revealed intact ventricular function. Brain MRI examination disclosed symmetrical high-intensity signals in the basal ganglias and thalamus. The EEG showed bilateral epileptic activity.
Results: With whole exome sequencing c.778-1G>A(IVS2-1G>A), a novel homozygous mutation in FASTKD2 gene was detected. Coenzyme Q10, biotin and riboflavin supplementation was initiated together with antiepileptics. Repeated neurological examination was compatible with cognitive decline, clumsy movements, abnormal balance tests, and horizontal nystagmus.
Discussion: It is not yet completely known how FASTKD2 mutations lead to oxidative dysfunction and neurological impairment. Further studies will be needed to better understand the pathogenic sequence initiated by rare mutations as well as more common genetic variants. Recent advances in next-generation sequencing enable the rapid and accurate diagnosis of mitochondrial diseases, thus facilitating appropriate counseling and the offer of preventive strategies, such as prenatal diagnosis.

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The clinical heterogeneity of the NARS2 c.822G>C mutation clearly illustrated in two siblings: epilepsy and myopathy

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Background: Pathogenic mutations in *NARS2* (mitochondrial asparaginyl-tRNA synthetase) reportedly were associated with a wide range of clinical phenotypes: intellectual disability, epilepsy, Alpers syndrome, Leigh syndrome, non-syndromic hearing loss and severe myopathy.
Case report: A 5 year old girl with mild developmental delay presented with hemiconvulsion-hemiplegia-epilepsy, evolving to therapy resistant epilepsy. Brain imaging showed FLAIR hyperintense lesions unilaterally in the right hemisphere. Two years after initial presentation she developed progressive exercise intolerance due to myopathy. Her brother showed mild developmental delay and an episode with seizures at age nine years. Subsequently, he developed progressive myopathy. No clear cognitive decline was observed in the girl although she had recurrent episodes with focal seizures.
Results: In both subjects, spectrophotometric analysis of respiratory chain complex activities revealed a combined complex I and IV deficiency in skeletal muscle. BN-PAGE confirmed the defect in complexes I and IV and showed the presence of a complex V subcomplex. Using whole exome sequencing, a previously reported homozygous mutation (c.822G>C) in *NARS2* was detected. The mutation causes skipping of exon 7 and part of exon 8 in *NARS2* mRNA.
Discussion: Here, we report two siblings harboring a homozygous pathogenic mutation in *NARS2*. Both siblings have a different clinical picture although they bear the same molecular defect resulting in an identical biochemical defect in analysed tissues. We previously reported two other siblings with the c.822G>C mutation who presented also with a different phenotype, i.e. (i) mild intellectual disability and epilepsy, and (ii) severe myopathy. This report highlights the clinical heterogeneity between subjects with *NARS2* mutations, and with mutations in mitochondrial aminoacyl-tRNA synthetase genes in general.

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Homozygous mutation in *NARS2* causes infantile-onset Alpers syndrome

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Background: Pathogenic mutations in *NARS2*, the gene encoding for mitochondrial asparaginyl-tRNA synthetase 2, have been associated with pronounced clinical heterogeneity; from non-syndromic hearing loss, mild intellectual disability with epilepsy and isolated mitochondrial myopathy to infantile-onset neurodegenerative disorder, Alpers syndrome and Leigh syndrome (MIM #616239). We have diagnosed and followed three patients of Scandinavian descent with mutations in *NARS2*.
Methods: Whole exome sequencing combined with muscle mitochondrial investigation were applied, along with clinical examination and follow-up. Here, we present the phenotype and natural history associated with a homozygous missense mutation in *NARS2*: c.641C>T, p.(Pro214Leu).
Results: Two opposite-sex siblings and one unrelated male patient have been identified. Signs of disease were present already in infancy, mainly failure to thrive, hypotonia, psychomotor regression and epileptic seizures. All patients developed mental retardation and spastic tetraparesis. Respiratory chain enzyme activities were affected in both examined patients (complex I deficiency and combined complex deficiencies respectively). Neuroimaging revealed cortical atrophy mainly supratentorially with variable involvement of the deep gray mater. One patient has survived into adulthood, while the other two deceased in childhood and adolescence.
Discussion: We present the phenotype and natural history of three patients of Scandinavian descent with the same homozygous mutation p.Pro214Leu in *NARS2*. Our patients presented with infantile-onset Alpers syndrome and similar phenotypic features but variable survival outcome.

P-280

Recurrent acute liver failure in alanyl-tRNA synthetase (AARS) deficiency

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Background: Aminoacyl-tRNA synthetases (ARS) are essential enzymes for protein synthesis. Variants in different ARS genes may cause variable clinical symptoms including early-onset neurodegenerative disorders and epileptic encephalopathy with a variable degree of liver involvement. Alanyl-tRNA synthetase (AARS) deficiency has been linked to axonal Charcot-Marie-Tooth disease type 2N and *early infantile epileptic encephalopathy* (Bansagi et al. (2005) Simons et al. (2015)).
Case report of a 5-y-old girl with *AARS* variants and recurrent acute liver failure.
Results: The patient presented with failure to thrive, microcephaly, developmental delay, and a squint. cMRI showed lesions within the occipital semioval center and a lactate peak on spectroscopy suggesting a mitochondrial disorder. At 24 months, she had a first episode of acute liver failure shortly after a gastrointestinal infection. She became symptomatic with hypoglycemia and a generalized seizure. Liver biopsy showed micro- and macrovesicular steatosis. She fully recovered but experienced similar episodes of acute liver failure at age 42, 48, 49, and 55 months. Whole exome sequencing revealed compound heterozygosity for two

variants within the gene of cytosolic AARS (*AARS* c.893A>T [p.Leu298Gln] and c.2251T>C [p.Arg751Gly]).
Discussion: Variants in mitochondrial and cytosolic ARS genes cause a growing spectrum of diseases. Our patient presented with a previously unknown phenotype. Multisystemic disease and acute liver failure/liver disease have been reported in patients with other cytosolic ARS defects (*IARS*, *MARS, LARS* and *YARS* by Kopajtichet al (2016); Casey et al. (2012); van Meel et al. (2013) and Nowaczyk et al. (2017), resp.). This is the first report that shows that also *AARS* variants may cause recurrent acute liver failure.

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Description of a cohort of 23 patients with 3243A>G mutation of the *MTTL1 gene* in mitochondrial DNA

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Background: 3243A>G mutationof *MTTL1* gene is one of the most frequent mitochondrial DNA mutations, usually present in heteroplasmy, with a variable proportion of pathologic mitochondrias in different tissues. The clinical manifestations are variable. Our objective is to describe the phenotypic alterations present in patients with this mutation so as to characterize the natural history of the disease.
Methods: Observational retrospective study including all the carriers of 3243A>G mutation of *MTTL1* gene with a follow up in the Internal Medicine Department at the University Hospital 12 de Octubre, Madrid (Spain) until January 2018.
Results: We retrieved a total of 23 patients, 74% female, with median age of 43 years (IQR: 37–47) at the end of the follow up. 18 patients (78%) had other relatives with the same mutation and 9 of the women (53%) had offspring. The most frequent manifestations were: migraine (52%), diabetes (52%), weakness (35%) and some degree of hearing loss (21%). 9 (39%) had myopathy and 3 (7.7%) cognitive impairment. Only 6 (26%) had MELAS syndrome. A brain MRI was obtained in 21 patients (91.3%) showing alterations in 12 of them (57%), with brain atrophy being the most common (83%), followed by hyperintense signal lesions (58%). Despite presenting cerebral lessions in MRI, 5 of them (42%) were asymptomatic. An echocardiogram was performed in 19 patients (83%), being pathological in 7 (37%). 6 showed hypertrophic and 1 dilated cardiomyopathy; 4 of them (57.1%) were asympthomatic.
Discussion:Mitochondrial cytopathies have great clinical heterogeneity, with hearing loss, diabetes, headache and muscle weakness as the most common manifestations. A significant percentage of patients present structural heart disease and brain alterations in MRI, even being asympthomatic. The diagnosis is complex so a better knowledge of clinical manifestations could facilitate an early suspiction of the disease, leading to a proper genetic counseling of these patients.

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WITHDRAWN

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Biallelic *VPS13D* variants cause severe movement disorder and a Leigh-like syndrome

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Background: *VPS13D* belongs to a family of ubiquitously expressed genes coding for proteins highly conserved in eukaryotic cells. Variants in *VPS13A* lead to the neurodegenerative disorder chorea-acanthocytosis while defects of *VPS13B* and *VPS13C* cause Cohen syndrome and early-onset Parkinson's disease, resp. *VPS13D* defects have recently been associated with a movement disorder and a Leigh-like syndrome. *VPS13D* codes for a ubiquitin-binding protein, which functions downstream of the mitochondrial fission factor DRP1 to control mitochondrial size and autophagic clearance in *Drosophila*. Mitophagy maintains mitochondrial homeostasis and cell health. We report the clinical and genetic findings of one of the seven patients from the original publication regarding *VPS13D* deficiency [1].
Case report of a 10 y-old female with early-onset developmental delay, axial hypotonia, hyperkinetic dystonia, and slowly progressive ataxia with spasticity of the legs. She is able to walk without assistance and attends a special school due to mental retardation and behavioral abnormalities.
Results: Brain MRI showed basal ganglia hyperintensities with no signs of progression within 3 years. CSF lactate was slightly increased. Trio whole exome sequencing revealed homozygosity for a *VPS13D* variant (c.12683G>A, p.R4228Q), predicting loss of VPS13D function. Both heterozygous parents are asymptomatic. A network of collaborators identified additional cases with overlapping phenotype and other biallelic *VPS13D* variants [1].
Discussion: These findings show that biallelic *VPS13D* variants cause a progressive movement disorder with mental retardation in children with a characteristic MRI pattern similar to Leigh syndrome. VPS13D deficiency is a new clinical entitiy affecting the basal ganglia that should be considered in the differential diagnosis of mitochondrial disorders.
[1] Gauthier et al., Ann Neurol. 2018

15. Mitochondrial disorders: mtDNA

P-284

Clinical evolution and diagnostic journey of Malaysians with mitochondrial DNA deletion syndromes - Report of 3 unrelated patients

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Background: Large-scale mitochondrial DNA (mtDNA) deletions (1.1-10 kb) predominantly result in three classical phenotypes- Kearns-Sayre syndrome (KSS), Pearson syndrome and progressive external ophthalmoplegia (PEO). Continuum of these phenotypes are increasingly recognized.
Methods: We report 3 unrelated Malaysians with mtDNA deletion syndromes.
Results: Patient A had a transient haemophagocytic syndrome requiring blood transfusions from age 3 to 13 months. At 4 years old, she presented with hypocalcaemic carpopedal spasm associated with hypokalemia, hypomagnesemia and aminoaciduria due to renal tubulopathy. She developed Leigh syndrome at 8 years. Mitochondrial DNA deletion of approximately 5.5 kb (MT-ATP6, CO3, ND3, ND4, ND5 and ND6 genes) with a high level of heteroplasmy (90%) was detected in her urine sample. Patient B had KSS with onset of PEO and retinitis pigmentosa at age

10 years old and heart block at age 13 necessitating a pacemaker insertion. He had 60% heteroplasmic deletion of 206 bp of the ATP8 gene detected in blood but a large 5.6 kb deletion (MT-CO2, ATP8, ATP6, CO3, ND3, ND4 and ND5 genes) was detected in urothelial cells. He progressed to develop cognitive decline, ataxia and muscle weakness. Muscle biopsy showed many ragged red fibres but no mtDNA deletion was detected in that muscle sample. Patient C also had KSS with PEO from age 10, followed by progressive cognitive decline, ataxia and muscle weakness. The common 5 kb mtDNA deletion (MT-ATP6, CO3I, ND3, ND4 and ND5 genes) was detected in >50% heteroplasmy in her urothelial cells, but not in blood. Patient A and C are still on follow up at age 14 and 15 respectively. Patient B passed away at age 24.
Discussion: The varied clinical evolution and atypical presentations of mtDNA deletion syndromes still make their diagnosis delayed or elusive. Simultaneous blood and urine testing of mtDNA deletions are two non-invasive tests that increased the diagnostic yield for our patients.

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Cerebrospinal fluid monoamines profile in patients with mitochondrial diseases

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Background: Mitochondrial diseases (MD) comprise a group of genetic disorders leading to a dysfunction of mitochondrial energy metabolism pathways. We aimed to assess the clinical phenotype and the biochemical cerebrospinal fluid (CSF) biogenic amine profiles of patients with different genetic diagnoses of MD.
Methods: We recruited 29 patients with genetically confirmed MD, harboring mutations in either nuclear or mitochondrial DNA (mtDNA) genes. Signs and symptoms of impaired neurotransmission and neuroradiological data were recorded. CSF biogenic amines and 5-methyltetrahydrofolate (5MTHF) concentrations were analyzed using high-performance liquid chromatography with electrochemical and fluorescence detection procedures. The mtDNA mutations were studied by SANGER, Southern blot, and real-time PCR, and nuclear DNA was assessed either by Sanger or Next Generation Sequencing.
Results:Five out of 29 cases showed predominant dopaminergic signs not attributable to basal ganglia involvement, harboring mutations in different nuclear genes. A chi-square test showed a statistically significant association between high homovanillic acid (HVA) values and low CSF 5-MTHF values (chi square 10.916; p 0.001). Seven out of the 8 patients with high CSF HVA values showed cerebral folate deficiency. Five of them harbored mtDNA deletions associated with Kearns-Sayre syndrome (KSS), one a mitochondrial point mutation at the mtDNA *MT-ATP6* gene, and one a *POLG* mutation.
Discussion: Dopamine deficiency clinical signs were present in some MD patients having different genetic backgrounds. High CSF HVA values, together with a severe cerebral folate deficiency is a characteristic profile for KSS patients but may also be present in other mtDNA mutation syndromes. Clinical and biochemical markers of dopamine deficiency may be impaired in mitochondrial disorders.

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The Needs and Emotional problems of Caregivers and Pediatric Patients with Mitochondrial diseases

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Background: Considering the persistent physical, neurocognitive and emotional difficulties for both patients and caregivers, we surveyed the symptoms and limitations in daily life which are most burdensome to pediatric mitochondrial patients and their parents, using developed survey for caregivers, to provide adequate interventions for patients and families.
Methods: A total of 83 caregivers of pediatric mitochondrial patients were recruited from the pediatric mitochondrial disease clinics of the Gangnam Severance Hospital in South Korea. Participants completed the survey about their problems and needs relating to mitochondrial disease during and after the diagnosis, and these clinical data were analyzed accordingly.
Results: Surveys from a total of 83 caregivers of patients were analyzed. Children with mitochondrial diseases were between 0 and 0.5 years of age at the time of first symptom onset (43%), and the duration of illness lasted more than 10 years in most cases (42%). Thirty-three had been diagnosed with nonspecific mitochondrial diseases, 19 with Leigh syndrome, and three with MELAS. Caregivers reported increased need for information relating to diseases, medical service, and intervention during the diagnostic process and the highest in intervention phase. The most frequent first symptom was developmental delay (46%) and then followed by seizures (22%), consequently suggesting the necessity of intervention including motor/cognitive rehabilitation therapy, language therapy, and parent education. For the caregivers' emotional experiences, the most common initial responses were 'Discouraged/despair,' 'Helpless/lethargic,' and 'Disconcerted', while "Anxiety" was reported as the most common emotional response for patients.
Discussion: A better understanding of the need and problems of these children and their family is essential for effective care planning for pediatric neurologists and might result in an improved quality of life.
Conflict of Interest declared.

P-287

Two cases of Leigh syndrome involving the m.13094 T> C mutation in the *MT-ND5* gene in Japan

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Background: The m.13094T>C mutation, in the *MT-ND5* gene, is considered as a rare pathogenic variant that has been previously reported to be associated with Leigh Syndrome (LS); however, the complete phenotypic spectrum is poorly understood. In this study, we report 2 Japanese cases involving LS.
Case Report & Results: Patient 1: A Japanese boy became bedridden because of status epilepticus and respiratory failure at 1 year of age, and required artificial ventilation throughout the day. He was diagnosed with LS due the presence of high lactic acid levels in the spinal fluid, and symmetric lesions in the brainstem and basal ganglia as revealed by brain MRI. At 14 years of age, he died of gastroenteritis and dehydration. He was found to have the m.13094T>C mutation in mitochondrial DNA

(mtDNA) with heteroplasmy levels of 49% and 45% in blood and muscle respectively. Patient 2: A Japanese boy developed divergent squint and ptosis at 1 year of age. He was diagnosed with LS due to the presence of high lactic acid levels in the spinal fluid, and symmetric lesions in the brainstem and basal ganglia as revealed by brain MRI. He was found to have the m.13094T>C mutation with heteroplasmy levels of 41% in the fibroblasts. At the time of the diagnosis, his development was normal, but at the age of 3 years, it was strongly suggested that he had autism spectrum disorder.
Discussion: The m.13094T>C mutation has highly variable neurological manifestations and is frequently associated with high disease burden and early mortality. This mutation has also been reported to be associated with various phenotypes; hence, it is necessary to carefully consider this diagnosis. This study proposes that the presence of an unexplained central nervous system disorder should raise clinical suspicion of a mitochondrial disorder.

P-288

Progressive External Ophthalmoplegia caused by a mutation in the *TWNK* gene: a case study

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Background: Progressive External Ophthalmoplegia (PEO) is the most common feature of a mitochondrial myopathy.
Case Report: We present a 45-year-old male without positive family history for neuromuscular diseases with PEO. The patient presented with bilateral ptosis (present since late adolescence) and external ophthalmoplegia with decreased ocular motility and impaired convergence.
Results: Muscle biopsy revealed numerous ragged-red fibers and cytochrome oxidase negative fibers, typical for a mitochondrial myopathy. Muscle mtDNA analysis revealed multiple deletions and subsequently molecular genetic testing of genes related to mtDNA depletion syndromes revealed a previously reported heterogyzous mutation, c.1342A>G (p.Asn448Asp) in exon 2 of *TWNK* gene.
Discussion: The *TWNK* (formerly C10orf2) gene, encodes the twinkle protein which is a mtDNA helicase. Several mutations in *TWNK* have been associated with autosomal dominant PEO, ataxia neuropathy spectrum, spinocerebellar ataxia, Perrault syndrome and mitochondrial DNA depletion syndrome. This specific mutation has been previously reported in a Chinese family with adPEO. Our case report supports the pathogenic nature of the heterozygous form of the c.1342A>G mutation and confirms its presence in the Greek population.

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Phenotypic spectrum of patients harbouring the m.3243A>G mutation

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Background: The phenotypes associated with the m.3243A>G mutation include clinical syndromes such as MELAS, CPEO, and MIDD, though the majority of patients have clinical features that do not fit any of these classifications. We aim to describe the phenotypic spectrum of three patients harbouring the m.3243A>G mutation.
Methods: Patients were identified in the research project during years 2015–2017. All patients underwent extended clinical, laboratory, imaging, neurophysiological and molecular studies.
Results: We identified three female patients with the m.3243A>G mutation. The mean ages of onset and diagnosis were 17.33 ±9.87 and 40.33 ±13.65 years, respectively. All patients developed short stature, ptosis, hearing impairment, dysarthria, dysphonia, dysphagia, exercise intolerance and muscle weakness as well as gastrointestinal dysmotility. Pigmentary retinopathy was detected in one patient. Liver impairment was observed in one patient, and liver biopsy showed macrovesicular steatosis. Endocrine dysfunction was observed in two patients, and was represented by T1DM and hypogonadism, and T2DM, respectively. None of the patients reported migraine. Sudden neurological deterioration was observed in two patients. NCV study results showed decreased amplitudes for sensory nerves in one. Muscle biopsies were performed in two patients and one showed RRF and COX deficiency affecting10% of myocytes. Neuroimaging showed diffuse cerebral and cerebellar atrophy in one patient, and subcortical focal abnormalities in two patients. One patient showed a lack of social interactions and emotional instability.
Discussion: We report detailed phenotypic and genetic characteristics of three unrelated Lithuanian patients with the m.3243A>G mutation. Although the patients harbour the same mutation, they demonstrate a heterogeneous phenotype. Supported by grant TAP LLT-02/2015.

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Early neonatal neurological distress revealing Succinyl-CoA ligase deficiency due to mutations in *SUCLA2*.

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Background: The encephalomyopathic mtDNA depletion syndrome with methylmalonic aciduria is associated with deficiency of succinate-CoA ligase, caused by mutations in *SUCLA2* or *SUCLG1*. We report a newborn with *SUCLA2* mutations presenting with early neonatal neurological distress in the context of perinatal asphyxia.
Case Report & Results: We report a male term newborn, from unrelated parents. There was evidence of perinatal asphyxia, with meconium stained liquor and poor extrauterine adaptation. He presented during the first hour of life with neonatal seizures, lethargy, hypotonia and severe respiratory distress requiring management in the neonatal resuscitation unit. Urine organic acids showed mildly elevated methylmalonate (16%). The acylcarnitine profile was not available. There was no lactic acidosis (in plasma and cerebrospinal liquid) or elevation in the lactate/pyruvate ratio. Cerebral magnetic resonance imaging showed cortical atrophy, cerebellar hypoplasia and increased signal in the basal ganglia on T1 images. The association of encephalomyopathy with an elevated urine methylmalonate suggested the diagnosis of mtDNA depletion

syndrome due to succinate-CoA ligase deficiency. A molecular study indicated the homozygous mutation in the gene encoding *SUCLA2* in the patient. The same heterozygous mutation was revealed in the two parents. The patient became deeply comatose & died aged four months.
Discussion: In this patient the mtDNA depletion syndrome had an antenatal onset as the newborn developed severe neurological distress rapidly after birth. The classic triad of encephalomyopathy, basal ganglia lesions and methylmalonate aciduria suggested the diagnosis, though this was delayed as symptoms were initially attributed to perinatal asphyxia.

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Cyclic vomiting in a subject with homoplasmic m.14674T>C alteration

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Background: For intramitochondrial protein synthesis 22 distinct mtDNA-encoded tRNAs are necessary. These mitochondrial tRNA genes are highly susceptible to point mutations, and are the leading cause of mitochondrial dysfunction. The associated clinical phenotypes are heterogeneous.
Case Report: A teenage girl presented with early onset repetitive cyclic vomiting syndrome and headache. In between she had no complaints and normal cognitive functions. Serum lactate concentration and urine organic acid profile were normal. Mitochondrial function was tested using measurement of activities of oxidative phosphorylation (OXPHOS) complexes, blue native polyacrylamide gel electrophoresis, oxygen consumption, visualization of mitochondrial membrane potential by JC-1, visualization of mitochondria by MitoTracker Red CMXRos fluorescence and OXPHOS complex integrity by immunostaining with subunit specific antibodies. The entire mtDNA was analyzed by massive parallel sequencing.
Results: Oxidative phosphorylation deficiencies were not found in skeletal muscle neither in cultured skin fibroblasts. In the latter, oxygen consumption rates were normal. Impaired build-up of the mitochondrial membrane potential was observed in subsets of fibroblasts. This heterogeneous staining pattern, suggestive of an mtDNA defect, was confirmed by immuno-staining for complex IV subunit I. A similar staining pattern was also observed in cultured skin fibroblasts obtained from the subject's mother. Genetic analysis revealed a homoplasmic m.14674T>C alteration in leukocytes from the proband and the mother.
Discussion: mtDNA 14674T>C alteration in the *MT-TE* gene ($tRNA^{Glu}$) was linked in previous reports to a unique benign syndrome termed reversible infantile respiratory chain deficiency. We here report a subject with cyclic vomiting, illustrating an alternative disease phenotype associated with this point mutation. Disease heterogeneity is also demonstrated in the subject's mother who suffered from severe myalgia and fatigue.

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Extraocular features in 414 German and Czech individuals with Leber hereditary optic neuropathy

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Background: Generally, eye is the only affected organ in Leber Hereditary Optic Neuropathy (LHON), but a small subgroup of patients with visual failure is believed to develop extraocular features. Descriptions of these include only isolated cases or small series, not considering the prevalence rate in the General Population (GP).
Methods: Data from 414 German and Czech individuals (216 symptomatic patients and 197 carriers) with one of the m.11778G>A, m.3460G>A and m.14484T>C were analysed and compared to GP in a retrospective double-centre study in order to provide overview on the occurrence of extraocular symptoms in LHON.
Results: The only symptom showing significantly higher prevalence in symptomatic females with LHON when compared to GP was peripheral neuropathy (PN; 28% vs. 4.5%). The overall prevalence of PN in LHON was not higher (12.7%) than in the GP (3.9 - 13.1% [1]). The risk of developing tremor, sensorineural hearing loss (SHL), depression, insomnia, migraines or thyroid disease was not elevated in LHON. The prevalence of cerebellar ataxia was high in female symptomatic patients (15.6%), but data on its prevalence in the GP are not available. In all, 6 patients (2.8%) manifested with Harding's disease, an association of LHON and multiple sclerosis and 8 (3.7%) patients developed Charles-Bonnet syndrome. The most remarkable feature was the dominance of extraocular symptoms in a family with m.11778G>A mutation. In 4 patients with myopathy and SHL or PN, visual impairment was completely absent. Neither Mitochondrial DNA sequencing nor Whole Exome Sequencing revealed any other pathogenic mutation.
Discussion: Except for PN in symptomatic females, the prevalence of extraocular symptoms in LHON does not seem to be higher than in the GP. One family illustrates the possibility of developing extraocular symptoms without visual symptoms in carriers of m.11778G>A. [1] Hanewinckel, R. et al. 2016 Neurology. Supported: AZV 16-32341A, VFN-RVO 64165, SVV 260367

16. Disorders of purines, pyrimidines, nucleic acids and porphyrias

P-293

EXPLORE: A prospective, multinational natural history study of acute hepatic porphyria patients with recurrent attacks

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Background: Acute hepatic porphyrias are rare, often misdiagnosed genetic diseases caused by a mutation in one of the enzymes of heme biosynthesis. This results in the accumulation of neurotoxic heme intermediates, aminolevulinic acid (ALA) and porphobilinogen (PBG), that can cause severe neurovisceral pain, life-threatening attacks and chronic debilitating symptoms (pain, nausea, and fatigue). We will be presenting updated ≥12-month data.
Methods / Case Report: Explore is a prospective, international, observational natural history study and clinical management of patients with hepatic porphyrias with recurrent attacks (≥3 attacks/year) or who receive prophylactic treatment to prevent attacks. Patient medical history, physical examination and porphyrin biomarkers, and questionnaires on porphyria activity were collected.
Results: 112 patients enrolled from 13 countries and were followed for 12 months. Mean patient age: 39 years, 89% female, 93% with acute intermittent porphyria, 4% variegate porphyria, and 3% hereditary coproporphyria. Patients reported a mean of 9.3 attacks in the 12 months prior to the study, pain being the most common symptom, occurring in 99% of attacks. Mean attack duration was 7 days. Chronic symptoms were reported by 65% of patients, pain being the most frequent symptom. On-study attack rate was 3.7 attacks/person/year, of which 69% required treatment with hemin or a healthcare visit. For those patients on hemin prophylactically, mean attack rate was 3.5 attacks/person/year. Mean ALA and PBG levels at screening (during non-attack) were markedly increased to 9 and 23 times the upper limit of normal, respectively.
Discussion: EXPLORE, the first international natural history study in patients with hepatic porphyria and recurrent attacks, demonstrates that patients suffer from attacks and chronic symptoms. Given morbidity and mortality, there remains an unmet need for novel therapies to prevent attacks and treat chronic symptoms.
Conflict of Interest declared.

P-294

Proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy as a diagnostic tool for purine/pyrimidine disorders

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Background: The timely and efficient diagnosis of IEMs remains challenging in third world countries due to limited awareness, logistical constraints as well as scarcity in expertise in the biochemical diagnosis thereof. Standard methods used for diagnosis of IEMs consist of GC-MS and/or LC-MS. ^{1}H-NMR spectroscopy is an established complementary analytical approach for screening of IEMs.The aim of this study was to determine the accuracy of quantitative results obtained by ^{1}H-NMR compared to collective results submitted by UPLC/UPLC-MS/MS-focused laboratories participating in an international EQC program for purine and pyrimidine metabolites.
Methods: This study consisted of the analysis of urine samples, provided by the European Research Network (ERNDIM) for evaluation and improvement of screening, diagnosis and treatment of IEMs. Sample preparation involved centrifugation, followed by the addition of a deuterated buffer, containing an internal standard, to the supernatant, before transfer to a 5mm NMR glass tube. Analysis proceeded on a Bruker Avance III HD 500 MHz NMR spectrometer.
Results: The ^{1}H-NMR specific purine/pyrimidine profiles were confirmed via pure compound library spectra. Most of the purines/pyrimidines were confidently quantified and compared well with the collective EQC results – z-score less than 1. Exceptions were: pseudo-uridine, thymidine and thymine, which were confidently identified but not confidently quantified. Omitted metabolites that were not detectable or clearly discernible were: 5-hydroxymethyluracil, dihydrothymine, orotidine, uracil and uric acid.
Discussion: This study demonstrates that relevant purine/pyrimidine metabolites can be accurately quantified via ^{1}H-NMR spectroscopy. ^{1}H-NMR proves to be an alternative analytical platform as a screening tool for potentially prevalent purine/pyrimidine related disorders. Furthermore, it may serve as a complementary application in combination with routine metabolic testing services.

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Dihydropyrimidinase deficiency due to novel *DPYS* mutations affecting protein structural integrity and catalytic activity

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Background: Dihydropyrimidinase (DHP) is the second enzyme of the pyrimidine degradation pathway and catalyses the ring opening of 5,6-dihydrouracil and 5,6-dihydrothymine. Here, we report molecular analysis of 4 newly-identified DHP deficient patients presenting with strongly elevated levels of 5,6-dihydrouracil and 5,6-dihydrothymine in urine and a highly variable clinical presentation, ranging from asymptomatic to infantile spasm and reduced white matter and brain atrophy.
Methods: The coding sequence of human DHP was re-cloned into the *BamHI-KpnI* site of the pcDNA3.1Zeo vector. The pcDNA3.1Zeo plasmid containing wild-type DHP was subjected to site-directed mutagenesis. HEK293 cells were transfected with pcDNA3.1Zeo-DHP (wild-type, variants). All transfections were performed at least in triplicate. DHP enzyme activity assay and western blot analysis was performed using transfected cell supernatants.
Results: Analysis of *DPYS* showed the presence of 8 mutations including 4 novel missense mutations and one novel deletion. Functional analysis of recombinantly-expressed DHP mutants carrying the p.M250I, p.H295R, p.Q334R, p.T418I and the p.R490H mutation showed residual DHP activities of 2.0%, 9.8%, 9.7%, 64% and 0.3%, respectively. The crystal structure of human DHP indicated that all point mutations were likely to cause rearrangements of loops shaping the active site, primarily affecting substrate binding and stability of the enzyme.
Discussion: We describe 4 Asian patients with a complete DHP deficiency presenting with a highly variable clinical phenotype, thus expanding the clinical spectrum of this inborn error of metabolism. Five novel/rare variants were detected in *DPYS,* affecting protein structural integrity and catalytic activity. The observation that the identified mutations were more prevalent in East Asians and the Japanese population indicates that DHP deficiency may be more common than anticipated in these ethnic groups.

P-296

Phase 1/2 and Open Label Extension Studies of Givosiran an Investigational RNAi Therapeutic, in Patients with Acute Intermittent Porphyria

Desnick R J[2], Sardh E[7], Harper P[7], Balwani M[2], Stein P[4], Rees D[4], Bloomer J[1], Bissell M[3], Park C[5], Phillips J[5], Bonkovsky H[6], Al-Tawil N[7], Dinh Q[8], Penz C[8], Chan A[8], Querbes W[8], Simon A[8], Anderson K[9]

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Background: Acute hepatic porphyrias (AHPs) are rare genetic diseases of heme synthesis. Induction of aminolevulinic acid synthase 1 (ALAS1) can lead to accumulation of the neurotoxic heme intermediates aminolevulinic acid (ALA) and porphobilinogen (PBG) that are causal for acute neurovisceral attacks and chronic symptoms. Givosiran is an investigational RNAi therapeutic targeting liver ALAS1 to reduce ALA and PBG accumulation in AHP patients and ameliorate disease manifestations.
Methods / Case Report: A phase 1, multinational, randomized, placebo-controlled, study was conducted in Parts A, B and C to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of subcutaneously administered givosiran in AIP patients. Part C also had exploratory analyses of clinical activity of givosiran in patients with recurrent attacks (ClinicalTrials.gov Identifier: NCT02452372). Patients completing Phase 1 were eligible to enroll in the open label extension (OLE) study (NCT0294983).
Results: As of February 2018, givosiran was generally well tolerated in Part C (Cohorts 1–3), with 2 patients with serious adverse events. In Phase 1, monthly dosing achieved approximately 60-70% ALAS1 mRNA silencing. With up to 22 months of total treatment in Phase 1/OLE, continuous dosing at 2.5 mg/kg monthly regimen potentially leads to enhanced clinical activity with >80% lowering of ALA and PBG. Patients treated with givosiran had mean reductions of 93% in annualized attack rate (AAR) (requiring hospitalization, urgent care, or hemin) and 94% in annualized hemin use observed in OLE relative to Phase 1 Run-in.
Discussion: Givosiran was generally well-tolerated and resulted in rapid, dose-dependent, and durable lowering of neurotoxic intermediates ALA and PBG. Importantly, this ALA and PBG lowering was associated with marked reductions in both the AAR and annualized hemin use. Complete Phase 1 study data will be presented along with interim study data from the Phase 1/2 OLE study.
Conflict of Interest declared.

P-297

Mass spectrometric analysis of purine de novo biosynthesis

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Background: Purine nucleotides have vital functions including nucleic acid synthesis, energetic homeostasis, cell signalling and others in both prokaryotes and eukaryotes. Supply of purines is provided by two pathways – salvage pathway and de novo synthesis (PDNS). Although PDNS activity varies during the cell cycle, it becomes an important source of purines especially for rapidly dividing cells. No current method exists for detailed study of PDNS due to analytical issues (sensitivity) and commercial unavailability of compounds.
Methods:The aim was to fully describe mass spectrometric fragmentation behaviour of newly-synthesized PDNS-related metabolites using liquid chromatography coupled to high resolution multistage mass spectrometry and to develop an analytical method.
Results:With the exception of four initial ribotide PDNS intermediates that preferred losing water, phosphate or cleaved forming base of the purine ring, all other metabolites studied cleaved the glycosidic bond in the first fragmentation stage. Fragmentation was possible to the third and sixth stages. A liquid-chromatography-high resolution mass spectrometric method was developed and applied in the analysis of CRISPR-Cas9 genome-edited HeLa cells deficient in individual steps of PDNS and the salvage pathway. Identity of newly-synthesized intermediates forming under pathological conditions of known and theoretical defects of PDNS was confirmed by comparing fragmentation patterns of synthesized metabolites of PDNS with those produced by cells.
Discussion:Use of stable isotope incorporation allowed confirmation of fragmentation mechanisms and potentionally provides data for future fluxomic experiments. The method may find a use in diagnosing of PDNS disorders, investigation of purinosome formation, cancer research, enzyme inhibition studies and other applications.
Supported by the MEYSCR LO1304, the MHCR (AZV 15-28979A), programmes PRIMUS/17/MED/6 and by IGA_LF_2018_010.

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Clinical manifestations and molecular aspects of phospho ribosylpyrophosphate synthetase superactivity in females

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Background: Phosphoribosylpyrophosphate synthetase (PRPS1) superactivity is an X-linked disorder characterized by urate overproduction Online Mendelian Inheritance in Man (OMIM) gene reference 300661. This condition is thought to rarely affect women, and when it does, the clinical presentation is mild. We describe a 16-year-old African American female who developed progressive tophi, nephrolithiasis and acute kidney failure due to urate overproduction. Family history included a mother with tophaceous gout who developed end-stage kidney disease due to nephrolithiasis and an affected sister with polyarticular gout. The main aim of this study was to describe the clinical manifestations of PRPS1 superactivity in women.
Methods: Whole exome sequencing was performed in affected females and their fathers.
Results: Mutational analysis revealed a new c.520 G > A (p.G174R) mutation in the *PRPS1*gene. The mutation resulted in decreased PRPS1 inhibition by ADP.
Discussion: Clinical findings in previously reported females with PRPS1 superactivity showed a high clinical penetrance of this disorder with a mean serum urate level of 8.5 (4.1) mg/dl [506 (247) μmol/l] and a high prevalence of gout. These findings indicate that all women in families with PRPS1 superactivity should be genetically screened for a mutation (for clinical management and genetic counselling). In addition, women with tophaceous gout, gout presenting in childhood, or a strong family history of severe gout should be considered for *PRPS1* mutational analysis.
Funding: This work was supported by NIH-NIDDK grant R21 DK106584 and by grants AZV 15–28979 A and AZV 17–29786 A from

the Ministry of Health of CR. Institutional support was provided by the PRIMUS/17/MED/6, UNCE 204011, UNCE 204064, PROGRES-Q26/LF1 and SVV 260367/2017 programmes of Charles University in Prague and by the Ministry of Education, Youth and Sports of CR [LQ1604 National Sustainability Program II].

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Other genetic factors associated with manifest AIP in a Spanish population with a founder mutation in *HMBS*

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Background: Acute intermittent porphyria (AIP) is a low-penetrant inherited disease caused by a disorder of haem biosynthesis. Over 10-20% of AIP carriers develop acute neurovisceral attacks, which are often associated with triggering factors such as prescribed drugs, endocrine factors, fasting or stress. However, the genetic factors underlying penetrance are still unknown. Drug-metabolizing cytochrome P450 enzymes (CYP) are haem-dependent proteins which play a role in haem demand, and, since the main hepatic CYP are polymorphic, it was decided to analyse whether the onset of acute attacks is related to the *CYP* genotype.
Methods: 50 Spanish AIP genetic carriers, from 21 different families, were included in the study. Most of them (78%) carried the founder pathogenic variant NC_000011.9 (NM_000190.3):c.669_698del in *HMBS* gene. Patients were deemed to have manifest AIP when they had had at least one attack of AIP. *CYP2C9**2, *3; *CYP2C19**2; *CYP2D6**4, *5; *CYP3A4**1B and *CYP3A5**3 defective alleles were genotyped. Urinary porphyrin precursors were also analysed in recent urine sample at an asymptomatic period.
Results: 52% had manifest AIP. Latent AIP was more frequent among defective *CYP2D6* allele carriers ($p < 0.05$). The urine PBG-to-creatinine ratio tended to be lower in individuals carrying defective alleles in *CYP2C9* and *CYP2D6* genes. However, this reduction in PBG levels was not attributed to the *CYP* genotype but was associated with the absence of history of acute attacks ($p < 0.05$).
Discussion: Our results suggest that *CYP2D6**4 and *5 alleles may be protective factors for acute attacks in AIP so *CYP2D6* may constitute a penetrance-modifying gene. Further studies in larger AIP populations are needed to validate this association and to obtain a more comprehensive overview. The confirmation of these findings would allow the acute attack risk profile for each AIP carrier to be predicted based on the *CYP* genotype and lead to progress in personalized medicine for porphyria carriers.

17. Peroxisomal, sterol, bile acid, lipid and lipoprotein

P-300 Universal screening for familial hypercholesterolemia in children - the Slovenian model

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Background: Familial hypercholesterolemia (FH) is the most common inborn error of metabolism and arguably also the most common monogenic disorder in humans, but severely under-diagnosed. Young adults with untreated FH have up to 100-fold elevated risk of cardiovascular complications as compared to unaffected individuals; early diagnosis and timely management substantially reduce this risk. Slovenia has gradually implemented the program of universal FH screening in pre-school children, consisting of two step approach: (1) universal hypercholesterolemia screening in pre-school children at the primary care level; (2) genetic FH screening in children referred to the tertiary care level according to clinical guidelines (with additional cascade screening of family members).
Methods: We analyzed the retrospective data (from 2012–2015), to assess the efficiency of the universal FH screening program.
Results: In that period, 280 children (59.3% female) detected were referred to our center for having TC level of either more than 6 mmol/L (231.7 mg/dL) or more than 5 mmol/L (193.1 mg/dL) with a positive family history of premature cardiovascular complications at the universal hypercholesterolemia screening. So far, 170 (57.1% female) of them were fully genotyped, 44.7% had a FH disease-causing variant (28.8% in *LDLR* gene, 15.9% in *APOB*, none in *PCSK9*); genetic analysis is still ongoing for one-third of patients. For every child with FH also one parent was detected (less than 5% of them had been previously diagnosed).
Discussion: Slovenia has a unique program of universal FH screening in pre-school children and in their family members, capturing the majority of FH cases in the generations screened.

P-301

Inflammatory profile in X-linked adrenoleukodystrophy patients with different phenotypes

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Background: X-linked adrenoleukodystrophy (X-ALD) is an inborn error of peroxisome metabolism. Hexacosanoic (C26:0) and tetracosanoic (C24:0) acids are saturated fatty acids that accumulate in tissues and body fluids. The exact mechanisms underlying brain damage in X-ALD are poorly elucidated, but some researchers have proposed that inflammation represents a hallmark in the pathogenesis of X-ALD. Objective: We aimed to investigate pro and anti-inflammatory cytokines in plasma from three different male phenotypes.
Methods / Case Report: Blood (plasma) taken from 24 X-ALD patients [8 CCER (cerebral form), 8 AMN (adrenomyeloneuropathy) and 8 asymptomatic patients] were included in this study. Cytokines measured in plasma were performed by an Invitrogen kit: Human Ultrasensitive Cytokine 10-Plex Panel.
Results: Asymptomatic patients showed higher levels of IL-1β, IL-2, IL-8 and TNF-α (pro-inflammatory cytokines) and TNF-α was also increased in AMN phenotype. Furthermore, asymptomatic patients presented higher levels of IL-4 and IL-10 (anti-inflammatory cytokines). AMN patients showed higher levels of IL-2, IL-5 and IL-4. We may infer that inflammation is related to plasma VLCFA concentration in X-ALD, because we observed, in asymptomatic and AMN patients, positive correlations between C26:0 plasma levels and pro-inflammatory cytokines. Also a negative correlation

between anti-inflammatory cytokine and C24:0/C22:0 (docosanoic acid) ratio was verified in AMN patients.
Discussion: The present work showed experimental evidence that there is an inflammatory imbalance associated with Th1, Th2 and macrophages response in the periphery of asymptomatic and AMN patients, and there is a relationship between VLCFA plasma levels and inflammation in X-ALD. It can also be inferred that the enhancement of cytokines in plasma from asymptomatic patients could be considered an early biomarker of brain damage and maybe also a predictor of X-ALD progression.

P-302

Differential diagnosis of ultra-rare peroxisomal disorders

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Background: The X-linked peroxisomal disorder CADDS "contiguous *ABCD1-DXS1357E* (*BCAP31*) deletion syndrome" was first described in 2002 in three patients. Subsequently, CADDS has been reported in two patients carrying a larger deletion, including the creatine transporter *SLC6A8* (CADDS-2). We describe a CADDS-2 patient and propose a novel diagnostic algorithm for the diagnosis of ultra-rare peroxisomal disorders, which integrates different omic platforms.
Methods / Case Report: A male infant died of acute liver failure at 6 months. He presented with hypotonia, dysmorphisms, cholestasis, hypothyroidism, hepatosplenomegaly, ambiguous genitalia, adrenal insufficiency, neonatal diabetes, basal ganglia lesions and leukoencephalopathy. Diagnostic investigations included multiplex LC-MS/MS method for simultaneous analysis of VLCFA, phytanic/pristanic, di/tri-hydroxycholestanoic, and docosahexaenoic acids (Semeraro et al. Clin Chim Acta 2016), LC-MS/MS plasma long-chain acylcarnitine analysis (Rizzo et al. Pediatr Res 2003), brain MRS, fibroblasts studies, and CGH array.
Results: Plasma VLCFA were elevated (C26=6.49, NV 0.01-0.9; C26/C22=0.11, NV 0.006-0.026 μmol/L) with normal values of all other metabolites; acylcarnitine analysis (μmol/L) showed increased dicarboxylic fractions (C14DC=0.04, NV < 0.03; C16DC=0.07, NV < 0.05; C18DC=0.11, NV < 0.03) and C26 monocarboxylic=0.72 (NV< 0.03). Brain MRS showed marked reduction of creatine peak. Fibroblasts studies showed elevated VLCFA, normal catalase reaction and absence of BAP31 protein. CGH array confirmed CADDS-2 syndrome.
Discussion: Our patient presented a combined increase of VLCFA and long-chain-acylcarnitines, which restricted the diagnosis to three diseases: ABCD5, ACOX, and CADDS. Brain MRS confirmed CADDS-2. This approach provides a new diagnostic algorithm for ultra-rare peroxisomal disorders, integrating multiplex LC-MS/MS analysis of peroxisomal biomarker with long-chain acylcarnitine analysis and brain MRS.

P-303

Homozygous familial hypercholesterolaemia in Greece: Epidemiological findings from 39 children, adolescents and young adults

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Background: Homozygous Familial Hypercholesterolaemia (Homo-FH) is a rare Inborn Error of Cholesterol Metabolism. The mode of inheritance is dominant and the frequency described as 1:1000000 births. Several genes can cause this disorder. The most frequent gene causes LDL-Receptor deficiency with about 1300 mutations. 47 mutations so far have been described in Greeks and Greek-Cypriots (2018). All of them have been found in our lab, 19 of them for the first time in Greece. 11 are novel.
Methods / Case Report: The Aim was to describe the characteristics of early diagnosed HomoFH. 3916 FH families were screened for 3–5 continuous generations each, over a period of 25 years (1992–2018). There was no consanguinity. The patients fulfilled the clinical inclusion criteria. Molecular analysis was performed by Sequencing and Nanochip Technology.
Results: We present the data from 39 Greek HomoFH patients (36 families, 1 with 3 and 1 with 2 siblings), age 1–36 years old today. Age of diagnosis varied from birth till 5 years. Cholesterol levels ranged from 347 to 2000 mg/dl. Tendon xanthomas presented in 39/39, corneal arcus in 25/39, stenosis of aorta 28/39 before the age of 8 years. 7 HomoFH patients died. 2 had successful liver transplantation. 18 mutations were found in the LDL-R gene, the most frequent being Genoa, Afrikaner-2, Greece-2, Greece-1, San Francisco and Sicily. 29 patients were true homozygous and 10 compound heterozygous.
Discussion: HomoFH is more frequent than expected. In Greece we estimate that of HomoFH is ≈1:300.000 births and HeteroFH ≈1:200–250 births.

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From biochemical to molecular genetic diagnosis of peroxisomal diseases and vice versa

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Background: The peroxisomal disorders (PDs) are a heterogeneous group of metabolic diseases caused by impairment of peroxisome biogenesis or one of the metabolic functions of peroxisomes. The phenotypic spectrum of PDs keeps widening, what intricates the clinical recognition of peroxisomal diseases.
Methods: Analysis of plasma VLCFA (C22:0, C24:0, C26:0, C24/C22, C26/C22), phytanic and pristanic acids were used for the first line biochemical tests. Molecular-genetic tests include direct sequencing or target NGS panel analysis for the second line test in most cases.
Results: The PD diagnosis was confirmed in 252 patients: 238 had - X-linked adrenoleukodystrophy (XALD), 12 - Zellweger Spectrum Disorders, and 2 had - D-bifunctional protein deficiency. In 2 cases biochemical data was normal or slightly elevated C24/C22, C26/C22 in plasma but XALD was confirmed by DNA-analysis. In 4 cases a non-classical clinical picture allowed firstly to provide the DNA sequencing and then we confirmed the diagnosis by VLCFA analysis. Two cases are briefly presented below. Case 1. 33y/o man, presented at 20 y/o with ataxia and spastic tetraparesis. MRI: bilateral periventricular white matter lesions and hypodensity TW2-changes in basal ganglia, slow progression of the disease. DNA-analysis: unreported mutation NM_00033.3: c.922C>T in *ABCD1*. Case 2. 8 y/o boy, sick from birth: failure to thrive, muscle hypotension, lack of speech, hepatosplenomegaly, and brachycephaly. MRI: myelination delay, 2 mutations in *PEX6*: NM_000287: c.G1817C, c.283_288del ware found by target sequencing.
Discussion: A complex approach is important in the diagnosis of PDs. Particularly in cases with nonclassical phenotype or in cases

with ambiguous laboratory data. Normal/slightly abnormal biochemical changes could not exclude PDs, and from the other side biochemical tests play an important role in the functional testing of novel mutations.

18. Lysosomal disorders: mucopolysaccharidoses, oligosaccharidoses, sphingolipidoses

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A novel mutation deep within intron 7 of the *GBA* gene causes Gaucher Disease

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Background: Gaucher disease is rare in Cyprus with only three patients (Type I) having been diagnosed in the last thirty years. Two patients have the genotype N370S/L444P while the third patient was negative for these two mutations, as well as for another eight common mutations. The objective of this study was to determine the genotype of this patient.
Case Report: The proband was referred to the Haematology clinic at the age of 42. She was found to have hepatosplenomegaly (liver 8cm, spleen 10cm), elevated acid phosphatase and gammaglobinaemia. Bone marrow biopsy showed the characteristic "Gaucher" cells. Diagnosis was confirmed by measuring glucocerebrosidase activity. She started ERT at the age of 53.
Methods/Results: All exons of the *GBA* gene, including exon-intron boundaries, as well as the 5'UTR and 3'UTR regions, were sequenced and no mutation was detected. To determine whether aberrant RNA splicing occurred, PCR amplicons of cDNA were generated and sequenced. Gel electrophoresis of the cDNA amplicon spanning exons 6–10 yielded an additional band of higher molecular weight. The DNA was extracted from the gel, cloned into a pGEM-TEasy vector and sequenced, revealing an insertion of 242bp between exons 7 and 8. Sequencing of genomic DNA of this region identified a novel mutation, g.7764C>A (c.999+242C>A), deep within intron 7. This mutation creates a new splice donor site leading to the insertion of the first 242 nucleotides of intron 7 in mRNA and resulting in a premature stop codon. The mutation was found in the homozygous state in the proband and heterozygous state in other family members.
Discussion: This study identified a novel mutation deep in intron 7 of the *GBA* gene. There are a few reports of mutations in deep intronic regions causing aberrant splicing of the pre-mRNA. This type of mutation mechanism has been previously described for other diseases but this is the first time, as far as we know, that it is described for Gaucher disease.

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Antibodies towards enzyme replacement therapy in Fabry disease: subclass analysis and impact on biochemical response to treatment

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Background: Fabry disease (FD) is an X-linked lysosomal storage disorder leading to premature mortality due to cardiac, renal and cerebrovascular complications. In male patients with classical FD, treatment with enzyme replacement therapy (ERT) is often complicated by anti-enzyme antibody formation. More knowledge regarding antibody subtypes, timing of antibody development and their effect on treatment efficacy is needed.
Methods: ELISAs measuring IgM, IgA and IgG1,2,3 and 4 anti-recombinant alpha galactosidase A (α-r-αGAL A) were developed (subclass analysis). A plasma pool of antibody positive FD patients served as a standard for inter patient comparison. Ig subclass analysis, was performed in 9 patients whose plasma inhibits recombinant enzyme activity *in vitro* (inhibitor positive), indicative of the presence of α-r-αGAL A antibodies and 3 inhibitor negative patients over a period of 8 years. *In vitro* plasma enzyme activity inhibition and lysoglobotriaosylsphingosine (lysoGb3) levels were determined in 259 samples of 39 treated classical male patients.
Results: IgG1 levels increased during treatment in plasma of all inhibitor positive patients and not in inhibitor negative patients. IgG4 α-r-αGAL A antibodies developed in 7 out of 9 inhibitor positive patients. Levels of anti-r-αGAL A IgG2, IgG3, IgA and IgM were either not present or too low to quantify. 20 out of 39 patients were inhibitor positive. 7 of these patients alternated between inhibitor-positive and inhibitor-negative status. Mean *In vitro* enzyme inhibition during treatment correlated with mean lysoGb3 (ρ_s=0.59, p=0.0001) as well as decrease in lysoGb3 from baseline ($\rho_s = -0.6$, p= 0.0001).
Discussion: The development of antibodies negatively affects the biochemical response to ERT in FD, indicative of insufficient treatment response with higher enzyme activity inhibition. The presence of both IgG1 and IgG4 anti-r-αGAL A antibodies is associated with *in vitro* enzyme inhibition.
Conflict of Interest declared.

P-307

Identification of novel mutations and copy number changes in patients with Tay-Sachs disease from India

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Background: Tay-Sach disease (TSD), one of the common lysosomal storage disorders in India, results from mutations in *HEXA* gene. According to HGMD database, nearly 170 different mutations in the form of single base-pair substitutions, deletions, insertions in *HEXA* gene have been described.
Methods: In the present study, Multiplex Ligation-dependent Probe Amplification (MLPA)-based approach (MRC-Holland, P199-B) were applied to investigate for the potential occurrence of large *HEXA* deletions/duplications in addition to common mutation screening and bidirectional sequencing of *HEXA* gene.
Results and Discussion: Overall analysis has identified 29 alleles in 39 individuals affected by TSD. Eighteen of which were novel, including 7 missense variants [V206L, Y213H, R252C, F257S, C328G, G454R, P475R], 3 nonsense variant [S9X, E91X, W420X], 3 splice site variants [c.347-1G>A, c.460-1G>A, c.1527-2A>T] and 2 small deletion [c.1349delC (p.A450VfsX3) and c.52delC (p.G18Dfs*82)]. Using the MLPA assay, 2 novel deletions and one novel duplication were also

identified. *In silico* analyses of all novel variants of *HEXA* gene were shown to be probably damaging with a deleterious effect on protein function. Protein homology modeling study was carried out to further establish the effect of novel variants. The sequence alignment of *HEXA* genes from various species reveals that all residues are highly conserved across the species.
Conclusion: This is the first global report showing large deletion and/or duplication in *HEXA* gene using MLPA. It can be concluded that MLPA needs to be considered as the second tier approach to identify deletions and/or duplication in the genomic sites where no variants are observed by conventional Sanger sequencing. Additionally the presence of E462V variant in 7/39 (~18%) unrelated patients further reconfirms this as a founder mutation in our population.

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Retina and optic nerve degeneration in alpha-mannosidosis

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Background: Alpha-mannosidosis is a rare, autosomal-recessive, lysosomal storage disease caused by a deficient activity of alpha-mannosidase. Clinical characteristics include facial coarsening, intellectual and motor and hearing impairment, as well as skeletal abnormalities. Opacities of the cornea or lens, strabismus, and ocular motility disorders were reported as typical ocular manifestation in alpha-mannosidosis. In contrast, retina and optic nerve degeneration have been rarely described.
Methods: We report ocular findings of 32 patients with alpha-mannosidosis and especially concentrated on retina and optic nerve abnormalities which we supported by posterior segment examination, fundus photography and Spectral-Domain optical coherence tomography (OCT).
Results: Retina degeneration with bone spicule formations in the peripheral retina or macular changes were seen in 3 patients (9.4%) on funduscopy; of these, 2 with optic nerve atrophy. 8 retinal images could be obtained by OCT or fundus photography; of these 6 showed thinning of the outer retinal layers on OCT. Overall, optic nerve atrophy was seen in 6 patients (18.8%); of these 4 with partial atrophy. 2 patients had partial optic nerve atrophy with no retinal abnormalities on funduscopy. Cataract was seen in 3 (9.4%), corneal haze in none of the patients. 3 patients (9.4%) had manifest strabismus, 4 (12.5%) with nystagmus, and in 5 patients (15.6%) impaired smooth pursuit eye movements were seen.
Discussion: Ocular pathologies are not exclusively confined to opacities of the cornea or lens, strabismus or ocular motility disorders but retina degeneration and optic nerve atrophy may be common eye pathologies in alpha-mannosidosis. OCT technology provides early diagnosis of retina degeneration by showing thinning of the outer retinal layers which can progress with age and potentially leads to vision loss over time.

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A-Synuclein dimerization in erythrocytes of patients and carriers of Sanfilippo syndrome

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Background:The link between Gaucher and Parkinson disease (GD, PD) is well established. However several studies indicate also a link between PD and other lysosomal storage diseases including Sanfilippo syndrome (San FS). a-Synuclein (a-Syn) has been linked to PD and its increased dimerization was reported in red blood cell (RBC) membranes of GD patients.
Methods :San FS patients (GrA; n=15, age 1–15 years), San FS carriers (GrB; n=11, age 8–58 years) and controls (GrC; n=38, age 4–65 years) were studied. RBCs were isolated from heparinized blood. Levels of monomeric and dimeric a-syn in RBC membranes were assessed using Western Immunoblotting and the C20 polyclonal antibody. Statistical analysis was carried out using the Kruskal – Willis test.
Results:No statistical difference was detected between the 3 groups studied with respect to monomer, dimer a-syn levels, as well as the dimer/monomer ratio.
Discussion:a-Syn aggregation has been described in the brains of PD and GD patients. Furthermore, increased dimerization of a-syn has been observed in RBC membranes in PD patients and PD patients with GBA mutations, as well as in patients with GD free of PD, suggesting that RBC a-syn could be a biomarker reflecting the brain situation in these patients. Relevant studies have not been reported in GD carriers without PD. a-Syn aggregation has also been described in the brains of San FS patients. However, in our study we did not detect increased dimerization of a-Syn in the RBC membranes of patients and/or carriers of San FS. Our findings indicate that different mechanisms may be operating in the above disorders.

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Cardiovascular imaging in MPS III patients reveals early left ventricular dysfunction

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Background: Mucopolysaccharidosis type III (MPS III, Sanfilippo disease) is characterized by progressive neurocognitive decline with limited somatic disease. As cardiovascular disease (CVD) has been observed in a few isolated cases, we aimed to investigate the presence of CVD among MPS III patients in the Netherlands, and to compare the myocardial function of MPS III patients to those of healthy controls.
Methods: In this cross-sectional study (2016–2017), echocardiographic studies including Speckle-tracking echocardiography (STE) and left ventricle ejection fraction (LVEF) were performed in 30 MPS III patients (21 patients < 18 years, 9 patients > 18 years). The results were compared to data from age and BSA matched controls.
Results: The mean global longitudinal strain (GLS) on STE, a marker for early myocardial dysfunction, was decreased in pediatric MPS III patients (−15.1%) compared to pediatric controls (−18.7%) (mean difference 4.3, 95% CI 2.53-6.07, p< .001), indicating a reduced myocardial deformation. The mean GLS of adult MPS III patients (−14.4%) was also decreased compared to adult controls (−16.2%) (mean difference 1.78, 95% CI .29-3.26, p=.021). The mean LVEF was lower in adult patients compared to adult controls (48.7% vs. 56.1%, p=.003). LVEF in pediatric MPS III patients and controls did not differ significantly.
Discussion: STE among both pediatric and adult MPS III patients shows signs of early LV dysfunction confirming cardiovascular involvement in

MPS III. In addition, STE is decreased in pediatric patients in the absence of LVEF abnormalities suggesting STE might be used as an early marker for cardiovascular involvement in MPS III patients. As a number of disease-modifying treatment options are now in trial, including gene therapy, this may become clinically important with increasing age of MPS III patients. Further studies are needed to assess a potential link with sudden death in MPS III.

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Psychosocial functioning in parents of MPS III patients

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Background: Mucopolysaccharidosis type III (MPS III or Sanfilippo syndrome) is a lysosomal storage disease resulting in progressive neurocognitive decline during childhood and early demise. Its diagnosis may have a great impact on parents, potentially leading to psychosocial problems such as anxiety, depression, parental distress, and posttraumatic stress. We aimed to assess the psychosocial functioning of mothers and fathers of Dutch MPS III patients.
Methods: Twenty-six mothers and 19 fathers of 34 MPS III patients completed the 'Hospital Anxiety and Depression Scale' (HADS), the 'Distress Thermometer for Parents'(DT-P) and the 'Self Rating Scale for Posttraumatic Stress Disorders' (SRS-PTSD). Independent-samples T-tests and Chi-Square tests were used to assess differences between parents of MPS III patients and reference groups regarding anxiety and depression (HADS), parental distress (DT-P), and posttraumatic stress (SRS-PTSD).
Results: Mothers met the criteria for clinically relevant anxiety (50%) and depression (34.6%) more frequently compared to reference mothers ($p<$ .01). Fathers more often met the criteria for clinically relevant depression (36.8%) compared to reference fathers ($p<$ .05). Clinically relevant distress was highly prevalent in mothers (84.6%) and fathers (68.4%) of MPS III patients compared to reference parents ($p<$ .01). Finally, the prevalence of PTSD was strikingly higher in both mothers (26.9%) and fathers (15%) than reported in the general Dutch population (respectively $p<$ .001 and $p<$ .05).
Discussion**:** We report a clinically relevant impact of parenting an MPS III patient on psychosocial functioning, which is demonstrated by high levels of anxiety, depression, parental distress and a remarkably high prevalence of PTSD. Structural monitoring of the psychosocial functioning of MPS III parents is therefore essential and may be beneficial for the whole family.

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Frequency of *IDUA* mutations reported for patients in the MPS I Registry

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Background: Mucopolysaccharidosis type I (MPS I) is a rare autosomal recessive disorder resulting from α-L-iduronidase (*IDUA*) gene mutations. Clinical phenotypes with variable times of symptom onset, severity, and progression, range from severe (Hurler) to attenuated (Hurler-Scheie, Scheie) syndromes. Therefore, defining phenotype at the time of diagnosis is essential for disease management. Phenotype can correlate with underlying *IDUA* mutations. However, over 200 pathogenic variants have been reported and phenotype prediction is possible for only a few known mutation combinations. Thus, refinement of genotype/phenotype relationships is of utility especially in the era of MPS I newborn screening initiatives.
Methods: The MPS I Registry contains the largest collection of MPS I patient genetic data. To determine *IDUA* mutation frequency, data from 538 patients (760 alleles from 380 severe cases; 316 alleles from 158 attenuated cases; individuals from Europe, 60%; North America, 35%; Latin America, 3%; Asia-Pacific, 2%) were examined.
Results: Median (IQR) age at diagnosis was 0.8 (0.6, 1.3) and 4.5 (2.8, 7.4) yr for individuals with severe and attenuated disease, respectively. 57% of patients had at least one nonsense mutation (71% severe, 21% attenuated), and 34% at least one missense (18% severe, 72% attenuated). The two most frequent mutations were nonsense mutations W402X (37%) and Q70X (15%). W402X and P533R were among the top five most frequent mutations in both severe and attenuated cases.
Discussion: Although P533R was more frequently observed in attenuated cases, occurrence in severe cases indicates that the presence of a single common mutation may not predict progression, making prognosis difficult. In many cases, clinical follow-up is required to properly define best management options. Thus, clinical decision making must be based on multiple factors, especially entire genotypes, since missense and nonsense mutations may be present in each phenotype.
Conflict of Interest declared.

P-313

Optimising the Nursing Care of Lysosomal Storage Disease (LSD) Patients: The Pre-anaesthetic assessment checklist.

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Background: Due to the multiple complications that are evident within lysosomal storage disorders, anaesthetics are particularly high risk and complex. They require a multi-disciplinary approach with a significant amount of careful planning with specialists, experienced professionals. Historically complications have arisen from anaesthetics within this patient cohort which have resulted in abandoned procedures, failed extubations and death.
Methods / Case Report: A pre-anaesthetic assessment checklist has been developed by an LSD nursing panel consisting of both adult and paediatric metabolic nurses from the UK and Ireland. The aims of this group are to enhance the LSD nursing community by sharing experience and encouraging discussion on clinical problem solving, innovation and

dissemination of best practice. During group discussions variations in clinical practice were highlighted throughout both adult and paediatric LSD centres and it was decided that a national approach to care was essential.
Results: Research topics were generated and protocols and procedures identified in order to develop consistency in the care delivered across the adults and paediatric LSD service within the UK.
Discussion:Many protocols are currently undergoing completion, including the production of a pre-operative worksheet for LSD patients. This has been devised in order to provide vital information to surgeons and anaesthetists to help to identify and communicate risk factors such as multi-level airway obstruction, cardiac abnormalities and an unstable c-spine. The incentive for producing this document includes previous incidents of serious anaesthetic complications. A case study will be included in the poster to illustrate the perceived benefit of the pre-op checklist. It is the view of the nursing panel that this checklist will now be used centre wide across the UK and Ireland.
Conclusion: It is the aim of the nursing panel to have this approved for inclusion as a protocol on the BIMDG website.
Conflict of Interest declared.

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Glucosylceramide species in red blood cell membranes of Gaucher disease patients.

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Background:Glucosylceramide (GlcCer) is the main substrate accumulating in Gaucher disease (GD). Its accumulation has been associated with different pathologies including a-synuclein aggregation in brain and red blood cell (RBC) membranes. Quantitative differences in GlcCer species in different tissues/regions that could influence the GD phenotypes have been described.
Methods: GD type I patients (GrA; n=39, age range: 6–69 years) and control individuals (GrB; n=49, age range: 4–76 years) were studied. RBCs were isolated from heparinized blood obtained on diagnosis. GlcCer species were isolated from RBC membranes by solid phase extraction and quantified by LC-MS/MS using GlcCer(C16:0)D3 as internal standard and GalCer(C15:0) as calibration curve standard. Statistical analysis was carried out using the Mann–Whitney test. Spearman's test was used for correlation studies.
Results: The GlcCer species detected in RBC membranes were C16:0, C18:0 and C24:1. In GrA the levels of C16:0 (pmoles/10^8 cells) [GrA : 6.0-23.7, median 14.2; GrB: 4.4-11.4, median 6.8; $p< 0.001$], C18:0 (pmoles/10^8 cells) [GrA: 0.14-0.54, median 0.3; GrB: 0.12-0.38, median 0.19; $p< 0.001$] and the sum of GlcCer (pmoles/10^8 cells) [GrA: 6.9-25.4, median 15.6; GrB: 5.0-12.8, median 7.6; $p< 0.001$] were significantly higher than those observed in GrB. In GrA a negative correlation, which was significant only for the sum of GlcCer species, was observed with age ($rs = -0.306$, $p = 0.037$).
Discussion: Our results show that the increased GlcCer levels in RBC membranes of GD patients result from the increase in the C16:0 and C18:0 GlcCer species. These changes could affect the biophysical properties of RBC membranes. The negative correlation with age may reflect differences in the severity of the disease, patients with mild disease being diagnosed later than patients with more severe phenotypes.

P-315

Natural history data for young subjects with Sanfilippo Syndrome Type B (MPS IIIB)

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Background: Sanfilippo Syndrome Type B (MPS IIIB) is a lysosomal storage disorder caused by deficiency of α-N-acetylglucosaminidase (NAGLU) and subsequent heparan sulfate (HS) accumulation in the brain. Sanfilippo B patients display progressive neurocognitive, behavioral, and motor decline and typically succumb to the disease in the second or third decades of life. To date few studies have comprehensively or quantitatively characterized Sanfilippo B progression in young patients. This information is essential for defining measures of therapeutic efficacy in treatment studies.
Methods: We initiated an observational study of Sanfilippo B patients 1–10 years of age (BMN 250–901) in an effort to better characterize disease progression in this population. All enrolled subjects had deficient NAGLU activity and a cognitive developmental quotient (DQ) ≥50 at the time of enrollment. Cognitive function and behavior is assessed every 12 weeks; measurement of heparan sulfate levels, abdominal and brain MRI imaging, assessment of hearing function and quality of life measures are conducted every 24 weeks.
Results: Our preliminary data demonstrate that CSF heparan sulfate levels are elevated in Sanfilippo B patients, but to varying degrees. Cognitive decline occurs in either a progressive linear or stepwise fashion as patients' age and is similar to that seen in young Sanfilippo A patients. Baseline measures of cognitive function are strongly correlated with caregiver assessments of adaptive function, demonstrating good construct validity of the utilized cognitive tests. Abdominal MRI imaging demonstrates baseline enlargement of the liver and spleen that tends to worsen over time. Whole brain volume decreased and ventricular volume increased over time.
Discussion: These data represent a systematic, quantitative evaluation of Sanfilippo B disease progression in young patients. This rich dataset will serve as an important comparison dataset for an ongoing treatment trial (BMN 250–201).
Conflict of Interest declared.

P-316

ICV-administered BMN 250 is well tolerated and reduces heparan sulfate accumulation in the CNS of subjects with Sanfilippo Syndrome Type B

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Background: Sanfilippo Syndrome Type B (MPS IIIB) is a lysosomal storage disorder caused by deficiency of the α-N-acetylglucosaminidase (NAGLU) enzyme and subsequent heparan sulfate (HS) accumulation in the brain. Sanfilippo B patients display progressive neurocognitive decline and typically do not live past the second or third decades of life. BMN 250 (NAGLU-IGF2) is a novel enzyme replacement therapy (ERT) for Sanfilippo B consisting of NAGLU enzyme fused to insulin-like-growth factor 2 (IGF2) to enhance lysosomal targeting. This report presents preliminary results from the first human study of BMN 250 (BMN 250–201; NCT02754076).
Methods: BMN 250–201 is a phase 1/2, open-label study with two parts. Part 1 consists of 3 dose-escalation periods (≥4 weeks) of intracerebroventricular (ICV) BMN 250 administered as a weekly isovolumetric bolus infusion. Subjects from Part 1 and from an ongoing observational study of Sanfilippo B patients (BMN 250–901; NCT02493998) will continue to Part 2, a 48-week treatment period to examine efficacy/safety at the maximum tolerated tested dose. For enrollment into Part 1, 1–10 year old subjects with Sanfilippo B must have deficient NAGLU activity at screening.
Results: ICV-administered BMN 250 was well tolerated with no treatment-emergent serious adverse events. All 3 subjects receiving BMN 250 demonstrated decreases in cerebrospinal fluid (CSF) total HS to normal levels. MRI imaging in untreated Sanfilippo B subjects demonstrated enlarged liver volumes, while treated subjects had liver volumes in the normal range. Improvement in developmental quotient (DQ) was observed in 2 of 3 BMN 250-treated subjects.
Discussion: These findings demonstrate that BMN 250 can be safely administered into brain ventricles via isovolumetric bolus infusion and that this treatment approach leads to a marked pharmacodynamic response in the CNS and somatic organs of Sanfilippo B patients. Impact on brain structure as assessed by MRI will also be discussed.
Conflict of Interest declared.

P-317

Effect of methylation in *GLA* gene: degree of methylation correlates to the severity of disease phenotypes

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Background: Fabry disease is a X-linked lysosomal storage disorder due to a defect in the *GLA* gene. Heterozygous Fabry females usually have an attenuated form of the disease, however, in rare cases, they can present with a severe phenotype.It has been well established that DNA methylation can influence gene expression. In general, DNA methylation represses transcription, and loss of methylation is associated with gene activation. We present here the methylation status of intergenic and intragenic methylation-sensitive restriction enzyme sites in *GLA* gene found to have clear correlation between the severity of phenotypes and methylation of non-mutated allele in heterozygous females.
Methods: 36 heterozygous females from 19 unrelated families were diagnosed based on their clinical and biochemical findings. α-gal A activity was measured in lymphocytes and skin fibroblasts (SF). Accumulation of sphingolipids was detected in urine, plasma and skin biopsy. Genomic DNA from different tissues was digested with methylation-sensitive restriction enzymes and respective mutation sites were amplified and DNA sequencing was performed. Methylation of restriction enzyme sites was confirmed with bisulphite modification kits.
Results: 16 moderate to severe female cases were detected based on clinical and biochemical data. α-gal A activity did not correlated to the severity of the disease. Excessive accumulation of sphingolipids correlated in severe cases. Non-mutated alleles of severely affected cases were found to be methylated, detected by non-digestion of intergenic and intragenic methylation sensitive restriction enzyme sites in *GLA* gene. However, moderately affected cases were being partially methylated.
Discussion: Our data suggest that DNA methylation study of *GLA* gene can be a future predictor for heterozygous females in affected families with Fabry disease and early initiation of therapy.
Conflict of Interest declared.

P-318

Analysis of glycosaminoglycans by UPLC/MS/MS in urine as biomarkers of diagnosis and monitoring of mucopolysaccharidoses

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Background: The mucopolysaccharidoses (MPS) are diseases of the catabolism of the glycosaminoglycans (GAGs), which are polymers characterized by specific dimers. Dermatan, heparan, chondroitin and keratan sulfates are the main (DS, HS, CS, KS). Urine profile of GAGs will depend on the disease. The aim was to develop a quantitative method by UPLC/MS/MS to analyse the dimers, for the diagnosis and monitoring of patients with MPS.
Methods: This method is based on the fragmentation of the GAGs into dimers by methanolysis in urine. The dimers are analysed according to their structure and MRM, in an Agilent LC1290/ABSciex Qtrap 4500. Calibration curves of the four GAGs are done also after methanolysis. Homemade deuterated standards are used.
Results: New dimers were searched to ensure the highest sensitivity. In addition, we studied which dimer has similar concentration between urine samples and commercial standards (shark, bovine or porcine sources) to improve accuracy. After validation, reference values in 4 age groups were stablished for the GAGs.
At present, ten MPS patients are monitored. Two MPS I-transplanted: one showed normal excretion, and the other only slight increase of DS. A third MPS I patient, under ERT, has elevated DS. Three untreated MPS II: HS is highly increased, DS only slightly. Three patients are untreated MPS IVA: two with elevation of KS, CS and HS, and the other only CS. One patient was a MPS IVA under ERT, who showed high CS, and slight DS and HS, except for the last sample, which is normal. In addition, we also analysed urine of patients with MPS III (increased HS), and VI and VII (increased DS, CS, HS in both cases). Increased excretion of KS was found in a patient finally confirmed as a GM1.
Discussion: This method allows quantitative analysis of DS, HS, CS and KS in contrast to biochemical conventional methods, like TLC. It is more reproducible and faster, and enables the biochemical diagnosis and follow up of the MPS patients.

P-319

Evaluation of biomarkers for differential diagnosis of Niemann-Pick diseases

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Background: Niemann-Pick disease type C (NPC) and acid sphingomyelinase deficiency (ASMD, Niemann-Pick disease type A/B) share many clinical similarities and the differential diagnosis often represents a challenge for clinicians. Recently, new disease biomarkers have been proposed for biochemical diagnosis of the two Niemann-Pick disease forms. These include oxysterols [cholestan-3beta5alpha,6beta-triol (triol) and 7-ketocholesterol (7-KC)], and lysosphyngolipids (lysoSLs) [lyso-sphingomyelin (lysoSM) and Lyso-sphingomyelin-509 (lysoSM-509)].
Methods: Analysis of plasma oxysterols and lysoSLs was performed by different LC-MS/MS methods in 57 samples from 16 patients with NPC, and in 18 samples from 4 patients with ASMD. Data are reported as median values.
Results: In NPC, both oxysterols (triol=81.9, nv< 21 ng/mL; 7-KC=120, nv< 40.4 ng/mL) and lysoSLs (lysoSM=21.4, nv< 15.4 nmol/L; lysoSM-509=22.7, nv< 3.8 MoM), were significantly increased compared to controls ($p< 0.001$).Biomarkers were also increased in ASMD (triol=83.2; 7-KC=99.1, $p< 0.0001$; lyso-SM=851; lysoSM-509=91.0, $p< 0.0001$). However, differently from oxysterols, which were similarly elevated in NPC and ASMD, plasma levels of lysoSLs were significantly higher in ASMD ($p< 0.001$). Positive correlations were found in NPC between oxysterols and lysoSM-509 (triol $P=0.413$; $p< 0.005$; 7-KC $P=0.380$; $p< 0.010$), and all three biomarkers negatively correlated with patient age ($p< 0.01$) and disease phenotypes. In ASMD, lysoSM significantly correlated with lysoSM-509 ($P=0.572$; $p< 0.05$) and with triol ($P=0.517$; $p< 0.050$), and all biomarkers negatively correlated with patient age ($p< 0.010$).
Discussion: This study shows that to discriminate between NPC and ASMD, lysoSLs represent the most reliable biomarkers, whereas oxysterols are sensitive but not specific in the different forms of NP disease. Correlations between biomarkers with patient's age indicate potential relationship with clinical phenotype and disease severity.
Conflict of Interest declared.

P-320

Agreement between the results of meta-analyses from case reports and from clinical studies regarding the efficacy of ERT in MPS-II patients.

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Background: Previous studies have shown a good rate of agreement between the results of case reports and clinical studies meta-analyses in patients with MPS-I (Sampayo et al., 2018).The aim of the present study is to confirm this previous result in another population of patients (MPS-II).
Methods / Case Report: A systematic review of case reports published up to March 2018 was conducted for MPS-II patients treated with ERT. We evaluated the same outcomes and population (males, any age, phenotype and ERT) that were analyzed in the meta-analysis of clinical studies (Bradley et al., 2017). Primary endpoint was the percentage of clinical cases reporting an improvement in an efficacy outcome, or a harm in a safety outcome, after ERT initiation. The primary analysis evaluates if the percentage of clinical cases reporting a modification in a specific outcome after ERT was statistically higher than 5%.We control multiple testing issues with a 10% false discovery rate. We correlated the number of clinical cases that report a modification in a specific outcome (case report meta-analysis) with the strength of evidence (SOE) score assigned to that outcome in clinical studies meta-analyses.
Results: The same outcomes identified as statistically significant in case report meta-analysis were scored with moderate SOE in meta-analysis of clinical studies (Urine glycosaminoglycans, liver volume and development of antibodies). Sensitivity, specificity, positive and negative predictive values between results of both meta-analysis reached the 100%. The rate of agreement (Rho = 0.82, 95%CI: 0.46 to 0.95) between the results of both meta-analysis was good.
Discussion: We found a good grade of agreement between the results of meta-analyses from case reports and from clinical studies in the efficacy of ERT in patients with MPS-II. Aggregating case reports quantitatively, rather than analyzing them separately or qualitatively, may improve conclusions in the field of rare diseases.

P-321

Critical care situations in adult patients with mucopolysaccharidosis (MPS)

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Background: Mucopolysaccharidoses (MPS) are rare, inherited disorders associated with enzyme deficiencies that result in glycosaminoglycan (GAG) accumulation in multiple organ systems. Management of MPS is evolving as patients increasingly survive to adulthood due to earlier diagnosis, treatment availability and multidisciplinary care. However, MPS can result in a range of critical clinical situations in adult patients. We present a series of critical care cases to highlight challenges and practical solutions to optimise care of adult MPS patients.
Case Reports: Nine cases (MPS I, II, IVA and VI; ages 21–37 years) were provided from four European leading inherited metabolic disease centres. Critical care situations included surgical procedures (spinal decompression, cardiac valve or corneal replacement, and tracheostomy), pregnancy and a thrombus in a port-a-cath.
Results: Major surgical challenges included managing complex cardiac and respiratory dysfunction, and short stature, in the context of limited clinical expertise in MPS. These were resolved by involving paediatric- and adult-care specialists with MPS expertise from external centres when needed. Medical challenges included disrupted enzyme replacement therapy (ERT) during pregnancy, resulting in increased risk of infection. This was addressed by regular follow-ups and antibiotics. The thrombus in the port-a-cath was resolved by

Hickman line insertion, which was managed through sterile methods, and support for the patient in disease management.
Discussion: These cases show the diverse considerations that need to be factored into coordinated MPS care plans. Where adult-care clinicians may have limited practical skills in managing MPS patients, expertise can be sought from more experienced paediatric teams. Standardised approaches, including input from paediatric and adult experts, familiar with the surgical and medical needs for MPS patients, and individualised support should be considered for every adult with MPS.
Conflict of Interest declared.

P-322

A Novel *PSAP* gene mutation causes a neuropathic form of Gaucher disease: A case series of 4 patients from the United Arab Emirates

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Background**:** Gaucher disease is classically caused by lysosomal B-glucosidase enzyme deficiency resulting in accumulation of glucocylceramide in the reticuloendothelial system. This results in hepatosplenomegaly, anemia, thrombocytopenia and bone disease. For normal enzyme function, an activator protein, saposin C, is additionally required. When deficient, a Gaucher-like presentation occurs.
Case Report: In this report, we present four patients with saposin C deficiency. They are two sets of siblings from two different consanguineous Emirati families. Two patients were diagnosed based on clinical symptoms, while the other two were diagnosed after genetic screening of family members. The average age of onset was 5 years. All patients had normocytic normochromic anemia, thrombocytopenia and hepatosplenomegaly at the time of diagnosis. Two of the older patients have difficult to control seizures, bone involvement as well as gall bladder disease requiring cholecystectomy. Molecular testing revealed a novel homozygous likely pathogenic variant; c.1014+1G>A in the PSAP gene. This variant is predicted to result in aberrant splicing in exon 10 which codes for the Sap C domain of the PSAP protein. A disease biomarker, Lyso-GB1 was significantly elevated in all patients and averaged 370 ng/ml (Normal < 4.8 ng/ml).
Results and Discussion: We describe the clinical and biochemical findings of four cases with a similar clinical phenotype to Gaucher type 3 disease due to sapocin-C deficiency. This information expands the clinical and mutation spectrum of the disease.

P-323

Effects of oral eliglustat on bone in adults with Gaucher disease type 1: long-term results from four clinical trials

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Background: Gaucher disease type 1 (GD1) is a multisystemic disorder resulting from deficient lysosomal enzyme acid β-glucosidase activity. Patients with GD1 are at risk of debilitating bone complications that decrease quality of life. Eliglustat, an oral substrate-reduction therapy, is a first-line treatment for adults with GD1 who have extensive, intermediate or poor CYP2D6 metabolizer phenotypes (>90% of patients).
Methods: We analyzed long-term bone mineral density data from 4 completed Sanofi-Genzyme sponsored clinical trials of 393 GD1 patients treated with eliglustat for 4–8 years: Phase 2/NCT00358150, ENGAGE/NCT00891202, ENCORE/NCT00943111, EDGE/NCT01074944.
Results: In treatment-naïve patients (Phase 2, N=26; ENGAGE, N=40), mean spine T-scores went from osteopenic at baseline to normal after 2 years of eliglustat and continued to improve over time. Mean±SEM spine T-score increased from −1.55±0.28 to 0.59±0.34 (n=14) after 8 years in Phase 2 and was −0.53±0.27 in ENGAGE patients with bone data at 4.5 years (n=9) (mean increase from baseline: 0.53±0.16). Femur T-scores improved over time in both trials, as did spine and femur Z-scores. In the ENCORE trial of patients stabilized after a mean of 10 years of enzyme therapy (ERT) (N=157), T- and Z-scores (spine and femur) remained in the reference range for up to 4 years; least square mean spine Z-scores improved by 0.29 ($P< 0.0001$). In EDGE (N=170, mostly ERT switch), patients stabilized on eliglustat during a 6- to 18-month lead-in period maintained normal T- and Z-scores (spine and femur). Eliglustat was well-tolerated in all trials; most adverse events (AEs) were mild/moderate (97%) and considered unrelated to eliglustat (86%); 2% of patients withdrew due to AEs considered drug-related.
Discussion: After long-term eliglustat treatment in 4 clinical trials, bone parameters showed continued improvement in treatment-naïve patients and remained stable and within normal ranges in previously treated patients.
Conflict of Interest declared.

P-324

Transition from paediatric to adult care in patients with mucopolysaccharidosis (MPS)

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Background: Mucopolysaccharidoses (MPS) are rare disorders associated with enzyme deficiencies, resulting in glycosaminoglycan (GAG) accumulation in multiple organ systems. As patients increasingly survive to adulthood, the need for a smooth transition into adult care is essential. Using case studies, we aim to share best practice strategies and highlight challenges and resolutions to optimise transition for patients with MPS.
Case Reports: Six cases were provided by four leading European metabolic disease centres, including patients with MPS I, IIIC and VI, aged between 15 and 18 years at first transition visit (age at transfer to adult care: 16–19 years).
Results: Strategies varied between centres in duration of transition, healthcare providers involved and documents used to support the patient and monitor readiness for transfer. At one centre, paediatric- and adult-care teams coordinated adult care in a children's hospital setting, while at others, patient care was transferred between teams in different hospital departments or sites. Transition processes were guided by national guidelines and institutional regulations. Challenges included unwillingness to attend appointments with unfamiliar healthcare professionals and

attachment to paediatricians. Limited knowledge of MPS in the adult-care team also concerned patients and parents. Challenges were resolved by starting transition at an early stage, regular communication with and reassurance of the patient and family, coordination by MPS experts, and including clinicians familiar with MPS in adults.
Discussion: These cases highlight the strategies that can be used to manage transfer from paediatric to adult care. Strategies should be comprehensive but flexible to ensure each individual patient's needs are met. Sufficient time should be provided to allow patients to understand the process. Effective communication between adult and paediatric teams, as well as between patients and carers, is key to ensuring smooth transition.
Conflict of Interest declared.

P-325

Neurodevelopmental Status and Adaptive Behavior of Pediatric Patients with Hunter Syndrome in a Two-year Observational Study

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Background: Two-thirds of patients with mucopolysaccharidosis type II (MPS II; Hunter syndrome), a rare lysosomal disease characterized by iduronate-2-sulfatase deficiency, have cognitive impairment (CI). The cognitive status and adaptive behavior of patients with MPS II over time was assessed.
Methods: Eligible patients in this observational, prospective, longitudinal study (NCT01822184) were aged 2–18 years with MPS II and mild-to-moderate CI, defined by a General Conceptual Ability (GCA) score of 55–85 as measured by the Differential Abilities Scales-II (DAS-II). Patients received IV idursulfase as standard of care during the study. DAS-II and adaptive behavior (Vineland Adaptive Behavior Scales, Second Edition, adaptive behavior composite (VABS-II ABC) tests were done at baseline, and at 3-month intervals for up to 24 months.
Results: Of 55 enrolled patients with a mean (SD) age of 5.60 (3.32) years, 32 discontinued (25 to enroll in a phase II/III-treatment study, 7 for other reasons). Mean (SD) DAS-II GCA (n=44) and VABS-II ABC scores (n=53) at baseline were 78.4 (19.1) and 83.7 (14.2), respectively. Change from baseline in mean (SD) DAS-II GCA score at month 12 (n=27) and month 24 (n=20) were −0.9 (9.4) and −3.8 (12.7), respectively. Change from baseline in mean (SD) VABS-II ABC score at month 12 (n=29) and month 24 (n=21) were −2.4 (7.6) and −2.0 (8.1), respectively. Least squared mean differences (LSMD) between DAS-II GCA scores for patients with baseline GCA scores of ≤70 (low cognitive function; n=18) and >70 (n=26) were −2.4 (p=0.5657) at month 12 and −7.4 (p=0.1461) at month 24. Similarly, LSMD between VABS-II ABC scores for patients with baseline GCA scores of ≤70 and >70 were −1.9 (p=0.5545) at month 12 and 0.3 (p=0.9484) at month 24.
Discussion: Overall, patients aged < 18 years with MPS II and mild-moderate CI had a stable cognitive and adaptive function over 24 months.
Conflict of Interest declared.

P-326

The unique neuroprotective effect and clinical characteristics of the G46E-*GBA* mutation in Korean Gaucher patients

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Background: Worldwide, most Gaucher disease (GD) patients are the non-neuronopathic type, but neuronopathic GD is more frequent in the Asia-Pacific area. The N370S mutation, which is known as a neuroprotective factor for GD, is absent in Japanese and Korean patients. The G46E mutation is known to be prevalent in Korean GD patients. As the G46E variant possibly indicates a neuroprotective mutation, we investigated the clinical characteristics of GD patients with this variant.
Methods: To determine the characteristics of GD patients with the G46E mutation, 17 Korean GD patients were enrolled. We assessed the patients' clinical manifestations, including the status of their neurology, haematology, visceromegaly and skeletal involvement.
Results: None of the patients displayed neurological symptoms but did present with severe visceromegaly and a tendency to show bone involvement at diagnosis. The mean age at diagnosis was 11 years (ranging from 15 months to 57 years). Twelve patients (70.5%) were diagnosed at less than 7 years old. All patients presented with splenomegaly and 4 patients (23.5%) had already undergone splenectomies during childhood. At diagnosis 4 patients (ages 11, 12, 12, and 57 years) showed femoral-neck avascular necrosis. One 2-year old girl displayed severe bone pain and bone involvement. The patients current ages ranged from 5 to 67 years, and no patients had developed neurological symptoms during the follow-up period (2 to 15 years). One boy of 19 years died due to acute bacterial meningitis.
Discussion: To the best of our knowledge G46E may be a unique variant identified in the Korean population. Despite the small number of patients, the G46E mutation seems to be a neuroprotective factor in our Korean Gaucher disease patients; they tend to be diagnosed with the disease early on due to their distinctive visceromegaly. Skeletal involvement is also frequent in G46E-GD patients. Further investigations of the founder effect of G46E in the Korean population will be needed.

P-327

Efficacy and safety of intrathecal idursulfase in pediatric patients with Hunter syndrome and early cognitive impairment

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Background: Two-thirds of patients with mucopolysaccharidosis type II (MPS II; Hunter syndrome), a rare lysosomal disease characterized by iduronate-2-sulfatase deficiency, have cognitive impairment (CI). The efficacy and safety of intrathecally-administered idursulfase (idursulfase-IT) in patients with early CI was tested in a phase II/III study.
Methods: Patients aged 3–18 years with MPS II and mild-to-moderate CI (assessed by the Differential Abilities Scales-II [DAS-II], General Conceptual Ability [GCA] score) who had tolerated IV idursulfase for ≥4 months, were included in a randomised, controlled, open-label, multicenter trial (NCT02055118). Patients were randomized (2:1) to receive once-monthly 10 mg idursulfase-IT, via either a surgically implanted intrathecal drug delivery device (IDDD) or lumbar puncture, or no intrathecal treatment for 12 months. Patients received IV idursulfase as standard of care during treatment. The primary endpoint was a change from baseline in DAS-II GCA score at Week 52. The key secondary endpoint was a change from baseline in Vineland Adaptive Behavioral Scales, Adaptive Behavior Composite (VABS-II ABC) score at Week 52. CSF glycosaminoglycan (GAG) levels, adverse events, anti-idursulfase antibody levels, and the safety of the IDDD were monitored.
Results: Of 49 randomized patients, 34 received idursulfase-IT, 32 of whom completed the study. The increase in DAS-II GCA scores with idursulfase-IT at week 52 (n=29) compared with no IT treatment (n=15) was not significant, with a least squared mean difference (LSMD) of 3.0 (p=0.5669). VABS-II ABC scores with (n=31) or without (n=14) idursulfase-IT treatment at week 52 were similar (LSMD of 0.3 [p=0.9218]). Idursulfase-IT treated patients had a 74% reduction from baseline in CSF GAG levels.
Discussion:Top-line data showed that primary and key secondary endpoints were not met. CSF GAG was markedly reduced in idursulfase-IT treated patients and there were no major safety issues.
Conflict of Interest declared.

P-328

Characteristics of humoral immunity in patients with Gaucher disease type I receiving long-term enzyme replacement therapy

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Background: Gaucher disease (GD) type I is the most common lysosomal storage disorder. It results from a deficiency in the lysosomal enzyme glucocerebrosidase and leads to the accumulation of glucocerebroside in macrophages. Immunoglobulin abnormalities are a typical feature of this disease.
Methods / Case Report: Here we have investigated the level of immunoglobulins (Ig) G, A, M and paraproteins in adult patients with GD type I receiving long-term enzyme replacement therapy (ERT). The study group included 42 adult patients with GD type I, all of which have been receiving ERT for at least 8 years. The study group consisted of 30 (71%) women and 12 (29%) men. The median age was 41 years. Immunochemical evaluation of serum proteins was performed twice: before the initiation of ERT and after 8–10 years of ERT.
Results: Before the initiation of ERT 27% (n=12) of patients had normal immunoglobulin levels, 73% (n=30) of patients had polyclonal gammopathy. The level of IgA was elevated in 50% of patients, the level of IgG was elevated in 31% of patients and the level of IgM was elevated in 23% of patients. After 8–10 years of ERT the percentage of patients with polyclonal gammopathy decreased to 47.5% ($p< 0.05$). The level of IgA was elevated in 38% of patients, the level of IgG was elevated in 26% of patients and the level of IgM was elevated in 14% patients. Monoclonal gammopathy was revealed in 7% (n=3) of untreated patients (median age – 50.5 years). After 8–10 years of ERT the level of monoclonal secretion in these patients either did not change or slightly increased but remained low (max – 12.7 g/l). One patient who did not have monoclonal secretion before ERT initiation developed minimal monoclonal secretion after 10 years of ERT.
Discussion: Immunoglobulin abnormalities in Gaucher disease are a consequence of macrophage activation. They respond to ERT and can be used to evaluate disease activity in combination with other clinical and laboratory indicator.

P-329

c-Abl kinase in Niemann-Pick type A disease: its implication in the pathogenic mechanisms leading to neurodegeneration

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Background: Niemann-Pick type A (NPA) disease is a lysosomal neurodegenerative disorder characterized by deficiency in acid-sphingomyelinase (ASM), sphingomyelin accumulation and autophagy alterations. We have described that the c-Abl kinase is activated in other neurodegenerative diseases. Interestingly, it has been shown that c-Abl inhibition promotes autophagy and cellular clearance. Our aim was to evaluate the participation of c-Abl in the autophagy alterations and neuronal pathology in NPA disease.
Methods: We used the ASM-deficient mouse and the SHSY5Y and H4-LC3-RFP-GFP cells treated with desipramine, an ASM inhibitor, and NPA human fibroblasts as *in-vivo* and *in-vitro* NPA models, respectively. We modulated c-Abl in NPA models using the c-Abl inhibitor Imatinib. In the NPA mice cerebellum we evaluated Purkinje cell loss, inflammation, autophagy markers and the c-Abl pathway activation. In *in-vitro* NPA models we evaluated viability, c-Abl levels and autophagy flux.
Results: We found progressive neurodegeneration, inflammation, autophagic flux alterations and activation of the c-Abl pathway both in the NPA mice cerebellum and cellular NPA models. Imatinib treatment improved the autophagic flux and reduced neuronal death and caspase 3 active levels in the cellular neuronal NPA models and preserved Purkinje neurons and reduced inflammation in the NPA cerebellum.
Discussion: c-Abl is activated and relevant in NPA neurodegeneration and autophagy alterations, supporting the potential use of Imatinib for clinical treatment of NPA patients.
Support: FONDECYT: 1150186 (SZ) and 1161065 (AA) CARE-Chile-UC/PFB12/2007 and CONICYT-PCHA/Doctorado Nacional/2015-150038

P-330

Expansion of enzyme testing in dried blood spots (DBS) for the diagnosis of 20 lysosomal storage disorders

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Background: Enzyme analysis is the gold-standard for the diagnosis of lysosomal storage disorders (LSDs). However, enzyme analysis in leukocytes requires that whole blood samples arrive to the testing laboratory within 24–48 hours of collection, and requires a relatively large volume of blood. This is problematic for the international shipment of specimens and for

testing infants. The adaptation of these enzyme assays for use in dried blood spots (DBS) has ameliorated these issues for a number of LSDs. The development of tandem mass spectrometry (MS/MS) substrates has allowed multiple enzyme reactions to be combined in a single assay, increasing efficiency. Here, we report validation results for a new 6-plex assay for the diagnosis of five Mucopolysaccharidosis (MPS) disorders (MPS II, IIIB, IVA, VI and VII) and neuronal ceroid lipofuscinosis type 2 in DBS using UPLC-MS/MS.
Methods / Case Report: Substrates were obtained from Perkin Elmer Inc. and the analyses performed using a Waters TQD UPLC MS/MS system. Ninety-nine samples with a confirmed diagnosis of one of the six disorders were used to evaluate clinical sensitivity and specificity. Two hundred thirty-six blood spots were obtained from unaffected controls or patients with a confirmed alternative diagnosis for the development of normal ranges.
Results: A clear separation between normal and affected patients was noted for all six enzymes providing 100% clinical sensitivity. Intra-day and inter-day precision was acceptable (< 20% CV). Additionally we show that by analyzing multiple enzymes, patients with Mucolipidosis types II and III and Multiple Sulfatase Deficiency will be detected.
Discussion: Our laboratory can now analyze 18 enzymes in DBS to diagnose 20 different LSDs. Molecular testing from the original DBS is available limiting the need for additional sampling. This expansion of DBS testing allows us to offer a seven enzyme DBS MPS panel which we hope will simplify the diagnosis of MPS worldwide.
Conflict of Interest declared.

P-331

Psychosine – A biomarker for newborn screening, follow up and monitoring of Krabbe disease

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Background: Newborn screening (NBS) for Krabbe disease (KD) is currently conducted in 6 US states (IL, KY, MO, NY, OH, TN) covering a total of ca. 730,000 live births per year (18% of US births). In most of these states, 1 in 3,500 to 6,000 newborns require follow up testing to exclude a possible diagnosis of KD. Infants with reduced GALC activity and genotypes of uncertain significance are subjected to long-term, time consuming, invasive and expensive monitoring when NY guidelines are followed (Pediatr Neurol. 2009;40:245–52). Psychosine (PSY) is a substrate of GALC and is markedly elevated in presymptomatic patients with early infantile KD and symptomatic patients with any KD variant (J Inherit Metab Dis. 2015;38:923–9). Here we report on the utilization of PSY in the follow up of NBS for KD.
Methods: Since March 2017, we received 180 specimens from infants born in IL, MO, NY, OH and TN either for 2nd tier NBS (IL, TN) or to follow up of a presumptive positive NBS result for KD. PSY was measured in dried blood spot and CSF specimens using liquid-chromatography tandem mass spectrometry.
Results: All but three infants had normal PSY concentrations and were considered unaffected because genotypes were also uninformative. Fraternal twins, now 9 months old and asymptomatic but with mild PSY elevations in blood and CSF are considered to have a late onset KD variant and continue to be followed. A now 4 months old infant had markedly elevated PSY levels in blood and CSF which began to decrease following a bone marrow transplant during the 5th week of life.
Discussion: These results support our hypothesis that PSY measurement can: a) serve as a 2nd tier assay in NBS for EIKD; b) simplify and reduce the cost of short-term follow up; and c) determine disease progression in asymptomatic cases with late onset KD variants. Additional longitudinal measurements of PSY in KD patients are required to confirm these findings.

P-332

90 Russian patients with Hunter syndrome: A five-year experience

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Background: Hunter syndrome is an X-linked recessive hereditary disorder caused by *IDS* gene mutations, leading to the accumulation of glycosaminoglycans (GAGs) in many tissues. This results in distorted growth or function.
Methods / Case Report: The study included 90 patients including 89 boys and 1 girl, with a diagnostic age ranging from 1 month to 45 years (the median age was 9 years old) The molecular genetic analysis included the analysis of all coding and adjacent intron regions of the *IDS* gene by Sanger sequencing. The recombinant alleles (inversions) were determined by the PCR-RFLP method.
Results: A total of 61 mutations in the *IDS* gene were detected. The most frequent 26 (43%) were missense mutations and 16 (26%) were frameshift deletions. Mutations in the first class of pathogenicity were detected in 42 patients and accounted for 53% of the total number of identified mutations. Twenty-six (43%) of the variants discovered by us were not previously described in the Human Gene Mutation Database. Analysis of the segregation of the detected variants allowed us to determine that only 67% of patients inherited mutations from their mothers, while 33% of cases were spontaneous. Interestingly among the patients included in the study, we identified one girl with clinical implications of Turner syndrome and Hunter syndrome, who had a deletion *c.1438_1442del* in the *IDS* gene on one X-chromosome and a partial monosomy of Xq22.1q24-Xq28 on the another X-chromosome.
Discussion: Among the revealed spectrum of mutations, 77% of the mutations were met once, which may indicate the absence of frequent variants typical for Russian patients with Hunter syndrome, which is typical for most X-linked diseases.

P-333

CRISPR generated reporter cell lines for discovering therapeutics for Sanfilippo lysosomal storage disorders

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Background: Mucopolysaccharidoses, such as the Sanfilippo syndromes, represent a group of lysosomal storage disorders where a deficiency of a lysosomal degradative enzyme leads to the accumulation of the enzyme's substrate, heparan sulphate (HS), in patient lysosomes. As a treatment modality, substrate reduction therapy (SRT) with the goal to prevent

accumulation of HS has been shown to be feasible by reducing the activity of the synthetic enzyme heparan sulfate N-deacetylase/N-sulfotransferase 1 (NDST1) that is involved in the formation HS.
Methods / Case Report: We used CRISPR-based genome editing to generate a luciferase-based cell reporter of endogenous NDST1 expression. These cell lines were used to screen libraries of drug-like molecules for compounds that reduce the expression of NDST1.
Results: In frame insertion of nanoluciferase at the C-terminus of NDST1 in Hek293, HeLa and neuronal-like BE2c human cell lines was effficiently achieved using CRISPR based homology directed repair. We also used a co-selection strategy based on Ouabain resistance to select for clones bearing an insertion of the luciferase reporter at the N-terminus of NDST1. Characterization of the tagged NDST1gene in terms of protein size, intracellular trafficking, Golgi localization and response to known SRT (SAHA) matched that of the native gene.
Discussion: These reporter lines have enabled screening for drugs that target the broader range of transcriptional and translational regulatory mechanisms controlling NDST1 expression.

P-334

An evaluation of the demographic and clinical characteristics of 17 patients with GM2 gangliosidosis

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Background: The purpose of our study is to analyse the demographic, phenotypic and diagnostic age characteristics of children with GM2 gangliosidosis.
Methods / Case Report: Patients with GM2 gangliosidosis who were referred to Ege University (Faculty of Medicine, Department of Paediatrics, Division of Paediatric Nutrition and Metabolism) between January 2004 and December 2016 were included in this study. Diagnosis was confirmed by serum β-hexosaminidase activity levels and genetic mutation analysis. The demographic and clinical features were reported for 11 patients with Tay-Sachs disease (TSD) and 6 with Sandhoff disease.
Results: The mean age at diagnosis was 21.3 months (ranging from 4 to 48 months) for TSD patients and 14.5 months (ranging from 8 to 36 months) in Sandhoff disease patients. The initial and main symptom in all patients were neurological problems such as developmental delay, developmental regression or both, seizures and macrocephaly. None of the patients exhibited evidence of organomegaly. Cranial MRI results were normal in 36% of the cases. 55% of the cases had bilateral thalami involvement presenting as T2 hyperintensity especially at the posterior thalami. 9% of cases had myelination delay.
Discussion: GM2 gangliosidosis disease diagnosis should be considered in children with developmental regression and/or delay. To prevent a delay in diagnosis, determination of β-hexosaminidase activity in serum and genetic mutation analysis should be undertaken in suspected cases. Curative gene therapy may be available in the future.

P-335

The utility of plasma lyso-GL1 level as a biomarker of Gaucher disease type 1 in 141 Russian patients

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Background: Gaucher disease (GD) is the most prevalent lysosomal storage disease, caused by mutations in the GBA gene which leads to accumulation of glucosylceramide (GL1) and its deacylated lipid, glucosylsphingosine (lyso-GL1) in different tissues. Currently plasma lyso-GL1 levels are a key biomarker for GD.
Methods / Case Report: Plasma lyso-GL1 levels in 141 patients with GD type 1 were measured by UPLC-MS/MS in dried blood spots (DBS) with reduced β-glucocerebrosidase activity. Molecular genetic testing was also carried out. As a control group for the lyso-Gl1 analysis, DBS samples from healthy patients with normal β-glucocerebrosidase and other lysosomal enzymes were also measured.
Results: Plasma lyso-GL1 levels in healthy controls averaged 2.5 ng/ml (range 1.4-3.6ng/ml; 95% CI). In patients with GD type 1 plasma lyso-GL1 level was significantly increased up to 100-fold compared to healthy controls 220.5 ng/ml (range 173.8-267.2ng/ml; 95% CI). Enzyme replacement therapy resulted in a significant reduction ($p< 0.001$) in plasma lyso-GL1 levels to 105.8 ng/ml (range 69.9–143.1; 95% CI). Plasma lyso-GL1 level correlated with chitotriosidase ($p< 0.001$), hepatomegaly ($p< 0.001$) and splenomegaly ($p< 0.001$). However, correlations between the level of lyso-Gl1 biomarker and the type of mutations in the GBA gene were not found.
Discussion: Correlations obtained from this work indicate that plasma lyso-GL1 level may be used to assess the severity of GD and the efficacy of enzyme replacement therapy in Russian patients with GD type 1

P-336

Clinical, biochemical and molecular characteristics of 16 patients with Mucopolysaccharidosis Type II in Western Turkey

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Background: Mucopolysaccharidosis Type II (MPS II, Hunter syndrome, OMIM 309900) is a rare X-linked lysosomal storage disease caused by the deficiency of iduronate-2-sulfatase (IDS) enzyme, one of the degradative enzymes of mucopolysaccharides. The purpose of the study is to present the clinical, biochemical and molecular characteristics of fifteen patients with mucopolysaccharidosis type II in Western Turkey.
Methods / Case Report: A retrospective study was carried out on sixteen patients with MPS II who had been followed up by the Paediatric Metabolic Diseases and Nutrition Unit, Medical Faculty, Ege University between October 2004 and January 2018.
Results: The age range of the patients enrolled in the study was between 11 months and 26.5 years at the time of diagnosis. The most common symptom was coarse facial features. On physical examination all patients presented with coarse facial features, macrocephaly and organomegaly. All but one patient had a severe phenotype. IDS activity was significantly decreased in all patients who underwent enzyme analysis. In this study one novel mutation was described.
Discussion: In this study clinical and molecular characterization of Turkish MPS II patients from a single centre were evaluated. The majority of patients had neurologic involvement with differing degrees of severity. The molecular analysis revealed one novel mutation and one recombination mutation.

P-337

Longitudinal brain volume changes in infantile and juvenile gangliosidoses

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Background: Infantile and juvenile gangliosidoses are inherited metabolic diseases in which specific enzyme activity deficiency leads to ganglioside accumulation in the central nervous system, progressive neurodegeneration and death during childhood. Brain structure atrophy is a common MRI finding, however, brain structure volumetrics have not been systematically studied. Feasibility of brain structure volumetrics in the infantile phenotype has been limited by undistinguished segmentation of grey and white matter cortical interface.Objectives: Explore quantitative brain structure volumetrics as a non-invasive marker of disease progression.
Methods: Yearly brain MRIs were performed in 14 patients with gangliosidosis (9 infantile, 5 juvenile). Volumes of cerebellum, caudate nucleus, putamen, corpus callosum, and basal ganglia were measured with BRAINS2 software. Age-matched controls were available for patients with juvenile phenotype.
Results: Marked reduction in brain structure volumes were found in both phenotypes, with the exception of cerebellar cortex volume in infantile GM2. The infantile phenotype showed macrocephaly, and rapidly increasing ventricular volumes, intracranial volume and total brain volume (without excluding ventricular volume) (all $P< 0.001$). Elevated CSF opening pressure was common in infantile disease and increased over time, without hydrocephalus. In contrast, in patients with juvenile disease, macrocephaly and elevated CSF opening pressure were absent, while total brain volume decreased with time ($P=0.004$).
Discussion: This study demonstrates the ability to perform brain structure volumetry with quantitative MRI in children with gangliosidosis diseases, including children under 3 years of age. The infantile and juvenile gangliosidoses are distinguished by rates in specific brain structures and total brain volume change. Quantitative MRI provides a non-invasive and reliable method for surveillance of CNS changes in the childhood gangliosidoses.

P-338

Neurological features in individuals with Mucopolysaccharidosis VI; an assessment carried out in a Reference Centre in Bahia, Brazil

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Background: Mucopolysaccharidosis VI (MPS VI) patients can present with cognitive impairment, visual or hearing impairment, sensibility loss, peripheral neuropathy, pyramidal signs, seizures and symptoms related to medullar or nerve root compression. The aim of the study was to describe neurological alterations identified in individuals with MPS VI, which was carried out in a reference centre in Salvador, Bahia, Brazil.
Methods / Case Report: We present a cross-sectional, observational and descriptive study based on a review of medical records. Patients cognitive functions were evaluated by the Wechsler Intelligence Scale for Children, fourth edition (WISC IV).
Results: Twelve individuals with MPS VI were evaluated, of which 75% were male. The mean age of onset of symptoms was 1.4 years old and the average age of patients was 13.3 years old. 83% of patients had visual impairment, 66% had hearing impairment, 42% had pyramidal sings, 8% had seizures, 16% had symptoms related to medullar compression. Cognitive evaluation was determined in seven patients; 86% of patients had intellectual disability and 14% had borderline performance. The indices of cognitive function showed that 100% the patients had processing speed deficit, 86% had verbal comprehension deficit, 71% had perceptual organization deficit and 71% had working memory deficit.
Discussion: The neurological features described among these patients were similar to results shown in other MPS VI studies. The cognitive profile showed impairment in all skills evaluated. This evaluation must include analysis of social, cultural and economic background factors as these may interfere in the results. The cognitive evaluation performed, represents new information about the neuropsychological development of patients with MPS VI. Medullary compression needs to be better evaluated by imaging examination.

19. Lysosomal disorders: others

P-339

Study of mutations in 58 genes causing lysosomal storage disorders in Parkinson's disease patients

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Background: Parkinson's disease (PD) is a progressive neurodegenerative disorder which affects the control of movements and is characterized by loss of dopaminergic neurons in the substantia nigra and intraneuronal aggregates of alpha-synuclein protein. Mutations in glucocerebrosidase gene (*GBA*), have been identified as a potential risk factor in PD. Likewise, substantial evidences highlight the importance of lysosomal mechanisms in PD susceptibility and pathogenesis. Therefore, our objective was to extend the genetic study of PD patients to all genes causing lysosomal storage disorders (LSD) with the aim to identify genetic variants potentially contributing to the risk of developing PD
Methods: DNA from 43 PD patients and 40 control subjects were analyzed through the NextSeq 500 platform and an enrichment protocol, Trusight One Sequencing Panel, created by Illumina for the simultaneous sequencing of all the exon regions of 4,813 clinically relevant genes. The variants present in 58 LSD genes were evaluated.
Results: We identified 26 rare and likely deleterious genetic variants in heterozygosity in 19 lysosomal genes in 20 of the 43 PD patients; 10 of these variants were pathogenic mutations previously described, 4 variants were of uncertain significance, and 12 were new potentially damaging variants. Among the identified variants, we highlight five patients with mutations in *GBA*.
Discussion: Our results corroborate the association previously described between PD and *GBA* mutations as a risk factor to develop PD. In addition, this association has been extended to other LSD genes. Our findings are in agreement with a recent publication (Roback et al., 2017, Brain) that describes a significant excessive burden of LSD gene variants in PD patients, reinforcing the importance of lysosomal regulatory mechanisms in PD pathogenesis.

These findings suggest that, in addition to *GBA*, mutations in many other lysosomal genes could predispose to PD.

P-340

Opening a window on lysosomal acid lipase deficiency: new biochemical and molecular insights

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Background:Lysosomal acid lipase deficiency (LAL-D) is an autosomal recessive disease caused by mutations in *LIPA*. LAL-D subtypes reflect lysosomal acid lipase (LAL) residual activity. The disease is characterized by multiorgan involvement including the liver, spleen, intestine and cardiovascular system. Owing to poor recognition, LAL-D is likely under-diagnosed.
Methods / Case Report: We have reviewed data generated from 681 clinical diagnostic samples (white blood cells (WBC) n=625, fibroblasts n=30, liver n=4, amniocytes=13, chorionic villus tissue=9) received for biochemical testing of LAL activity over a 15-year period. A cut-off of 12 pmol/min/mg protein has been used to diagnose LAL-D. Biochemical data were collected and analyzed with respect to age of patients at time of evaluation. *LIPA* molecular test was performed for 47 patients.
Results: LAL activity was deficient in 37 cases (5.92%). The average LAL activity was 62.92 ±3.98 for individuals designated as normal, 19.32±0.86 and 5.90±1.42 for subjects designated as having reduced and deficient activity. The average age at diagnosis for LAL-D was 23.6 years (0.0625-82 years). The correlation between the age at diagnosis and residual LAL activity showed a moderate direct correlation ($r=0.45$, $p< 0.005$). Out of 47 patients, 7 were found to carry homozygous or compound heterozygous pathogenic variants (detection rate 14.9%), while 4 subjects had only one mutation. The average LAL activity in molecularly confirmed patients was 3.5±2.73; in molecularly negative patients, the value was 13.886±4.41 ($p< 0.0001$). 22 different mutations were identified, including two novel and likely pathogenic variants (c.309C>A and c.856G>C).
Discussion: LAL activity in WBC is a validated tool for diagnosis of LAL-D. Higher residual enzymatic activity might result into a milder phenotype and postpone disease onset and diagnosis. A cut-off below 13.886 pmol/min/mg protein might be useful to discriminate patients with LIPA mutations. Two novel mutations were found.

P-341

Cognitive functioning in Fabry disease: relation with stroke, depression and disease phenotype

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Background: Despite a high prevalence of subjective cognitive complaints, robust data on objective cognitive impairment (OCI) in the different subpopulations of patients with Fabry disease (FD) are lacking. The aim of this study was to investigate the relationship between OCI, subjective cognitive complaints and depressive symptoms in men and women with classical and non-classical FD.
Methods: Cognitive functioning was assessed using a neuropsychological test battery and T-scores calculated using extensive normative data. Subjective cognitive complaints were assessed using a structured interview. Assessment of depressive symptoms was performed using The Centre for Epidemiological Studies-Depression scale (CESD) and disease severity by the Mainz Severity Scale Index (MSSI). The associations between OCI, stroke, depressive symptoms, sex, phenotype, disease severity and subjective cognitive complaints were analyzed.
Results: Eighty-one patients were included (mean age 44.5±14.3, 35% men, 74% classical phenotype). Subjective cognitive complaints were reported by 64% of all patients. OCI was present in thirteen patients (16%), predominantly in men with classical FD. Thirty-one patients (38%) scored ≥16 on the CESD, indicating high likelihood of depression. Male sex (OR, 10.4; 95%CI, 2.6-56.2; p=2.0*10-3) and a history of stroke (OR, 5.8; 95%CI, 1.1-35.7; p=4.3*10-2) were independently positively associated with OCI. The CESD score (one point increase: OR, 1.07; 95%CI, 1.02-1.13; p=3.3*10-3), a history of depression (OR, 2.7; 95%CI, 1.1-7.3; p=3.9*10-2) and the MSSI general score (one point increase: OR, 1.3; 95%CI, 1.1-1.5; p=5.5*10-3) were independently positively associated with subjective cognitive complaints.
Discussion: OCI is present in 16% of FD patients, predominantly in men with classical disease. In contrast, 64% of FD patients report subjective cognitive complaints, which show a relation to depressive symptoms. Treatment of the latter is therefore highly relevant.
Conflict of Interest declared.

P-342

Cognition and brain involvement in patients with infantile Pompe disease

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Background: There is limited understanding of central nervous system (CNS) involvement in Pompe disease, a lysosomal storage disorder caused by deficiency of acid-alpha glucosidase. With the availability of enzyme replacement therapy (ERT) for Pompe disease, patients with infantile Pompe disease (IPD) are surviving longer, making an assessment of CNS involvement possible as well as timely. Furthermore, such an assessment is vital to understand disease progression in the CNS, facilitate anticipatory surveillance and institute appropriate rehabilitation measures. This research is the first attempt to link neuroimaging findings in IPD patients with cognitive outcomes. This study aims to 1) use neuroimaging to determine the nature of brain involvement in IPD and 2) explore the relationship between cognitive functioning and brain involvement.
Methods: Nine patients (ages 6–17 years) with IPD and on long-term ERT had brain MR imaging. Eight completed cognitive testing using age-appropriate Wechsler scale and/or Leiter-3. MRI images were read by a trained radiologist.
Results: MRI images were normal in 4 patients (mean age 11.60 years, median FSIQ = 116). However, mild hyperintense white matter (WM) lesions on FLAIR and T2-weighted images in the centrum semiovale were found in 3 individuals (mean age 11.94 years, median FSIQ = 98); and two patients had abnormal periventricular and frontal white matter signal and/or diffuse abnormality of supratentorial white matter

(mean age 10.58 years, FSIQ = 88 for one patient). The median nonverbal IQ for the 3 patients in the entire sample administered the Leiter-3 was 96. Median time between imaging and cognitive testing was 17.5 days (range 1–132 days).
Discussion: While there were suggestive trends in executive function, our study also examined association between MR findings and other cognitive domains. In view of initial interesting findings in our sample, further exploration is warranted using a larger patient population.

P-343

The second case of Saposin A deficiency and altered autophagy

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Background: Deficiency of saposin A results in Krabbe-like manifestations. Mouse studies have undergone autophagic flux alterations in lysosomal storage disorders but there has been very little examination of saposin A deficiency and its effect on autophagy.
Methods / Case Report: A seven-month-old infant girl presenting with progressive neurodegeneration was suspected of having Krabbe disease. GALC gene analysis did not show any pathogenic variant. Prosaposin (PSAP) gene analysis was performed with a preliminary diagnosis of saposin A deficiency, which is known to follow a similar clinical course to that of Krabbe disease. A novel homozygous pathogenic c.209T>G (p.Val70Gly) variant in the PSAP gene was identified. We investigated ganglioside patterns and autophagic flux in patient's and heterozygous carrier relatives' fibroblasts.
Results: We detected significant increases in the levels of GalCer, LacCer, Cer, and GlcCer in the proband fibroblast cells compared to the control group. Fluorescence microscopic imaging of lysosomal-associated membrane protein (LAMP1) revealed increased numbers of lysosomal vesicles in proband fibroblasts. We determined a 2-fold enhancement in light chain 3 (LC3) and p62 proteins in the proband fibroblasts. Autophagosome maturation was confirmed by co-localization of p62 and LC3. We showed that the extent of LAMP1/LC3A co-localization was reduced in the proband fibroblasts compared to control. Autophagosome-lysosome fusion was impaired in saposin A deficient fibroblasts.
Discussion: Saposin A deficiency is one of the rarest forms of inborn errors of metabolism. Our data suggests that accumulation of autophagosomes in saposin A deficiency is due to defective clearance caused by impaired autophagosome-lysosome fusion. This finding is important to demonstrate which step autophagy is altered. To the best of our knowledge, this is the second case following Spiegel et al.'s case report published in 2005 which also showed the alteration of autophagy in a case of human saposin A deficiency.

P-344

Cross-talk between the c-Abl kinase and RIPK3 pathway-involved in neuronal necroptosis in Gaucher Disease

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Background: Gaucher disease (GD) is an inherited metabolic disorder, caused by mutations in the *glucocerebrosidase* (*GBA*) gene that lead to deficiency of the lysosomal glucocerebrosidase (GCase) enzyme and the accumulation the glucocerebroside (GlcCer). It has been shown that necroptosis, mediated by the RIPK3 protein, is a key mechanism involved in GD neurodegeneration. Here, our goal was to address whether the c-Abl kinase, which is involved in the activation of cell death in a variety of neurodegenerative diseases and has been recently linked with necroptosis, is activated in GD promoting necroptosis through RIPK3 modulation.
Methods: We used GD in vitro and in vivo models: i) GD pharmacological models: SHSY5Y cells and primary cortical neurons treated with the GlcCerase inhibitor, conduritol-β-epoxide (CBE) at 150 mM and ii) GCase null mice: cerebral cortex slices derived from $Gba^{flox/flox}$;nestin-Cre mice. To modulate c-Abl we used the c-Abl inhibitor Imatinib or cortical neurons obtained from wild-type and c-Abl null mice embryos. We evaluated GCase activity and ClcCer accumulation by conventional protocols. The activation of the c-Abl and RIPK3 pathways were followed by the levels of phosphorylated c-Abl and CRKII, and phosphorylated RIPK3 and MLKL, respectively. To determine c-Abl-RIPK3 interaction was performed a coinmunoprecipitation.
Results: Our results show that: i) CBE decreases GCase activity and increases GlcCer accumulation; ii) the c-Abl pathway is activated in the GD neuronal models; iii) c-Abl interacts with RIPK3, and iv) c-Abl inhibition decreases the levels of the p-RIPK3 and p-MLKL, the final effector of necroptosis, in neuronal GD models.
Discussion: Our results suggest that the cross-talk between the c-Abl and RIPK3 pathways is required for neuronal necroptosis in GD.
Support: FONDECYT N°3170710 (MJY), N°1150186 (SZ), N°1161065 (AAR) and REDES N°150082.

P-345

Monitoring neuropsychological function in CLN2 disease

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Background: CLN2 disease, a rare, pediatric-onset, neurodegenerative lysosomal storage disorder caused by TPP1 deficiency, is characterized by a delay in early language development, refractory seizures, progressive dementia, movement disorders, motor and visual deterioration, and death by mid-adolescence. While management strategies for CLN2 disease have recently been published, there is currently no standardized approach for monitoring neuropsychological functioning in affected individuals.
Methods: A panel of neuropsychologists and NCL experts convened to discuss and develop recommendations for neuropsychological monitoring in CLN2 disease.

Results: Cognitive and adaptive behavior decline are recognized hallmarks of CLN2 disease. Important considerations in monitoring neuropsychological function in CLN2 disease are that patients progressively lose the ability to speak, become blind, and lose manual dexterity. These are each key considerations in the selection of specific tools to assess neuropsychological functioning, as well as the mode of administration. Experts recommend a core battery of adaptive function, behavioral and emotional function, and IQ assessments, including the Vineland Adaptive Behavior Scale, Achenbach Child Behavior Checklist, and Bayley Scales of Infant and Toddler Development/Wechsler Preschool and Primary Scale of Intelligence /Wechsler Intelligence Scale for Children. The type of tool selected will be dependent on the stage of disease and the patient's developmental age.
Discussion: Long-term, sensitive and objective neuropsychological monitoring of patients with CLN2 disease can be accomplished with existing standardized measures, but with consideration for the specific phenotype and impairments of individuals. These assessments are critical for elucidating natural history, planning appropriate interventions, and assessing the impact of available therapies.
Conflict of Interest declared.

P-346

Lysosomal acid lipase deficiency in Greece: Enzymatic and molecular analysis of 31 patients using DBS Screening

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Background: Lysosomal acid lipase (LAL), encoded by the LIPA gene, catalyses hydrolysis of cholesteryl esters (CE) and triglycerides. The enzymatic deficiency is rare, underdiagnosed and results in CE accumulation in liver, spleen and cells of the macrophage-monotype system. It can cause cirrhosis and early atherosclerosis.
Methods / Case Report: The aim of the study was to use DBS screening for enzyme analysis in all members of 29 families (2 have two homozygous each) and compare the accuracy of the method for homo and hetero LAL-D members with DBS molecular screening. LAL activity was measured by fluorometric analysis in presence of specific inhibitor Lalistat-2. In all members molecular analysis of LIPA gene was also carried out by direct sequencing.
Results: In all 31 patients low LAL activity (≤0.04 mmol/punch/hr) and mutations of LIPA gene were detected (19 true homozygous and 12 compound heterozygous). Five mutations were detected: C.894G>A (51.2%), C.193C>T (2.2%), C.796G>T (5.7%), C.1024G>4 (12.8%) and the novel mutation C.361A>G (28.1%).
Discussion: DBS screening is an easy and safe way for detection of enzymatic and molecular abnormalities in LAL-D. The enzymatic method may need improvement for detection of borderline heterozygous. LAL-D is highly underdiagnosed in all age groups. The relatively small number of mutations demonstrates a homogeneity in the Greek population.
Conflict of Interest declared.

P-347

Experiences of late infantile neuronal ceroid lipofuscinosis type 2 (CLN2 disease) diagnosis in the UK

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Background: CLN2 disease (late infantile neuronal ceroid lipofuscinosis type 2) is a rare, progressive disorder caused by a deficiency of the lysosomal enzyme tripeptidyl peptidase 1 (TPP1), leading to neuronal loss and neurological symptoms, including seizures, language and motor deficits, cognitive decline and vision loss. The most commonly noted presenting symptom is unprovoked seizures, although language deficits and motor defects are also reported at this stage, often before 3 years of age. CLN2 disease is frequently associated with diagnostic delays due to its rarity and limited disease awareness among clinicians assessing patients at early stages. As CLN2 disease progresses rapidly, early diagnosis is key to ensuring optimal patient care through prompt treatment, multidisciplinary planning and family support. We present the experiences of CLN2 disease diagnosis across the UK, through an examination of testing strategies and diagnostic databases, to provide information on the mean age of diagnosis and raise awareness of CLN2 disease.
Methods: Four diagnostic centres in the UK provided information on testing strategies and age of diagnosis.
Results: Diagnostic centres had between 4 and 20 years of experience in testing for CLN2 disease, and number of patients diagnosed at each centre ranged from 1 to over 100. Testing strategies included standard lysosomal screening panels that incorporate TPP1 activity, and stand-alone tests combining TPP1 and PPT1 analysis that require a specific request from a referring clinician. The mean age of diagnosis was 4.47 years, although in siblings, diagnosis was frequently before 1 year of age.
Discussion: The results indicate that there is frequently a delay of approximately 1.5 years between the onset of symptoms and diagnosis of CLN2 disease. By raising awareness of CLN2 disease, diagnostic tests can be administered at an early disease stage, reducing diagnostic delays and allowing optimal disease management plans to be initiated.
Conflict of Interest declared.

P-348

The 6-minute walk test reveals early onset of motor decline in CLN3 disease

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Background: CLN3 disease (OMIM #204200) is a severe lysosomal storage disorder with childhood onset affecting both the retina and the brain. We recently showed that – contrary to common belief - onset of cognitive decline parallels onset of vision loss. Analogously, we hypothesized that motor decline may also have its onset earlier than commonly thought.
Methods: Motor function was assessed using the 6-minute walk test (6MWT), a clinical evaluation tool applied in various clinical populations but not yet in CLN3 disease or in visually impaired populations in general. From 2012–2017, the 6MWT was repeatedly assessed in 17 patients with CLN3 disease. In addition, the 6MWT was assessed under similar circumstances in a control cohort of visually impaired children. For data-analysis, we assessed correlation of 6MWT scores with age –including multi -level modelling allowing assessment of imbalanced repeated measurements – and with unified Batten disease rating scale (UBDRS) scores.

Results: In CLN3 disease patients, 6MWT scores showed a moderate inverse correlation (r-0.64, p< 0.0001) with age, with immediate impairment and start of decline from diagnosis. This linear deterioration was confirmed by multilevel analysis ((Y= 491(59)-14(4)*age) and correspondingly, a moderate inverse correlation was seen between 6MWT and UBDRS summary scores (r-0.60, p0.0001) (Figure 2). These results contrasted a positive correlation with age seen in the visually impaired control cohort who showed, compared to healthy sighted children, near-normal 6MWT results.
Discussion: Using the 6MWT, we show that motor function declines from early on in CLN3 disease. Although official reference values for the visually impaired were not available, the unaffected 6MWT results from the blind but otherwise healthy control group support our hypothesis that early motor impairment in CLN3 disease reflects early onset of primary neurological deterioration.

P-349

A cross-sectional quantitative analysis of the natural history of free sialic acid storage disease

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Background: Free sialic acid storage disease (SASD, OMIM 604369) is an ultra-orphan progressive multisystemic neurodevelopmental lysosomal storage disorder caused by the deficiency of the proton-driven carrier SLC17A5. Hard clinical endpoints for future clinical trials remain to be defined.
Methods: We quantitatively analyzed published cases with SASD (N=116). Main outcome variables were survival and diagnostic delay. As potential predictor of disease onset and survival, the influence of the amount of free sialic acid storage was investigated. Moreover, major disease features and geographical patient distribution were explored. The analysis was performed in compliance with STROBE criteria.
Results: Median age at disease onset was 0.17 years. Median age at diagnosis was 3 years with a median diagnostic delay of 2.5 years. Median survival was 11 years. The biochemical phenotype clearly predicted the disease course: Patients with an urinary free sialic acid excretion below 6.37-fold or an intracellular free sialic acid storage in fibroblasts below 7.37-fold of the mean of normal had a later onset of disease and survived longer than patients with biochemical values above these thresholds. Cluster analysis of disease features suggested a continuous phenotypic spectrum. Patient distribution was panethnic.
Discussion: The combination of neurologic symptoms, visceromegaly and dysmorphic features and/or nonimmune hydrops fetalis should prompt specific tests for SASD, reducing diagnostic delay. The present quantitative data inform clinical studies and may stimulate and accelerate development of specific therapies. Biomarker-phenotype association is particularly important for both counseling parents and study design.

P-350

Expanding the phenotype of the rare Neuronal Ceroid Lipofuscinoses (NCL) 10

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Background:NCL type 10 is caused by a deficiency in cathepsin D (*CTSD*), a lysosomal protease involved in proteolytic degradation, cell invasion and apoptosis, which results in a severe congenital phenotype with rapid progression to exitus and a milder form with a later onset with ataxia, dementia and retinopathy.
Case Report: This 3,5-year-old girl was born from consanguineous parents after a pregnancy complicated by fetal ascites since the 6th month. Previous pregnancies were normal. She presented development delay during the first months of life. Neurological regression was observed at 26 months of life. At 28 months she showed: facial dysmorphism, acquired microcephaly, apostural status with profound hypotonia, unresponsiveness to visual stimulation. EEG showed a multifocal epileptic pattern. Brain MRI showed a generalized cortical atrophy. On CSF examination folate was low (43.2 nmol/L; r.v. 63–111). An extensive neurometabolic work-up failed to detect any alteration. She eventually developed optic atrophy with "salt and pepper" retinopathy. ERG were not evocable. Echocardiogram revealed a slight thickening of the interventricular septum. Ultrastructural analysis of the skin detected vacuolated fibroblasts, osmophilic deposits, fingerprint-like structures, lipopigment storage in the cytoplasm of a lymphocyte, suggesting NCL.
Results: SNP-array analysis was negative for CNVs but disclosed a high rate of regions of homozygosity(about 9%; ROH), among which a ROH of about 3.5 Mb within 11p15.511p15.4 genomic region,including NCL10 locus (*CTSD* gene). NGS panel for NCL detected a homozygous causative variant in *CTSD* gene (c.308T>A; p. L103Q).
Discussion: With respect to other patients so far reported (11) our case presents an intermediate phenotype and age of onset. Heart involvement, reported in several NCL types, is emerging as a possible second target of the disease. Further study is required to support CSF folate as a metabolic biomarker of the disease.

P-351

High risk screening for lysosomal storage disorders using new technologies

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Background: Lysosomal storage disorders (LSDs) includes more than 50 different types of inherited metabolic disorders. Modern methods for enzyme and metabolite analysis in dried blood spots (DBS) improve the efficiency of diagnosis for some LSDs. The aim of this study was to compare results of LSD diagnoses before and after applying new methods for high risk screening.
Methods: From 1998 to 2007 fluorimetric enzyme assays in leukocytes and urine glycosaminoglycans (GAG) analysis were used for biochemical LSDs testing. Since 2014 mass spectrometry analysis of 6 lysosomal enzymes has been used and since 2016 lysosphingolipids and the inhibitor assay for acid lipase in dry blood spot (DBS) were included to the list of diagnostic tests. Sanger sequencing and Next-generation sequencing (NGS) technology has been used for DNA analysis to confirm the diagnosis, and are first-line tests for neuronal ceroid lipofuscinoses Types 3, 6, 8.
Results: Data from two periods (1998–2007 and 2008–2017) of LSD diagnosis from a Russian reference laboratory are compared. The number of tests has gradually increased (from 2000 to 10000 tests per year) due to the initiation of high risk screening in some hospitals and medical genetic units. The number of diagnoses has also increased though not so notably from 902 (1998–2007) to 1351 (2008–2017). In both periods mucopolysaccharidoses were the most frequent group of LSDs (42% and 36% respectively) followed by Gaucher disease (25% and 23%

respectively). Some changes in the frequency of the various LSDs were observed: increased frequency of Fabry disease (from 2% to 10%), NPC (from 0.3% to 3%), NP A/B (from 0.9% to 3.2%) and Pompe disease (from 0.5% to 1.2%) and decreased frequency for GM1-gangliosidosis (from 5% to 0.8%) and Alpha-mannosidosis (from 3% to 0.6%).
Discussion: Mass spectrometry analysis in DBS for treatable LSDs is important for the screening of high risk populations. To determine the true prevalence of the various LSDs and to define a diagnosis it is necessary to carry out differential diagnostic tests for various LSDs.

P-352

Miglustat treatment is associated with stabilised disability scores in patients from the International NPC Registry

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Background: The International NPC Registry is a multicentre, prospective, observational study in patients with Niemann-Pick disease Type C (NP-C) (EUPAS4622). It describes the natural history, disease course, clinical outcomes and treatment experience in real-world settings.
Methods: Patients diagnosed with NP-C were eligible for enrolment; we report on the subset of patients who were continuously treated with miglustat for ≥12 months. A modified NP-C scale with 4 domains (Pineda et al. Mol Genet Metab 2009;98:243–9) was used to assess disability (range: 0 [no disability] to 1).
Results: 472 patients were enrolled in 22 countries. A subset of patients (241/472) were treated continuously with miglustat (median observation period [range]: 3.29 [0.11–7.62] years [y]), 216 of which had received miglustat prior to enrolment. In the continuously treated subset, mean age (±SD, y) at enrolment was 20.0±12.4, at neurological onset 11.2±10.2 (patients by neurological onset category: < 2 y: 9.4%; 2–< 6 y: 29.5%; 6–< 15 y: 36.2%; ≥15 y: 25.0%), and at diagnosis 15.0±11.9. More than 50% of patients had ataxia, vertical supranuclear saccade palsy, dysarthria and cognitive impairment prior to enrolment; a neurological abnormality (any) was observed in 81.4% patients with data available. Overall mean composite disability score at enrolment (±SD) was 0.38±0.26: the < 2 y category mean score was highest (0.59±0.35) and ≥15 y category lowest (0.32±0.16). Overall, 70.5% of patients had improved or stable disease, with ≥3 of the 4 domains (ambulation: 67.8%; manipulation: 69.2%; language: 73.9%; swallowing: 71.3%) having a lower score or remaining unchanged from enrolment to last follow-up. No new safety concerns were identified.
Discussion: The NPC Registry describes long-term outcomes in patients with NP-C in all ages of onset. The findings are consistent with our current understanding of NP-C and its management. The disease of most continuously treated patients was stabilised.
Conflict of Interest declared.

P-353

Does miglustat treatment confer a benefit on survival in NP-C? Insights from a large observational cohort study

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Background: Miglustat has been indicated for the treatment of Niemann-Pick disease Type C (NP-C) in a number of countries for almost 10 years. The aim of this study was to assess the effect of miglustat on the survival of patients with NP-C.
Methods: For this observational study we collected and combined clinical and treatment data for 789 patients from five large national cohorts (Brazil, Czech Republic, France, UK, US) and from the NP-C Registry. Analyses compared miglustat treated and untreated patients at all ages and within previously defined categories according to age-at-neurological-onset: early-infantile (< 2 years), late-infantile (2 to < 6 years), juvenile (6 to < 15 years) and adolescent/adult (≥15 years). Survival was analysed from the time of first neurological manifestation (Neuro Onset group) and from diagnosis (Dx group) using a Cox proportional hazard model adjusted for covariates including age-at-neurological-onset, cohort and sex.
Results: Overall, 669 and 590 evaluable patients comprised the Neuro Onset and Dx groups. Miglustat treatment was associated with a significant reduction in mortality risk in both groups (Neuro Onset group, Hazard ratio [HR]=0.49; Dx group, HR=0.42; both p< 0.001). The effect was observed and consistent in all age groups (HRs=0.3 to 0.6), and was significant for late-infantile patients in both groups (Neuro Onset group, HR=0.36, p< 0.05; Dx group, HR=0.32, p< 0.01), and juvenile patients in the Dx group only (HR=0.30, p< 0.05).
Discussion: Miglustat treatment indicated improvement in survival of patients with NP-C in all age groups. Improvement was statistically significant in the late-infantile and juvenile groups. Study limitations, such as the differences in data collection among cohorts and missing key variables, an inability to adjust for disease severity, variable medical practices across time periods and within/among cohorts and miglustat treatment duration, urge cautious interpretation of these observational findings.
Conflict of Interest declared.

P-354

Large-scale expansion of human iPSC-derived skeletal muscle cells for Pompe disease modeling and cell-based therapeutic strategies

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Background: Although skeletal muscle cells can be generated from human iPSCs, transgene-free protocols include only limited options for their purification and expansion.

Methods / Case Report: iPSCs and myogenic progenitors were generated as described by us previously (van der Wal et al. Mol Ther Nucleic Acids. 2017 Jun 16;7:101–115). Gene correction using CRISPR/cas9 was performed in iPSCs using a donor vector that allows expression of the *GAA* cDNA into a safe harbor. Multinucleated myotubes were generated *in vitro* using a minimal medium. Myogenic progenitors were engrafted into NSG immunodeficient mice, and formation of human myofibers was assessed using human-specific antibodies for lamin A/C, dystrophin, and spectrin.
Results: We found that FACS-purified myogenic progenitors generated from healthy controls and Pompe disease iPSCs can be robustly expanded as much as 5 x 10^{11} fold. At all steps during expansion, cells could be cryopreserved or differentiated into myotubes with a high fusion index. I*n vitro*, cells were amenable to maturation into striated and contractile myofibers. Insertion of the acid alpha-glucosidase cDNA into the *AAVS1* locus in iPSCs using CRISPR/Cas9 prevented glycogen accumulation in myotubes generated from a patient with classic infantile Pompe disease. *In vivo,* the expression of human-specific nuclear and sarcolemmar antigens indicated that myogenic progenitors engraft into murine muscle to form human myofibers.
Discussion: This protocol is useful for modeling of skeletal muscle disorders and for using patient-derived, gene-corrected cells to develop cell-based strategies.
Conflict of Interest declared.

P-355

CLN8 deficiency alters the motility of lysosomes in a hippocampal neuronal model

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Background: CLN8p is an Endoplasmic Reticulum (ER) transmembrane protein, in which mutations cause CLN8 disease, one of thirteen forms of Neuronal Ceroid Lipofuscinosis. CLN8p shuttles between the ER and Golgi Apparatus and is related to sphingolipid biosynthesis, in spite of its role remaining unknown. We aim to study the effects of CLN8 deficiency on lysosomal dynamics and protein distribution in a neuronal model.
Methods: Hippocampal rat neurons of 7 d.i.v. were transfected with pYFP, pCLN8wt (overesxpression) or pshCLN8 plasmids to modulate *CLN8* expression. Dendritic morphology was evaluated by Sholl analysis. For kymographs, lysosomes were marked with Lysotracker. For protein distribution, LAMP1, transferrin receptor (TfR) and TMEM106b were overexpressed and specifically marked. Distribution was expressed as polarity index ($I_{dendrites}/I_{axon}$). Images were analysed by ImageJ-Fiji.
Results: Sholl analysis revealed dendritic shortening and diminished ramification in deficient cells. Kymographs of dendritic segments showed that the number of moving lysosomes is increased both in *CLN8* overexpressed and deficient neurons; however, direction was not altered. Moreover, trajectory and speed of lysosomes were significantly increased in dendrites of deficient cells ($p< 0.001$). Regarding protein distribution, both LAMP1 and TfR polarity indexes tend to decrease in deficient cells, not so for TMEM106b.
Discussion: CLN8p deficiency alters dendritic pathways involved in lysosomal motility, increasing their movement regardless of direction. This could explain dendritic shortening in CLN8 silenced neurons. One hypothesis suggests that movement impairment may be caused by mis-sorting of proteins. The results of protein distribution showed that traffic of proteins could be altered, but it may not be the only explanation (TMEM106b, involved in lysosome motility, is not affected). Further analyses is needed to uncover the CLN8p involvement on lysosomal motility in neurons.

20. Lysosomal disorders: treatment, enzyme replacement therapy

P-356

Pharmacokinetics, pharmacodynamics and safety of a mannose-terminated α-galactosidase (moss aGal) in patients with Fabry disease

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Background: Moss-aGal is a plant-made version of human α-galactosidase developed for enzyme replacement therapy (ERT) in patients with Fabry disease. Manufactured in a moss (*Physcomitrella patens*), the enzyme exhibits a homogenous N-glycosylation profile with more than 90% mannose-terminated glycans. In contrast to mammalian cell produced α-galactosidase, moss-aGal does not rely on mannose-6-phosphate receptor mediated endocytosis but targets the mannose receptor (MR) for tissue uptake.
Methods: Between January and November 2017, we conducted a phase 1 clinical trial with moss-aGal in six patients with confirmed diagnosis of Fabry disease during a 28-day clinical schedule. Recruiting took place in Mainz and Budapest. All patients received a single dose of moss-aGal with 0.2 mg/kg by i.v. infusion. Primary endpoints of the trial were safety and pharmacokinetics; secondary endpoints were evaluation of pharmacodynamics by analysing plasma and urine Gb3 and lyso-Gb3 concentrations.
Results: In all six patients, the drug was well tolerated, and no safety issues were observed. No anti-drug antibodies were formed. Pharmacokinetic data revealed a stable non-linear profile with a short plasma half-life of moss-aGal of 14 minutes. After one single dose of moss-aGal, median lyso-Gb3 plasma concentrations decreased by 3.8% and Gb3 by 11% from baseline to day 28. A significant and prolonged decrease in urinary Gb3 concentration was shown, with a decrease up to 23% 7 days and up to 60% 28 days post-dose.
Discussion: These data reveal that moss-aGal was safe and well tolerated. As shown previously in *in vivo* and *in vitro* experiments, moss-aGal is taken up via the MR, which is not only expressed on macrophages but also on endothelial and kidney cells. Our data now confirm that also mannose terminated α-galactosidase efficiently targets kidney cells. After these promising results, phase 2/3 clinical trials for moss-aGal are in preparation.
Conflict of Interest declared.

P-357

Comparison of bone marrow fat-fraction values to liver and spleen volumes in measuring ERT response in Gaucher disease

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Background: Deficiency of β-glucocerebrosidase in Gaucher disease results in bone marrow infiltration by abnormal macrophages that compromise bone with complications including impaired growth, pain, osteonecrosis, osteopenia and fractures. Liver and spleen volumes are

used along with laboratory values as markers of disease severity, but it is unclear if these adequately reflect disease activity including marrow involvement for initiation and monitoring enzyme replacement or substrate reduction therapy. Magnetic resonance spectroscopy (MRS) measures bone marrow quantitatively with lower fat-fractions representing worse disease.
Methods: Patients with Type I Gaucher disease were imaged using MRI for liver and spleen volumes as well as MRS of the lumbar spine and femoral neck prior to or early in treatment and at follow-up on enzyme replacement therapy. Differences were assessed between initial and follow-up using paired Wilcoxon signed ranks tests.
Results: This study included 5 patients (1 female, 4 male) with initial and follow-up imaging (mean age 8.7 years, mean follow-up duration 4.6 years). Fat-fractions increased with treatment (femur: 0.24 vs. 0.38 p=0.06; lumbar: 0.12 vs. 0.20 p=0.06). Organ volume decreased during treatment (liver: 1.33 vs. 1.18 multiples of normal, MN p=0.47; spleen: 8.79 vs. 4.31 MN p=0.47). Annual percentage increases were greater for femur and bone marrow (femur:+778%, lumbar:+674%) than annual decreases in organ volumes (liver:-10%, spleen: −51%).
Discussion: A small group of Gaucher disease patients increased marrow fat-fractions and decreased organ volumes in treated patients, albeit without statistical significance. These results raise the question of whether bone marrow fat-fraction may be more sensitive to treatment change than organ volumes, which may be slower to change and have other interactions. A larger study is needed to assess if bone marrow MRS may provide additional information in guiding therapy decisions in Gaucher disease.
Conflict of Interest declared.

P-358

Therapeutic goals in Fabry disease: European expert consensus recommendations based on current clinical evidence

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Background: Fabry disease, an inherited lysosomal X-linked disorder ranging from classic early-onset to non-classic later-onset phenotypes, can result in multi-organ pathology, substantial morbidity, and reduced life expectancy. To effectively manage patients with Fabry disease, physicians need therapeutic goals which are evidence-based, individualized to patient characteristics, and aligned with disease variant/status.
Methods: A European, multidisciplinary expert panel convened to develop organ-specific therapeutic goals and a disease-management algorithm based on consensus opinion and evidence obtained from a systematic literature review of 269 publications.
Results: The outcomes highlight the need to choose optimal treatment strategies depending on therapeutic goals, to start disease-specific therapy early to delay or slow disease progression, to acknowledge the potential negative burden of treatment, and to use non-specific adjunctive therapies in preventing or treating effects of organ damage on quality of life. The first step in the algorithm is to confirm diagnosis of Fabry disease, followed by a comprehensive initial patient assessment of organ pathology and clinical signs/symptoms of Fabry disease and by the establishment of appropriate individualized therapeutic goals and medical care plan. Monitoring should be performed regularly to assess whether the patient is achieving the specified therapeutic goals. If goals are missed, this should prompt review and optimization of the patient's therapeutic goals or treatment plan.
Discussion: The Therapeutic Goals Initiative evidence-based consensus recommendations and algorithm facilitate personalized medicine for different Fabry patient populations (male, female, pediatric) and help the multidisciplinary team to manage patient and physician expectations of treatment outcomes, reassess individual therapeutic goals and medical care plans in order to provide the best care possible. Funding: Sanofi Genzyme.
Conflict of Interest declared.

P-359

Early access to new treatments in France : the experience with velmanase alfa enzyme replacement therapy for alpha mannosidosis

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Background: Alpha-mannosidosis (AM) is a lysosomal storage disorder displaying a significant clinical heterogeneity. Before 2018, no treatment was approved for AM. Velmanase alfa is an enzyme replacement therapy (ERT) for the treatment of non-neurological manifestations of mild to moderate alpha-mannosidosis, not yet available. Temporary authorisation for use(ATU) is a France national process that allows early access to unlicensed drugs.
Case Report: Four patients in Lyon started receiving velmanase alfa 1 mg/kg weekly via intravenous under ATU. Patient 1 is a boy aged 12yrs, treated for 13 months; patient 2 is a boy aged 8yrs treated for 13 months; patient 3 is a male aged 35yrs treated for 8 months and a last patient 4, a female aged 43yrs just started ERT. All patients suffered from motor disability, hearing loss, neurocognitive impairment, pain, recurrent or severe infections. Patients are assessed every 6 months with administration of neuro motor test (BOT-2, LEITER), 3-Minutes Stair Climb Test (3MSCT), 6-Minute Walk Test (6MWT), lung test, cardiac test, audiometry test, urine oligosaccharides.
Results: velmanase alfa is well tolerated, with no safety concern until today. Urine oligosaccharides decreased with profile improved. Patients and caregivers are reporting improvement since start of therapy in particular in global motor coordination, less pain, less infectious events, stronger endurance. The most clinically relevant improvements were found in balance, global coordination. In particular patients 1 and 2 presented with improved global coordination, less infectious episodes, that was of particular burden before treatment. The 3-MSCT, 6MWT improved in patient 1 and 2. Discussion: Velmanase alfa was well tolerated by our patients, and led to improvements in clinical manifestations and patients daily living. Patients will be kept under monitoring for longer follow-up assessments.
Conflict of Interest declared.

P-360

Very early treatment for Pompe disease contributes to better outcomes: 10-years of experience in Taiwan

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Background: Newborn screening is the only way to initiate the very early diagnosis and treatment of Infantile onset Pompe disease (IOPD). The Taipei Veterans General Hospital began Pompe newborn screening from

2008, testing approximately two-thirds of the newborn population in Taiwan. There were more than 1 million newborns who were transferred from confirming diagnosis to our hospital. By 2010, we had established an effective newborn screening program with rapid diagnostic strategies, and almost all of the suspected IOPD infants could be diagnosed correctly and receive first time ERT within 4 hours of admission. To evaluate whether very early treatment in our IOPD patients would result in better clinical outcomes, comparing to our Late onset Pompe disease (LOPD) patients who were diagnosed before NBS program started and other IOPD study groups. This is a ten-year cohort study.
Methods: We diagnosed 26 IOPD & suspected LOPD in 48 of these newborns. Five LOPD patients were diagnosed by clinical evaluation before NBS program started and were treated in our hospital. We diagnosed and treated forty percent of Pompe patients in Taiwan.
Results: After 2010, the mean age at first ERT was 9.6 days old. Our patients had better biological, physical and developmental outcomes and lower anti-rh acid α-glucosidase antibodies after 2 years of treatment. Our IOPD patient needed invasive ventilation support. The mean age for independent walking was 11.6 ± 1.3 months, the same age as normal children. Furthermore, comparing to the LOPD patients who were diagnosed by clinical evaluation and conforming diagnosis were around 12-year-old, our IOPD patients who had more severe form of this disease have better clinical outcomes.
Discussion: ERT for patients with IOPD and LOPD should be initiated as early as possible, before irreversible damage occurs. Our results indicate that early identification of patients with Pompe disease allows for the very early initiation of ERT. Starting ERT even a few days earlier might lead to better outcomes.

P-361

Distal muscle function in treated classic infantile Pompe patients.

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Background: The introduction of enzyme replacement therapy (ERT) has significantly improved the prospects for patients with classic infantile Pompe disease. We now learn the clinical phenotype of patients surviving on ERT. Although Pompe disease is known to cause predominantly proximal muscle weakness, we have now noticed the development of substantial distal weakness in these patients. In this study we focus on the development of distal muscle weakness in relation to general motor development.
Methods: We included treated classic infantile Pompe patients with a follow-up time of ≥3.0 years who had learned to walk. Comprehensive examination of motor function was conducted every 3 months and recorded on video. Qualitative motor development and function was independently assessed by three reviewers using these recordings. Achievement of motor milestones was scored during regular outpatient visits.
Results: Fifteen patients were included. Median follow-up time was 6.0 years (3.1-18.2). Median age at which motor milestones were achieved: sitting 10.6 months (8.3-13.7), standing 12.2 months (9.2-16.5), and walking 17.2 months (14.0-21.3). Five patients lost the ability to walk and stand, all of whom had proximal weakness of the legs. We found early weakness of foot dorsiflexors in 14 patients, leading to a foot drop during walking. Weakness of the hands leading to loss of pincer grasp was found in 4 patients, this only occurred in patients with general muscle weakness.
Discussion: We found that ERT treated classic infantile patients, despite improvement of proximal muscle strength and function, developed moderate to severe weakness of the dorsiflexors of the feet at early age. A typical phenotype emerges with distal as well as proximal weakness, that differs from patients with non-classic Pompe disease. The etiology of this new phenotype of muscle weakness is currently unclear. Further investigation is required.
Conflict of Interest declared.

P-362

Mucopolysaccharidosis VI enzyme replacement therapy outcomes across disease spectrum: Findings from the MPS VI Clinical Surveillance Program

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Background: Mucopolysaccharidosis VI (MPS VI) is a rare lysosomal storage disorder with progressive glycosaminoglycan (GAG) accumulation. Disease manifestations vary across a spectrum of phenotypes. In general, the classical phenotype is associated with urinary GAG (uGAG) levels over 200 μg/mg creatinine while levels under 100 μg/mg creatinine are associated with a non-classical phenotype.
Methods: A voluntary observational study, the MPS VI Clinical Surveillance Program (CSP), collects data on individuals with MPS VI, including long-term efficacy and safety outcomes of enzyme replacement therapy with galsulfase. Long-term outcomes were evaluated for CSP participants at either end of the disease spectrum, as determined by pre-treatment uGAG level.
Results: Sixty-eight and 39 patients with at least 6 months of treatment exposure had pre-treatment uGAG levels over 200 and under 100 μg/mg creatinine, respectively. For the high and low uGAG groups, median ages at diagnosis were 2 and 10 years, median pretreatment baseline ages were 7 and 17 years, median baseline heights were 97.5 and 143 cm, and median treatment durations were 11 and 8 years. From pretreatment baseline to last follow-up, mean uGAG decreased by 82% (SD=19%, n=64) and 59% (SD=40%, n=36); mean 6-min walk test distance increased by 41 m (SD=165 m, n=31) and 49 m (SD=151 m, n=23); mean 3-min stair climb test results increased by 30 stairs/min (SD=45 stairs/min, n=23) and 17 stairs/min (SD=33 stairs/min, n=12); and mean forced vital capacity increased by 36% (SD=44%, n=27) and 30% (SD=43%, n=19) for the high and low uGAG groups, respectively. In the high uGAG group, 199 serious adverse events were reported in 49 patients (9% possibly or probably related to galsulfase); 97 were reported in 30 patients in the low uGAG group (7% possibly or probably related to galsulfase).
Discussion: Based on these findings, galsulfase appears to have similar safety and efficacy profiles regardless of disease severity.
Conflict of Interest declared.

P-363

Enzyme replacement therapy in patients with mucopolysaccharidosis VI: Updated findings from the MPS VI Clinical Surveillance Program

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Background: Clinical trials of galsulfase, approved for mucopo lysaccharidosis VI (MPS VI), have shown galsulfase as efficacious with a tolerable safety profile.
Methods: In 2005, MPS VI Clinical Surveillance Program (CSP) was initiated to characterize disease progression and evaluate long-term efficacy/safety of galsulfase. Previously published interim results corroborated clinical trial observations. Updated findings from the CSP are reported.
Results: As of March 2017, 223 patients were enrolled; 13 patients never received galsulfase and 48 initiated treatment in clinical trials. For all patients (n=219), median age at CSP enrollment was 12.6 years (range 0.8–65.0). For those already receiving galsulfase (n=206), median age at first infusion was 9.9 years (0.1–63.3) and treatment duration was 9.2 years (0.08–16.2). Outcomes were assessed for patients with pretreatment baseline and last follow-up assessment. From a mean (SD) last follow-up of 7.1 years (3.36), uGAG (n=132) decreased by 250.2 (262.17) μg/mg creatinine. Improvements in the six-minute walk test (6MWT; n=74) and three-minute stair climb test (3MSCT; n=40) were observed: mean (SD) increase of 48.2 (145.43) meters on 6MWT with a mean (SD) follow-up of 6.7 (3.20) years and an increase of 23.7 (42.05) stairs/min on 3MSCT with a follow-up of 4.3 (3.35) years. Pulmonary function also improved [FEV_1 mean (SD) increase 0.30 (0.53) L, n=55; FVC increase 0.39 (0.63) L, n=58] with a mean (SD) last follow-up of 6.9 (2.89) years. 79% reported ≥1 adverse event (AE), most frequently reported as MPS-related complications. Nine (4.3%) patients experienced drug-related serious AEs, with the most common being infusion-related reactions in two patients (1%). 32 patients discontinued for reasons unrelated to galsulfase. No new safety concerns were observed.
Discussion: Galsulfase was associated with a tolerable safety profile and long-term maintenance of endurance and pulmonary function, suggestive of ongoing clinical benefit.
Conflict of Interest declared.

P-364

Mucopolysaccharidosis VI enzyme replacement therapy initiated in adulthood: Findings from the MPS VI Clinical Surveillance Program

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Background: Mucopolysaccharidosis VI (MPS VI) is a rare lysosomal storage disorder caused by deficient arylsulfatase B activity which results in progressive glycosaminoglycan (GAG) accumulation. Galsulfase is available as an enzyme replacement therapy (ERT) for this condition.
Methods: The MPS VI Clinical Surveillance Program (CSP) is a voluntary observational study which collects data on individuals with MPS VI, including long-term efficacy and safety outcomes of galsulfase ERT. Considering the progressive course of the disease, a sub-analysis of the CSP data was performed to elucidate the impact of treatment initiation in adulthood.
Results: Of the 223 patients who have enrolled in the CSP, 51 started ERT at ≥16 years old and have been receiving treatment for ≥6 months. For these patients, median age at ERT initiation was 23 (range 16–63) years, median age at diagnosis (n=42) was 12.5 (range 2–54) years, median baseline urinary GAG level (n=30) was 65 (range 0–584) μg/mg creatinine, and median baseline height (n=48) was 149 (range 90–183) cm. Mean (SD) treatment duration was 9 (4) years. From pretreatment baseline to last follow-up, mean urinary GAG decreased by 66% (SD=45%, n=29), mean 6-min walk test distance decreased by 17 m (SD=107 m, n=23) from a baseline of 301 m, mean 3-min stair climb test results increased by 26 stairs/min (SD=33 stairs/min, n=14) from a baseline of 99 stairs/min, and mean forced vital capacity increased by 5% (SD=22%, n=19) from a baseline of 1.9 L. Ninety-six serious adverse events were reported by 36 patients. Only one, a seizure of mild severity, was reported as possibly related to galsulfase.
Discussion: Overall, galsulfase was well tolerated in the adult population, urinary GAG results were similar to those seen in the clinical trials, and endurance and pulmonary function outcomes were suggestive of long-term disease stabilization with prevention of deterioration, despite late treatment initiation.
Conflict of Interest declared.

P-365

The efficacy of intracerebroventricular ERT with IDS-β in MPS II murine model

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Background: Mucopolysaccharidosis II (MPS II) is caused by a deficiency of iduronate-2-sulfatase, results in accumulation of glycosaminoglycans (GAG) including heparan sulfate (HS) which is considered to contribute to neuropathology. We examined the efficacy of intracerebroventricular (ICV) enzyme replacement therapy (ERT) of idursulfase-beta (IDS-β) and evaluated the usefulness of HS as a biomarker for neuropathology in MPS II mice.
Methods: We first examined the efficacy of single ICV injections of three different doses (3, 10, and 30 μg) of IDS-β in MPS II mice. After single-injection study,its long-term efficacy was elucidated with 30 μg of IDS-β ICV injections repeated every 4 weeks for 24 weeks. The efficacy was assessed by the HS content in the cerebrospinal fluid (CSF) and the brain of the animals along with histologic examinations, and behavioral tests.
Results: In the single-injection study, the 30-μg of IDS-β ICV injection showed significant reductions of HS content in brain and CSF and maintained for 28 days. Furthermore, HS content in CSF was significantly correlated with HS content in brain. In the long-term repeated-injection study, the HS content in the brain and CSF was also significantly reduced and correlated. The histologic examinations showed a reduction in lysosomal storage. A significant improvement in memory/learning function was observed in open-field and fear-conditioning tests.
Discussion: ICV ERT with 30-μg of IDS-β produced significant improvements in biochemical, histological, and functional parameters in MPS II mice. Furthermore, we firstly demonstrate that the HS in the CSF had significant positive correlation with brain tissue HS and GAG levels, suggesting HS in CSF as a useful clinical biomarker for neuropathology.

P-366

Pregnancy outcome after exposure to migalastat: a case study

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Background: Fabry disease is a rare, X-linked, lysosomal storage disorder caused by deficiency of α-galactosidase A (α-Gal A), encoded by the *GLA* gene. Migalastat, a pharmacological chaperone, binds to *amenable* mutant forms of α-Gal A, restoring lysosomal trafficking and enzyme activity. In rabbits, developmental toxicity was observed at maternally toxic doses. Migalastat is not recommended during pregnancy.
Case Report: We describe a Caucasian woman with Fabry disease who became pregnant, despite hormonal contraceptives, while receiving migalastat during the phase 3 ATTRACT trial (NCT01218659).
Results: Prior to Fabry disease diagnosis, she delivered a 50-cm, 3.4-kg healthy boy (*GLA* WT) via emergency caesarean section due to proteinuria, preeclampsia, and pelvic abnormalities. One month later (Oct 2005), she was diagnosed with Fabry disease based on kidney biopsy and mutational analysis (*GLA* p.R112H) prompted by persistent proteinuria (1.4 g/24 h) during the postpartum period. At diagnosis, the patient had a >10-yr history of recurrent headache, nausea, vomiting, and vertigo. Enzyme replacement therapy was initiated in 2009. The patient enrolled in ATTRACT and started migalastat in May 2012. Proteinuria (2.2 g/24 h) without hypertension (131/68 mmHg) recurred 2 yrs after initiating migalastat; a urine-based pregnancy test taken at that time was false negative. A month later, proteinuria (protein/creatinine ratio 1755 mg/g) prompted kidney biopsy; serum pregnancy test was positive; ultrasound confirmed 18 wks gestation; the woman was 37 yrs old. Migalastat and hormonal contraceptives were stopped. Fetal MRI was normal at ~29 wks of gestation. In Oct 2014, the patient delivered a 45-cm, 2.29-kg healthy girl (*GLA* WT) via caesarean section. The pregnancy was uneventful, but birth weight was low.
Discussion: Except for low birth weight, pregnancy outcome was normal despite exposure to migalastat for 18 wks. Migalastat therapy during pregnancy is not advised.
Conflict of Interest declared.

P-367

Intracerebroventricular cerliponase alfa in children with CLN2 disease: Two year results from an ongoing multicenter extension study

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Background: CLN2 disease, a rare, pediatric, neurodegenerative storage disorder caused by TPP1 deficiency, is characterized by seizures, language and motor function loss, blindness and early death. An open-label study (NCT01907087) demonstrated that intracerebroventricular (ICV) infusion of 300 mg cerliponase alfa (recombinant human TPP1) qow for 48 weeks slowed deterioration in motor and language (ML) function. This extension study (NCT02485899) assesses long-term safety and efficacy for up to 240 weeks.
Methods: Subjects who completed the open-label study continued 300 mg cerliponase alfa qow in this open-label extension study. Cumulative data were used to evaluate long-term safety (adverse events (AEs) frequency) and efficacy (changes in CLN2 clinical rating scale ML domains).
Results: Of the 24 subjects in the open-label study (9 male, 15 female, mean (SD) age 4.3 years (1.24)), 23 enrolled in the extension study (96–161 weeks total exposure, median 116 weeks). All had AEs; most were Grade 1–2. Common AEs included pyrexia, vomiting, and convulsion. Twenty (83%) subjects had at least one serious AE, mostly consistent with neurodegenerative disease in a pediatric population. Significant attenuation of the rate of decline in ML score (mean (95% CI): 0.27 (0.12, 0.42) points/48 weeks, $p < 0.0001$) was observed compared with a rate of decline of 2.0 points/48 weeks in untreated patients. The responder (< 2 point loss/48 weeks) rate at 96 weeks (87%, $p < 0.0001$) was stable compared to 48 weeks.
Discussion: These data suggest that ICV-administered cerliponase alfa has an acceptable safety profile and a sustained treatment effect.
Conflict of Interest declared.

P-368

Evaluation of the long-term treatment effects of idursulfase using statistical modelling: data from the Hunter Outcome Survey (HOS)

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Background: Treatment for mucopolysaccharidosis type II (MPS II; Hunter syndrome) is available in the form of intravenous enzyme replacement therapy (ERT) with idursulfase (Shire, Lexington, MA, USA). This analysis used statistical modelling to evaluate the long-term treatment effects of idursulfase on selected clinical parameters based on data from the Hunter Outcome Survey (HOS), a global, observational registry (Shire, Lexington, MA, USA).
Methods / Case Report: Mixed modelling was used to analyse data from male patients followed prospectively in HOS who had received idursulfase for 5–8 years and who had information available for two or more timepoints, of which one was pre-ERT. Data from patients with only pre-ERT information available, and those who had received a bone marrow transplant or had enrolled in an idursulfase clinical trial were excluded. Time since start of ERT was included as a covariate and results were modelled for up to 8 years of treatment. For the prediction of values for percent-predicted forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV_1), and the 6-minute walk test (6MWT), only data from patients without cognitive impairment were used. A sensitivity analysis was conducted to assess robustness of the model using information from patients who had data for five or more timepoints.
Results: Urinary glycosaminoglycan levels decreased over time and palpable liver size also decreased. Left ventricular mass index was stable for up to 8 years of treatment while there was a slight decrease in percent-predicted FVC and FEV_1 and a gradual increase in distance walked in the 6MWT. Similar results were observed in the sensitivity analysis, indicating that this model provides reliable estimates.
Discussion: The nature of this analysis means that these findings are descriptive only. However, our results support those of previous studies and indicate that idursulfase has a positive effect on somatic manifestations of MPS II.
Conflict of Interest declared.

P-369

Infusion-related reactions in patients with mucopolysaccharidosis type II on idursulfase in the Hunter Outcome Survey

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Background: Enzyme replacement therapy (ERT) with intravenous idursulfase alleviates many of the somatic signs and symptoms experienced by patients with mucopolysaccharidosis type II (MPS II; Hunter syndrome). However, infusion-related reactions (IRRs) are a commonly reported, treatment-related adverse event.
Methods: Using data from March 2018, the occurrence of IRRs was investigated in patients with MPS II receiving idursulfase and enrolled in the Hunter Outcome Survey (HOS) observational registry (Shire, Lexington, MA, USA; NCT 03292887). Prospective patients who started idursulfase treatment at or after enrolment in HOS and for whom there was at least 1 year of follow-up data available (n = 250/908 treated patients; 1084 prospective patients enrolled) were included in this analysis. Data on frequency and timing of reported IRRs during the first year of therapy were analysed. AEs were coded in HOS using terminology in the Medical Dictionary for Regulatory Activities (MedDRA; version 16.0). In addition to MedDRA preferred terms, IRRs were defined as events occurring within 24 hours of an infusion and with evidence of a causal relationship with idursulfase.
Results: Median age at ERT start was 7.6 years (range, 0.2–48.0 years). In total, 142 IRR events were reported during the first year of treatment in 69 patients (27.6%). For most patients (n = 58/69; 23.2% of total population), the initial IRR occurred within the first 3 months of therapy. Only 11 patients (4.4% of total population) experienced their first IRR after 3 months of treatment. For six of these patients, their first IRR occurred more than 6 months after starting idursulfase; only one patient experienced their first IRR after more than 9 months of treatment.
Discussion: In conclusion, our analysis confirms that initial IRRs are most likely to occur within the first 3 months after starting idursulfase and are rare after more than 6 months of therapy. Shire sponsors HOS and funds medical writing support.
Conflict of Interest declared.

P-370

NEO1/NEO-EXT: Long-term safety of repeat avalglucosidase alfa dosing in late-onset Pompe disease patients for 3.5 years

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Background: NEO1 (NCT01898364) evaluated safety, tolerability, PK, pharmacodynamics and exploratory efficacy of repeat avalglucosidase alfa (neoGAA) dosing (5, 10 or 20 mg/kg qow) for 6 months in late-onset Pompe disease patients either treatment-naïve (Naïve Group) or previously receiving alglucosidase alfa for ≥9 months (Switch Group).
Methods: After NEO1 completion, all participants were invited to enter the NEO1 extension phase, NEO-EXT (NCT02032524), an ongoing study primarily evaluating long-term safety and PK of repeat avalglucosidase alfa dosing over 6 years. In 2016, all participants switched to avalglucosidase alfa 20 mg/kg qow.
Results: 8/10 Naïve and 11/14 Switch Group participants entered NEO-EXT; currently 18/19 are enrolled. Mean ages at NEO1 enrolment were 44.8 (SD 20.3; range 20–78) and 46.7 (SD 14.1; range 21–68) years for the Naïve and Switch Groups, respectively. By May 2017, mean (SD) avalglucosidase alfa exposures were 817 (469) and 931 (437) days for the Naïve and Switch Groups, respectively, and mean (SD) number of infusions administered were 59 (33) and 67 (31) for the Naïve and Switch Groups, respectively. Across both studies, treatment-emergent adverse events (AEs) were generally mild across all doses; most common treatment-related AEs were nausea (3/24 patients), headache (3/24), fatigue (3/24), dizziness (2/24), erythema (2/24), muscle spasm (2/24), myalgia (2/24), dyspnea (2/24) and rash (2/24). 1 participant discontinued NEO1 due to a serious AE and infusion-associated reaction of respiratory distress and chest discomfort at the 9th infusion and 1 discontinued NEO-EXT for personal reasons. No deaths/life-threatening serious AEs have been reported. No patient developed IgE antibodies or tested positive for enzyme activity inhibition over the analysis period.
Discussion: The safety profile of avalglucosidase alfa in NEO-EXT is consistent with the first 6 months of treatment in NEO1. Funding: Sanofi Genzyme.
Conflict of Interest declared.

P-371

Vestronidase alfa stabilizes or improves disease manifestations in subjects with MPS VII

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Background: Vestronidase alfa is an approved enzyme replacement therapy for patients with Mucopolysaccharidosis (MPS) VII, a heterogeneous, ultra-rare, debilitating, lysosomal disorder.
Methods: CL203 (NCT02418455) is an open-label study investigating the efficacy and safety of vestronidase alfa in subjects with MPS VII < 5 yrs old. Eight subjects (1.7-5 yrs old, mean weight 13.5 kg) enrolled and received vestronidase alfa 4mg/kg IV every other week; 5 subjects completed 48 treatment weeks.
Results: Urinary glycosaminoglycans (uGAG) decreased from baseline (BL) by a LS mean (SE) of 64% (5%) at week 4, and this reduction was sustained through Week 48 (W48). Mean standing height increased from 86 cm at BL to 92 cm at W48; Z score remained stable from BL (mean −2.27) to W48 (−2.12). Mean (SD) growth velocity was 5.06 (1.89) within 2 yrs pre-treatment (n=5); post-treatment increased to 6.84 (1.58) cm/yr (n=5). BL ultrasound showed hepatomegaly in 3 subjects and splenomegaly in 2 subjects. Hepatomegaly and splenomegaly resolved at W48 in 2 and 1 subject, respectively. Overall, clinical global impression score, ranging from −3 (worsening) to +3 (improvement), showed improvement or no worsening. Only 2 subjects had reliable BL and W48 functional development evaluations and both improved. The number of subjects able to achieve all motor function evaluation milestones increased from 0 at BL to 2 at W48. Six subjects developed anti-drug antibodies; 2 had neutralizing antibodies, 1 of whom had a Grade 1 anaphylactoid

reaction during 2 consecutive infusions, managed with antihistamine. ADA was not linked to a reduction in efficacy. No subject died or permanently discontinued therapy due to an AE.
Discussion: Vestronidase alfa-treated subjects with MPS VII < 5 yrs old showed decrease in uGAG, stabilization or improvement of disease manifestations, and a similar safety profile to older subjects. Longer treatment will provide better insight into the potential for growth improvement.
Conflict of Interest declared.

P-372

Sustained efficacy and safety of long-term vestronidase alfa (rhGUS) enzyme replacement therapy in patients with MPS VII

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Background: Vestronidase alfa (rhGUS) is an approved enzyme replacement therapy for mucopolysaccharidosis (MPS) VII, a heterogeneous ultra-rare disease. We sought to determine the long-term efficacy and safety of vestronidase alfa in patients (pts) with MPS VII.
Methods: 12 pts, ages 8–25 y, average weight 51.3 kg, completed a Phase 3, randomized, placebo-controlled, blind-start, single-crossover study (UX003-CL301; NCT02230566), received 24–48 wk of vestronidase alfa 4mg/kg IV Q2W, and achieved a 65% reduction in urinary GAG (uGAG) after 24 wk of treatment ($P< 0.0001$). All 12 pts enrolled in the open-label extension (UX003-CL202; NCT02432144). Efficacy measures in CL202 include uGAG (LC-MS/MS), 6-min walk test (6MWT), pulmonary function, fine and gross motor function, fatigue, shoulder flexion, visual acuity, and patient-reported outcomes.
Results: We report interim results for 9 pts who received ≥48 wk of treatment. Continuous treatment resulted in sustained uGAG reduction and in most pts improvement in clinical response including 6MWT, visual acuity, and fatigue. One pt discontinued due to noncompliance after receiving only 1 infusion: uGAG gradually returned to baseline levels, but the pt had continued improvement in fine motor precision and manual dexterity. One pt showed new sustained improvement in fatigue score (Wk 32 to 80), and a 73-meter increase in 6MWT (Wk 8 to 80) in CL202 not observed in CL301. Manual dexterity and balance decreased in some pts, and others had continued improvements in fine and gross motor function. One pt had treatment-related Grade 2 urticaria and bronchospasm (Wk 50), managed with diphenhydramine and corticosteroid premedication on subsequent infusions. No deaths or discontinuations due to AEs were reported. Six of 9 developed antidrug antibodies with no visible impact on drug safety/efficacy.
Discussion: These interim data show durable clinical efficacy and safety in 9 pts with MPS VII during long-term vestronidase alfa treatment.
Conflict of Interest declared.

P-373

Differential effects of agalsidase alfa and agalsidase beta in Fabry outcomes: 10 year outcomes from the Canadian Fabry Disease Initiative

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Background: We compare the efficacy of agalsidase alfa (AGALα 0.2 mg/kg) relative to agalsidase beta (AGALβ 1 mg/kg) in treatment naïve patients with Fabry disease (FD).
Methods / Case Report: The CFDI is a prospective multicenter study of ERT in FD in Canada. Treatment naïve patients who meet treatment guidelines (Cohort 1b) are assigned to AGALα or AGALβ. We report clinical outcomes of patients started on therapy between 2007 and January 2017
Results: 132 patients (56 AGALβ 76 AGALα) were followed for median time of 99 (range 5–123) months. There were no significant differences between the groups in baseline characteristics (age, gender, comorbidities, proteinuria, or concomitant medication use including renin angiotensin system (RAS) blockade). The rates of cardiac or neurological events or death did not differ between the two treatment groups. RAS blockade was used by 81.5% of AGALα and 59.1% of the AGALβ males (p=0.12) and 67.4% AGALα and 64.7% of AGALβ females (p=0.82). There were more renal events (renal replacement therapy, doubling of serum creatinine, proteinuria>3.5 g/day) in males receiving AGALα than males receiving AGALβ (1.1 versus 0.31 events/100 patient months IRR 0.24 p=0.006) and the rate of decline of eGFR tended to be lower in male patients on AGALβ than those on AGALα (−1.98 vs. -4.15 ml/min/1.73m^2/year; p=0.09). No difference in renal events, or the rate of decline in eGFR was seen between the two treatment groups in females.
Discussion: The use of AGALβ is associated with a trend to a slower rate of decline in renal function in males than the use of AGALα and significantly fewer renal events over 99 months of follow up. The drugs did not differ in their effects in women or on cardiac or neurologic events or death in men.
Conflict of Interest declared.

P-374

Late-onset Krabbe disease treated with haemopoietic stem cell transplantation: Outcomes two years after engraftment.

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Background: Krabbe disease results from a deficiency in the lysosomal enzyme galactocerebrosidase. Late onset disease presents in children and adults with progressive spasticity, ataxia and cognitive impairment due to progressive demyelination and the subsequent loss of neurons.
Case Report: Monozygotic twins born at 28 weeks gestation had motor and cognitive delay at 2 years. This was assumed to be secondary to prematurity until 7 years of age when a progressive decline was noted. Their MRI scans showed leucodystrophy and they had low levels of galactocerebrosidase activity (measurement of radiolabelled natural substrate in leucocytes). A pathogenic compound heterozygous mutation was

identified (common 30kb exon 11–17 deletion and c.956A>G mutation in exon 10). Both twins received haematopoietic stem cell transplants (HSCT). They had pre and post HSCT assessments which included: MRI of the brain, neurophysiology, nerve conduction studies, neuropsychology assessments and leucocyte enzyme activity.
Results: Successful primary engraftment was achieved for twin 1 using cord blood whilst twin 2 required a secondary haploidentical rescue transplant. Two years post-transplant galactocerebrosidase activity is in the normal range. Radiologically an improvement has been observed in the MRI for both twins. A reduction in spasticity was observed for both twins whilst their intellectual ability and speech has remained stable. Nerve conduction studies were stable for twin 2 whilst in twin 1 an improvement was seen in motor function yet a decline in sensory function.
Discussion: In the case of these twins, HSCT has been shown to improve the radiological changes caused by late onset Krabbe disease. Clinically we have observed stabilisation of functions of the central nervous system whilst the effect on the peripheral nervous system was more variable.

P-375

Investigator-initiated Clinical Trial of Intra-cerebroventricular enzyme therapy for sever type mucopolysaccharidosis II

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Background: Mucopolysaccharidosis type II (MPSII) is a lysosomal storage disease caused by deficiency of iduronate 2-sulfatase. We followed 13 patients with MPSII treated by ERT for more than 7 years and found that significant decrease of developmental age started when they were 3–5 years old, suggesting that intravenous administration of enzyme is not effective for neuronal regression of MPSII. To solve this problem, we have explored intra-cerebroventricular (ICV) enzyme replacement therapy.
Methods: An open labelled phase I/II clinical trial was carried out. Six Japanese patients have been enrolled. idursulfase (IDS)-β (1 to 30mg) was administered every 28 days using cerebrospinal fluid (CSF)-reservoir. We injected 13 times in 52 weeks. Primary endpoint is reduction of heparan sulfate (HS) concentration in CSF and secondary endpoint is changing of developmental age evaluated by K style scale.
Results: Significant reduction (50-70%) of HS concentration in CSF was observed in 5 treated patients. Cognitive impairment did not progress in all examined patients.
Discussion: Before starting this clinical trial, we performed an animal study using IDS-KO mice. Thirty μg of IDS-β were given via ICV injection once a month for 6 months. The accumulated heparan sulfate (HS) in the brain and in the CSF was reduced in the treated IDS-KO mice. The HS level in brain was correlated with that in CSF. We also demonstrated that ICV-ERT treatment ameliorated the pathological features in brain, and the open-field test showed improvement of brain functions. These results indicate that HS level in CSF is a possible surrogate biomarker for this study, and we selected HS concentration in CSF as a primary endpoint of the study. The results of this clinical trial suggest that intra-cerebroventricular enzyme replacement therapy for neuronopathic mucopolysaccharidosis type II is promising.
Conflict of Interest declared.

P-376

The relation between anti-drug antibody status and proteinuria in Fabry disease patients with a progressive loss of kidney function

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Background: Fabry disease (FD) is an X-linked multisystem lysosomal storage disorder affecting males and females caused by the deficient of α-Gal-A activity. Disease manifestations include progressive renal failure, hypertrophic cardiomyopathy, cardiac rhythm disturbances, stroke and death. The clinical benefit of the commercial enzyme replacement therapies (ERT) agalsidase-alfa and beta may not be as robust as anticipated, which may be explained by combination of factors.
Methods: In an effort to identify FD patients for the BALANCE study, a blinded, head-to-head comparison of pegunigalsidase-alfa to agalsidase-beta, with change in estimated glomerular filtration rate (eGFR) as the primary end-point (NCT02795676), the baseline characteristics, including anti-drug antibody (ADA) and serum-mediated inhibitory activity in patients who continue to progressively lose kidney function despite receiving ERT were analyzed.
Results: Differences observed between males and females in proteinuria (669±579 and 45±25 mg/g UPCR, respectively) and ADA status having 15/27 (56%) males ADA-positive to agalsidase-beta (titer: 469–127933) vs. 0/10 females. These males had 84±11% serum-mediated inhibition to agalsidase-beta. Lower ADA titers (66–46688) with lower serum-mediated inhibition (62±17%) to pegunigalsidase-alfa was shown. Combined analysis of ADA, proteinuria and eGFR show that more ADA-positive males (10/14=71%) had significant proteinuria than ADA-negative males (5/12=42%).
Discussion: The BALANCE screening identified FD patients with severe progressive nephropathy despite long-term ERT. Severe proteinuria was more frequently observed in ADA-positive males. Serum-mediated inhibition may contribute to the progressive loss of kidney function in FD patients, which may limit the success of ERT. The data show that pegunigalsidase-alfa activity is less inhibited by serum-mediated inhibition, suggesting a potential of treating with pegunigalsidase-alfa to control renal function.
Conflict of Interest declared.

P-377

Expert-agreed, modified-Delphi consensus recommendations for management of patients with MPS IVA/VI

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Background:Management of mucopolysaccharidosis (MPS) is complex, therefore robust independent guidance is required. This program used a modified-Delphi consensus methodology to develop evidence-based, expert-agreed recommendations for the management of patients with MPS IVA or VI.
Methods: To ensure credibility, the program was led by an international, multidisciplinary Steering Committee (SC) of MPS experts and managed by an independent secretariat. Four co-Chairs, appointed using a systematic ranking of professional activity, recommended 25 additional SC members, including 3 from Patient Advocacy Groups (PAGs); conflicts of interest were provided. The SC defined clinical questions and search terms for a systematic literature review, which was conducted by 3 Bibliographic Fellows using PRISMA. Oxford evidence levels were applied, and summaries assessed by the SC. Interviews with 5 PAGs

ensured the patient voice was represented. Statements were ratified using a blinded modified-Delphi online survey, in which they were rated using a Likert scale. Disagreements were explained and amendments suggested. Consensus was reached when ≥75% respondents agreed with a statement.
Results: 117 statements were developed for general management, routine monitoring/assessment, anaesthetics and disease-modifying/surgical interventions for MPS IVA/VI. Round 1 was completed by 103 physicians from 82 institutions in 20 countries; 7 submissions did not meet the minimum experience threshold and were excluded. 104 of the 117 statements reached consensus. Those that did not were amended by the SC using respondent feedback and sent for re-voting. Round 2 was completed by 71 physicians from 53 institutions in 18 countries; 2 submissions were excluded. Consensus was reached on all remaining statements.
Discussion:This guidance will be independently assessed against the AGREE II tool. It is envisaged that the publication of these guidelines will improve the outcomes of patients with MPS IVA/VI.
Conflict of Interest declared.

P-378

High dose genistein aglycone in Sanfilippo syndrome: results of a randomized, double-blinded, placebo controlled clinical trial

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Background: Genistein aglycone is thought to reduce glycosaminoglycan (GAG) synthesis and to cross the blood–brain barrier, making it a potential substrate reduction therapy for MPS III. Urine GAG and plasma heparan sulfate (HS) reduction with low dose genistein has been demonstrated in MPS III patients, and high doses have been shown to be well tolerated. This study is the first formal assessment of efficacy of high dose genistein aglycone.
Methods: In this phase III, randomized, double-blinded, placebo-controlled trial, enrolled participants received either placebo or oral genistein aglycone 160mg/kg/day for 12 months. All participants then received genistein aglycone for a further 12 months. The primary outcome measure was CSF HS. Secondary outcome measures included plasma HS, urine GAG and neuropsychological tests.
Results: 20 participants with MPSIIIA, B and C completed the placebo-controlled phase. From baseline to 12 months, CSF HS decreased by a mean of 34.7ng/ml in the genistein group and increased by 80.7 ng/ml in the placebo group. Adjusted for baseline, CSF HS at 12m was 7% lower in the genistein group compared to placebo (95% CI: 16% lower to 4% higher). Urine GAG decreased in both groups: mean reduction 11 mg/mmol creatinine (genistein), 5 mg/mmol creatinine (placebo).Plasma HS decreased in both groups: mean reduction 192ng/ml (genistein), 75ng/ml (placebo). Bayley developmental quotient (DQ) scores deteriorated in both groups: mean decline 3 points (genistein), 9 points (placebo). Vineland DQ also deteriorated in both groups: mean decline 4 points (genistein), 9 points (placebo). No statistically significant differences were observed.
Discussion: Biomarker reduction in CSF, plasma and urine was marginally greater in the genistein group compared to placebo. However, the degree of reduction is unlikely to be of clinical relevance. Cognitive scores declined in both groups, consistent with natural history, suggesting a lack of clinical effect.
Conflict of Interest declared.

P-379

Baseline characteristics of alpha-mannosidosis patients in the velmanase alfa enzyme replacement therapy clinical development program

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Background: Alpha-mannosidosis (AM) is a progressive lysosomal storage disorder. Velmanase alfa (VA) is an enzyme replacement therapy for the treatment of non-neurological manifestations of mild and moderate AM patients.
Methods: A total of 33 patients (19 paediatric, 14 adults) affected by AM participated in the VA clinical development program. All patients were assessed at baseline before receiving weekly administrations of VA. Assessments included 3-Minute Stair Climb Test (3MSCT), 6-Minute Walk Test (6MWT), Forced Vital Capacity percentage of predicted (FVC %), Childhood Health Assessment Questionnaire (CHAQ), Disability Index (DI) and Visual Analogue Scale (VAS) pain, and hearing ability among others. Descriptive statistics was used to describe two age subgroups.
Results: in paediatric patients, the 3MSCT median (range) was 55 (16.7-83.3) steps per minute, the 6MWT 454 (180–586) metres, the FVC 83 (50–99) %, CHAQ DI scores 1,25 (0–2.4) and CHAQ VAS Pain 0.21 (0.0 -1.5). In adult patients, the 3MSCT median (range) was 48.0 (37.7-71.3) steps per minute, the 6MWT 466 (335–690), the FVC 97.50 (51.0-119.0) %, the CHAQ DI scores 1.56 (0.6-2.6) and CHAQ VAS Pain scores 0.54 (0.0-2.5). Ninety-seven percent of the study population already demonstrated impaired hearing (26–55 dBHL; best ear bone conduction) or seriously impaired hearing (≥56 dBHL; best ear bone conduction). The range of values across the baseline domains was wide and no significant differences were observed between male and female patients.
Discussion: AM patients present a broad degree of afflictions in different domains. The AM population studied in the clinical programme of VA is one of the largest cohorts ever assessed. The disease presentation is very heterogeneous and no single aspect of disease manifestation alone can be considered a representative marker of the degree of disease severity suffered by the patient.
Conflict of Interest declared.

P-380

Applicability of alpha-mannosidosis classification by phenotype severity in a patient cohort participating to velmanase alfa clinical trials

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Background: Alpha-mannosidosis (AM) is a lysosomal storage disorder with a significant clinical heterogeneity. A classification of severity has been proposed dividing patients into “Mild” (Type 1), “Moderate” (Type 2), and “Severe” (Type 3). Velmanase alfa (VA) is an enzyme replacement therapy for the treatment of non-neurological manifestations of mild and moderate AM patients. METHODS: 33 AM patients participated in VA clinical trials; their baseline characteristics were analysed to assign each patient to one category of AM classification. No severe Type 3 patients were enrolled.
Results: heterogeneity in severity was observed, not only between patients, but also within patients (different severity and age of onset for different symptoms). Eleven patients (33.3%) were identifiable as Type 1. However, 10 of them had a non-mild degree of impairment in other manifestations not included in the classification, like motor (n=9) or pulmonary (n=5) dysfunction, and all presented some degree of mental impairment. Seven patients (21.2%) could be allocated to Type 2 given the simultaneous presence of ataxia and skeletal abnormalities. Of the 7 type 2 patients, ataxia was present in almost all paediatric cases (4 out of 5) regardless the fact that the classification foresees the onset of ataxia only in adults. All these patients had motor impairment and two had pulmonary dysfunction. Some degree of mental impairment was present. Three of these 7 cases, all paediatric, had more serious impairments. Finally, for 15 patients out of 33 (45.5%), it was not possible to formally assign one of the two phenotypes since they either presented with ataxia but not with skeletal abnormalities, or vice versa.
Discussion: AM patients show significant clinical heterogeneity, without falling into a clear category in almost 50% of cases. Most patients show features of both mild and moderate AM severity. A new disease severity categorization may be needed.
Conflict of Interest declared.

P-381

Glucosylsphingosine a useful biomarker for monitoring treatment in Gaucher disease

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Background: Gaucher disease (GD) is an autosomal recessive lysosomal disorder caused by impaired activity of B-glucocerebrosidase. Difficulties in identifying rate biomarkers in patients with GD are due to the diverse natural history of the disease and wide variability in patient response to Enzyme Replacement Therapy (ERT). Recently, an optimal diagnostic biomarker of GD has been established in glucosylsphingosine (Lyso-Gb1), the deacylated form of glucosylceramide. The aim of our study was to evaluate the efficacy of LysoGb1 in the long-term monitoring of patients treated with different therapies as ERT and oral substrate reduction therapy (SRT).
Methods: 7 GD patients (6 GD type 1, 1 GD type 3, 4 females and 3 males, median age at diagnosis 14 yrs, range 2–65 yrs) diagnosed by enzymatic assay and molecular analysis. Plasma Lyso-Gb1 was assayed by LC-MS/MS (n.v. 1.12- 3.00 nmol/L) (Polo et al. 2017). Patients on ERT (n=6) were treated with the dose of 60 U/kg bimonthly; one patient (GD Type 1) with a new SRT drug (Eliglustat) at the dose of 100 mg/die as first-line treatment. The Lyso-Gb1 was determined at diagnosis and after 12 and 24 months of therapy.
Results: At diagnosis all patients showed high levels of plasma LysoGb1 (median 316 nmol/L, range 131.1-649.9). After 12 months of ERT, Lyso-Gb1 was reduced of 72% (mean 124.1 nmol /L, range 83.5-233.2) and at 24 months, the decay reached the 83% (mean 77.9 nmol / L, range 18. 8–218). Interestingly, in 2 patients in whom a rate escalation protocol was started (from 60 to 45 U/kg), we observed in a rather short period of time an increased of Lyso-Gb1 value of 20 and 25% respectively. After one month, the patient treated with SRT showed a sharp reduction of LysoGb1 from 316 to 196 nmol/l (38%).
Discussion:Lyso-Gb1, which is presently the most specific and sensitive diagnostic biomarker of GD, can be used as useful biomarker for therapy monitoring of patients treated with ERT or SRT.

P-382

Childhood Health Assessment Questionnaire Disability Index (CHAQ-DI) in alpha-mannosidosis and velmanase alfa enzyme replacement therapy

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Background: Alpha-mannosidosis (AM) is a lysosomal storage disorder characterised by a wide heterogeneity of symptoms making it difficult to assess patient severity. Velmanase alfa (VA) is an enzyme replacement therapy for the treatment of non-neurological manifestation of mild and moderate AM.
Methods: Disease burden is not defined in AM; the CHAQ-DI (by severity of impairment category) was evaluated in 33 patients participating in the VA clinical development program and considered as a proxy measure of disease burden. It was explored whether a differential response to treatment exists depending on the degree of baseline CHAQ-DI score. Score ranges were 0–3 with higher values indicating greater disability. Patients were exposed to VA for a mean (SD) of 890 (461) days. A post-hoc Global Treatment Response (GTR) was evaluated by the aggregation of single endpoints into disease relevant domains: pharmacodynamics (reduction of serum oligosaccharides), functional (3-Minute Stair Climb Test, 6-Minute Walk Test, Forced Vital Capacity % of predicted), and quality of life (CHAQ DI and

CHAQ pain). Patients were considered responders in one domain if the Minimal Clinical Important Difference (MCID) established for each endpoint was exceeded in at least one endpoint of the domain; GTR is reached by patients that are responders in at least 2 domains.
Results: at baseline, 12 patients were scored in the mild range of disability (0–1), 13 in the moderate range (1–2), and 8 in the severe range (2–3). GTR was reached in 91.6% of patients scoring 0–1 on CHAQ-DI, 84.6% scoring 1–2, and 87.5% scoring 2–3. A response in all three domains was observed in 58.3%, 30.8%, and 50.0%, respectively.
Discussion: a treatment effect was seen across all severity categories at baseline, regardless of the initial burden of the disease. Notably, a response in all three domains was equally achieved in those patients more severely affected (scoring 2–3) as well as those with milder disability (scoring 0–1).
Conflict of Interest declared.

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The Lysosomal Disease Network global studies

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Background: The Lysosomal Disease Network (LDN) has created a community of clinical investigators, patient advocacy groups (PAG), and other interested parties, to become a synergistic research and educational consortium advocating advancement of treatment for these diseases. In the past three decades, lysosomal diseases have been a test bed for some of the most innovative therapeutic modalities.
Methods: In current activities, this program includes 9 longitudinal studies of natural history and/or treatment, and 2 pilot studies for novel ideas under the central theme of "clinical trial readiness". Because central nervous system (CNS) disease is the most challenging to measure and treat, there is a major emphasis on quantitative analysis of CNS structure, function and biomarkers for relevant conditions: mucopolysaccharidoses (MPS), mucolipidosis IV, Batten disease, gangliosidoses (Tay-Sachs, Sandhoff and GM1 gangliosidosis diseases), globoid cell leukodystrophy. Projects are (a) evaluating immune modulatory factors affecting treatment response in Pompe disease; (b) assess bone disease in MPS, (c) correlating renal structure and function in Fabry disease; (d) search for undiagnosed Fabry disease in high-risk populations; (e) determining outcomes of newborn screening for Krabbe disease; and (f) shortening the diagnostic odyssey. In addition, the LDN: (a) supports at least postdoctoral fellows each year; (b) organizes a scientific meeting patient-oriented and scientific meetings (bracketing the WORLDSymposium) and (c) is extending its reach with internet-based studies.
Results: In the past 7 years of NIH funding (U54NS065768), the LDN has accelerated knowledge acquisition in the field — with more than 60 MyNCBI cited publications — and furthered the development of therapeutic options.
Discussion: Global communication is provided by a list-serve with more than 3,000 subscribers, webinars, and the website Lysosomal DiseaseNetwork.org.

21. Glycosylation disorders/CDG, protein modification disorders

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A change of focus: determining phenotypic specificity facilitates understanding of pathophysiology in ultra-rare genetic disorders

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Background: Data scarcity impedes progress in recognizing, understanding and treating the rapidly growing group of ultra-rare genetic disorders. We hypothesized that looking beyond frequencies of phenotypic descriptions, into the specificity of a phenotypic feature, will facilitate recognition and - based on the principle that clinical similarities may be indicative of shared pathophysiology – will provide unsuspected insights in the underlying pathophysiology.
Methods: We explored this strategy by studying subunit deficiencies of the Conserved Oligomeric Golgi (COG) complex, a subgroup of Congenital Disorders of Glycosylation (CDG). Using available data with maximal efficiency, we describe a four-step strategy focusing on phenotypic specificity: 1) aligning phenotypic descriptions using the Human Phenotype Ontology, 2) delineation of frequently occurring phenotypic features, 3) determining phenotypic specificity by studying occurrence in other genetic diseases and 4) studying pathophysiological insights in diseases sharing highly specific phenotypic features.
Results: In contrast to prioritization based on phenotypic frequency, prioritization based on phenotypic specificity was highly informative. It captured not only phenotypic features commonly associated with CDG but also phenotypic features not seen in any other CDG. These features, among which episodic fever, indicate presently underappreciated functions of the COG complex. Interestingly, the COG complex is implicated in the autophagy pathway, as are more than half of the genes underlying diseases that present with episodic fever. This suggests that whereas many COG-CDG phenotypic features are caused by disrupted glycosylation, episodic fever might be caused by disrupted autophagy.
Discussion: In conclusion, we demonstrate that our new, four-step strategy focusing on phenotypic specificity can facilitate progress in understanding underlying pathophysiology of ultra-rare genetic disorders.

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A new DMP3-CDG (CDG-Io) Greek patient. A hot spot for the disease?

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Background: Congenital disorders of glycosylation (CDGs) are genetically and clinically heterogeneous diseases that can affect different glycosylation pathways.
Case Report: A 10 year old girl, born full term to consanguineous parents, referred following an incidental finding of elevated serum creatine kinase (CK) and mild raise of aminotransferases levels. Medical history was unremarkable except for subclinical hypothyroidism under therapy.

There was no family history of neuromuscular disorder Physical examination revealed mild bilateral gastrocnemius pseudohypertrophy, negative Gower sign and normal neurological examination. Manual muscle testing, Doppler echocardiography, fundoscopy and cerebral MRI imaging were normal. At the age of 10.5 years she developed impaired systolic function (ejection fraction: 57%). Furosemide and captopril treatment was initiated.
Results: DNA testing for Limb Girdle Muscular Dystrophy 2A and for mutations in the dystrophin gene was negative. α-Glucosidase activity was normal excluding Pompe disease. A quadriceps muscle biopsy showed a dystrophic pattern and loss of α-dystroglycan expression with the monoclonal antibody against α-dystroglycan (VIA41; Santa-Cruz Biotechnology). Transferrin isoelectric focusing revealed an abnormal CDG type I profile. *DPM3* sequencing revealed the homozygous mutation c.254T>C, p.L85S (NM_153741.1), also detected in heterozygosity in both parents.
Discussion: This is the third case of CDG-Io reported worldwide. Interestingly our patient originates from the same island and has the same genotype as the first patient (Lefeber et al. 2009). The case illustrates the importance of including CDG disorders in the diagnostic algorithm of patients with myopathy and abnormal CK levels.

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Whole-Body muscle MRI in 15 patients suffering from glycogen storage disease type III

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Background: Glycogen storage disease type III (GSDIII) is an autosomal recessive disorder caused by mutations in the AGL gene coding for the glycogen debranching enzyme. The pattern of muscle involvement with the whole-body muscle MRI has never been reported in this condition compared to other glycogen storage disease such as Pompe disease.To describe muscle involvement on whole-body MRI scans in adults at different stages of glycogen storage disease type III (GSDIII), fifteen patients aged 16 to 59 were examined. Three required a cane to walk.
Methods / Case Report: Whole-body muscles MRI were performed on a 3T system. The examinations consisted of coronal T1 weighted images and axial T1 weighted or fat images from 3 points Dixon sequence. MRI examinations were scored for 47 muscles using Mercuri's classification. Muscle changes consisted of internal bright signals of fatty replacement.
Results: Distribution across muscles showed predominant impressions in the lower limbs and postural muscles which was consistent with the overall clinical presentation and the results providing in the Heat-Maps scores. Careful review of the MRI scans collected provided new informations regarding recurrent muscle changes particularly in the soleus, gastrocnemius medial head and thoracic extensors muscles.
Discussion: These results demonstrate whole-body MRI provides a very evocative pattern of muscle involvement in GSDIII in adults. Further correlations with clinical and functional parameters might lead to the development of a severity score which might contribute to improving patient management.

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ALG 13 Deficiency: A treatable CDG Type I causing X-linked mental retardation

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Background: *ALG13* and *ALG14* encode a UDP-GlcNAc transferase and are essential during N-glycosylation. The UDP-GlcNAc transferase catalyses the transfer of the second N-acetylglucosamine to the dolichol linked oligosaccharide precursor. By disrupting this pathway deficiencies in ALG13 causes a congenital disorder of glycosylation (CDG) type 1 with X-linked mental retardation as main clinical phenotype.
Methods: We performed screening for CDG in two brothers presenting with mental retardation, dystrophy, retrognathia as well as muscular hypotonia by using high performance liquid chromatography (HPLC), mass spectrometry and isoelectric focusing. Sanger sequencing was performed to identify the genetic cause. In order to monitor our therapy, we again performed HPLC.
Results: As genetic cause we identified a hemizygot variant c.1886 A>G (Y629C) in *ALG 13* in both brothers. Their mother and grandmother are heterozygous carriers for this variant. The screening for CDG was positive by revealing a hypoglycosylation, while mass spectrometry confirmed the missing of the second N-acetylgylosamine in the oligosaccharide chain of transferrin. After one year of treatment the brothers present in daily life more awareness of their surroundings, improving motor abilities and the younger one with progress in his talking abilities, while there is still hypoglycosylation present in HPLC.
Discussion: There are many diseases presenting with X-linked mental retardation, while there are only a few treatable causes. A deficiency in ALG13 is one of these few when treated with oral N-acetylglycosamine substitution result in an improved clinical outcome.

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Deficiency of lysosomal acid alpha-glucosidase in a patient with dystroglycanopathy due to *GMPPB* deficiency

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Background: GDP mannose pyrophosphorylase B (GMPPB) deficiency is one of several dystroglycanopathies, a heterogeneous group of neuromuscular disorders characterized by aberrant glycosylation of alpha-dystroglycan (ADG). GMPPB catalyzes the formation of GDP-mannose, required for mannosylation and glycosylation of ADG. The phenotypic spectrum of GMPPB deficiency is broad and includes a progressive myopathy, brain and eye abnormalities, and intellectual disability.
Case Report: In a patient with GMPPB deficiency due to *GMPPB* gene mutations (p.Q234X/p.T153I), presenting with hypotonia, elevated serum creatine kinase, psychomotor delay, seizures, and bilateral cataracts, we found a profound deficiency of the lysosomal acid alpha-glucosidase (GAA).

Results: GAA activity in fibroblasts was 2.61 nmol/mg prot/h (NV 64.4 ±22.9). The molecular analysis of the *GAA* gene in this patient was negative. A western blot analysis showed impaired processing of GAA in fibroblasts. GAA was mostly detectable as the 110kDa precursor isoform, with near-complete deficiency of the mature 70 and 76 kDa peptides. Conversely, exogenous recombinant human GAA (Myozyme) was normally internalized and processed into the mature GAA. In peripheral lymphocytes GAA activity and maturation were normal, likely due to the different intracellular processing and trafficking of the enzyme. In addition, we found reduced activities of beta-galactosidase, alpha-galactosidase, alpha-mannosidase, beta-glucosidase and alpha-fucosidase, suggesting an impairment of multiple lysosomal enzymes.
Discussion: Our results suggest aberrant processing of endogenous GAA in our patient's fibroblasts, possibly due to defective glycosylation and mannose-6-phosphate generation. We speculate that defective GMPPB function impacts not only on ADG, but also on glycosylation of other glycoproteins, including lysosomal enzymes. It is possible that secondary GAA deficiency contributes to the pathophysiology of muscle disease in GMPPB deficiency.

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Integrating glycomics and genomics uncovers SLC10A7 as essential factor for bone mineralization, protein transport and glycosylation

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Background: Abnormal protein glycosylation has been associated with all major human diseases, however, the underlying mechanisms remain poorly understood.
Methods / Case Report: We here combined genomics and plasma glycomics in 99 patients to identify novel genetic factors and associated disease phenotypes.
Results: In a subgroup of patients with teeth and skeletal abnormalities and a discriminative glycomics signature, deficiency of *SLC10A7*, a gene of unknown function, was found as cause of disease. The patients' phenotype consisted of amelogenesis imperfecta, skeletal dysplasia, and decreased bone mineral density compatible with osteoporosis. The patients' phenotype was mirrored in slc10a7 deficient zebrafish. Furthermore, alizarin red staining of calcium deposits in zebrafish morphants showed a strong reduction in bone mineralization. Cell biology studies in fibroblasts of affected individuals showed intracellular mislocalization of glycoproteins and a defect in post-Golgi transport of glycoproteins to the cell membrane. In contrast to yeast, human SLC10A7 localized to the Golgi apparatus.
Discussion: Our combined data indicate an important role for SLC10A7 in bone mineralization via regulating post-Golgi protein transport and glycosylation.

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Dolichols as non-invasive marker for rare types of CDG

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Background: Dolichols (Dol) are chain polyisoprenoid alcohols mainly composed of 17–21 isoprene units (IU) in mammalian cells. The phosphorylated derivative of Dol participates as lipid carrier for N-linked protein glycosylation. In recent years, human defects have been identified in Dol biosynthesis genes within the group of Congenital disorders of glycosylation (CDG). This has increased interest in Dol metabolism. Furthermore, Dol biosynthetic pathway shares identical initial steps with cholesterol and coenzyme Q10.
Aim of our study was to introduce the analysis of Dol in urine and tissues with a view to extending spectrum of non-invasive screening methods for suspect CDG patients.
Methods: Material for this study consisted of urine samples from 76 controls in age from 2 months to 82 years and 6 patients with CDG (NUS1-CDG, SRD5A3-CDG, DPAGT1-CDG, PMM2-CDG, PGM1-CDG); and samples of frontal cortex, liver, muscle and heart from 2 NUS1-CDG patients and controls. Dol were analysed by LC-MS/MS (UPLC Agilent 1290 Infinity LC System and SCIEX API 4000 LC-MS/MS System AB/MDS). Dol with 17, 18, 19 and 20 IU were captured to determine the Dol-18 /Dol-19 ratio.
Results: In controls, significant correlation between Dol-18/Dol-19 and age was found (P< 0,005). The Dol-18/Dol-19 control range was defined for different age categories (means+SD): < 1 year: 0.76 ± 0.17, 1–6 years: 0.76 ± 16, 6–18 years: 0.62 ± 0.16, > 18 years: 0.56 ± 0.12. Gender specific differences were no found. It has been verified that ratio of Dol-18/Dol-19 was significantly increased in NUS1-CDG urine and tissues in comparison with controls. Interestingly, levels of Q10 were significantly decreased in tissues of NUS1-CDG as well.
Discussion:The results show a new possibility of diagnosing patients with rare types of CDG that cannot be captured by routine screening methods.
Supported: by MZ CR AZV-16-31932A, AZV-15-28208A, RVO-VFN64165

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Combination of plasma glycomics and genomics for subtyping of Congenital Disorders of Glycosylation type-1

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Background: Previously, total plasma glycomics revealed abnormalities that are diagnostic for ALG1-CDG and elevated levels of 3- and 4-mannose containing N-glycans in PMM2-CDG and MPI-CDG. Here, we investigate the potential of total plasma N-glycoprofiling to identify characteristic features for CDG-I subtypes
Methods / Case Report: PGC-chip-QTOF profiling of N-glycans was performed in 71 CDG-I patients with different gene defects. In addition, smMIPS panel sequencing was established for fast and cost effective sequencing of candidate genes.
Results: In addition to the literature reported CDG-I subtypes, differential glycomics profiles were observed for the dolichol-P-mannose synthesis defects, and several ALG glycosyltransferases. Application of glycomics in a cohort of 30 unsolved CDG-I cases showed characteristic profiles for ~75% of the patients. Application of smMIPS sequencing solved ~2/3 of the patients with predicted pathogenic mutations.
Discussion: We propose total plasma N-glycomics in combination with panel sequencing for facilitated diagnostics of CDG-I.

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Disruption of the responsible gene in a phosphoglucomutase 1 deficiency patient by homozygous chromosomal inversion

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Background: Phosphoglucomutase 1 (PGM1) deficiency is a recently defined disease characterized by glycogenosis and a congenital glycosylation disorder caused by a recessive mutation in the PGM1 gene.
Methods / Case Report: We report a case of a 12-year-old boy with first-cousin parents who was diagnosed with a PGM1 deficiency due to significantly decreased PGM1 activity in his muscle. However, Sanger sequencing revealed no pathogenic mutation in the PGM1 gene in this patient. As this case presented with a cleft palate in addition to hypoglycemia, elevated transaminases and creatine kinase, karyotyping was performed and identified homozygous inv(1)(p31.1p32.3). Based on the chromosomal location of the PGM1 gene at 1p31, we analyzed the breakpoint of the inversion.
Results: Fluorescence in situ hybridization (FISH) combined with long PCR analysis revealed that the inversion disrupts the PGM1 gene within intron 1. Since the initiation codon in the PGM1 gene is located within exon 1, we speculated that this inversion inactivates the PGM1 gene and was therefore responsible for the patient's phenotype.
Discussion: When standard molecular testing fails to reveal a mutation despite a positive clinical and biochemical diagnosis, the presence of a gross structural variant that requires karyotypic examination must be considered.

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Clinical spectrum of Congenital Disorders of Glycosilation: experience of a Portuguese center

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Background: Congenital disorders of glycosylation (CDG) constitute a rapidly growing group of diseases, with a very wide spectrum of manifestations. Serum transferrin isoelectrofocusing (IEF) or carbohydrate deficient transferrin (CDT) are still the screening methods of choice for N-glycosylation defects, but they fail to other CDG, usually diagnosed by genetic studies.
Methods / Case Report: In our center, were identified 10 patients with 7 different CDG subgroups.
Results: − 4 patients with PMM2-CDG, who showed neurological involvement and cardiac involvement in 3, with persistent pericardial effusion, requiring albumin infusion and, in one, the performance of a pericardial window surgery. - 1 female, with GALNT3-CDG, formerly known as familial hyperphosphatemic tumoral calcinosis, who presented severe hyperphosphatemia with painful and incapacitating hyperostosis. - 1 male with COG4-CDG, who showed intellectual disability, recurrent infections, scoliosis and liver cytolysis.- 2 males with defects in the V-ATPase complex: one with ATP6V0A2-CDG, who presented cutis laxa, mental retardation and ocular abnormalities; and another with a mutation in ATP6AP1, a X-linked disease, with hypogammaglobulinemia, hypercholesterolemia, hepatopathy and normal cognitive level.- 1 male with PGM1-CDG, a treatable disease with galactose, who showed hypoglycaemia, liver cytolysis and background of submucosal cleft palate, without neurologic problems. - 1 female patient with an unidentified defect of glycosylation (CDG-x). She presented after birth with hepatopathy and coagulation disturbances, and then developed diabetes, scoliosis and nephropathy. CDT quantification was persistently elevated and there was a type 2 pattern on serum transferrin IEF.
Discussion: The diversity of presentations and the difficult to confirm diagnosis must compel us to be alert and persistent, considering that even in our centres many other patients could not yet have been recognized.

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Clinical and radiological features of SLC35A2-CDG patients

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Background: SLC35A2-CDG was first described in 2013. It is caused by mutations in *SLC35A2* that encodes for a UDP-galactose transporter. Although classified as a type II CDG, the screening of transferrin isoforms may be false negative. Previously reported patients had severe neurological symptoms and often early-onset epileptic encephalopathy.
Methods: The clinical data of 15 patients from eleven different countries was collected. In nine, a brain MRI (at the age of 10 months to 18 years) was available for re-evaluation.
Results: The cohort included 4 males and 11 females (present age 4 to 28 years) of which one patient was deceased. All the patients showed moderate to severe psychomotor developmental delay and only two can walk independently. None of the patients had normal cognitive development. Epilepsy was present in 80%, muscular hypotonia in all. Hypsarrhythmia and epileptic encephalopathy were described in 82% of patients with seizures. MRI showed mild brain atrophy, short and thin corpus callosum and delayed myelination with T2 intensities in parieto-occipital periventricular white matter and dorsal mesencephalon. Many patients (79%) had

short stature, and skeletal anomalies were common. More than half of the patients had ophthalmological findings (strabismus, refractive error, cerebral visual impairment). Notably, six patients had normal transferrin isoform analysis.
Discussion: The clinical findings in our cohort support the previous reports by showing that SLC35A2-CDG mainly affects the nervous system and symptoms are mostly severe. Based on the data of 15 patients, the diagnosis of SLC35A2-CDG can be challenging as the neurological syndrome with psychomotor developmental delay, hypotonia and seizures, together with short stature is rather unspecific. Of note is that negative CDG screening does not exclude the diagnosis. Though early diagnosis is important for genetic counseling and possible treatment with oral galactose.

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Proteostasis regulators as a new therapeutic approach for PMM2-CDG

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Background: PMM2-CDG, the most common glycosylation disorder for which up to date there is not treatment available, has been suggested to be a conformational disease. The misfolded nature of most of the disease-causing mutations makes the use of stabilizing molecules like pharmacological chaperones (PCs) or proteostasis regulators (PRs) the most straightforward and promising therapeutic strategy for this disease. The main aim of this work is to identify the effect of the PR celastrol in some PMM2 folding mutants and evaluate its synergistic effect with a PC previously identified in our group as able to enhance the stability and activity of different unstable PMM2 mutations.
Methods / Case Report: Patient-derived fibroblasts transduced with their own PMM2 folding mutation (p.Asp65Tyr, p.Arg162Trp, p.Thr237Met and p.Pro113Leu) were treated with celastrol or with a combination of celastrol and a selected PC. Amount and activity of PMM2 were respectively evaluated by western blot and enzymatic assays. mRNA expression of molecular chaperones was assessed by qRT-PCR and western blot.
Results:This work reports the identification of a PR, celastrol, which restores PMM2 protein of different unstable mutants in a cellular model of PMM2-CDG. In this model, celastrol treatment correlates with a significant increase in the expression of Hsp27, Hsp40, Hsp70 and Hsp90. Additionally, the mutant p.Arg162Trp was treated with pifithrin-μ, an Hsp70 inhibitor, and 17-AAG, an Hsp90 inhibitor, revealing a significant reduction in PMM2 protein levels indicating the possible implication of both molecular chaperones, Hsp70 and Hsp90 on the p.Arg162Trp folding. Finally, we show the positive effect of the co-application of celastrol and a selected PC for PMM2 (p.Arg162Trp) showing a synergistic effect on several PMM2 mutants.
Discussion: Preliminary results suggest that the stabilizing effect of celastrol on PMM2 folding mutants acts through the modulation of the proteostasis network.

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Twenty years outcome of treatment with low dose oral mannose in a patient with MPI-CDG

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Background:Congenital disorders of glycosylation are hereditary multisystem disorders characterized by the hypoglycosylation of glycoproteins. MPI-CDG (CDG type Ib) due to phosphomannose isomerase (MPI) deficiency is the one of disorders in this group that responds to treatment with mannose. The long-term outcome is unknown, but several patients experienced progressive liver fibrosis. We describe the outcome of 20 years of treatment with low dose mannose in a patient with MPI-CDG.
Methods / Case Report:The patient presented at 8 months of age with hypoglycemia during an intercurrent illness. At 2 years of age she developed sagittal sinus thrombosis, severe coagulopathy and protein losing enteropathy. Liver biopsy showed ductal plate malformation with features of congenital hepatic fibrosis. Isoelectric focusing of transferrin in serum showed marked undersialylation of transferrin. PMI activity in leukocytes and skin fibroblasts was low. Her psychomotor development and growth were normal.
Results:Therapy with oral mannose in dose 0.15 g/kg/dose 4 times a day was initiated and her biochemical findings almost completely normalized. Over the subsequent years she had mildly elevated HgbA1c (up to 7%) and the dose of mannose was gradually decreased to 0.10 g/kg/day. On this dose the patient remains clinically well, in almost complete biochemical control and without evidence of liver fibrosis.
Discussion:Recommended initial dose of for mannose is 0.2 g/kg 4 to 6 times a day, and dosing for chronic treatment is based on sustained high blood levels of mannose, but testing is offered in only a few specialized laboratories, which is inconvenient for daily practice. Long term consequences of high blood mannose levels are not known. Biochemical markers of metabolic control in PMI deficiency may be used for dose adjustment in chronic treatment. The outcome of our patient treated with 0.1 g/kg/day indicates that lower dose of mannose may be efficient to maintain metabolic control in MPI-CDG (CDG1b).

P-397

Glycogen storage disease associated with glycogenin-1 deficiency

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Background: Glycogen, a storage form of glucose, is essential for energy supply and glucose homeostasis. Glycogenin is a glycosyl transferase that catalyzes the formation of a short glucose polymer of approximately ten glucose residues from UDP-glucose in an auto-glucosylation reaction, which is followed by elongation and branching of the polymer, catalyzed by glycogen synthase and branching enzyme, to form glycogen. There are two glycogenin isoforms in humans. Glycogenin-1, encoded by *GYG1*, is ubiquitously expressed, whereas glycogenin-2 is the main isoform in liver.
Methods:We have previously discovered and characterized a glycogen storage disease caused by defective glycogenin-1, either by truncating *GYG1*mutations or by missense mutations inhibiting the auto-glucosylation. The disease manifests as either a dilated cardiomyopathy necessitating heart transplantation or a pure skeletal myopathy with adult onset muscle weakness. We have investigated the expression of glycogenin-1 and glycogenin-2 by western blot analysis in liver, heart and muscle from controls and in heart and muscle from patients with biallelic *GYG1*mutations.
Results: Muscle and heart biopsy demonstrated focal intracellular storage of abnormal glycogen with a filamentous structure. There was also accumulation of apparently normal glycogen. Individuals with cardiomyopathy expressed glycogenin-1 with missense mutations whereas patients with pure myopathy in most cases expressed no or very low levels of glycogenin-1 due to truncating *GYG1*mutations. It has been speculated

that glycogenin-2 may be expressed and upregulated to compensate for glycogenin-1 deficiency in muscle cells but our investigations demonstrated that this was not the case.
Discussion: Our results challenge the generally accepted concept that glycogenin is essential for glycogen synthesis. Investigations on the composition of the stored glycogen may help to understand the pathogenesis and be a basis for development of specific treatment.

P-398

Outcome of one year galactose trial in patient with UDP-Galactose Transporter deficiency

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Background: UDP–galactose transporter deficiency is a rare congenital disorder of glycosylation resulting from mutations in the SLC35A2 gene. Clinically it manifests with developmental delay, epilepsy, and brain abnormalities. Currently, there is no causative treatment for this condition. We present results of a 1-year galactose treatment trial in a patient with UDP-galactose transporter deficiency.
Methods / Case Report: The patient is 5 years old, with severe developmental delay, microcephaly, hypomyelination, cerebral and cerebellar atrophy, seizure disorder and dysmorphic features. Metabolic investigations were remarkable for an abnormal transferrin isoform (TIEF) pattern at 1 year of age, which subsequently normalized. Exome sequencing via EPGEN study showed a de novo c.466_468delTCC (p.Ser156del) *SLC35A2* variant.
Galactose trial was initiated at 0.5gr/kg/day, increased to final dose of 1.5 gr /kg/day. Outcome measures were severity of epilepsy and development. Monitoring during the trial included clinical assessments (0, 4, 6, 12 months), abdominal ultrasound (0, 3, 12 months) and biochemical parameters: Gal-1phosphate, urine galactitol, TIEF, hematology profile, liver and kidney function tests.
Results: Seizure monitoring and EEG studies prior to initiation of treatment and at 12 month follow up did not show improvement in seizure control. Developmental assessment revealed overall functioning to be below the 1st percentile at baseline and at 12 months follow up. Biochemical monitoring showed transient increase in urine galactitol.
Discussion: A 5 years old patient with UDP-Gal transporter deficiency was treated with galactose for a period of one year. The treatment was tolerated well and monitoring of biochemical parameters showed a good safety profile. There was no improvement in the study outcome parameters of seizure control and development. Parents and health caregivers reported increased patient's alertness.

P-399

Clinical and long-term outcome data in 96 French patients with PMM2-CDG and review of the literature

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Background: PMM2-CDG is one of Congenital Disorders of Glycosylation (CDG), an inborn error of metabolism with multisystem features.
Methods: Data on clinical, biochemical, molecular parameters and outcome of 96 patients (86 families, 41 males and 55 females) with PMM2-CDG diagnosed in France were examined.
Results: The patients were born between 1963 and 2011. Diagnosis of PMM2-CDG was made at a mean (SD) age of 6.8 (8.5) years. The presenting signs and symptoms were mostly neurological (hypotonia, intellectual disability, cerebellar syndrome). A total of 38 patients (14 males, 24 females) exhibited, in addition to neurological signs, visceral features including at least one of these: feeding difficulty requiring a nutritional support (n=23), cardiac features (n=20; pericarditis: 14, cardiac malformation: 9, cardiomyopathy: 2), hepato-gastro-intestinal features (n=12; chronic diarrhea: 7, exsudative enteropathy: 1, ascitis: 3, liver failure: 1, portal hypertension: 1), kidney (n=4; nephrotic syndrome: 2, tubulopathy: 2), and *hydrops fetalis* (n=1). While the presence of neurological signs was not associated with visceral failures, a trend could be observed for epilepsy. Twelve patients died at a mean age of 3.8 years (especially from pericarditis and other cardiac issues). Biological presentation mostly included elevated transaminases, and abnormal coagulation parameters. Hypothyroidism, hypocholesterolemia, hypoalbuminemia and elevated transaminases were associated with the visceral phenotype. A great genotypic heterogeneity was observed.
Discussion: The clinical phenotype is remarkably heterogeneous in terms of clinical course and outcome, with no formal cleavage between neurological and visceral presentations. Hypotonia and cerebellar ataxia are present in most of the patients.

22. Neurotransmitter and creatine related disorders

P-400

Pregnancy management and outcome in patients with four different tetrahydrobiopterin disorders

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Background: Inborn errors of tetrahydrobiopterin (BH_4) biosynthesis or recycling are a group of very rare neurometabolic diseases. Following growing awareness and improved availability of drug treatment the number of patients with BH_4 disorders reaching adulthood is constantly increasing. Management of neurological symptoms and treatment of female patients with these disorders during pregnancy is one of new challenges.
Methods: This retrospective study summarizes clinical and biochemical monitoring data of 16 pregnancies in 7 women with various BH_4 metabolism disorders for the first time. It also evaluates treatment regimens

before and during pregnancy in relation to the obstetrical outcome and paediatric follow-up.
Results:Worsening of pre-existing neurological symptoms or occurrence of new symptoms during pregnancy was not observed in most of the cases. Treatment regimens remained mostly unchanged. Pregnancies were not complicated by disease-specific features. Organ abnormalities, miscarriage, prematurity, IUGR and chromosomal changes were occasionally reported, without showing any association with the standard drug treatment for BH_4 deficiencies.
Discussion:Our data did not reveal any specific adverse effects of established drug treatment regimens on pregnancy course or obstetrical and paediatric outcomes in 7 out of 16 pregnancies. Patients with BH4 deficiencies should be monitored intensively prior to, throughout and post-partum period by multidisciplinary team, as these longitidunal studies are limited.

P-401

IPSCs as a model to study DNAJC12 deficiency

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Background: Heat shock proteins (HSPs) play a central role in protein homeostasis and folding and exist as 6 major families: HSP40 (DNAJs), HSP60, HSP70, HSP90, HSP100, and small HSPs. DNAJs comprise 3 subfamilies (DNAJA, DNAJB, DNAJC) sharing the J domain, the HSP70 interacting domain. Together, HSP70s and DNAJs constitute the HSPA system. The initial publication on patients with biallelic mutations in *DNAJC12* defined a phenotype consisting of intellectual disability, dystonia and hyperphenylalaninemia, responding to BH_4 and/or neurotransmitter precursor supplementation. Follow-up on patients with unexplained HPA broadened the clinical phenotype with very mild neurological symptoms (autistic features and hyperactivity) and early-onset Parkinsonism. DNAJC12 seems to interact with aromatic amino acid decarboxylases, but the exact pathophysiological mechanisms of *DNAJC12* deficiency resulting in its heterogeneous neurological phenotype remain unclear. To overcome these limitations we have generated hiPSCs from a patient with *DNAJC12* deficiency to study the pathophysiology of the disease.
Methods: Fibroblasts of a patient with *DNAJC12* deficiency have been reprogrammed and characterized for marker gene expression, karyotype, transgene silencing and differentiation potential. Deficient iPSCs have been differentiated to neural progenitor cells (NPCs) and characterized extensively.
Results: *DNAJC12* deficient iPSCs express classical pluripotency marker genes, show transgene silencing, a normal karyotype and differentiate into cells of all three germ layers. Differentiated NPCs express classical marker genes. *DNAJC12* mRNA and protein is barely detectable in the deficient cells.
Discussion: Mutations in *DNAJC12* do not affect reprogramming or undirected differentiation. Although deficient iPSCs are slower in growth compared to healthy wildtype controls, they are a helpful tool to broaden our understanding of DNAJC12 deficiency and its underlying pathophysilogical processes.

P-402

Caffeine inhibits the enzyme guanidinoacetate-methyltransferase (GAMT)

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Background: Caffeine is a competitive antagonist on adenosine receptors, but the knowledge on the mode of action of this widely used psychotropic substance is partial and incomplete. An exploratory analysis based on an interventional trial in which healthy volunteers were analyzed by cerebral MR spectroscopy before and after caffeine administration led to the observation that cerebral guanidinoacetate (GAA) levels slightly increased and cerebral creatine (Cr) levels decreased after ingestion of caffeine. This led to the hypothesis that caffeine inhibits guanidinoacetate methyltransferase (GAMT).
Methods / Case Report: In order to test the aforementioned hypothesis, a confirmatory study with complementary laboratory diagnostics was designed. Healthy volunteers ingested caffeine at a dose of 7.5 mg/kg body weight. Repeated blood plasma samples as well as urine were collected over a period of 5 hours after caffeine ingestion on one day and compared to specimen collected without caffeine exposure on another day. All samples were analyzed for GAA, Cr and creatinine by liquid chromatography-tandem mass spectrometry.
Results: In urine, Cr normalized to creatinine significantly decreased after caffeine ingestion ($p = 0.024$). No significant difference in GAA was observed. In blood plasma, normalized Cr was lower after caffeine administration, but the effect was not significant. No difference in GAA was observed.
Discussion: Caffeine is frequently used to treat apnea of prematurity. As demonstrated here in healthy adult volunteers, caffeine inhibited GAMT and thus endogenous Cr production. RESULTS were more conclusive in urine than in plasma. In a follow-up study, urine may thus be collected from neonates and preterm infants in order to assess their Cr metabolism under caffeine treatment. Caffeine, both as a drug in neonatal care and as a substance widely used in everyday life, may furthermore be harmful in patients with defects in Cr synthesis or transport.

P-403

CRLF1 controls thermoregulation through regulation of central nervous noradrenaline synthesis

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Background: Crisponi syndrome (CS) is a rare autosomal recessive disorder characterized by a variable phenotype with a high lethality during infancy. Characteristic clinical features include hyperthermia, camptodactyly, paroxysmal muscular contractions as well as swallowing difficulties. Surviving CS individuals show a spontaneous improvement of hyperthermia and swallowing difficulties, but develop paradoxical sweating induced by cold temperatures during adolescence associated with elevated plasma noradrenaline (NA) levels. Mutations in the *Cytokine Receptor Like Factor 1* (*CRLF1*) gene, a ligand for the CNTF receptor, can cause CS. However, the pathophysiological role of CRLF1 is poorly understood. In this study, we investigated if CRLF1 regulates thermoregulatory processes in the central nervous system.
Methods / Results: To investigate the localization of Crlf1 during and after embryogenesis, we performed *in situ* hybridization and immunohistochemistry on paraffin embedded mouse embryos and brains of P10 mice. Crlf1 was localized in all neuronal tissues during embryogenesis as well as in neurons of the hippocampus, the cortex and the hypothalamus, the control center of thermoregulatory processes in the brain postnatally at day10. In isolated murine neuronal cells of wild type and Crlf1 deficient embryos, deficiency of Crlf1 was associated with an increased expression of

noradrenergic genes, such as tyrosine hydroxylase and dopamine beta hydroxylase resulting in increased central nervous NA levels.
Discussion: A disturbed thermoregulation is the main clinical feature of CS. Our results implicate that CRLF1 modulates the function of thermosensitive neurons and thermoregulatory processes by regulation of NA synthesis in the central nervous system.

P-404

Functional analysis and therapeutic approach for three new deep intronic variations identified in two PTPS-deficient patients

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Background: The use of splice antisense therapy is highly promising for treatment of a variety of disorders. In PTPS-deficiency this approach may be a potential new treatment.
Methods / Case Report:we report two new PTPS-deficient patients. The study included genetic analysis (massive parallel sequencing and/or *PTS*-specific transcript studies). Minigenes were used for functional splicing analysis. Splice switching antisense oligonucleotides (SSO) directed to the 5' splice site were used to block pseudoexon inclusions in the mRNA.
Results: MPS allowed the detection of only one pathogenic variant in case 1 (c.243+3A>G). RT-PCR analysis in case 2 revealed the presence of a new pseudoexon insertion between exon 1 and 2. Subsequent sequencing of *PTS* intronic regions revealed the presence of three new deep intronic variants: c. 164-672C>T in case 1 and c.83+658C>G and c.83+758T>A (both in maternal allele) in case 2. All three changes are predicted to be pathogenic. c.164-672C>T variant creates a potential 5´ss (75.95 splice score according to HSF), c.83+658C>G creates a potential 3´ss (68.72) and c.83+758T>A activates a potential 5´ss (71.86 to 76.94). None of the three variants were identified in gnomAD. Minigene analysis showed that the variant c.164-672C>T leads to the inclusion of three overlapping intronic cryptic exons of 93, 118 bp and, to a much lesser extent, inclusion of a previously described one of 70 bp. The insertion of the new pseudoexon in intron 1 needs the synergistic effect of both the c.83+658C>G and c.83+758T>A changes. Transfection with SSO resulted in the recovery of normal transcript.
Discussion: Transcript analysis is needed for diagnosis in combination with genomic NGS for deep intronic mutations. Our results demonstrate the need for minigene functional analysis for identification/confirmation of the pathogenic variants. Of note, the PTS gene seems particularly prone to activation of cryptic exons which can be corrected with SSO.

P-405

Correlation between salivary, platelet and CSF serotonin levels in pediatric population

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Background: Serotonin (5HT) is a neurotransmitter synthesized in both the CNS and in enterochromaffin cells of the gut. 5HT biosynthesis is mediated by two separate pathways and is spatially and biochemically segregated between the periphery and the CNS by the blood brain barrier. The mechanism responsible for any observed correlations between centrally and peripherally measured 5HT remains to be elucidated. The gold standard measurement of central 5HT is 5-hydroxyindoleacetic acid in CSF via lumbar puncture. Previous experiments have sought a non-invasive surrogate marker of central 5HT, including 5HT in whole blood, plasma, platelets, saliva and urine. Differences in study design however, yield conflicting results.
Methods: We recruited 26 patients between the ages of 0.5 and 15 years, with various neurologic presentation. Their clinical care included lumbar puncture for CSF collection for neurotransmitters. An additional salivary and blood sample was obtained at the same time. 18 patients had suitable samples, of which quantitative measure of serotonin was performed. We calculated the bivariate correlation between the 5HT levels in CSF, platelets and saliva.
Results: There was no correlation between plasma and CSF serotonin levels (Pearson's coefficient of correlation 0.010) or between salivary and CSF serotonin (0.258). There was strong negative correlation between salivary and platelet serotonin (−0.679).
Discussion: Contrary to previous reports we have not found correlation between salivary and CSF 5HT levels. The source of salivary serotonin is not well understood. Animal studies suggest an autocrine-paracrine action of 5HT in salivary glands, with treatment of 5HT decreasing salivary flow rate. This opposing effect of plasma 5HT on salivary flow rate and subsequently the level of salivary 5HT may explain the negative correlation seen in our study. Our findings suggest that salivary 5HT measurement is not a suitable non-invasive marker for measuring central serotonin turnover.

P-406

Effect of guanidinoacetate on brain creatine uptake in Agat and Gamt mice from birth to 20 weeks

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Background: Creatine (CT) replenishment in brain following CT treatment is more complete in patients with AGAT deficiency compared to those with GAMT deficiency.
Hypothesis: GAA accumulation in GAMT prevents CT uptake in the brain.
Objective: To assess brain CT during development comparing Agat- (as model of low GAA) with Gamt (as model of high GAA) mice on CT-free and CT-containing mouse chow.
Methods: Agat and Gamt mice, 2 WT, 2 HET, and 2 MUT each strain, were fed with regular chow (**CT**$^{+}$, ~0.1% CT) or vegetarian chow (**CT**^{0}, CT-free). At 2, 4, 6, 8, 10, and 20 weeks after birth, following whole body perfusion and brain dissection, CT and GAA concentrations were determined in homogenates via cation exchange chromatography with post-column derivatization and normalized for wet mass of brain tissue.
Results: The data confirm expected brain biochemical phenotypes, in Agat^{MUT} decreased CT and GAA, in Gamt^{MUT} decreased CT but increased GAA. Unlike previous studies using significantly higher CT doses and leading to complete CT replenishment, in our study brain CT levels in both strains fail to normalize on **CT**$^{+}$ chow and remain low throughout the observation period. Starting at six weeks, however, it appears that Agat^{MUT} on **CT**$^{+}$chow have higher CT levels than Gamt^{MUT}. Furthermore, both mutant mice on **CT**^{0} chow consistently have lower CT levels compared to those on **CT**$^{+}$ chow. Of note, Gamt^{WT} mice have elevated brain GAA at 2 weeks (likely effect of maternal Gamt^{HET}) that normalizes afterwards.
Discussion: The higher CT levels in Agat^{MUT} compared to Gamt^{MUT} mice after six weeks of age on a **CT**$^{+}$ diet confirms the hypothesis of

an inhibitory effect of GAA on CT uptake in the brain. Using adult mice to avoid cofounding variation our experimental setting will be applicable for pre-clinical studies assessing the efficacy of GAA lowering drugs on improving brain CT uptake in mice.

P-407

Treatment response in secondary neurotransmitter deficiencies

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Background: Despite numerous reports of secondary CSF neurotransmitter deficiencies in genetic disorders, pathophysiology is still not fully understood. Reports on benefits of treatment with L-dopa/carbidopa and 5-hydroxytryptophan (5HTP) in secondary biogenic amine deficiency are emerging, although still controversial.
Case Reports: We present 11 patients with various neurological presentations and low CSF levels of biogenic amines. They were given a trial of L-dopa and 5HTP. They were enrolled in Whole Exome Sequencing.
Results: Variants classified as pathogenic, likely pathogenic and of uncertain significance were found in 9 patients in the genes: *KCNJ6, SCN2A, CSTB, NRXN1, DLG4, PAK3* and *KIF1A*. No results were found in 3 patients. 10 patients had favorable response to L-dopa, 5HTP treatment, manifested in improvement of seizures, psychiatric and/or movement disorders. One patient had worsening dyskinesia and treatment was discontinued.
Discussion: The complex pathophysiology of secondary neurotransmitter deficiencies is not understood. We propose the following hypotheses: impaired vesicular release because of electrochemical ion gradient imbalance; defective phosphorylation through PKC, preventing activation of rate-limiting enzymes in the biogenic amine synthesis; apoptosis and neuronal death; complex cytoskeleton architectural changes preventing cytoplasmic vesicular transport. Further studies and in vitro experiments are needed for validating these hypotheses, but with making connections between the dynamically interacting mechanisms of chemical neurotransmission, we'll come to better understanding of secondary neurotransmitter deficiencies, which may improve future therapy.

P-408

Autosomal dominant *GCH1* mutations causing spastic paraplegia at disease onset

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Background: Autosomal dominant *GTP cyclohydrolase 1* gene (*GCH1*) mutations are well known to cause dopa-responsive dystonia (DRD) and can also lead to adult-onset parkinsonism. In this case series, we confirm a deviant phenotype, characterized by a pyramidal-predominant presentation at disease onset.
Methods: The patients were referred to our tertiary movement disorder expert center for diagnostic evaluation because of suspected hereditary spastic paraparesis (HSP). *GCH1* mutations were identified by exome sequencing or targeted Sanger sequencing. Clinical trajectories are described.
Results: All 4 patients presented with spastic gait and leg hypertonia. This preceded the emergence of extrapyramidal symptoms (dystonia and/or parkinsonism) by decades in all patients; the youngest patient (now 28 years) has no clear extrapyramidal symptoms to date. All patients had diurnal fluctuations. All patients responded to low-dose levodopa/carbidopa with improvement of gait and of extrapyramidal signs, while leg hypertonia remained present in all.
Discussion: Autosomal dominant *GCH1* mutations may cause a spastic paraplegia phenotype rather than (classic) DRD, with extrapyramidal symptoms developing only after decades. In order not to miss this treatable condition, *GCH1* should be included in HSP gene panels and its testing is pivotal in patients with spastic paraplegia and concomitant extrapyramidal signs and/ or diurnal fluctuation.

P-409

Ascertaining molecular components of the regulatory network controlling creatine biosynthesis

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Background: High extracellular levels of creatine (CT) (10 mM) lead to a more than 2-fold reduction of steady state levels of *AGAT* mRNA in HeLa cells, consistent with previous publications demonstrating CT mediated transcriptional repression in human, mouse and chicken. AGAT is the first and rate-limiting step in endogenous CT synthesis, thus, CT controls its own synthesis via negative feedback loop. We hypothesize that the transcriptional regulation of AGAT is mediated by a network consisting of CT-sensor(s), second messenger(s), and transcription factor(s).
Methods: Mapping and bioinformatic examination of the responsive element (mRE) in the *AGAT* promoter involved in CT mediated transcriptional repression by deletion scanning mutagenesis.
Results: We show that a minimal promoter consisting of 1 kb of DNA upstream of the *AGAT* start codon, driving firefly luciferase activity is subject to CT mediated transcriptional repression in HeLa and HEK293. Deletion scanning mutagenesis suggest the mRE is ~8-96 nucleotides upstream of the transcription start site. We have identified a number of potential transcription factor (including MyoD1, TCF3, MAZ and NFYB1) binding sites based on high quality ChIP-seq. We have also identified ClinVar SNPs and eQTLs that could affect *AGAT* expression and map in the vicinity of the mRE. Additional ChIP-seq suggests that TCF12 a basic-loop Helix transcription factor that recognizes the E-box motif binds to this region of the *AGAT* promoter.
Discussion: High resolution mapping and mutagenesis with CRISPR-mediated genome editing of the mRE in the *AGAT* genomic locus together with our expertise in ChIP-PCR will be used to evaluate the role of these transcription factors and polymorphisms in CT mediated transcriptional repression.

23. Disorders of vitamins, cofactors and trace elements

P-410

Treatment of low CSF 5-methyltetrahydrofolate with leucovorin improves seizure control and development in PCDH19-related epilepsy

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Background: PCDH19-related epilepsy is a complex syndrome characterized by medically intractable seizures, intellectual disability and behavioral dysregulation. Seizures may be fever-related and occur in clusters. This condition is X-linked but primarily affects females.
Case Report: A six year old girl presented for assessment of intractable seizures, developmental delay and behavioral concerns. Since developing clusters of seizures at age 2, the patient had tried multiple medications and was on oxcarbamazepine, topiramate, valproic acid and lacosamide at the time of her assessment. Global developmental delay was noted. Her family history was significant with a 10 month old sister with seizures but normal development. A comprehensive neurometabolic evaluation was performed including a lumbar puncture for 5-methyltetrahydrofolate, B6 metabolites, neurotransmitters, glucose, amino acids and lactate. An epilepsy gene panel was also sent.
Results: Neurometabolic evaluation was normal with the exception of the cerebrospinal 5-methyltetrahydrofolate which was 37 (normal 40–128). Leucovorin 25 mg daily was started for one week and then increased to 25 mg twice daily (2 mg/kg/day). In addition, the epilepsy panel found a pathogenic mutation in PCDH19 (c.1502_1509delCTGTCTTCinsG; p.Pro501ArgfsTer66). Her sister shared this mutation. She was also started on leucovorin. Parental testing could not determine parent of origin suggesting paternal gonadal mosaicism. At the most recent visit, at age 8 years 8 months, the patient had been seizure-free for almost 2 years and had improved development. Her sister was seizure-free with normal development.
Discussion: Treatment of low cerebrospinal fluid 5-methyltetrahydrofolate with leucovorin improves seizure control and development in PCDH19-related epilepsy.

P-411

A new case from Turkey with homozygous *C19ORF12* mutation

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Background: Neurodegeneration with brain iron accumulation (NBIA) comprises a group of disorders with a progressive extrapyramidal syndrome and excessive iron deposition in the brain, particularly globus pallidus and substantia nigra. NBIA is considered to be a rare disease group, with a prevalence of less than 1/1,000,000 in the general population. Ten genes were associated with different NBIA subtypes. Only two of these genes (FTL and CP) encode proteins that play a direct role in iron metabolism, while the remaining eight (PANK2,PLA2G6, C19orf12, WDR45, FA2H, ATP13A2, DCAF17 and COASY) encode proteins involved in lipid metabolism, mitochondrial function, coenzyme A (CoA) metabolism, and autophagy. The most common forms of NBIA are pantothenate kinase-associated neurodegeneration (PKAN), PLA2G6-associated neurodegeneration (PLAN), and beta propeller protein-associated neurodegeneration (BPAN). Mitochondrial membrane protein-associated neurodegeneration (MPAN) occurs less frequently. It is inherited in an autosomal recessive fashion. MPAN is caused by mutations in *C19ORF12*, which encodes a protein of the mitochondrial membrane.
Case Report:Herein we present a patient with progressive, early-onset optic atrophy, spastic ataxia, and cognitive decline after normal development during infancy,undiagnosed for 19 years until detailed genetic analysis revealed mutations in *C19orf12*.
Results:Whole exome studies identified a homozygous 11 base pair deletion, c.171_181delCGGGGGGCTGT, predicted to cause a frameshift and premature termination of the C19orf12 protein (p.Gly58Argfs*10).The segregation analysis of the parents showed that they were both heterozygous.
Discussion:While clinical diagnosis of NBIA is a challenge, analysis of both clinical findings, including age at onset, family history, and presence of optic atrophy, and characteristic imaging abnormalities, allows accurate diagnosis of most of the NBIA subtypes and guides the genetic testing.

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A case of hypomagnesemia with secondary hypocalcemia caused by *TRPM6* gene mutation

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Background:Hereditary hypomagnesemia with secondary hypocalcemia (HSH) is a rare autosomal recessive disease caused by mutations in the transient receptor potential melastatin 6 (TRPM6) gene. Affected individuals present in early infancy with seizures caused by the severe hypocalcemia and hypomagnesemia. By presenting this case report, we also aimed to highlight the need for molecular genetic analysis in familial cases with hypomagnesemia.
Case Report: An offspring of marriage between two first cousins presented with seizures developed on the 40(th) day of life. The physical examination of the case was normal. In laboratory results, Ca(+2) level was 5.7 mg/dl, Mg(+2): 0.48 mg/dl (1,3-2,1), PTH: 35.8 pg/ml (12–92), and P-: 4.5 mg/dl. The case was diagnosed as hypomagnesemia with secondary hypocalcemia (HSH) and TRPM6 gene mutation analysis revealed a homozygous mutation of c.3178A>T(p.Ile1060Phe).
Results:Intravenous Mg2+ sulfate was administered, and he was discharged with a treatment schedule of oral magnesium (elemental magnesium oxide 40 mg/kg/day) and calcium gluconate. Calcium therapy was stopped when the normal calcium levels were achieved. During his follow-up, he showed an age-appropriate physical and neurological development
Discussion:Accurate genetic diagnosis is crucial for estimating the prognosis, detecting complications in organs other than the kidneys, and for directing genetic counseling. The developed flowchart for identifying responsible genes for hypomagnesemia was useful for diagnosing inherited hypomagnesemia.

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Menkes disease- a single centre case series: the clinical disease spectrum

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Background: Menkes Disease (MD) is an X-linked, neurodegenerative disorder of copper metabolism caused by alterations in the *ATP7A* gene.

We present a single centre case series of patients with MD reviewing their symptoms, complications, treatment and outcomes.
Methods: Clinical, laboratory, radiological and outcome data for all children with MD were collected from hospital database from 2012–2018.
Results: Nine patients (Eight male) from six families. Median age at diagnosis was five months (birth-17months). All patients demonstrated hair/skin changes, hypotonia, and developmental delay. Seven had failure to thrive, and five had recurrent chest infections (two developed oxygen requirement). Five patients developed seizures. Diagnosis was confirmed in all with genetic analysis.
Seven patients commenced copper therapy (median age three months (Day 2–22 months)). Daily dosage of copper ranged from 150- 800micrograms. Pre-treatment median copper and caeruloplasmin levels were 2umol/L (range 0.61-12umol/L) and 0.06g/L (range 0.03-0.21g/L) respectively. Post-treatment showed a mean increase in serum copper of 7.8umol/L. Three patients had poor compliance and one experienced minor irritation at injection site. Two patients receiving early (< 10days) copper treatment did not develop any seizures. Whilst three patients who received copper post-seizure onset showed no improvements in seizure activity. Remaining two patients (one female) commenced copper at 22 months and remained seizure-free. Connective tissue complications were observed in five patients (two hernias, one with bladder diverticulum, two with renal hydronephrosis).
Average follow-up duration 26months. Four patients have died.
Discussion: Despite limited efficacy of copper therapy the majority of our patients received treatment. Copper therapy was well tolerated but did not improve seizure activity in those who developed seizures prior to commencing copper. Mortality remains high.

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Plasma Coenzyme Q10 status is impaired in selected genetic conditions.

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Background: The identification of diseases displaying chronic plasma Coenzyme Q10 (CoQ) deficiency may be important to prevent deleterious consequences of this antioxidant impaired status. The aim of this study was to retrospectively evaluate plasma CoQ concentrations in a large cohort of paediatric-young adult patients.
Methods / Case Report: We evaluated plasma CoQ values in 597 individuals (age range 1 month - 43 years, average 11.0 years), studied during the period 2005–2016. Patients were classified into 6 different groups; control group of healthy participants, phenylketonuric (PKU), patients with mucopolysaccharidoses (MPS), patients with other inborn errors of metabolism (IEM), patients with neurogenetic diseases and individuals with neurological diseases with no genetic diagnosis. Plasma total CoQ was measured by reverse-phase HPLC with electrochemical detection and UV detection at 275 nm.
Results: Anova with Bonferroni correction showed that plasma CoQ values were significantly lower in the PKU and MPS groups when compared with controls and neurological patients. IEM group showed intermediate values but were not significantly different when compared with the controls. In PKU patients, Chi-Square test showed a significant association between the fact of having a CoQ deficiency and the fact of being classic PKU patients. The percentage of neurogenetic and other neurological patients with low CoQ values was low (below 8%).
Discussion: Plasma CoQ monitoring in selected groups of patients with different IEM (especially in PKU and MPS patients, but also in IEM under protein restricted diets) seems advisable to prevent the possibility of a chronic blood CoQ deficiency in such groups of patients.

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CSF-5MTHF levels in patients with recurrent seizures

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Background: Many IEM are known to have low CSF 5MTHF levels. Some of these disorders present with recurrent seizures.
Methods / Case Report: We studied 106 patients with recurrent seizures, with normal levels of ammonia, blood sugar, ketones, keto-acids and ABG. Disorders under these criteria are: B6/PLP/folinic acid dependencies, Glycine encephalopathy, Pterins Defects (GTPCH deficiency, Dihydropteridine reductase deficiency, Phenylketonuria, Tetrahydrobiopterin biosynthesis defect), Serine Synthesis Defect, Aspargine Synthetase Deficiency, SO/XO, Peroxisomal, GLUT1 deficiency. Biochemical diagnosis - qualitative and quantitative analysis were done by UPLC, GCMS, LC-MS-MS.
Results: Out of 106 patients screened for CSF-5MTHF, 74 were deficient (69.81%). Deficiencies were divided in 3 groups depending on the severity: Severe (1-20nmol/l) - 28/74 (37.84%), moderate (21-40nmol/l) – 29/74 (39.19%) and mild (41–60 nmol/l) – 17/74 (22.97%). Severe 5MTHF Deficiency was seen in 22/57(38.59%) – pyridoxine / pyridoxal phosphate / folinic acid dependency, 2/17 (11.76%) glycine encephalopathies, 1/11(9.09%) – pterins metabolism defects had severe, 1/9(11.11%) – serine synthesis defect, 1/3(33.33%) – asparagine synthesis defect, 1/4(25%) – peroxisomal defects. Normal 5 MTHF were observed in 18/57 (31.58%) pyridoxine / pyridoxal phosphate / folinic acid dependency, 9/17 (52.94%) Glycine encephalopathy, 3/11 (27.27%) Pterin metabolism defects, 1/4 (25%) SO/XO Deficiency, 1 Glut1 deficiency. Rest of the patients had mild to moderately low 5 MTHF.
Discussion: On studying 5MTHF levels in patients with recurrent seizures, Pyridoxine / pyridoxal phosphate / folinic acid dependency was the prominent group with 38.59% of patients having severe deficiency. Folinic acid is available and shows good result. One patient with serine synthesis defect was had moderate deficiency (46.18 nmol/l) responded to Folinic acid supplementation and normalized to (80.43 nmol/l) with good clinical improvement.

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Ataxia and developmental delay as presenting phenotype of *COQ4* defect

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Background: Homozygous or compound heterozygous mutations in *COQ4* cause primary CoQ10 deficiency, a treatable respiratory chain defect that leads to a severe mitochondrial encephalomyopathy. We report a new clinical presentation of the disorder.
Case Report: Patient (Pt) 1, a 30-month-old child presented by the age of 22 months with development delay. At our observation, generalized hypotonia, sialorrhea, ataxia with postural instability and dysmetria were evident. Brain MRI at age of 22 months was normal. Heart ultrasound showed initial parietal thicknesses of the left ventricle. EEG showed focal paroxysmal focal anomalies without clinical manifestations. Pt 2, a 19-year-old girl with mild-to-moderate cognitive impairment presented since age 5 with a slightly progressive neurological disorder characterized by dysmetria, head ballottement, gait ataxia and, over the following three years, with spastic paraparesis. At the age of 18 years, she presented tonic-clonic fits. Brain and spine MRI at age 15 showed a discrete enlargement of pericerebellar sulci but no focal abnormalities in supratentorial structures. Skeletal muscle biopsy showed mild defects of oxidative metabolism.
Results: Using a targeted multigene sequence panel covering the coding exons of over 200 genes associated with inherited ataxias and related disorders, we identified compound heterozygous mutations in *COQ4* (NM_016035): c.577C>T/p.Pro193Ser and c.718C>T/p.Arg240Cys in Pt1; c.284G>A/p.Gly95Asp and c.305G>A/p.Arg102His in Pt2.
Discussion: Mutations in *COQ4* usually result in a severe neonatal presentation with early fatal outcome and characterized by encephalopathy or cardiomyopathy and lactic acidosis, or both. These two new cases we reported expand the phenotype associated with *COQ4* defects showing a later onset characterized by cerebellar dysfunction and developmental delay. We suggest to consider mutations in early ataxia and developmental disorders.

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PROSC and vitamin-B6-dependent epilepsy

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Background: Vitamin-B_6-dependent epilepsies are treatable disorders involving a number of genes including *PROSC,* encoding a pyridoxal 5'-phosphate (PLP) binding protein, important for intracellular PLP regulation.
Case Reports: Case 1: A female infant born to consanguineous Indian parents (head circumference and BW ^{2nd} centile) developed refractory seizures on day 1 of life. Lactate was elevated. Investigations included raised urinary vanillyllactate and plasma 3-O-methyldopa (3OMD 1030nmol/L). MRI brain showed simplified gyral pattern, lack of myelination and diffuse white matter oedema. On day 8, IV pyridoxine 50mg/kg/day was given resulting in hypotonia, respiratory depression, decreased seizures and normal lactate. CSF showed raised threonine (116umol/L) with low PLP (< 4nmol/l). Substituting PLP 35mg/kg/day further improved seizures. *PROSC* genotyping: homozygous c.524T>C, p.L175P mutation.
Case 2: A male child born to consanguineous Pakistani parents (HC 9th, BW 3rd centile) had refractory seizures within minutes of birth with raised lactate and acidosis. Urinary vanillyllactate and plasma 3OMD (3310nmol/L) were raised. He was treated with PLP 20mg/kg/day (day 3) with resultant reduced respiratory drive, hypotonia and resolution of seizures and lactate. MRI brain showed prominent ventricles, wide extracerebral spaces and thin corpus callosum. *PROSC* genotyping: homozygous c.676T>C; p.S226P mutation.
Results: Patient 1 is now 6 years old with global delay and autism. Seizures are well controlled with PLP and 2 antiepileptic drugs. Patient 2 is 6 months old with age-appropriate development and good seizure control on PLP and Levetiracetam. In both cases, plasma 3OMD normalised with treatment.
Discussion: Early onset seizures with lactic acidosis and raised urinary vanillyllactate should prompt consideration of a vitamin B_6-dependent epilepsy. Under development of white matter may suggest a PROSC mutation. Plasma 3OMD is a marker of treatment effect.

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Accidental diagnosis of Molybdenum cofactor deficiency type A in a neonate and cPMP rescue prior to irreversible brain injury

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Background: Molybdenum cofactor deficiency (MoCD) may be suspected if a newborn presents with a combination of encephalopathy, seizures and dystonia. The diagnosis needs to be confirmed with specific metabolic investigations and is usually made after significant and irreversible brain injury has already occurred.
Methods / Case Report: A newborn baby manifested with hypoglycaemia and raised lactate at the age of 10h. Routine laboratory work-up identified a depletion of L-Cystine in plasma and measurement of urinary S-sulphocysteine was added and found to be grossly increased. Upon finding a decreased plasma urate, a diagnosis of MoCD was made and substitution with recombinant cyclic Pyranopterin Monophosphate (rcPMP) initated at the age of almost 5 days, before the patient developed seizures or severe encephalopathy.
Results: When rcPMP was started, the child showed moderate irritability and the cranial MRI demonstrated typical signs of sulphite toxicity. We observed a prompt biochemical and clinical response to treatment and the MRI later normalised. The diagnosis of MoCD type A was subsequently confirmed by demonstrating biallelic pathogenic mutations in the *MOCS1* gene, the previously described *MOCS1* c.1027C>T, p.R343X and the novel mutation *MOCS1* c.251-2A>G, which is predicted to abolish an acceptor splice site.
Discussion: The early diagnosis allowed to source recombinant cyclic pyranopterin (cPMP) and initiate treatment at an early stage in the disease process. The case demonstrates the value of careful and systematic evaluation of unexpected laboratory findings. This is the first index patient with MoCD type A who could be rescued without severe brain injury.

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Potentially Treatable Neurodegenerative Disorder

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Background: Manganese (Mn) serves as a co-factor for numerous enzymes in different metabolic pathways. It is absorbed in the intestine and excreted through the biliary system, maintaining very tight homeostasis. High level of Mn is neurotoxic due to the accumulation of Mn in the brain, mainly the basal ganglia. Hypermanganesemia with Dystonia-2 (HMNDYT2) (OMIM 617013), is a rare neurodegenerative disorder. It is due to an abnormality in the Mn pathway as a result of a homozygous mutation in the *SLC39A14* gene. Patients will present in infancy or early childhood with developmental regression and progressive dystonia, leading to loss of independent ambulation. Chelation therapy with disodium calcium edetate (Na2CaEDTA) has shown to improve symptoms and gaining the ability to ambulate.
Case Report: We report a 13- month- old boy with developmental regression and dystonia. He was gaining his milestones appropriately. At 8 months of age, he became irritable with excessive crying. He lost the ability to sit independently. He had dystonia of upper limbs; more on the right side with exaggerated DTR. MRI brain showed T1 hyperintensity in the basal ganglia with T2 and FLAIR hypointensity. His blood Mn level was elevated at 45.6 ug/L (normal < 2.5 ug/L). We started chelation therapy with Na2CaEDTA.
Results: Whole exome sequencing showed a novel homozygous variant c.776_777insAT p.(Glu261Leufs*14) in *SLC39A14*. It is classified as likely pathogenic (class 2) according to the recommendations of ACMG.
Discussion: Here, we report the youngest patient with HMNDYT2 who was started on chelation therapy in literature, as far as we know. This is one of very few treatable neurodegenerative disorders. Our patient presented typically with loss of milestones and dystonia as well as the MRI brain changes. He had a normal level of Zn and Fe with high Mn level. We expect an improvement in his symptoms after several months since the treatment started at an early age.

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Late onset methylmalonic acidemia with homocystinuria (cblC) mimicking a demyelinating disorder.

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Background: cblC is the most frequent intracellular error of cobalamin metabolism. Among the pleomorphic clinical manifestations, spastic paraparesis is emerging as a relatively frequent feature in adolescent/adult patients. We aimed at focusing on this clinical association.
Methods: We describe 2 new CblC patients and reviewed the clinical characteristics of 24 previously reported patients with associated spastic paraparesis.
Results: The age at onset in our 2 subjects was 14 and 10 (current age 17 and 14 years, respectively). Presenting symptoms were spastic paraparesis with cognitive decline in case 1 and ataxia in case 2. They eventually developed a progressive spastic palsy of lower limbs and optic atrophy. Neuroimaging showed in both multifocal areas of brain and medullary alterations leading to the diagnosis of demyelinating disorders (Multiple sclerosis and Devic's syndrome, respectively) and steroid therapy. Sensory-motor peripheral neuropathy was detected in case 2. Case 1 was heterozygous for c.482 G>A variant (MLPA under study) and case 2 homozygous for c.347T>C variant. Combining data with 24 cblC patients from the literature, spasticity was the presenting symptom in 12/26; cognitive decline, peripheral neuropathy, psychiatric symptoms and optic atrophy were detected in about 60% of the patients. C.482G>A variant in exon 4 was found in 15/20 patients.
Discussion: Spastic paraparesis, peripheral neuropathy, cognitive decline, psychiatric symptoms, and optic atrophy seem strictly associated with c.482G>A variant. If recognized, this alteration could optimize clinical and instrumental follow-up of patients detected through NBS. Clinical and neuroimaging findings suggested the diagnosis of demyelinating disease in a number of patients with cblC. To avoid delay in diagnosis and unappropriated treatment, cblC should be a consideration in differential diagnosis of early onset spastic paraparesis and enclosed in genetic panels aimed at the molecular diagnosis of these conditions.

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Improved neurodevelopmental outcome using L-arginine in children with pyridoxine-dependent epilepsy

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Background: Pyridoxine dependent epilepsy (PDE) usually presents in the neonatal period with seizures that are resistant to antiepileptic medications. Introduction of pyridoxine results in both clinical and EEG improvements. Mutations in the *ALDH7A1* gene cause a defect in an enzyme involved in the catabolism of lysine. Whilst pyridoxine improves seizures, poor developmental outcomes are common, probably due to the accumulation of toxic metabolites such as aminoadipic semialdehyde (AASA). Use of dietary lysine restriction with arginine supplements is increasingly common but the contribution of each is unclear. Here we look at the effect of starting arginine supplements for 12 months before dietary restriction.
Case Report: All three children in this study presented in the first 48 hours of life with seizures that responded only to pyridoxine. Mutations in the *ALDH7A1* gene were identified in two of the subjects and is awaited in the third. L-arginine was commenced in all 3 patients in addition to their regular pyridoxine. A cognitive and developmental assessment was performed prior to commencement and again after 12 months on treatment. Urinary AASA was performed pre and post treatment for some of the patients.
Results: Following the introduction of L-arginine, improvements were seen in the cognitive and developmental assessments in all aspects aside from verbal IQ. All patients had a raised AASA level prior to commencement of treatment. Where AASA was repeated after L-arginine introduction, a decrease was observed.
Discussion: L-arginine is thought to compete with lysine for uptake into the brain. Its use in patients with PDE aims to reduce the production of lysine's toxic by-products. It has previously been shown, when used concurrently with a lysine restricted diet, to improve neurodevelopmental outcomes. In this study we saw an improvement in most neurodevelopmental areas for all patients when L-arginine was used.

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Menkes case.Usefulness of biomarkers

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Background: We hereby report a case of a boy who showed a progressive neurologic delay from the 3rd month of life and he was diagnosed of Menkes disease (MD). It is caused by a mutation in the *ATP7A* gene, which codes for the copper-transporting ATPase in the cell organelles, resulting in low concentrations of copper in some tissues and causing multiple effects on the CNS. Copper and ceruloplasmin levels can be low even up to 6 months of age and in these cases other biomarkers can be useful in order to establish the diagnosis certainly.
Methods / Case Report: The male proband was born at term, at 3 months he rejected meals and exhibited abnormal movements with bilateral palpebral clonus. Loss of head control and social smile were observed. EEG showed several temporal focal crisis becoming later multifocal epilepsy that didn't respond to therapy. MRI revealed white matter hyperintensity.
Results: The biochemical findings showed increases of lactate in cerebrospinal fluid (CSF) and blood, alanine in CSF was high. He had low ceruloplasmin (2–16 mg/dL) and copper (< 10 μmol/L). CSF 3-orthomethyldopa was 217 nM (20–162), 3-methoxy-4-hydrophenylglycol (MHPG) 11 nM (30–124), HVA 704nM (354–1328), CSF HVA/MHPG 64. Urine cathecolamine: HVA/ vanillylmandelic acid (VMA) >4.
Discussion: The early diagnosis was made based on clinical and biochemical findings and lead to establishment of copper L-histidinate treatment but it was stopped at 48 hours due to neurological deterioration. In MD dopamine β-hydroxylase (copper-dependent enzyme) is partially inhibited, causing a decrease of the amount of dopamine that is transformed into norepinephrine. Cathecolamine and their metabolites concentrations in CSF, plasma and urine tend to be abnormal at all ages and should be taken into account as more reliable biomarkers for MD. There are few studies about urine HVA/VMA and hardly any of them about CSF HVA/MHPG. We propose more investigations in this field and the inclusion of these ratios in the screening of MD.

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Menkes syndrome, an inherited copper transport defect in an extended family.

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Background: Menkes disease (MD) is an X-linked recessive disorder where, due to deficiency of ceruloplasmin, copper level is deficient in brain and liver. This disease results from defective functioning of copper transport P-type ATPase, ATP7A, which encodes a transmembrane protein transporting copper ions across the cell membranes. Clinical signs of MD usually present at the age of 2–3 months as developmental delay, seizures and hair changes as *pili torti.* A positive effect with copper histidine subcutaneous injections have been achieved in some patients.
Methods / Case Report: To present a variable clinical presentation of MD in a large pedigree.
Results: **Patient 1** was first seen by a geneticist at the age of two years two months because of peculiar hair texture. His development was age-appropriate and as first-line analysis revealed normal results nonsyndromic *pili torti* was diagnosed in patient. His brother, **patient 2**, was referred to a geneticist at the age of two years for moderate developmental delay, ataxia, *pili torti* and complicated pedigree. By this time his older brother was 6 years old and mild mental retardation was diagnosed in him too. Biochemical analysis of both patients revealed low serum copper and ceruloplasmin concentrarion in blood and subsequent molecular analysis of *ATP7A* gene revealed hemizygous mutation c.3097G>A. Pedigree was complicated: five female heterozygous carriers in three generations were detected and at least four affected men with the same disorder have been identified among relatives.
Discussion: **Conclusions.** Isolated *pili torti* can be the first sign of MD therefore measurment of copper and ceruloplasmin in serum of those patients is indicated. Extended pedigree analysis is necessary in X-linked disorders causing progressive mental deteoration for appropriate genetic counselling of at-risk individuals.

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New insights in the prevalence of molybdenum cofactor deficiency from the Exome Aggregation Consortium

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Background: Molybdenum cofactor deficiency (MoCD) is a rare, recessive inborn error of metabolism characterized by severe, fast progressing neurodegeneration and intractable seizures usually resulting in early childhood death when untreated. The first case of MoCD was described four decades ago (Duran et al. 1978) and since then major progress has been achieved in both developing an effective treatment for type A MoCD and understanding the underlying disease mechanism. Nevertheless, certain central aspects of this disease including its prevalence still remain elusive. In this study we address the disease incidence of the treatable type A MoCD deriving from mutations in *MOCS1* utilizing biochemical and bioinformatical methodology.
Methods: Exomes of over 60000 unrelated individuals deposited in the Exome Aggregation Consortium were analyzed for *MOCS1* mutations yielding over 50% of all so far described MoCD type A causing missense-mutations, all heterozygous. Overall, more than 280 missense-mutations could be identified (over 20% of sequenced alleles), most of which are of yet unknown effect. To address a possible role of the novel *MOCS1*-mutations in MoCD, their probability to cause MoCD was estimated *in silico*.
Results: Subsequent biochemical analysis of 50 mutations including positive and negative control mutations to probe the prediction did not validate the disease probabilities, at first. Therefore the obtained biochemical data was utilized as a training set, creating a second prediction, which was again probed biochemically by analyzing an additional set of 15 mutations. Since the biochemical data of the second set of mutations were in line with the bioinformatical prediction, we next validated the prediction against an additional database.
Discussion: Finally we were able to estimate the MoCD type A incidence based on identified loss of function-mutations, published MoCD causing missense-mutations and novel MoCD causing miss-sense mutations found in this study.
Conflict of Interest declared.

P-425

Assessment of ascorbate (vitamin C) in cerebrospinal fluid (CSF) and its application to inborn errors of metabolism.

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Background: Ascorbate is a key antioxidant and has a crucial role in protecting against oxidative damage. Ascorbate is also a co-factor for enzymes, including dopamine β-hydroxylase (DBH), which converts dopamine to noradrenaline.
Methods / Case Report: A validated method for the assessment of CSF ascorbate by HPLC with electrochemical detection was developed.
Results: Ascorbate was measured in 159 diseased control CSF samples and a reference range established (118–246 μmol/L). To highlight the possible usefulness of CSF ascorbate measurement, three patients with low CSF ascorbate were followed up in more detail. Patient 1 mitochondrial complex IV deficiency. Ascorbate, 17μmol/L. Patient 2 MPSIIIa. Ascorbate,102μmol/L. This in association with low CSF pyridoxal phosphate and increased dopamine and serotonin turnover. Patient 3 Superficial Siderosis. Ascorbate, 26μmol/L. This in association with increased dopamine turnover.
Discussion: The patients described above illustrate the possible usefulness of measuring CSF ascorbate in patients with suspected or confirmed inborn errors of metabolism. Oxidative stress is implicated in the pathology of mitochondrial and lysosomal storage disorders and this is corroborated by the low CSF ascorbate observed in patients 1 and 2. Ascorbate is also a co-factor for DBH and low levels of ascorbate may be expected to reduce the rate of DBH catalyzed conversion of dopamine to noradrenaline. A relationship between CSF ascorbate and reduced noradrenaline synthesis/increased dopamine catabolism is suggested by the elevated dopamine turnover observed in patients 2 and 3. Measurement of ascorbate in CSF will be useful to help identify possible inborn errors in ascorbate transport and metabolism and will also be a useful marker of co-factor status and oxidative stress, which has been implicated as a key disease causing mechanism in a wide range of diseases. Supplementation with ascorbate in such cases may also be a treatment option.

P-426

Neurodegeneration with brain iron accumulation (NBIA) spectrum – a fifteen-year experience from a genetic centre in India.

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Background: The unique group of NBIA disorders is caused by mutations in at least 10 genes known so far, and present with characteristic features of progressive dystonia, spasticity, parkinsonism, neuropsychiatric abnormalities, and optic atrophy or retinal degeneration. Most of these disorders have identifiable neuroimaging features, like the 'eye of the tiger sign' for Pantothenate kinase-associated neurodegeneration (PKAN), and cerebellar atrophy for PLA2G6-related neurodegeneration (PLAN), and hypointensities in globus pallidi.
Methods: Clinical and molecular study data was collected from all patients diagnosed with NBIA, attending genetic clinic, since year 2003 till date. Case records, MRI and genetic analysis reports were reviewed.
Results: Of 46 patients with NBIA, 32 were diagnosed with PKAN, 12 with PLAN, and one each with *FA2H*-gene related neurodegeneration (FAHN) and Woodhouse-Sakati Syndrome (WSS). Mean ages of onset / diagnosis were 4.4 /8.3 years for PKAN, and 2.7 / 5 years for PLAN. Patients with FAHN and WSS were 8.5 years and 22 years at diagnosis. All patients showed neurological symptoms – motor regression, or spasticity/ataxia or dystonia. PLAN patients had optic atrophy and WSS patient had multi-systemic involvement. Eighteen PKAN patients belonged to a single community of Agarwals. Mutations in *PANK2* gene revealed homozygous mutation, c.545_546insA, in all 16 of 18 patients tested from Agarwal community. None of the non-Agarwal patients showed c.545_546insA mutation. In *PLA2G6* gene, both truncating and missense mutations were noted with no common or repetitive mutation. One patient each showed homozygous mutation in *FA2H* and *DCAF17* gene. Prenatal diagnosis was performed in fourteen families in the group.
Discussion: Molecular analysis is useful for confirmation of type of NBIA and can subsequently be used for carrier or prenatal testing in other family members. Presence of a common mutation in PKAN simplifies the diagnostic algorithm.

P-427

Sagittal sinus thrombosis and developmental regression in one of twin patients with CblG deficiency, and two-year follow up

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Background: Cobalamin G deficiency (cblG) is a rare inborn error of cobalamin metabolism resulting from mutations in the *MTR* gene. We report the more severely affected of twin infants with cblG deficiency presenting with sagittal sinus thrombosis and a two-year follow up.
Methods / Case Report: The twins were born at 35 weeks gestation. Twin A presented at the age of 11 months with failure to thrive; developmental regression with loss of motor, language and social cues; hypotonia and dyskinetic movements; marked pancytopenia, and macrocytosis. Brain MRI showed diffuse cerebral and mild cerebellar atrophy, delayed myelination, and sagittal sinus thrombosis with collateral formation. Twin B manifested mild developmental delay, hypotonia, and mild macrocytic anemia. Brain MRI showed mild delayed myelination and abnormal deep white matter with T2 hyperintensities.
Results: Metabolic investigations in twin A revealed low-normal plasma methionine 5 μM, elevated plasma homocysteine 79 μM, and low serum B12 78 pM. Twin B had normal plasma methionine 57 μM, but abnormal homocysteine and B12. Molecular testing in both twins revealed heterozygous variants in the *MTR*: c.1771C>T (known pathogenic mutation) and a VUS c.2738A>T. Treatment with betaine, hydroxycobalamin, and folinic acid was initiated in both twins. A follow up brain MRI at 3 years of age for twin A showed improved brain appearance with decreased atrophy and multiple foci of signal abnormality present in the subcortical and posterior periventricular white matter. Clinically, both twins showed good growth; moderate language delay is present in twin A.
Discussion: To our knowledge this is the 2nd reported case with cblG deficiency and sagittal sinus thrombosis. The patients showed reversal of the neurological findings and significant brain MRI improvement in the more affected twin. The clinical improvement was maintained upon switch to cyanocolbalamin with only minimal increase in homocysteine.

P-428

Mitochondrial thiamine pyrophosphate carrier deficiency: a treatable disease

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Background: The metabolism involving thiamine as cofactor is crucial for cellular functions, especially in neural tissue which requires a high level of oxidative phosphorylation. Thiamine deficiency can lead to fatal outcomes without therapeutic intervention. Genetic defects involve cell membrane transporters encoded by *SLC19A3*, *SLC19A2,* and mitochondrial carrier encoded by *SLC25A19*. Regarding *SCL25A19*, two phenotype-related diseases have been described. Amish lethal congenital microcephaly results in severe encephalopathy with CNS malformations and microcephaly. The milder type is characterised by bilateral striatal damage with a progressive neuropathy.
Methods / Case Report: A seven year-old Turkish boy born to consanguineous healthy parents presented with sudden behavioral changes, visual hallucinations and bilateral extrapyramidal symptoms. Cerebral MRI revealed bilateral Gadolinium-enhanced and T2 weighted hyper-intense lesions of caudate and lenticular nuclei. Treatment with high dose thiamine, biotine and L-Carnitine led to complete resolution within ten days. He did not suffer any relapse during the following years under long term vitamin supplementation.
Results: Blood and CSF lactate were increased up to 5.1 and 2.5 mM respectively. Metabolic screening including amino and organic acids was not specific. Exome sequencing revealed bi-allelic *SLC25A19* variants: c.[240A>C], p.(Lys80Asn). The younger brother, who complained since early childhood about painful legs, was further identified with the same genotype and both parents were heterozygous carriers.
Discussion: We describe a new mutation in *SLC25A19* revealed by a recognizable clinical phenotype including encephalopathy and psychiatric features. Symptoms may occur before appearance of typical neuroradiological signs and are amenable to successful treatment by high dose thiamine.

P-429

Low function of natural killer cells in Menkes disease

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Background: Menkes disease (MD) is an X-linked, multisystem lethal disorder of copper metabolism resulting from mutations in the ATP7A gene. Features such as Ehler Danlos syndrome, trichopoliodystrophy, urologic and skeletal changes have been reported. We present a case of classic MD treated with copper infusions who suffered from natural killer (NK) cell dysfunction.
Methods / Case Report: NK cytotoxic assay was performed using the TopCount scintillation Counter to assess the amount of Chromium released by K562 cells when patient NK cell were added to a preparation of K562 cells./ A 2-year-old, Caucasian male child presented at 8-month-old of age with persistent hypotonia, kinky hair and developmental regression. Diagnosis of MD was based on low serum levels of copper {5 mg/dL (18–37)} and ceruloplasmin {18 ug/dL (75–153)} and gene sequencing studies revealing exon 12 deletion in *ATP7A* gene. Brain MRI showed mild hypoplasia of the cerebellar vermis and vascular tortuosity typical of MD. Copper chloride treatment was immediately initiated. Child became more alert with excellent eye contact and purposeful movements. The child was hospitalized for recurrent respiratory infections, each time caused by enterovirus as confirmed by bio fire. In addition he was also admitted for multiple episodes of fever of unknown origin.
Extensive immunologic studies were negative.
Results: Lysis of the K562 targets by patient's NK cells was markedly reduced on multiple occasions (0.6 NK lytic Units; N >2.6).
Discussion: We postulate that the reduced cytotoxicity of the NK cells of our patient with classic MD is due to a deficient incorporation of copper in endoplasmic reticulum leading to an abnormal Fenton chemistry within phagosomes.

24. Miscellaneous / New Disease Group

P-430

Old roads, new connections: glutathionuria caused by a large homozygous intragenic deletion in *GGT1*

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Background: γ-glutamyl transpeptidase (γ-GT) deficiency (glutathionuria, OMIM #231950) is a rare disease first described in 1975 but with only 6 patients reported in the literature. All except one presented mild mental retardation as the predominant clinical feature. The biochemical hallmarks of this condition are high concentration of glutathione in urine and serum and low γ-GT activity in plasma. So far, no genetic defect has been coupled to this condition.
Case Report: We report two siblings, a 10 year old male and a 25 year old female, that were referred to our Department for investigation because of mild psychomotor developmental delay and mild neurological symptoms.
Results: Analysis of amino acids in the urine from both siblings showed a markedly increased excretion of glutathione. A very low γ-GT activity in serum supported the diagnosis of glutathionuria. Whole Genome Sequencing (WGS) on an *in silico* panel comprising all expressed genes of the human γ-GT gene family (*GGT1, GGT2, GGT5, GGT6, GGT7, GGTLC1, GGTLC2* and *GGTLC3*) revealed the presence of a 16.9 kb homozygous deletion in *GGT1* together with a 13bp insertion at the deletion junction.
Discussion: This is the first report of a genetic variant causing glutathionuria. In addition, genetic characterization of the patients' parents and a healthy sibling has provided direct genetic evidence regarding the autosomal recessive nature of this condition.
The difficulty to identify glutathione with conventional amino acid analysis based on cation-exchange chromatography, the presence of multiple genes sharing homology with *GGT1* and a diffuse clinical picture may have hindered the diagnosis of this disease.
Careful interpretation of amino acid profiles, or implementation of amino acid analysis by mass spectrometry, inclusion of serum γ-GT activity in the screening of metabolic diseases and the use of WGS in routine genetic investigation will increase the chances of identifying patients with glutathionuria.

P-431

Tetrahydrobiopterin improves hippocampal nitric oxide-linked long-term memory

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Background: Tetrahydrobiopterin (BH4) is synthesized by the combined action of three metabolic pathways, namely *de novo* synthesis, recycling, and salvage pathways. The best-known function of BH4 is its mandatory action as a natural cofactor of the aromatic amino acid hydroxylases and nitric oxide synthases. Thus, BH4 is essential for the synthesis of nitric oxide, a retrograde neurotransmitter involved in learning and memory. METHODS: We investigated the effect of BH4 (4–4000 pmol) intracerebroventricular administration on aversive memory, and on BH4 metabolism in the hippocampus of rodents. Memory-related behaviors were assessed in Swiss and C57BL/6J mice, and in Wistar rats. **RESULTS:** It was consistently observed across all rodent species that BH4 facilitates aversive memory acquisition and consolidation by increasing the latency to step-down in the inhibitory avoidance task. This effect was associated with a reduced threshold to generate hippocampal long-term potentiation process. In addition, two inhibitors of memory formation (N(ω)-nitro-L-arginine methyl ester - L-Name – and dizocilpine - MK-801 -) blocked the enhanced effect of BH4 on memory, while the amnesic effect was not rescue by the co-administration of BH4 or a cGMP analog (8-Br-cGMP). Discussion: The data strongly suggest that BH4 enhances aversive memory by activating the glutamatergic neurotransmission and the retrograde activity of NO. It was also demonstrated that BH2 can be converted into BH4 by activating the BH4 salvage pathway under physiological conditions in the hippocampus.This is the first evidence showing that BH4 enhances aversive memory and that the BH4 salvage pathway is active in the hippocampus.

P-432

Characterization of acylpeptide hydrolase sequence variants – potential implications for valproic acid efficacy and Alzheimer disease

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Background: Acylpeptide hydrolase (APEH) catalyzes the hydrolysis of *N*-acylated peptides, which results in the formation of an acyl-amino acid and a peptide with a free *N*-terminus. Apart from its role in amino acid recycling, APEH was shown to exert hydrolytic functions in amyloid-β degradation and valproic acid (VPA) metabolism. APEH hydrolyzes VPA-glucuronide (VPA-G) allowing reabsorption of VPA. Recently, an *APEH* sequence variant was identified which results in increased VPA elimination by reducing VPA-G hydrolysis rate thus leading to alleviated therapeutic VPA effects (Wen et al. Pharmacogenomics 2016; 17: 1219–25). We assume that other *APEH* polymorphisms may have similar effects and search for additional *APEH* variants with potential implications for VPA and/or amyloid-β metabolism.
Methods: Novel *APEH* variations were selected based on minor allele frequency and pathogenicity predictions. Point mutations were introduced into *APEH* by site-directed mutagenesis, confirmed by sequencing and APEH variants were overexpressed in HEK293 cells. Protein expression was analyzed by immunoblotting and APEH function was assessed by performing an APEH activity assay.
Results: Immunoblotting revealed that all APEH variants and wild type APEH were efficiently expressed to similar extents. Overexpression of an APEH variant known to affect VPA metabolism resulted in reduced APEH activity. Interestingly, two novel APEH variants predicted to be pathogenic also displayed significantly decreased enzyme activity levels.
Discussion: Here, we have characterized several *APEH* variants and have identified two novel sequence variants which result in reduced APEH activity. We suggest that these mutations will affect APEH function in VPA metabolism or amyloid-β degradation.

P-433

Characterizing the molecular architecture of mitochondrial energy metabolism

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Background: Fatty acid oxidation (FAO) and electron transfer chain (ETC.) co-exist in mitochondria. Trifunctional protein-generated NADH and ETFDH-generated QH_2, which are products of FAO, are known to transfer to the ETC' complex I and complex III, respectively. However, the means by which allow these products to transfer safely and effectively to their destination are uncertain.
Methods: The main methods used in this study include: blue native gel electrophoresis (BNGE); second-dimensional electrophoresis; sucrose gradient centrifugation; western blotting, cross-linking; co-immunoprecipitation; mass spectrometry; immunogold electron microscopy; stimulated emission depletion microscopy.
Results: This study reports the following non-inclusive evidence:
1. BNGE, 2D electrophoresis, and western blotting showed that the FAO enzymes, TFP and ETFDH, co-migrated with ETC. supercomplexes;
2. Cross-linking and co-immunoprecipitation demonstrated that TFP interacted with the NADH-binding domain of complex I of the ETC. It also showed that ETFDH of FAO interactions with the core II of complex III of the ETC.;
3. Immunogold labeling electron microscopy visualized TFP being located on complex I where the NADH-binding domain can be found. ETFDH was found to be bound to the matrix side of complex III;
4. Stimulated emission depletion microscopy captured the physical image of FAO proteins co-localizing, or overlapping, with ETC. proteins.
Discussion: This study shows that FAO enzymes physically interact with ETC. supercomplexes through two interactions. These findings provide first view of an integrated molecular architecture for the major energy generating pathways in mitochondria, and offer insight into clinical ramifications of patients with genetic defects in these pathways.

P-434

French Registry of Glycogenosis Type III: phenotypic and genotypic description

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Background: Glycogenosis type III (GSDIII) is a rare autosomic recessive metabolic disorder, due to a deficit in the debranching enzyme, encoded by the *AGL* gene, and is of both hepatic and muscular expression. Its evolution is typically two-phased: a pure metabolic impairment in childhood, improving at puberty; and later on muscular impairment characterized by exertion intolerance followed by permanent muscular weakness. Cardiac involvement is often encountered, without correlation with the severity or duration of muscular disease.
Methods / Case Report: The French registry was created in 2014, constituted by nine centers. It prospectively retrieves genetic data, muscular, cardiac and clinical features, CPK levels in blood, and glucose tetrasaccharide (Glc4) levels in urine. This allows us, in a first time, to establish a genotypical and phenotypical description of a large cohort of GSDIII patients, and, in the future, to describe the natural history of the disease.
Results: 44 patients have been included, with a mean age of 26 ± 15 [4–66]. 19 patients were under 20 years old. Molecular analysis showed a great variability, with a large majority of private mutations, except for a few ethno-geographic groups. Mean MFM score was 88.40% (± 17.07, 32–100). Mean 6-minute walking-test was 472 meters (±133, 50–677). 5 patients (11%) had difficulty walking, 3 were wheelchair-bound. Muscular involvement was more severe in older patients, but did not correlate with CPK or Glc4 dosages. Hypertrophic cardiomyopathy, the most frequent cardiologic finding (56%), was associated with higher levels of CPK and Glc4.
Discussion: This registry aims to collect epidemiologic, clinical, biological and therapeutical data on glycogenosis type III patients, and to further and better describe the disease and its evolution. Baseline analysis of the patients already allows us to identify some of the disease's characteristics, and prospective data will help us to determine its natural history.

P-435

Further confirmation of the clinical phenotype in patients with bi-allelic mutations in *TANGO2*: a report of 6 new cases

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Background: *TANGO2* (transport and golgi organization 2) related disease is a newly delineated cause of recurrent crises with rhabdomyolysis, arrhythmias and metabolic disturbance that can also present with developmental delay. The precise function of *TANGO2* is unknown however it is hypothesized to function in loading of newly synthesized secretory proteins in the endoplasmic reticulum. Bi-allelic pathogenic variants resulting in loss of protein function is the likely cause of this disorder.
Case Report
Results:Here we present six patients with bi-allelic variants of the *TANGO2* gene. Three had homozygous, previously described exon deletions, whilst the remainder were homozygotes or compound heterozygotes for three new variants that were predicted to be pathogenic. The clinical phenotype was characterized by developmental delay and regression often associated with viral illness. Ataxia, dysarthria and signs of spastic diplegia were also present with progression during childhood.
The majority of patients had metabolic crises associated with catabolism which were characterized by decreased consciousness, ataxia and weakness. Hypoglycaemia, hyperlacticaemia and hyperammonaemia were frequently present initially with subsequent development of rhabdomyolysis. Investigations showed variably increased dicarboxylic acid excretion and abnormal acylcarnitine profiles. Cardiac evaluation during crises showed prolongation of QTc and ventricular tachycardia. Three patients died, in one of whom a diffuse disorder of neuronal migration was found on autopsy.
Discussion:We report six cases of bi-allelic mutations in *TANGO2* which both confirm the existing phenotype and expand it. This brings the total number of reported cases to 21 since 2016. New information in the form of a neuronal migration disorder indicates the developmental deficits seen are not solely related to the cumulative effects of repeated crises and instead may have an origin during development of the nervous system.

P-436

Patients experiences are essential for improving the quality of health care

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Background: Metabolic diseases (MD) are rare and specific care for them is insufficiently developed. Because of the complexity and severity of MD, patients' opinions about care needs are essential. We inventoried the patient's perspective on health care and centres of expertise (CEs) in The Netherlands through an online survey as part of the project Expertise Mapped. This provides a picture of the organisation of care for rare diseases from the patients' perspective. From these data we have distilled shared opinions to advocate the needs of patients with MD.
Methods: Between 2013–2018 401 Dutch patients with 97 different MD filled in the online survey.
Results: 38.9% of patients experiences problems regarding their care. Their top 3 problems are: 1) a lack of knowledge among healthcare professionals regarding their disease (62.8%); 2) a lack of collaboration between specialists/healthcare professionals (42.9%); 3) the administrative burden of arranging their medical care (30.8%). Problems identified by the entire group are: insufficient support regarding the organisation/coordination of care (29.8%) and insufficient psychological support (39.8%). Of all respondents, 46.4% is aware of the existence of a CE. 57.6% is willing to travel to always see an expert.
Discussion: CEs should increase their visibility for patients, emphasize their added value and involve patients in the organisation of care. When a larger proportion of patients is seen in a CE, the knowledge of MD will increase and the quality of care will improve. Simultaneously, experts must pass along their expertise to other healthcare professionals to decrease the knowledge gap. Support for patients in the CEs in the coordination of their care is indispensable and collaboration between healthcare professionals should be stimulated. All these data suggest there is a larger role for the CEs, and possibly for the MetabERN as well, to fill the gaps that patients experience. See also www.expertisemapped.org.

P-437

Renal dysfunction in respiratory chain disorders

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Background: Renal impairment has high prevalence within mitochondrial disorders being responsible for significant comorbidity. Kidney disease, either glomerular or tubular, may have secondary impact in growth and development, hence its early detection becomes of greatest importance.
Methods: We retrospectively reviewed clinical records and annual assessments of 37 patients with a definite diagnosis of mitochondrial disorder and a minimum 2-year follow-up at our center (except for those decesead in the first year of life). Renal evaluation included serum creatinine, urea, sodium and phosphate, urinalysis, urinary protein/creatinine, fractional excretion of sodium (FENa) and tubular reabsorption of phosphate on occasional urine samples.
Results: Our sample has a median age of 13,3 years, 24% older than 18 years-old and 8% decesead in the first years of life (under the age of 5). Average follow-up time was 8 years.
Within our sample 76% have renal dysfunction of any kind with only 6% present at the time of diagnosis. The majority (73%) has tubulopathy with 43% of patients with isolated tubular damage. Renal insufficiency affects 8% of cases.
In those with evidence of tubulopathy, the inability to acidify and/or concentrate urine was the most common finding affecting 46% of patients, followed by phosphaturia in 27%, a low FENa in 22%, tubular proteinuria in 19% and glycosuria in 11%.
Proteinuria was found in 27% of patients, only 5% with glomerular without tubular proteinuria. Despite evidence of tubular proteinuria, these cases have other evidence of tubular disease.
Discussion: Kidney disease in mitochondrial disorders arises in various forms and degrees and glomerular and tubular dysfunction are often associated. The inability to acidify and/or concentrate urine, phosphaturia and a low FENa appear to be early indicators of tubular dysfunction. These findings should warrant further investigation and close follow-up as overt tubulopathy may arise.

P-438

Targeted Metabolomics in Children with Epilepsy under Ketogenic Diet: Altered Levels of Sphingolipids and Glycophospholipids

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Background: Ketogenic diet (KD) is an established therapeutic option in pharmacoresistant epilepsy. The mechanism of this nutritional intervention remains elusive, though many different theories like changes in energy metabolism or changes in lipid composition were proposed. We performed targeted metabolomics in children with epilepsy who were switched to KD in an attempt to identify potential metabolites mediating the antiepileptic effect.
Methods: Blood samples of patients were taken before and under KD. Samples were analyzed in a mass spectrometric targeted metabolomics screen using AbsoluteIDQ® p180 Kit (Biocrates Life Science). The analyses included quantification of 188 substances from the following substance classes: acylcarnitines, amino acids, glycophospholipids, sphingomyelins and biogenic amines.
Results: Apart from well-known changes in amino acids and acylcarnitine profiles under ketogenic diet, a significant number of sphingomyelins were increased and a wide range of phosphatidylcholines were reduced under KD. Analyses of biogenic amines showed an increase of histamine, methionine sulfoxide, spermine and a reduction of dimethylarginine. Calculations of ratios between different substances revealed increased systemic oxidative stress. Calculated ratios also confirmed increased fatty acid oxidation and fatty acid uptake.
Discussion: Interestingly, the metabolomics studies showed altered levels of sphingolipids and phosphatidylcholines in children under KD diet. These substances are important constituents of biomembranes, especially in the brain. Alterations in blood may be linked to modified biophysical and biochemical properties of neuronal biomembranes which may contribute to the antiepileptic effect of KD. Oxidative stress is known as inter- and intracellular messenger regulating various physiological processes.

P-439

U-IMD: Unified European Registry for Inherited Metabolic Disorders as a patient database for MetabERN

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Background: Inherited metabolic disorders (IMD) are a prominent group of over 700 monogenic rare diseases with an estimated total birth frequency of at least 1:500. In 2017, the European Commission established the non-profit European Reference Network for Rare Hereditary Metabolic Disorders (MetabERN), which connects 69 health care providers in 18 EU countries and delivers care to approximately 43,000 patients with IMDs. An important instrument to improve diagnosis, treatment and wellbeing of patients is a systematic collection of data in a registry.
Methods, Results, Discussion: The Unified European Registry for Inherited Metabolic Disorders (U-IMD) project started in February 2018. The project has three major activities: a/to establish a patient registry for the MetabERN based on the common data elements of the European Platform on Rare Disease Registration; U-IMD will be the first unified European registry that encompasses all IMDs, b/to upgrade already existing IMD registries to the standard of U-IMD, starting with the registry of the International Working Group on Neurotransmitter Related Disorders (iNTD) and c/to develop a standard for minimal core data sets shared by the MetabERN and the European Rare Kidney Disease Reference Network (ERKNET). The diverse nature of the heterogeneous etiological and clinical spectrum of the IMDs necessitates collection of a minimal set of common data elements and the usage of controlled and standardized vocabularies such as the Human Phenome Ontology or the WHO ATC classifications for the description of the clinical phenotype and treatment strategies, respectively.

Acknowledgement: This abstract is part of the project / joint action '777259 / U-IMD' which has received funding from the European Union's Health Programme (2014–2020).

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Use of carglumic acid in a tertiary pediatric hospital.

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Background: Carglumic acid (CA) is indicated by the European Medecines Agency in treatment of hyperammonemia (HA) due to N-Acetylglutamate synthase (NAGS) primary deficiency, isovaleric acidemia, propionic acidemia and methylmalonic acidemia. However, its use in clinical practice is more widespread. The aim of this study is to describe the use of CA in our Centre.
Methods / Case Report: Observational and retrospective study in a Tertiary Pediatric Centre (< 18 years) with long term follow-up of neonatal metabolic screening. Demographic and clinical data of patients in whom CA was employed, between January 2006 and February 2018, was analysed.
Results: During this period of time, forty children were treated with CA. Half of them were girls. Mean age was 7.3 years (SD 7.3). In 25 patients (63%) HA was secondary to valproic acid (VPA); 22 of them (91.6%) had concomitant treatment with other antiepileptic drugs (AEDs), most of them in polytherapy. Two suffered from HA due to AEDs different from VPA: topiramate and phenobarbital. Six out of forty (15%) presented HA due to liver failure secondary to Hematopoietic Stem Cell Transplantation (HSCT). Other uses were: propionic acidemia (three patients), and four patients with unexplained HA (in two an Ornithine transcarbamylase deficiency was subsequently diagnosed, in the other two a prompt diagnosis could not be made). In three of the patients CA was prescribed as ambulatory maintenance therapy: two with propionic acidemia and difficult-to-manage episodes of HA and one with refractory epilepsy in treatment with VPA, who had secondary elevations of blood ammonia.
Discussion: Use of CA in our centre is broad and involves different pediatric departments. Main indications in our centre are: HA due to VPA or other AEDs, liver failure secondary to HSTC, Inherited Metabolic Disorders and unexplained HA. CA should be considered as a maintenance therapy in selected patients.

P-441

Evaluation of inflamatory markers and blood parameters in patients with inherited metabolic disorders

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Background: Inherited metabolic disorders (IMDs) are mostly chronic and progressive diseases, involving multiorgan systems. Inflamation is thought to be an important indicator in the clinical picture, as DNA damage and elevated interleukin 6 levels were shown. Mean platelet volume (MPV) is a measure of platelet function and activation and it gives information about the inflammatory processes. Therefore, the aim of this study is to evaluate blood parameters, including MPV, in patients with different IMDs.
Methods/Case Report: 191 patients with IMDs [75 small molecule diseases, 55 lysosomal storage disorders (LSDs) and 61 other IMDs (hyperlipidemia, peroxisomal disesases etc.)] and 64 healthy controls were enrolled in this cross-sectional descriptive study. Blood parameters of the study and the control subjects at their first hospital admission were assessed.
Results: Mean ages of the patient (48,2% female) and the control groups (53,1% female). There were no significant differences in age and gender between the two groups. Mean hemoglobin, leukocyte and platelet counts, CRP and MPV values of the patient group were 12,26 g/dL, 10104/mm^3, 308760/mm^3, 9,6 mg/L and 8,38 fL respectively. The same values for the control group were as follows; 13,53 g/dL, 7992/mm^3, 297150/mm^3, 17,6 mg/L and 9,27 fL respectively. Differences in MPV, hemoglobin and leukocyte values were significant between the two groups (p values in order; 0,001, 0,001 ve 0,002). The lowest MPV values were determined in the LSD patients.
Discussion**:** In conclusion, low hemoglobin and MPV values with high leukocyte counts led us to think that these disorders were possibly accompanied with inflammatory vascular complications. Our results are important in terms of suggesting that inflammation could be an important process in the pathophysiology of these disorders. Therefore, hematologic parameters of these patients should be followed carefully, as to define the inflamation at the early stages of the diseases.

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***DHDDS de novo* mutations cause a new metabolic disorder at a crossroad between polyprenols biosynthesis and N-glycosylation**

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Background: *DHDDS* encodes for the dehydrodolichol diphosphate synthase,an enzyme working at a crossroad between different biochemical pathways (i.e. dolichol, cholesterol, and CoQ10),involved in the biosynthesis of dolichol monophosphate (Dol-P),essential lipidic carrier for N-glycosylation. Four N-glycosylation defects related to Dol-P biosynthesis have been described due to recessive mutations in *NUS1*, *DHDDS*, *SRD5A3*, *DOLK*.These defects are almost constantly associated with altered levels of isoprenoids, polyprenols and dolichol species, with clinical features ranging from classic CDG phenotype to pure neurologic forms. A homozygous *DHDDS* mutation (p.K42E) was also reported in retinitis pigmentosa.Recently we contributed to the identification of recurrent *DHDDS de novo* missense mutations (DNMs) as responsible for a complex neurological syndrome, transmitted as an AD trait [Hamdan, 2017].
Methods:To better characterize the clinical,biochemical and genetic phenotype of patients harbouring *DHDDS* DNMs,a cohort of 7 patients (aged 5–35 years;3 previously in Hamdan et al.,4 unreported) from 7 unrelated families was further investigated.
Results: Developmental disorders and epilepsy were associated with a mixed movement disorder encompassing cortical tremor, ataxia, dystonia, and parkinsonism. Fluctuating catatonia was reported. Transferrin isoelectric focusing testing resulted normal.Three mutations were identified (p.R37H;p.R37C;p.R211Q),falling in conserved regions corresponding to the catalytic domain (p.R37H;p.R37C) and the substrate binding region (p.R211Q).

Discussion:Our findings support the causative role of DHDDS DNMs in a complex neurological syndrome without obvious signs of glycosylation deficits.Given the multiple functions of dolichol species, and the position of DHDDS between different pathways,further studies are needed to elucidate if the pathophysiology of this new condition relies on isoprenoids accumulation,glycosylation deficit,or secondary effects on contiguous pathways.

P-443

Reduction of complexity: explaining inborn errors of metabolism and their treatment to children and adolescents

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Background: Inborn errors of metabolism (IEM) encompass numerous rare genetic conditions typically affecting enzyme functioning. Organ and neurological sequelae and/or potential risk of life-threatening crises demand long-term therapy. Patients reaching school age face new challenges in handling their disease. They often have limited knowledge of their condition because communication with the metabolic team has entirely been handled by their parents. Knowledge about disease and treatment constitute pillars of self-responsible disease management. Not many written patient education materials on IEM are available and due to the complexity of IEM, these are often hard to understand. We developed and tested comprehensible, age-appropriate patient education materials for school-aged children and adolescents with IEM.
Methods: Informative texts and illustrationswere developed by an international network of metabolic care professionals together with a graphic artist and experts for easy-to-read text. The materials were presented to healthy children and patients with IEM from four metabolic centres in standardized group training sessions. Knowledge-gain was assessed by pre- and post-testing.
Results: Knowledge-gain was significant ($t(73)$ = 11.16, p = .000, *Cohen's d* = 0.84). Following the standardized teaching intervention, test questions were answered correctly by >90% of participants. In healthy children, this effect was independent from age and teacher-assessed concentration or cognitive capacity.
Discussion: The newly developed patient education materials are a powerful tool to enhance disease-related knowledge and facilitate communication with school-aged children and adolescent patients with IEM.
Conflict of Interest declared.

P-444

The role of diagnostic laboratories in the early identification of hyperammonaemia – an opportunity not to be missed

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Background: Diagnostic laboratories have a critical role in identifying patients with hyperammonaemia. Urgent recognition and treatment of such patients, especially in the neonatal period, is vital as if left untreated, morbidity and mortality is high. Unfortunately blood ammonia is unstable *in vitro* and ammonia may be mildly raised as a result of delayed separation, haemolysis and contamination. There is a concern that samples which do not comply with stringently applied acceptance criteria are being rejected by labs.
Methods: A questionnaire on the ammonia analysis was piloted in the 18 MetBio.Net stakeholder labs in the United Kingdom in order to review current practice and inform best practice. The 18 labs were asked whether they require samples to be unhaemolysed, sent on ice, within a defined time interval and whether they test vacutainers for contamination.
Results: All 18 labs responded. Out of the three laboratories that test for contamination, only one laboratory has found contamination. All but 1 laboratory recommends that samples are sent within a specific time interval; 6 labs reject samples that they consider too old for analysis. Thirteen labs recommend that samples are sent on ice; 2 laboratories reject samples that they are not received on ice. All labs assess the haemolysis status of sample sent for ammonia analysis. Five labs reject samples which they consider to be haemolysed whilst 12 labs reject samples which they consider to be grossly haemolysed. Thirteen labs who reject samples request repeat samples.
Discussion: The data shows that a proportion of samples sent to labs for ammonia analysis are rejected. As a result, opportunities for identifying patients with significant hyperammonaemia may be missed which may lead to significant morbidity and even death. The MetBio.Net guidelines for the investigation of hyperammonaemia are being revised and will recommend more lenient sample acceptance criteria.

P-445

Glycerol – 3 – Phosphate Dehydrogenase 1 deficiency - a new differential diagnosis for Hepatic Storage Diseases

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Background: Glycerol - 3- phosphate dehydrogenase 1 (GPD1) deficiency is a newly described and not yet fully understood metabolic disorder with only 18 published patients, characterised by infantile hepatomegaly, steatohepatitis, hypertriglyceridaemia. It is not associated with recurrent hypoglycaemia or myopathy.
Methods / Case Report: All three children of a non-consanguineous couple presented from early infancy with large hyperchogenic livers, increased ALT, mixed hyperlipidaemia with pronounced hypertriglyceridaemia and mildly increased lactic and uric acid. Hypoglycaemia was suspected but could not be confirmed. A liver biopsy at 4 months of age in the oldest child revealed severe lipid and moderate glycogen storage. Glycogen debrancher activity was absent in liver tissue and a diagnosis of Glycogen storage disease type 3 was made. All siblings were managed for presumed GSD3 with late meals and overnight feeding to prevent morning hypoglycaemia and all later displayed symptoms of exercise intolerance and muscle pain without an increase of CK.
Results: After 12 years of dietary management and follow up, genetic testing revealed a normal sequence of the *AGL* gene. Genetic panel testing for Glycogen storage diseases was normal, apart from a heterozygous pathogenic mutation in *PYGL* found in all three siblings. This puzzling family was recruited to the NHS England 100,000 Genomes study which finally revealed compound heterozygosity for two pathogenic mutations in the *GPD1* gene in all three affected children, namely the novel mutation c.361G>A p.(Gly121Arg) and the previously described mutation c.686G>C p.(Arg229Pro).

Discussion: GPD1 deficiency is a new important differential diagnosis of infantile hepatomegaly with lipid accumulation and can be mistaken for a hepatic glycogen storage disease.

P-446

Aminoacyl-tRNA synthetase deficiencies: in search of common themes

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Background: By attaching amino acids to their cognate tRNAs, aminoacyl-tRNA synthetases (ARSs) play an important role in protein translation. Mutations in genes encoding these enzymes are increasingly associated with human disease. Clinical features of autosomal recessive ARS deficiencies appear very diverse and without apparent logic.
Methods: We searched for all patients with recessive ARS mutations in literature (July 2017). Clinical and laboratory findings of all patients were categorized. Additionally, we deep-phenotyped five patients with novel IARS, KARS, LARS and QARS mutations seen in our own hospital. We considered symptoms to be common if they occurred in > 30% of individual ARS deficiencies.
Results: We identified 107 patients with homozygous or compound heterozygous mutations in the cytosolic genes AARS, DARS, HARS, IARS, LARS, MARS, RARS, SARS, VARS and YARS, and the combined cytosolic and mitochondrial genes GARS, KARS and QARS. All presented with abnormalities of the central nervous system and/or senses, and nearly all with failure to thrive. Other common symptoms included feeding problems, dysmaturity, liver symptoms, and various endocrine abnormalities. All 3 combined mitochondrial and cytosolic and several cytosolic ARS deficiencies showed signs of mitochondrial dysfunction and facial dysmorphisms. Symptoms were most severe in the first year of life and during infections. Deep-phenotyping of our five patients revealed anemia, interstitial lung disease and renal tubulopathy as additional common symptoms.
Discussion: Deep-phenotyping of reported and new patients with recessive ARS deficiencies enabled us to discern a common clinical phenotype, putatively resulting from insufficient aminoacylation activity to meet translational demand in specific organs or periods of life. Assuming residual ARS activity, adequate protein/amino acid supply seems essential instead of the traditional replacement of protein by glucose in patients with metabolic diseases.

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Biochemical and molecular characterization of a South African Trimethylaminuria cohort and the description of a novel FMO3 variant

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Background: Trimethylaminuria (TMAU) is characterized by systemic trimethylamine (TMA) accumulation due to compromised Flavin-containing monooxygenase 3 (FMO3) activity. The disorder does not have detrimental pathophysiological consequences, but patients develop psychological problems due to the debilitating "fishy" odor.
Methods: 7 Patients with phenotypical TMAU gave informed consent for relevant metabolite and genetic testing. A TMA loading protocol was applied with urine collected at assigned intervals. TMA and trimethylamine-oxide (TMAO) levels were measured via ^{1}H-NMR spectroscopy to determine the FMO3 capacity. PCR and Sanger sequencing of the coding regions of the *FMO3* gene (exons 2–9) were performed on isolated DNA.
Results: Patient 1 had significantly compromised FMO3 capacity (37%, pathological value < 43%), and had 3 homozygous missense variants: a novel p.I8T as well as two known variants (p.E158K, p.E308G) associated with mild TMAU. Patient 2–4 had attenuated FMO3 capacity (78-89%, pathological value 71-92%). Patient 2 and 3 were heterozygous for the benign p.V257M variant and/or the mild p.E158K variant. An altered metabolite profile was noted in patient 4, but only the benign p.V257M variant was detected. Patient 5–7 had normal FMO3 capacity. Patient 5 was heterozygous for p.E158K. Patient 6 was homozygous for p.E158K and heterozygous for p.E308G. For patient 7 no FMO3 variants could be detected in the coding region.
Discussion: A phenotype-genotype correlation was noted in the severely affected patient with the novel homozygous variant. This was not the case in the rest of the cohort. All patients however complained of the "fishy" odor. Our findings may be explained by non-compliance during the loading protocol as well as the presence of primary (due to FMO3 variants) and/or secondary (due to gastrointestinal aberrations) TMAU. Physiological and biochemical assessment are required to ascertain the origin of the odor, to treat accordingly.

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Fever induced recurrent acute liver failure as a dominant feature in congenital disorders of intracellular trafficking

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Background:Acute liver failure remains unexplained in up to 50% of the pediatric cases. Whole-exome sequencing has revealed new genetic disorders causing liver disease. Among these, mutations in *NBAS* and *SCYL1* genes, coding for two proteins involved in cellular retrograde trafficking regulated by t-SNARE Syntaxin complex, were linked to syndromes with variable liver disease.
Methods:We analyzed comparatively clinical and biochemical data in two patients, one with NBAS and one with SCYL1 deficiency (CALFAN), presenting a history of recurrent acute liver failure (RALF).
Results: In both infants RALF was triggered by common febrile illnesses and occurred within the first 2 years of life. Episodes were characterized by vomiting and dehydration. The NBAS patient died at the age of 40 months and suffered from 3 episodes in total; the last was associated with marked hypoglycemia (4 mg/dl), lactic acidosis (10 mmol/L), hyperammoniemia (345 umol/L) and hyperferritinemia. Urine organic acids showed massive elevation of lactate, 3-hydroxybutyrate and 4-hydroxyphenylacetate, mimicking an hepatic mitochondrial disorder. The SCYL1 patient had a milder course, with 3 RALF episodes with hyperferritinemia, severe hyperbilirubinemia, moderate hypoglycemia and abnormal transferrin isoforms (T0=15%); at follow-up he displayed cognitive impairment but improvement of liver disease. Genetic analyses

showed in pt.1 mutations in *NBAS* (c.1550G>A/c.6805G>T) and in pt.2 a homozygous mutation (c.1314C >T) in *SCYL1*. Western blot analysis confirmed strongly reduced expression of NBAS and SCYL1 proteins in patients' fibroblasts.
Discussion: Our observation indicates that congenital disorders of intracellular trafficking may present with a dominant feature of fever induced RALF. Interestingly, also Lipin-1 disease, another defect related to SNARE protein machinery, is responsible for fever induced rhabdomyolysis, highlighting the role of these proteins in regulating cell response to body temperature changes.

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Management of hyperammonemia in a patient with *TANGO2* mutations suggests a role for defective ureagenesis

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Background: *TANGO2* mutations lead to encephalopathy, tachyarrhythmia, episodic rhabdomyolysis, hypoglycemia, hyperammonemia and lactic acidosis during catabolism. Despite metabolic crises resemble fatty acid oxidation defects; global developmental delay and epilepsy are distinguishing features that suggest other mechanisms may be involved. *TANGO2* belongs to the transport and Golgi organization family; its function is unknown. The role *TANGO2* plays in the mitochondria and endoplasmic reticulum remains unclear. Herein, we describe management of hyperammonemia in a patient with *TANGO2* mutations.
Methods / Case Report: An 11 y/o female was admitted with fever, respiratory distress, diarrhea, dehydration and altered mental status. She had a history of global developmental delay, epilepsy, recurrent pneumonia, but no rhabdomyolysis. CXR showed pneumonia, EKG and echo heart were normal. After antibiotics and hydration with dextrose and normal saline were started, her urine flow and symptoms improved, and she tolerated g-tube feeds.
Results: Homozygous pathogenic deletions of exon 3–9 in *TANGO2* were detected by prior WES. Throughout admission: CPK, acylcarnitines, glucose and electrolytes were normal. Her free carnitine was low. BUN was 6mg/dl, NH3:72umol/l, lactate peaked at 2.9mM/L. After supportive care, NH3 continued to rise to 118umol/l and BUN dropped to 1mg/dl. Serum aminoacids showed increased Gln and Ala. Arginine (2g/m^2) was started, and two days later, her NH3, and BUN normalized.
Discussion: Elevated NH3, Gln with a low BUN suggests defective ureagenesis. Caloric increase by adding dextrose, fat, protein, and replacing carnitine were insufficient to normalize NH3 and BUN. Following recovery from acute illness, arginine was discontinued; NH3 remained normal, suggesting *TANGO2* mutations may be associated with urea cycle dysfunction during stress, requiring treatment with arginine. These findings may reflect a broader mitochondrial dysfunction than previously recognized.

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Sirtuin levels in blood from patients with glycogen storage disease type 1

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Background: Glycogen storage disease typ 1 (GSD 1) is due either to a deficiency of glucose 6-phosphatase (GSD 1a) or the glucose 6 phosphate transporter (GSD 1b). In GSD, glycogen breakdown and mitochondrial uptake of long-chain fatty acids are compromised due to the accumulation of glucose 6-phosphate. Impaired hepatic fatty acid oxidation is responsible for reduced ketogenesis. Hypoketotic hypoglycaemia as a biochemical hallmark results and leads to cerebral energy deficiency. Accumulation of malonyl CoA is the biochemical basis of impaired mitochondrial fatty acid-uptake. Sirtuins are essential regulators of cellular metabolism. SIRT 4 is known to regulate the mitochondrial uptake of long-chain fatty acids via malonyl CoA. Malonyl CoA decarboxylase is inhibited by SIRT 4 which leads to malonyl CoA accumulation, hence reduced fatty acid oxidation. SIRT 1 and SIRT3 have a direct effect on glycogenolysis and gluconeogenesis.
Methods: We analyzed relative transcript levels, protein amount and enzymatic activities of SIRT1, SIRT3, SIRT4 and SIRT5 in blood from children with GSD 1 and age-matched healthy controls.
Results: SIRT 4 was down-regulated in plasma from patients with GSD 1 at protein-level. SIRT1 was not significantly altered at protein level while enzyme activity of SIRT1 was slightly decreased. Measurement of maximal SIRT3 enzyme activity showed no alterations while the SIRT3 protein levels were slightly decreased. While the protein levels of SIRT5 were significantly increased in most patients, the maximal SIRT5 enzyme activity was slightly decreased compared to the healthy controls.
Discussion: Decreased levels of SIRT 4 result in activation of malonyl CoA decarboxylase, hence activation of mitochondrial fatty acid uptake and hepatic ketogenesis. This may be interpreted as a compensatory mechanism to rescue ketogenesis. Pharmacological inhition of SIRT 4 may result in better metabolic control in GSD 1-patients and prevent cerebral energy deficiency.

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Mental health influences the quality of life in parents of children with an inborn error of metabolism

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Background: Inborn errors of metabolism (IEM) often require medical monitoring along with a restrictive diet and/or drug treatment. Such constraints likely decrease the quality of life (QoL) in affected patients and their families. However, this topic remains largely unexamined. In this study, we examined the factors that influence QoL in parents of children with IEM.
Methods: We assessed self-reported QoL in parents of 30 children (M_{age}=8.82 years; 10 girls) using an adaptation of the Phenylketonuria Quality of Life questionnaire. Additionally, parents responded to questions about mental health (Hospital Anxiety and Depression Scale), stress related to their child's IEM (Pediatric Inventory for Parents), coping strategies (Cognitive Emotion Regulation Questionnaire) along with questions about their child's mental health (Strengths and Difficulties Questionnaire). Treating physicians evaluated the severity of the IEM (adaptation from Intermed).
Results: QoL did not vary according to the severity of the child's IEM ($p>0.05$). The factor that influenced most the QoL scores was parental mental health: parents who passed the HADS depression/anxiety cutoff (n=7) reported more child symptoms ($p< 0.001$) and a heavier impact of their child's IEM on their emotional, social and practical life ($p< 0.001$). Additionally, they reported more frequently stress related to their child's

IEM (p< 0.001) as well as more internalizing (p=0.026) and externalizing (p=0.039) symptoms in their children.
Discussion: QoL is a self-report of how a disorder affects psychosocial functioning. Our results suggest that QoL in parents of children affected by an IEM is not influenced by objective factors, such as the severity of the child's IEM. QoL seems to be more affected by intrinsic psychological factors, such as parental depression/anxiety. Special care should be addressed to identify mentally vulnerable parents of children affected by an IEM already at diagnosis and to offer supportive interventions.

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The European Reference Network Program and the MetabERN (ERN for Rare Hereditary metabolic Diseases)

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On behalf of the MetabERN Members

Background: The European Reference Network for Hereditary Metabolic Diseases (MetabERN) has been set up in response to the European Commission call to establish European Reference Networks (ERNs) following the Directive on patients' rights in cross-border healthcare. MetabERN represents the first most comprehensive, pan-metabolic, pan-European, patient-orientated platform aimed to transform how care is provided to patients with inherited metabolic diseases (IMDs) in Europe.
Methods / Case Report:
Results: MetabERN represents 69 certified Healthcare Providers (HCP) from 18 European countries, 44 Patients Organisations and is endorsed by the SSIEM.
MetabERN involves 1681 experts 52% of which are specialized medical doctors. It manages 42.427 patients, 68%of which are pediatric. More than a half of the patients are affected by lysosomal disorders or amino acid and organic acids related diseases (23% and 39% respectively).
The group of more than 700 ultra rare metabolic diseases is structured in 7 subnetworks for different metabolic diseases and divided in 8 workpackages.
Discussion: MetabERN aims to accommodate and interconnect expertise; harmonise data collection; optimise prevention, diagnostics, management and treatment; develop and implement guidelines; stimulate cross-border research and innovative treatments; develop training and education opportunities and interact with patients. Patient organizations will work in close collaboration with HCP to map and understand patients' needs and identify solutions.
MetabERN will is facilitating and harmonizinge patients' access to diagnosis and best treatment across the EU. By implementing collaboration with academia, politics, insurance companies and industry it aims to capture the most innovative medical advances and tailors them to patient needs. It is also fostering collaboration with other ERNs in areas where overlapping exists.

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MetabERN (ERN for Rare Hereditary metabolic Diseases): first year deliverables

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On behalf of the MetabERN Members

Background: The European Reference Network for Hereditary Metabolic Diseases (MetabERN) has been organizing activities and expected outcomes into a set of work packages (WPs). We aim to report the main achievements per WP related to the first year of MetabERN activity.
Methods & Results: **WP1 Coordination and management:** Setting up of the main structure of the network and all the meetings that enable a smooth running of cooperation, coordination, management and communication.
WP2 Dissemination: Set up of MetabERN website (www.metab.ern-net.eu), social media channels, monthly newsletter, press releases and patient brochure.
WP3 Evaluation: A policy on continuous monitoring system, an approach for periodical self-assessment and a Conflict of Interest policy within the ERN-Coordinators group are being written.
WP4 Guidelines and Care Pathways: Development of an online accessible information platform on clinical pathway recommendations (CPR).
WP5 Virtual Counseling Framework: Launching ofClinical Patient Management System (CPMS) by the EC and incentivation of it's use.
WP6 Research, transitional activities and clinical trials: Surveys collection to identify expertise to generate research projects and identify gaps. Setting up of a structure for a Grant Office with potential opportunities for MetabERN to apply for grants.
WP7 Capacity building and training: Generation if a platform for education/ trains and collaboration with the SSIEM academy(http://metabern-educ.eu)
WP8 Continuity of Care: Mapping of diagnostics procedures on metabolic diseases
Discussion: During the first year of activities MetabERN has constructed a solid basis for connecting the most specialised centres to promote prevention, accelerate diagnosis and improve standards of care across Europe for patients living with IMDs. In particular, with the support of the SSIEM we will further encourage the knowledge transfer and support specialized education of professionals and patients.

P-454

A new disease and its pathomechanisms - anhidrotic ectodermal dysplasia with immunodeficiency caused by *ORAI1* mutation and abolished SOCE

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Background: Store-Operated Ca^{2+} Entry (SOCE) is an important pathway for increasing intracellular Ca^{2+} regulated by stromal interaction molecule (STIM) & Ca^{2+} channels formed by ORAI proteins. *ORAI1* mutations abolish SOCE thus lead to insufficient & excessive immunity & nonimmunological symptoms.
Case Report: Patients had Anhidrotic Ectodermal Dysplasia (EDA), Immunodeficiency (ID), Lymphoproliferation, Autoimmunity & Congenital Myopathy (CM) caused by novel homozygous *ORAI1* mutations that prevented expression of a functional protein (p.V181SfsX8) or suppressed protein expression by interfering with its stability (p.L194P, p.G98R) which led to abolished SOCE.
Results: Patients had dry/exfoliate skin, thin/brittle hair, heat intolerance/ thermoregulatory instability with attacks of facial flushing, tachycardia, tachypnea, hypertension, anhidrosis, impared eccrine sweat glands due to Ca^{2+}-activated chloride channel TMEM16A dysfunction & severe enamel defects. Conventional T/B cells numbers were preserved compared to their function. T cells showed reduced/absent proliferation in response to PHA, decreased IL-2, IL-22, TNF-α & IFN-γ production contributing to ID. Patients had lymphadenopathy, hepatosplenomegaly, autoimmune pancytopenia, antiphospholipid syndrome, loss of naive CD45RA1 T cells & concomitant expansion of CD45RO1 or HLA-DR1 activated T cells, decreased % of CD251FOXP31 Treg cells & CD251CD127low T cells, reduced Treg cells in CD45RA1FOXP3low naive & CD45RA2FOXP31 activated Treg cells suggesting that SOCE is required for immunological tolerance. Generalized CM & iris hypoplasia was due to mitochondrial dysfunction.
Discussion: ORAI1 mutations were associated with impaired T-cell function, reduced numbers of invariant NK T cells & regulatory FOXP31 Treg cells, altered composition of gd T-cell & natural killer cell subsets. We propose that ORAI1 mutations that abolish SOCE constitute a new form of EDA-ID, distinct from EDA-ID observed in patients with impared NF-κB pathway.
Conflict of Interest declared.

P-455

Deficiency in perilipin 5 results in encephalopathy with cerebellar ataxia and recurrent episodes of ketoacidosis

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Background: Perilipin 5 (Plin5) is a protein that coats lipid droplets and is highly expressed in oxidative tissues such as heart, liver, and skeletal muscle. The proposed physiological role of Plin5 is the regulation of intracellular lipid storage according to metabolic demand. Under basal condition, Plin5 reduces lipolysis and sequesters fatty acids (FA) into lipid droplets, while under increased energy demand it stimulates lipase interaction on lipid droplets and directs FA to mitochondrial beta-oxidation. Mice deficient in Plin5 show reduced lipid droplet formation and enhanced FA metabolism in oxidative tissues, and develop cardiomyopathy, liver damage, and insulin resistance.
Methods / Case Report: We report a case of a 10 year old boy with a compound heterozygous mutation in the PLIN5 gene. The patient presented with congenital cerebellar ataxia, severe encephalopathy with psychomotorical retardation and recurrent hyperketotic acidosis (pH < 7.00) triggered by infections. Interestingly, he had no clinical signs of cardiac dysfunction, liver injury or altered insulin sensitivity.
Results: Respiratory chain complex activities were in the lower normal range in skeletal muscle and fibroblasts. Whole exome sequencing revealed two mutations in the *PLIN5* gene predicted to be deleterious (p.R145C; p.P370L). Direct assessment of basal palmitate oxidation and oxygen consumption in patient-derived fibroblasts were normal, a finding that is in accordance with the low expression of Plin5 in fibroblasts.
Discussion: Plin5 deficiency has so far not been associated with a disease phenotype in humans. Although Plin5 deficiency could explain the disinhibited FA oxidation and hyperketosis observed in this patient, we still lack functional proves for its pathogenicity in cells. Further investigations are ongoing in induced pluripotent stem cell-derived hepatocytes.

P-456

Biallelic mutations in *LZTR1*: a new inheritance mode of the syndromic hypertrophic cardiomyopathy

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Background: Hypertrophic cardiomyopathy (HCM) is the most frequent cardiovascular defect in children, following mainly autosomal dominant inheritance. Recently, recessive (AR) form of a novel phenotype, including HCM as a cardinal feature, caused by *LZTR1* mutations has been suggested. Additionally, in two reported patients mitochondrial dysfunction comprising complex I and III deficiency was observed.This study is the second report of biallelic mutations in *LZTR1* associated with a severe cardiac phenotype.
Methods / Case Report: A boy, presenting at birthwith HCM, hypotonia, failure to thrive and short stature was analyzed long-term for a metabolic disease without a conclusive diagnosis. Newborn screening results were normal and Pompe disease was ruled out. Biochemical study at 2 and 4 years excluded other enzymatic blocks. Echocardiography revealed ASD, LVOTO, longQT and episodes of paroxysmal supraventricular tachycardia. Because of the dysmorphic craniofacial features which manifested with patient's age, the syndromic occurrence of HCM was suggested. Despite the satisfactory improvement of his condition after a myectomy, he died suddenly, at the age of 9, due to the circulatory and respiratory failure. Postmortem analysis of a cardiac multigene sequencing panel was performed.
Results: Biallelic novel *LZTR1* mutations, c.257A>T and c.2044A>G, corresponding to a new recessive phenotype of Noonan syndrome (NS), were identified and suggested as a cause of the sudden cardiac death in this patient. NS has been described solely as a dominant disease so far.
Discussion: Our result confirm recently discovered *LZTR1* molecular defect as a novel ARinheritance mode of the syndromic cardiomyopathy. The phenotypic overlap with mitochondrial disorders makes the diagnosis difficult and time-consuming. The molecular autopsy allowed to establish the cause of death, direct risk stratification and clinical management of the surviving family.
Supported by projects: S149/2016, S140/2014.

P-457

Synaptic metabolism: a tentative to describe new categories of diseases in inborn errors of neurotransmission

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Background:Inborn errors of neurotransmitters (IEN) have been classically considered as synthesis, catabolism and transport defects. The synapse is a highly specialized structure devoted to neurotransmission (NTs). The presynaptic side hosts the machinery needed to release and recycle synaptic vesicles (SV). The postsynaptic side responds to the released vesicle contents and triggers downstream cellular responses. Specific metabolic pathways regulate this complex process and are disturbed in an important number of classic and potentially new IEM.
Methods: We retrospectively analyzed patients recruited in our center with abnormal CSF monoamine and GABA levels without classic IEN defects (the already mentioned synthesis, catabolism and transport pathways) of these molecules. Based on the final genetic etiology we aimed to detect new pathophysiological categories leading to abnormal NTs.
Results: A series of 312 patients with abnormal HVA, 446 with abnormal 5-HIAA, and 37 with abnormal GABA CSF values were recruited from our database of patients with neurological diseases and CSF studies. A final genetic diagnosis excluding "classic IEN" was found in 30% of patients. Channelopathies, mitochondrial disorders and complex molecule metabolism defects that regulate intracellular vesiculation, trafficking, processing, and quality control were the most common diagnosis, and in particular those diseases affecting SV dynamics.
Discussion: In the last recent years the description of new diseases within the context of the –omics revolution is leading to an extended concept of IEM. Other than the classic concept of IEN, diseases of complex molecule processing are becoming increasingly important in the field of neurometabolic diseases and together with energy defects are amongst the most common IEM involved in disturbed NTs. This approach provides new insights into biomarkers and therapies.

P-458

Possible mechanisme of action for liver failure in X-linked protoporphyria caused by gain of function mutations in *ALAS2*

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Background: X-linked protoporphyria (XLP) caused by gain-of-function mutations in *ALAS2* is the most recently described form of porphyria. Clinically it mimicks erythropoietic protoporphyria with affected subjects presenting an important cutaneous photosensitivity. XLP patients however present a higher incidence of liver disease.
Case Report: We present a 2.5 year aged boy with XLP who was admitted to the emergency ward with somnolence and hypotonia. First investigations showed a deep hypoglycemia (glucose 11mg/dl), hyperammoniema (216 μg/dl) and elevated lactate (78 mg/dl). These values in addition with elevated liver transaminases and prolonged prothrombin time were suggestive for acute liver failure. He was treated by supportive treatment with elevated glucose supply and packed red cells. After normalisation of clotting, liver was biopsied for further evaluation of causes and importance of liver damage. Attempting to prevent further decompensation events, chronic treatment was designed to promote wash out of porphyria intermediates by cholestyramine and deoxycholic acid treatment, to promote complete heam synthesis by iron and zinc supplementation.
Results: Spectrophotometric evaluation of oxidative phosphorylation complexes in liver tissue showed an important decrease of complex I, II and III, which is a hallmark for a deficiency in the iron-sulfur biosynthesis pathway. In contrast, OXPHOS analysis in cultured skin fibroblasts showed normal activities, arguing in favour of a synthesis defect limited to liver tissue. Light microscopic evaluation of liver tissue showed massive presence of apoptotic and necrotic hepatocytes as sign of acute liver disease.
Discussion: This boy with XLP presented an acute liver failure. OXPHOS analysis showed evidence of an iron-sulfur cluster deficiency which we assume to be triggered by iron deprivation due to the constitutivly active *ALAS2* enzyme. We propose a therapeutic approach to keep a stable metabolic condition.

P-459

Results from 16 years of a pioneer service for aid with inborn errors of metabolism issues in Brazil.

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Background: The Inborn Errors of Metabolism Information Service (SIEM) provides (free of charge) information and guidance for diagnosis and management on Inborn Errors of Metabolism (IEM). Once contacted by a health professional, a form is completed with relevant clinical information, which is recorded in a database and analyzed by a metabolic specialist who will suggest diagnostic hypotheses, will guide the investigation, and if necessary, will propose emergency management measures.
Methods: This cross-sectional study aimed to analyze the results obtained by SIEM from October 2001 until April 2018.
Results: 3592 cases were recorded in the period. Most requests (88.9%) were made by health professionals who sought diagnostic and management assistance, followed by 207 requests (5.7%) looking for information on IEM and 137 requests (3.8%) looking for guidance in cases that had already been diagnosed as IEM. The purpose of the contacts was mostly related to patients with a suspicion of IEM (86.2%); the most frequent symptoms were developmental delay (46.6%), seizures/hypotonia (41.3%), hepatomegaly (22.5%) and GI disturbances (21.3%). The onset of symptoms was below 1 year in most cases (54.2%). 339 cases (10.2%) were eventually confirmed as an IEM of: amino acids (142, 41.9%); lysosomes (61, 18%); energy metabolism (40, 11.8%); carbohydrates (35, 10.3%); fatty acids/ketone bodies (21, 6.2%); other (40, 11.8%).
Discussion: IEM diagnosis is challenging, mainly due to non-specific symptoms, lack of awareness by health professionals and difficult access to diagnostic tests; however, when a diagnosis is reached, several measures could be taken to modify the natural course of the disease. In a large country with scarce and unevenly distributed resources, a service like SIEM could be an useful tool. One in each ten cases that were submitted to SIEM had a diagnosis of IEM eventually confirmed, indicating the importance and usefulness of this service in a country like Brazil.

P-460

Clinical characterization of individuals with epileptic syndrome secondary to an inborn error of metabolism – Bahia, Brazil.

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Background: Inborn Errors of Metabolism (IEM) are uncommon causes of epilepsy, but epileptic seizures are a common feature in several IEM. The aim of this research is to identify IEM with epilepsy, and to recognize the most frequent type of epileptic seizures and associated neurological syndromes.
Methods: This study was carried out in a University Hospital in the city of Salvador/Bahia, Brazil. Data about age, gender, types of seizures, neurological manifestations and diagnosis was collected.
Results: 28 patients were included with a mean age of 12.8 years. The diagnoses identified were as follows: Tay-Sachs disease, Mucopo lysaccharidosis, Niemann-Pick C, Krabbe's disease, Non-ketotic hyperglycinemia, Lipofuscinosis Ceroide Neuronal, Aciduria 2-Hydroxyglutaric, Glutaric Aciduria Type I, X-Linked Adenoleu kodystrophy, Deficiency of Coenzyme Q10, Deficiency of Sulfite-Oxidase, Maple Syrup Urine Disease, Phenylketonuria, Gangliosidosis GM1, Hyperargininaemia, Homocystinuria and Cerebral Tendinea Xanthomatosis. Generalized tonic-clonic seizures were more frequent (96.4%), followed by myoclonic (39.2%), tonic (17.8%), focal (10.7%), atonic, absence (1%) and infantile spasms (1%). The associated neurological syndromes identified were cognitive and pyramidal (67.8% each), regression of neuropsychomotor development (60.7%), dyskinetic (35.7%), cerebellar (28.5%), global developmental delay, psychiatric disorders (17.8%), lower motor neuron syndrome (7.1%) and hypotonic child (3.5%). Among the patients, 64.2% were in crisis control.
Discussion: Generalized epileptic seizures are more common in individuals with IEM, especially tonic-clonic and myoclonic seizures, corresponding to diffuse involvement of central nervous system in metabolic diseases. IEM, although rare as causes for epilepsy, should be suspected when generalized seizures are associated with other neurological manifestations, especially cognitive, pyramidal and refractory to conventional anticonvulsants.

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WITHDRAWN

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Management of two patients with Infantile liver failure syndrome type I due to *LARS* mutations: an under recognized reversible hepatopathy

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Background:Infantile liver failure syndrome type I (ILFS1) is a multisystem hepatopathy caused by recessive mutations in cytoplasmic leucyl-tRNA synthetase (LARS). LARS is a leucine sensor in mTORC1 signaling, which controls cell growth,autophagy, and protein synthesis. The phenotype was characterized in Irish travelers; leucine supplementation was not helpful. We report the clinical course, management, and follow up of 2 non-Irish patients identified during workup for liver transplantation.
Methods / Case Report: *Patient 1:* 7 y/o female, hospitalized at 17 mo for vomiting, anemia and acute liver failure. *Patient 2*: 4.5 y/o male, NBS showed elevated methionine- due to liver dysfunction. He had a prolonged NICU course due to complications of prematurity and liver failure. On discharge; he had global developmental delay, severe visual impairment, and required a g-tube for feeding. During a fever episode he developed status epilepticus, and occipital cortical dysplasia was noted. Both children were born prematurely, with IUGR and failure to thrive.
Results: Diagnostic workup during acute episodes showed elevated transaminases, cholestasis, coagulopathy, chronic microcytic anemia, aminoaciduria, and abnormal liver imaging. Liver biopsies showing steatosis and iron deposition. Whole exome sequencing revealed novel compound heterozygous mutations in LARS genes. Acute and chronic treatment using high protein (3–3.5g/kg) was associated with normalization of liver functions and growth parameters.
Discussion: ILFS1 is a metabolic disorder with reversible liver disease that should be considered in the differential diagnosis of infantile hepatopathy regardless of ethnic background. Clinical suspicion should prompt early molecular testing to confirm the diagnosis because, unlike other causes of hepatic encephalopathy, high protein intake reverses acute liver dysfunction, while chronically improving growth, and decreasing the frequency and length of hospitalizations.

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A case of 5-fluorouracil rechallenge after 5-fluorouracil-relatedhyperammonemic encephalopathy.

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Background:-Fluorouracil (5-FU) is a key drug for many tumor types for several years, used alone or in combination. The most common side effects are gastro-intestinal disorders, mucositis, myelosuppression, hand foot syndrome and rarely cardiac toxicity. These toxicities are deeply enhanced with Dihydropyrimidine dehydrogenase (DPD) deficiency. Hyperammonemic encephalopathy, manifesting as altered mental status with elevated ammonia levels is a very rare toxicity following 5-FU chemotherapy. This side effect can probably be explained by impairment of ATP-and glutamate-dependent urea cycle by direct inhibition of Krebs' s Cycle by fluoroacetate upstream of alpha-ketoglutarate production.
Case Report:We report here one case of 5-FU related hyperammonemic encephalopathy occurring after FOLFIRINOX - Bevacizumab (oxaliplatin, irinotecan, bevacizumab, folinic acid and 5-fluorouracil) chemotherapy to treat a metastatic gastro-intestinal cancer of unknown primary, in a context of partial DPD deficiency.
Results:With a first described protocol based on ammonium chelators and Krebs and Urea cycle intermediates, patient was successfully rechallenged by the same chemotherapy regiment for more than 6 months.
Discussion:We propose a new protocol to prevent 5-FU related hyperammoniemic encephalopathy, thus helping to keep 5-FU as a first line key drug for various tumor types.

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Severe hypoglycemia and seizures as the first signs in NBAS deficiency

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Background: Hypoglycemia in infants may be the first symptom, which leads to the diagnosis of an inborn error of metabolism, like hyperinsulinism or organic acidurias. This can also be the first sign for acute liver failure (ALF).

Methods / Case Report: We report a 16 months old child presenting with severe hypoglycemia (13 mg/dl/ 0.7 mmol/l) and seizure starting one day after onset of fever and vomiting. No lactic acidosis (1.6 mmol/l – ref. < 2), no hyperammonemia (38 μmol/l – ref. < 48), but highly elevated transaminases (ASAT 9678 U/l – ref. < 55 and ALAT >1000 U/l – ref. < 46) and coagulopathy (*PT*28 % – ref. 70–125, INR 2.1, aPTT 45.8 s – ref. 30.0- 45.0, thrombin time 20.9 s – ref. < 21.0) were found. Quick recovering –within 8 days- after performing of anabolic management (high glucose and lipids) and administration of antipyretics. Metabolic workup showed unremarkable acylcarnitines and amino acids in plasma and urine. Organic acids showed traces of lactate. The child was neurological totally normal. Besides of failure to thrive and short stature since birth, normal mental and motor development was noted. No prove of Pelger-Huët anomaly. Meanwhile patient shows full recovery of the liver function and normal mental development.

Results: Whole exome sequencing revealed compound heterozygosity for 2 missense variants c.2535G>T (p.Trp845Cys) and c.5761 G>C (p.Ala1921Pro) in the *NBAS* (NM 015909.3)-gene. These variants are phylogenetically conserved but have not been reported in patients till now. Functional characterization of NBAS protein in fibroblasts is currently pending.

Discussion: NBAS is meanwhile a known heterogeneous disease causing recurrent acute liver failure (RALF) and can manifest with multisystemic symptoms. NBAS should be included in the hypoglycemia work-up. Early diagnosis and antipyretic treatment can prevent life-threatening ALF and improve the quality of life of patients and their families.