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ABSTRACTS

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01. Inborn errors of metabolism: general, adult

O-001

Suitability of nitisinone in alkaptonuria - a dose response study

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Background: Homogentisate-lowering therapy was investigated for alkaptonuria (AKU). Nitisinone decreases homogentisic acid (HGA) but the dose–response relationship has not been previously studied.
Methods: SONIA 1 was an international, multicenter, randomized, open-label, no-treatment controlled, parallel-group, dose–response study. The objective was to investigate the effect of different doses of once daily nitisinone on 24-h urinary HGA excretion (u-HGA_{24}) in patients with AKU after 4 weeks of treatment. Forty patients were randomized to either no treatment, or 1, 2, 4 or 8 mg of nitisinone (8 per group).
Findings: Nitisinone and u-HGA_{24} showed a clear dose–response. At 4 weeks, the adjusted mean u-HGA_{24} was 31152, 3846, 1668, 686 and 327 mmol for the 0, 1, 2, 4 and 8 mg doses, respectively. The 8 mg daily dose, decreased mean u-HGA_{24} by 99.4 % compared to baseline. Serum HGA was below limit of detection. Increased serum tyrosine levels were seen at all doses to at least 10 times baseline values. No serious or severe adverse events were reported over the 4 weeks of nitisinone therapy.
Conclusion: In SONIA 1, nitisinone therapy significantly decreased u-HGA_{24} in a dose-dependent manner and was well tolerated within the studied dose range in AKU patients.
Conflict of Interest declared.

O-002

Delineation of new IEM phenotypes via an integrated - omics approach

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Introduction: Genomic sequencing provides the exciting opportunity to further delineate the clinical and biochemical spectrum of known inborn errors of metabolism, and to identify the etiology of IEM-mimics.
Methods: As part of our TIDEX discovery study, 15 carefully characterized patients in 13 families with unexplained biochemical/mitochondrial phenotypes were selected according to our TIDEX criteria for whole exome sequencing. Trio data were analyzed via our customized pipeline; candidate genes with rare, damage variants following Mendelian pattern of inheritance validated via Sanger validation +/− in vitro studies.
Results: Aside 7 novel genes, 6 novel phenotypes of recently discovered human diseases were discovered: low HVA and 5-HIAA in SCN2A epileptic encephalopathy (dopamine/OH-tryptophan responsive); intermittent metabolic crises with status dystonicus, and respiratory chain complex III deficiency in UQCRC2; renal failure, hearing loss, complex I & IV deficiency in RMND1; neonatal hypotonia with mitochondrial complex I,II,IV deficiency in SCN4A; cortical neurodegeneration with infantile onset in AIMP1; atypical demyelination pattern in PLP1; myoclonic epilepsy, retinitis pigmentosa, hearing loss, complex IV deficiency in PIGA.
Discussion: A combined phenomics-genomics approach identified novel phenotypes in 45 % of families studied (including respiratory chain enzyme deficiencies secondary to non-mitochondrial gene defects) with identification of 1 immediate treatment target.

02. Novel diagnostic/laboratory methods

O-003

Rapid quantification of underivatized amino acids in plasma by hydrophilic interaction chromatography (HILIC) coupled with tandem mass-spectrometry

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Background: Aminoacidopathies are a class of inborn errors of metabolism (IEM) that can be diagnosed by analysis of amino acids (AA) in plasma. Current strategies for AA analysis include post-column ninhydrine derivatization or the use of perfluorinated acid such as ion-pairing agents. Major drawbacks are time-consuming procedures, costs, problems with retentions and MS-sensitivity. Here we report a method for analysis of 24 underivatized AA in plasma to detect defects in AA metabolism. The presented method is adapted from Guo and co-workers (J Agric Food Chem;61:2709–2719; 2013), who analyzed AA in fruit.
Methods: A rapid, sensitive and specific method was developed for the analysis of AA in plasma without derivatization using hydrophilic interaction chromatography (HILIC) coupled with tandem mass-spectrometry (Xevo, Waters). Results: Excellent separation of 24 AA in plasma was achieved on an Acquity BEH Amide column (2.1 mm x 100 mm, 1.7 μm) in a single MS run of 12 min. Plasma samples of patients with a known IEM in AA metabolism were analysed and patients were identified. The method was validated according to ISO-15189 accreditation for medical laboratories.
Conclusion: The reported method is rapid, sensitive and specific and is applicable to study defects in AA metabolism in plasma.

O-004

Zebrafish as a disease model for inborn errors of metabolism

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Background/Objectives: Next generation sequencing is increasingly providing candidate genes associated with human disease. Hence, it is imperative to uncover the role of these genes to understand disease pathogenesis. We have chosen zebrafish to study inborn errors of metabolism with the aim of using this experimentally tractable model organism to shed light on disease mechanisms and provide a route for drug discovery.
Methods/Results: State of the art genome editing technologies such as TALENs and the CRISPR/Cas system were used to target highly conserved regions within zebrafish genes that are orthologues of human disease genes. RNAs were injected into one cell stage embryos and the rate of mutagenesis assessed using High Resolution Melting Analysis. Heterozygous carriers from the F1 generation were selected for disruptive mutations and in-crossed for further studies. For instance, we have generated a zebrafish mutant harbouring a frameshift mutation (p.L100Sfs*7) in SLC30A10, the gene affected in inherited hypermanganesaemia associated with dystonia/Parkinsonism. Work is currently underway to characterise the mechanisms of manganese toxicity and identify novel therapeutic targets.
Conclusion: We have established a pipeline to model inborn errors of metabolism in zebrafish in order to study the function of disease genes and provide a platform for drug screening.

O-005

Urine screening using tandem mass spectrometry: wide-ranging, rapid, high-throughput screening for inborn errors of metabolism

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Urine tandem mass spectrometry screening (UMSMS) using targeted multiple reaction monitoring is an effective tool for diagnosing almost all amino and organic acidurias. Many other metabolites can be analysed in parallel and our laboratory has been successively adding other IEMs to extend the panel of disorders detected by UMSMS. Patient samples can be used for validation and a multiple-of-median analysis used to flag abnormal samples when the relevant marker metabolite is not available. The advantages of UMSMS are exemplified by prospective diagnoses of disorders such as cerebrotendinous xanthomatosis, creatine transporter defect, infantile sialuria and deficiencies of steroid 21 hydroxylase (late onset), antiquitin, purine nucleoside phosphorylase, adenylosuccinase, GAMT and FBP'ase. Early treatment of several of the above disorders was a significant benefit. Most of these disorders were not suspected by referring clinicians and would not have been diagnosed during their work-up unless UMSMS had been used. A rapid (~30 min) UMSMS modification has also been developed for the diagnosis of acutely presenting IEMs.

O-006

Hepatocyte transfection of naked DNA by hydrodynamic intraportal injection in domestic pigs

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Background: Liver is an attractive organ for gene delivery in order to correct several different genetic (metabolic) diseases. Hydrodynamic vein injection of naked DNA/minicircles devoid of viral backbones was demonstrated (e.g. in murine PKU) to allow effective transduction of liver cells. We challenge here the hypothesis that successful hydrodynamic injection in mice via the tail vein can be adapted to portal vein injections in newborn pigs after weaning.
Materials and Methods: A surgical method allowing direct hydrodynamic portal vein injections of naked DNA in small pigs with long-term survival of the animals was developed. Efficiency at 10 and 28 days after portal vein injections of naked DNA was evaluated by PCR in liver biopsies of sacrificed pigs.
Results: Surgical procedure and intraportal hydrodynamic injectionsof minicircles with portal vein pressure up to 90 mmHg were well tolerated by newborn pigs (n=5) after weaning. PCR analysis showed that 25 % of liver samples were PCR-positive four weeks after the intervention.
Conclusion: We accomplished positive transfection of liver cells in a dose dependent effect upon hydrodynamic injection of naked DNA vectors in newborn pigs. Further work must aim at increasing the efficacy of the procedure.
Grant: CRPP radiz - Rare Disease Initiative Zurich

O-007

NAD profiling: an innovative tool for the characterization of mechanisms behind disease and pharmacological approaches

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Nicotinamide adenine dinucleotide (NAD^+), its phosphorylated ($NADP^+$) and reduced (NADH, NADPH) forms are known for their essential role in energy metabolism, reductive biosynthesis and antioxidative pathways. Deficiency of NAD^+, consequence of decreased dietary precursors or enzymatic defects, has been associated with neurodegenerative diseases, diabetes, cancer and epilepsy. Quantitative information on the status of these metabolites in Inborn Errors of Metabolism (IEM) has not been gathered yet. This work reports novel sensitive UHPLC-MS/MS-based

methodologies for the analyses of NAD (P)$^+$, NAD (P) H and nicotinic acid. Limits of quantification ranged from 2–200 pmol and linearity was confirmed up to 25 nmol ($0.87<R^2<0.98$). Procedure validation was undertaken in a model of drug-induced liver injury (tissue) and in human fibroblasts. The developed methods enabled an accurate characterization of NAD (P)(H) and the detection of NAD$^+$ precursors in rat liver and in human control fibroblasts. The levels of these redox signaling intermediates in rat liver samples revealed a significant reduction of NAD$^+$ and NADP$^+$ (30 %, $p<0.05$) subsequent to in vivo treatment with a steatogenic drug. Application of these methodologies to human samples will account for a more comprehensive understanding of the pathophysiological mechanisms behind disease, namely involving IEM or oxidative stress, and to assess rescuing therapeutic approaches.

03. Newborn screening

O-008

Newborn screening for Pompe disease: a comparative effectiveness study

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Newborn screening (NBS) for one or more LSDs has been implemented in NY, MO and Taiwan by enzyme assay using either tandem mass spectrometry (MS/MS) or fluorometry. Other assays making use of Luminex technology have also been proposed. To prevent a recurrence of the significant variability in performance that characterized the application of MS/MS in NBS, we implemented three high-throughput screening assays for the simultaneous measurement of multiple biomarkers in dried blood spot (DBS) specimens for the detection of Pompe disease, 12 other LSDs, Friedreich Ataxia, Wilson disease and X-adrenoleukodystrophy. These assays were tested in a prospective comparative effectiveness study using nearly 100,000 leftover NBS samples to determine the most efficient and effective approach to NBS for these conditions. In addition, samples from affected patients were analyzed to assess the various methods and to establish disease ranges. Following the analytical phase of the study, we have begun to develop web-based post analytical tools to maximize screening performance as was previously done as part of the Region 4 Collaborative project (http://www.nbstrn.org/research-tools/lab-performance-database/). We found all assays to be sensitive for Pompe disease but only the development and application of a post-analytical tool achieved an acceptable false positive rate for all assays (<0.01 %).

04. Dietetics and nutrition

O-009

Improvement of cardiomyopathy after high fat diet in two siblings with glycogen storage disease type IIIa

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Glycogen Storage Disease type III (GSDIII), autosomal recessive disorder due to amylo-1,6 glucosidase deficiency, causes limit dextrin storage in liver, skeletal muscle and heart. Cardiomyopathy is quite frequent in GSDIIIa with variable severity and progression of manifestations. It is unclear whether diet manipulation may interfere with progression of cardiomyopathy. Ketogenic diet seemed effective in few cases, however the compliance to ketogenic diet is difficult in the long term. These two 7- and 5-year-old siblings affected with GSDIIIa developed severe and rapidly deteriorating left ventricular hypertrophy. They were then treated with high fat (60 %) and protein (25 %), low carbohydrate (15 %) diet. After 12 months exertion dyspnea disappeared and biochemical blood tests, cardiac enzymes and congestive heart failure markers improved (CK 3439>>324, 1304>>581 U/L; NT-proBNP 2084>>206, 782>>135 pg/mL respectively); ultrasound assessment showed a relevant reduction of the thickness of interventricular septum (30>>16, 16>>11 mm respectively) and left ventricle posterior wall (18>>7, 13>>8 mm respectively) and an improvement of the outflow obstruction in both patients. A diet rich in proteins as well as fats and poor in carbohydrates could be a beneficial therapeutic choice for GSDIII cardiomyopathy.

O-010

Management and outcomes of glycogen storage disease (GSD) type VI and IX from a single centre in the United Kingdom

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Background: GSD VI and IX are the commonest hepatic GSD diagnoses with limited published literature.
Method: The dietary management and outcomes of GSD type VI and IX patients currently under treatment were reviewed.
Results: Thirty-eight patients (11 GSD-VI, 17 GSD-IXa2, 3 GSD-IXb, 5 GSD-IXg, 2 GSD-IX unknown subtype) were identified. Diagnosis was confirmed by enzymology and/or mutation analysis. Median age of presentation was 2.7y (IQR 1.4-4.0). All presented with hepatomegaly, five had documented hypoglycaemia prior to diagnosis. Twelve patients require overnight tube feeding to supress ketosis (median carbohydrate 0.35 g/kg/h). Twenty-eight patients receive starch (median 1.2 g/kg, 2 doses). Sixteen patients have reported feeding difficulties and 32 patients require vitamin and mineral supplementation. Outcomes: At diagnosis patients were short (median height SDS −2.0) with increased BMI (median SDS 1.1). With treatment height SDS increased by 0.97 and BMI SDS decreased by 0.49. Initially, 24 patients had elevated liver enzymes (ALT or ALP) reducing to 12 patients after treatment (0 GSD-IXg normalised). Sixteen patients had bone density scans, nine were abnormal. Fractures were reported in five patients. Sixteen patients have experienced Vitamin D deficiency.
Conclusions: This large series showed treatment improves growth and liver inflammation however bone health is a concern.

O-011

The nutritional adequacy of tube feeds for children with organic acidaemias

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Introduction: Children with organic acidaemias (OA) are commonly dependent on enteral tube feeds (TF's) and nutrient intake is rarely reported.
Aim: A retrospective analysis of nutrient intake in TF children with OA.
Method: Intake of energy, protein (natural and precursor-free L-amino acids (PF-LAA), vitamins, minerals, and fibre were analysed in 14 subjects (8 PA, 5 MMA, and 1 IVA).

Results: Median % energy from 1 to 10y was 76 (63–131) of EAR, with intake decreasing by 25 % between 6 m to 3y ($p<0.05$), and 40 % between 6 m to 5y ($p<0.05$). The ratio of PF-LAA increased from 3 m to 5y ($p=0.03$), providing a median of 30 % total protein intake. The % median intake of RNI (from age 3 m to 10y) for sodium, potassium and magnesium was 58 (42–97), 91 (46–125), and 85 (48–116) respectively. Fibre intake was low (median 4 g/day). The intake of calcium, selenium and iron decreased below RNI with increasing subject age, particularly from age of 10 years. Median weight and height z scores from 1-10y was +0.6 and −1.2 respectively.
Conclusions: Stable OA patients on TF's had low energy intakes, leading to inadequate intake of essential minerals. Current formula does not meet the nutritional requirements in children aged >1y.

O-012

Taste preferences and food neophobia in children with phenylketonuria

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Background/Objectives: In PKU, little is known about the effect of dietary restriction and L-amino acid (bitter taste) exposure on taste preference development. This observational, controlled, prospective study aimed to determine the flavour preferences of children with PKU compared with healthy control children.
Methods: 35 children with PKU and 35 age/gender matched control children aged 4-13y (median: 8.5y), tasted 10 blinded puree foods (apple, banana, strawberry, custard, broccoli, cauliflower, carrot, sweet potato, lemon, coffee) in random order, rating them using a 7-point pictorial hedonic scale (super yummy to super yucky) and then ranking them in preferential order. Carers completed a neophobia and food frequency questionnaire.
Results: Children with PKU rated sweet potato ($p=0.007$), carrot ($p=0.008$) and custard ($p=0.001$) higher whilst apple was rated higher by control children ($p=0.02$). This was mainly attributable to children aged 4-8y. Children with PKU had more overall neophobia (uncomfortable in unfamiliar environments) and were untrusting/fearful of new foods. Children with PKU consumed 50-100 % more than control children of high energy: sugar containing drinks, sweets, chips, sweet biscuits, crisps, and butter/margarine.
Conclusions: Children aged ≥4y did not prefer bitter tasting foods associated with the taste of L-amino acids but were suspicious of new foods and tastes.
Conflict of Interest declared.

O-013

Ketogenic diets (KDs) in children - efficacy, side effects and metabolic changes

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Background: Different types of KDs are used for treatment of intractable epilepsy and disorders of brain energy metabolism. We report data regarding efficacy, changes of laboratory parameters and side effects in patients with classical ketogenic diet (CKD) and modified Atkins diet (MAD).
Patients: In a retrospective review data of 31 children (0.1 - 15.7 years (median 5.5, mean 5.5), CKD (16) or MAD (14), one with both) were analyzed. 30 patients had intractable epilepsy, in one GLUT-1-deficiency was diagnosed. Investigations before and after initiation of ketosis were clinical examination and laboratory tests, eventually EEG, ECG, abdominal/cardiac ultrasound (every 3–6 months).
Results: KDs were well tolerated and led to reduction of seizures to less than 50 % in 17/31 patients (3 months after initiation of KD). Most common clinical side effect was constipation which was anticipated with soluble fiber supplementation. Bromine level in one patient with Dravet-syndrome increased to toxic levels without clinical symptoms. In one patient with Rett-syndrome slightly elevated QT-time was observed in initiation phase and resolved after termination of ketosis. Hypercalciuria (urine calcium/creatinine-ratio >0.59) was observed in 12/22 patients but none developed nephrolithiasis.
Conclusion: KDs are effective and safe. In our patient group serious side effects were absent.

O-014

Dietary management of branched chain ketoacid dehydrogenase kinase deficiency (BCKDKD)

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Background: BCKDKD has recently been described causing neurobehavioural deficit and autistic features in paediatric patients.
Case Report: A child of healthy non-consanginous parents presented aged 3 years with developmental delay, acquired microcephaly, autistic features and broad-based gait. Further investigation revealed consistently low branch chain amino acids (BCAA) in plasma, urine and cerebrospinal fluid (CSF). The rate decay constants for BCAA were calculated to be 5.0 to 5.9 times higher than observed in a normal subject. Diagnosis of BCKDKD was confirmed by mutation analysis. Protein rich diet and oral BCAA supplements (Solvil®,Vitaflo) mixed with yogurt were commenced. Each sachet provided 2.5 g leucine, 1.24 g isoleucine and valine. Supplements were adjusted based on regular monitoring of plasma BCAA. Optimal results were achieved giving 1 sachet between main meals and at 11 pm and 4 am (maximum 5 h fast at night).
Results: On BCAA supplements pleasing developmental progress was seen with improvement in speech, gait, growth and social interaction.
Conclusion: Further work is needed to establish effective dietary treatment targets by measuring paired CSF and plasma BCAA at key points throughout the day to ensure CSF BCAA are maintained within normal range. Early diagnosis and treatment is crucial to improve neurocognitive outcome.
Conflict of Interest declared.

05. New metabolic disease groups

O-015

Rabenosyn-5 (ZFYVE20) deficiency in a female with intractable seizures and evidence of defective endocytic trafficking

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Background: Rbsn-5 (ZFYVE20) regulates receptor bound internalization and recycling and the ability of integrins to control cellular motility. ZFYVE20 deficiency has not been described in humans.
Case report: A 6.5 year-old female presented with intractable seizures, microcephaly, craniofacial dysmorphism, dysostosis, megaloblastoid erythropoiesis, transient cobalamin deficiency, severe hypertriglyceridemia upon ketogenic diet, microalbuminuria.
Methods and Results: Whole exome/Sanger sequencing revealed a homozygous mutation GGG-aGG in ZFYVE20 (frequency: 0.003987) resulting in a G425R substitution, as the only mutation segregating with disease in the family. Fibroblast studies revealed expression and localization of Rbsn5-G425R in wild-type manner, but a 50 % decrease in transferrin accumulation (a biomarker for endocytic internalization), which was corrected by wild-type allele transfection. Furthermore, fibroblasts displayed an impaired proliferation rate, and cytoskeletal abnormalities. Partial cathepsin D deficiency (35 %) was associated with an inversed relation of precursor to mature protein on Western blotting.
Conclusion: Results are consistent with a functional defect in early endocytosis. Cathepsin D findings are most likely due to altered subcellular availability of mannose-6-phosphate receptors affecting its biosynthetic transport from Golgi to the lysosome (Naslavsky 2009). Patients with similar manifestations should be screened for ZFYVE20.

O-016

Inositol monophosphatase 1 deficiency causes non-syndromic severe intellectual disability

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Intellectual disability (ID) affects up to 3 % of world population and it is estimated that more than 1,000 genetic conditions can be associated to ID. In a highly inbred region of Northeast Brazil, we evaluated siblings with non-syndromic severe ID. Homozygosity-by-descent analysis disclosed a 20.7 Mb region in 8q12.3-q21.2, which gave a lod score of 3.11. Whole exome sequencing of one patient recognized a single rare homozygous deleterious variant in inositol mononphosphatase 1 gene (IMPA1) in the candidate region, consisting of a homozygous 5 bp duplication leading to a frameshift and premature stop. This variant co-segregates with disease within the family; further investigations confirmed 3 additional affected individuals belonging to the same expanded consanguineous family. Brain myo-inositol peak, measured by magnetic resonance spectroscopy in one patient, was normal. Plasma and urine myo-inositol and phospho-myoinositol were also in the normal range in 4 affected individuals. IMPA1 gene product is responsible for the final step of biotransformation of the second messenger inositol-polyphosphates. Homozygous IMPA1 knockout mice have fetal death, but can be rescued by maternal supplementation of myo-inositol during pregnancy and early life. Deficiency of IMPA1 might represent a new group of IEM, affecting inositol phosphate biotransformation.

06. Phenylketonuria: general

O-017

Mapping the functional landscape of frequent phenylalanine hydroxylase genotypes

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Background: Solid experimental data on enzymatic phenylalanine hydroxylase (PAH) function associated with full PAH genotypes is not available.
Methods: We expressed 6 homozygous and 24 compound heterozygous genotypes in a cellular model, assessed PAH function at a wide range of phenylalanine and tetrahydrobiopterin concentrations, and depicted results as activity landscapes.
Results: Specific genotypes are associated with different patterns of the functional area of PAH enzyme activity in the metabolic (phenylalanine) and therapeutic (tetrahydrobiopterin) space. (i) a shift of peak activity to higher substrate concentrations, which is potentially associated with low dietary phenylalanine tolerance at low phenylalanine concentrations, (ii) narrow landscapes suggesting the need for strict dietary control and the risk for phenylalanine fluctuations, and (iii) a shift of peak activity to higher cofactor concentrations indicating a need for increased tetrahydrobiopterin supply and possibly explaining the occurrence of inconsistent clinical phenotypes.
Discussion: The analysis of activity landscapes provides clinically relevant new insights into genotype-related impaired PAH function beyond the known link between residual activity of single mutations and clinical phenotype. This work provides a tool to implement personalized medicine strategies for dietary regimes and pharmacological treatment in phenylketonuria patients. A related web application warrants continuous extension, up-dates, and research on demand.
Conflict of Interest declared.

O-018

Methylome repatterning in maternal PKU syndrome provides pathophysiological insight

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Background and Objectives: Maternal PKU syndrome results from in utero PHE exposure. Numerous toxic insults induce repatterning of the methylome. We hypothesized in utero PHE toxicity induces methylome repatterning in offspring.
Materials and Methods: $PAH^{enu2/enu2}$ females on normal or PHE restricted diet were mated to C57BL/6 males. Brain and heart were dissected from E18-19 fetuses, DNA prepared, libraries constructed, α-5-methylcytosine immunoprecipitation enriched methylated DNA, and paired end sequencing was performed. Bioinformatics assessed methylation patterns. Expression was investigated and related to methylation.
Results: Methylome repatterning in heart and brain is extensive in offspring of PHE unrestricted females while attenuated in offspring of PHE restricted females. Differential promoter methylation impacts genes recognized as causal in neurological and/or congenital heart defects. Differentially methylated gene coding regions primarily targeted non-coding

RNA genes. Microarray analysis demonstrated methylation alters expression patterns.
Discussion: In utero PHE toxicity alters the methylome and subsequently gene expression. Promoter methylation is observed in genes with recognized involvement in congenital heart defects and/or neurological deficit suggests mechanisms of disease pathophysiology. Methylome modification of noncoding RNA genes alters their expression with secondary impact on expression of target genes. Methylome modification alters gene expression which contributes to pathophysiology of maternal PKU.

07. Phenylketonuria: treatment, BH4

O-019

Pterin-4a-carbinolamine dehydratase deficiency causes neonatal hyperphenylalaninemia with MODY-like diabetes

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Deficiency of pterin-4a-carbinolamine dehydratase (PCD) or dimerization cofactor (DCoH1) was initially described as a benign form of hyperphenylalaninemia. It is caused by variants in the PCBD1, coding PCD and DCoH1. These patients are detected by the newborn screening for PKU and with primapterinuria. So far 26 patients with 10 different PCBD1 variants are tabulated in the BIODEFdb (www.biopku.org/). Most of these patients did not require treatment. Recently we detected a novel deletion in PCBD1 in a consanguineous family with early-onset antibody-negative diabetes. Subsequent investigation of patients tabulated in the BIODEF database revealed three additional cases, who all developed maturity onset diabetes of the young (MODY) at or after puberty. MODY-like diabetes was reported in 4 out of 7 families, suggesting that recessive variants in PCBD1 might increase the risk for MODY-like diabetes. Three patients below age 9 years presented without diabetes. This is the first report of a linkage between a recessively inherited disorder in amino acids metabolism and MODY-like diabetes. These patients can be detected by NBS for PKU, need genetic counseling, and can be treated with oral sulphonylureas instead of insulin. PCD/DCoH1 dysfunction might disturb the early pancreatic transcriptional network to induce MODY-like diabetes.

O-020

Erythrocytes as carriers of phenylalanine ammonia lyase in phenylketonuric (BTBR-Pah^{enu2}) mice

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Phenylketonuria is a genetic disease caused by the defect of phenylalanine hydroxylase gene. Preclinical and clinical investigations suggest phenylalanine ammonia-lyase (PAL) as a possible enzymatic substitutive treatment for phenylketonuria. The aim of this study was to investigate erythrocytes (RBCs) as a possible vehicle of PAL (rAvPAL) focusing on: in vivo biochemical efficacy; possible antibody inactivation under repeated administrations; treatment safeness.
Methods: Three groups of BTBR-Pah^{enu2}mice were treated with a single intravenous injection of PAL-loaded RBCs at different doses (1.0, 0.5, 0.25 IU/mouse, respectively). Then, the dosages of 1.0 and 0.5 IU/mouse were administered at 9–10 day-time intervals for 11 weeks in 2 groups of mice. Blood Phe and anti-rAvPal antibody were monitored during the entire trial.
Results: Both dosages caused a relevant decline of blood Phe which persisted for the first 5 days after the infusion and slowly returned to basal levels (21 % and 25 % of their respective basal values on day 8). The effect was persistent under repeated injections and not affected by the generation of antibodies induced by the recombinant enzyme. No side effect was observed.
Conclusions. The loading of proteins into RBCs opens new perspectives for the enzyme substitution/replacement therapies for disorders involving enzymatic deficiencies.
Conflict of Interest declared.

O-021

Non-viral minicircle vectors for long-term gene expression to treat liver diseases

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Non-viral minicircle (MC)-DNA vectors devoid of bacterial and viral sequences provide sustained transgene expression, whereas corresponding parental plasmids are silenced. We have reported long-term correction of hyperphenylalaninemia in the C57Bl/6-Pahenu2 mouse model of human phenylketonuria (PKU) following liver-directed gene transfer via hydrodynamic tail vein injection with MC-DNA vectors (Viecelli et al., 2014, Hepatology). Here we improved the efficacy of transgene expression and investigated the fate of MC vectors in mouse liver. The dose of MC-DNA required to correct hyperphenylalaninemia could be lowered 10-fold by using MC-DNA vectors consisting of a codon-optimized murine phenylalanine hydroxylase (mPah) cDNA with a 5' truncated intron 1. MC-DNA vector employing different liver-specific promoters, including the endogenous mPah promoter, were also evaluated for in vivo efficacy. There was no significant difference in blood L-Phe clearance efficacy in treated mice whether using MC-DNA vector purified by standard DNA vector purification (Qiagen) or by GMP grade purification. Following 70 % partial hepatectomy, MC-vector-treated PKU mice showed normal liver regeneration and blood L-Phe concentration increased to pretreatment levels, which corroborate previous observations that episomal MC-DNA does not integrate. In summary, MC-based hepatic gene delivery to PKU mice provides an ideal system to investigate improvement and efficacy of liver gene therapy.

08. Sulphur amino acid disorders

O-022

Hydrogen sulfide production by mutant CBS enzymes

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Background: The important gaseous signaling molecule hydrogen sulfide (H_2S) is produced by three enzymes including the cystathionine beta-synthase (CBS). To explore whether H_2S homeostasis may be altered in homocystinuria we determined the H_2S producing activities of recombinant CBS mutants.
Methods: Purified wild-type enzyme and nine pathogenic CBS mutants (p. P49L, p. P78R, p. A114V, p. R125Q, p. E176K, p. R266K, p. P422L, p. I435T, and p. S466L) were incubated with combinations of the substrates homocysteine (Hcy), cysteine (Cys) and serine (Ser). As a measure of H2S synthesis by alternative CBS reactions we determined by LC-MS/MS the production of cystathionine from Hcy+Cys, and of lanthionine or serine from Cys.
Results: The canonical activity (Hcy+Ser) of the wild-type CBS enzyme was 59.5 μmol/mg/h; the H_2S-producing activities from condensation of Hcy+Cys, Cys+Cys and by Cys desulfhydration were 26 %, <1 % and <1 % of canonical activity, respectively. The nine CBS mutants exhibited canonical activities between 38.1 and 206 μmol/mg/h and the H2S-producing activities from Hcy+Cys and Cys condensation in the range 18.4- 32.9 % and <1 % of the canonical activity, respectively.
Conclusion: H_2S production from cysteine and homocysteine by CBS mutants is altered implicating possible derangement of H_2S metabolism in homocystinuria. Acknowledgement: This work was supported by the grant No. LD14082 from the Ministry of Education.

O-023

Long-term treatment of severe molybdenum cofactor deficiency type A with cyclic pyranopterin monophosphate: report on the first cohort of patients

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Molybdenum cofactor deficiency (MoCD) is characterized by rapidly progressing encephalopathy and intractable seizures, leading to severe disability and early death. We report the outcome of the first cohort of patients substituted with cyclic pyranopterin monophosphate (cPMP). cPMP (80-320 μg/kg daily IV) was started in 16 neonates diagnosed with MoCD (11 type A and 5 type B) and continued in 8 type A patients for up to 5 years. Safety and efficacy were regularly assessed. We observed no drug-related serious adverse events for >6000 doses. Urinary S-sulfocysteine, xanthine and urate returned to near normal concentrations in all type A patients within two days and consistently remained normal for up to five years on continued cPMP substitution. Eight type A patients became more alert and convulsions were either suppressed or markedly reduced under treatment. Three early-treated patients remain seizure-free and show near-normal long-term development. We detected no biochemical or clinical response in type B patients. cPMP substitution is the first effective therapy for MoCD type A patients. It results in an improved neurodevelopmental outcome when started early enough. Every newborn with signs of encephalopathy should be tested for urinary sulfite and, if positive, considered for immediate cPMP substitution, to maximize treatment benefits.
Conflict of Interest declared.

O-024

Studies of methylenetetrahydrofolate reductase (MTHFR) deficiency

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MTHFR deficiency is the most common inherited disorder of folate metabolism and causes severe hyperhomocysteinaemia. We performed extensive enzymatic and molecular genetic investigation of 72 patient cultured skin fibroblast cell lines. Mutational analysis revealed a deleterious variation on each of the 144 alleles, with one allele harbouring 2 mutations. Of these, 65 different mutations (44 novel) were detected, including 43 (30 novel) missense mutations. Using an enzyme assay with the natural substrate in the physiological direction which allows precise detection of low levels of residual MTHFR activity (1-2 % of the mean control values) we tested the cell lines for activity, kinetic abnormalities and cofactor-responsiveness. 42 cell lines had residual activity (1.2-42.2 % of control). Of these, 25 showed reduced affinity for NADPH, 1 reduced affinity for Methylene-THF, 5 FAD-responsiveness and 23 abnormal kinetics of AdoMet inhibition. We found a good correlation between enzymatic abnormalities and location and type of detected mutations. Expression of 14 novel and 6 known missense mutations in an expression system, employing immortalized fibroblasts of a MTHFR deficient patient homozygous for a nonsense mutation corroborated the results found in primary patient fibroblasts. Characterization of patients in this way provides a basis for possible new treatments e.g. by chaperones.

O-025

Molecular basis of S-adenosyl-L-methionine binding to cystathionine beta synthase: insight into enzyme activation and disease mutations

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Background: Cystathionine β-synthase (CBS) is a key enzyme in sulphur metabolism, and its inherited deficiency causes the protein misfolding disorder, homocystinuria. The enzyme is activated by S-adenosyl-L-methionine (SAM) binding to its regulatory domain. Pharmacological chaperone (PC) therapy targeting this domain has been suggested, prompting the need to establish the molecular mechanism of SAM binding and activation.
Results: The 1.8 Å structure of the CBS regulatory domain in complex with SAM reveals one SAM molecule bound per domain, within a cleft formed by the interface of the Bateman1 and Bateman2 motifs. SAM binding at this surface accessible site is accompanied by a rotational rearrangement of the Bateman1 motif relative to Bateman2. This conformational change, supported by solution data, recruits an otherwise distant conserved loop (Phe443-Asp444-Gln445) into the SAM binding site. A majority of disease-causing missense mutations in the regulatory domain localize to the Bateman1 motif, and likely prevent formation of the SAM-bound conformation.
Conclusion: We propose that binding of the activator SAM at the regulatory domain communicates to the catalytic domain via conformational

changes, and mutations in the regulatory domain likely disrupt this transduction mechanism. Our data also provide the molecular framework to develop PC molecules as a therapy for homocystinuria.

09. Other amino acid disorders

O-026

Asparagine synthetase deficiency: a new inborn error of metabolism

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Background: Asparagine synthetase deficiency (ASD) is a novel inborn error of metabolism just described recently in 9 individuals from four families presented with progressive microcephaly, global developmental delay, early intractable seizure, axial hypotonia with severe appendicular spasticity in all cases. The diagnosis confirmed by finding of homozygous or compound heterozygous mutations in ASNS gene.
Case report: In 2 and 3 year old brother and sister, who had presented with neonatal/early intractable onset seizure, progressive microcephaly and spastic quadriplegia, ASD was confirmed through whole exome sequencing analysis. The MRI findings, consistent with the first report of this disorder, showed decreased cerebral volume and size of pons, in addition to gyral simplification and microcephaly. There are no other systems involved. Extensive metabolic/genetic testing showed unremarkable results apart from slightly low asparagine level in CSF studies of one of the patients.
Conclusion: ASD should be considered in any infant with early intractable onset seizure, progressive microcephaly and spastic quadriplegia.

10. Urea cycle disorders

O-027

Phase I/II multicenter trial of liver derived mesenchymal stem cells (HepastemR) for the treatment of urea cycle disorder and Crigler Najjar syndrome. interim analysis at 6 months post infusion.

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Background: Heterologous Human Adult Liver-Derived Progenitor Cells (HHALPC)(HepastemR) synthetise urea, engraft and differenciate into hepatocyte like cells.
Patients & Methods: This open label, phase I/II trial evaluates safety of one cycle of HHALPC in 14 urea cycle disorders (UCD) and 6 Crigler Najjar syndrome (CN). 0.3 to 4 % of liver mass was infused. Secondary endpoints included 13C incorporation into urea (area under the curve (AUC-120), 0 to 120 min after oral Na [1-13C] acetate, or bilirubin. Donor/recipient chimerism was evaluated.
Results: Adverse events were more common within 14 days following infusion, a.o abnormal lab values, non specific symptoms and transient metabolic decompensation in 5 UCD. Two patients had a thrombotic event. [13C] blood urea AUC-120, highly variable at baseline, increased by 30 %(ns) at 3 months and by 90 % (p=0.01) at 6 months versus baseline (n=13). Compared to 1 year pre-infusion available data, median ammonium post-infusion tended to decrease in ‹12yo UCD patients and median bilirubin decreased in 3/6 CN (−42,-28 &-11 %). Donor specific gene sequences were detected in 3/13 patients.
Conclusion: One single injection of 0.3 to 4 % of liver mass is safe and can bring functional metabolic changes and tissue engraftment.
Conflict of Interest declared.

O-028 Withdrawn

11. Organic acidurias: branched-chain

O-029

Krebs cycle enzymes interact with methylmalonyl mutase as a complex associated with the mitochondrial membrane

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Background: Although complex formation of the Krebs cycle enzymes has been proposed about 30 years ago and malate dehydrogenase and citrate synthase were shown to interact at that time, the complex which may be present derived from Krebs cycle enzymes which may be more mitochondrial membrane-located and has been less studied.
Materials and Methods: We are using immunoprecipitation to identify enzymes in the mitochondrial membrane associated portion of the Krebs cycle.
Results: Here, we show interactions of several enzymes including methylmalonyl CoA mutase, oxoglutarate dehydrogenase, and succinyl CoA ligase by immunoprecipitation. In addition, propionyl CoA carboxylase, beta subunit also has been shown to interact with methylmalonyl CoA Mutase as one would expect.
Discussion: A complex of enzymes connecting the Krebs cycle and the propionate pathway should change our approach to therapeutic options.

O-030

Pancreatitis as a complication of organic acidaemias: the experience in a UK paediatric centre

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Background: Pancreatitis is a well recognised complication of the branch-chained organic acidaemias (OA). We report our experience of pancreatitis and associated complications in this patient group.
Methods and setting: Of the 126 patients followed in our centre with a diagnosis of branch-chain OA we identified and reviewed medical notes of nine patients with pancreatitis. Pancreatitis was recognised by symptoms, biochemical markers, radiological findings, or autopsy.
Results: 9 children with pancreatitis were identified among 126 children with OA of which 2/9 had methylmalonic and 7/9 had propionic acidaemia. Median age at presentation was 5 years (3–14). All surviving

patients (4/9) had more than one episode of acute pancreatitis. 1/4 developed chronic pancreatitis. There were 6 deaths: 4/6 died as a result of pancreatitis, 2/6 had pancreatitis found on post-mortem. 4/9 patients developed pancreatic pseudocysts. 6/9 patients had an abdominal ultrasound as a first radiological modality with 3/6 requiring further imaging. The mean peak serum amylase was 703U/l (317–1599) and average time to normalise (<100U/l) was 2.5 days.
Discussion: Pancreatitis evolves as a significant complication of OA's natural history and poses challenges for diagnosis, management and poor outcomes. Further work is required to understand pathophysiology of pancreatitis in this patient group.

O-031

A novel mouse model of methylmalonic acidemia circumvents neonatal lethality

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Methylmalonic acidemia (MMAemia) is an autosomal recessive disorder of propionate metabolism caused by a de?ciency of methylmalonyl-CoA mutase (MUT), which catalyzes the reversible isomerisation of L-methylmalonyl-CoA to succinyl-CoA in the mitochondria. MMAemia patients often present in the newborn period with ketoacidosis, lethargy, repeated vomiting, coma or even death, while survivors may suffer severe long-term complications such as renal failure and neurological impairment. Proper mutase function is also vital in mice, since Mut knock-outs (KO) display neonatal lethality. To circumvent this, we have created a constitutive Mut knock-in (KI) mouse model carrying the Mut p. M698K mutation, based on the human p. M700K patient mutation which causes an intermediate MMAemia phenotype. In vitro expression of constructs carrying these mutations in MUT-deficient human fibroblasts revealed comparable enzymatic activity (~3.5% of wt) and KM for adenosylcobalamin (~55 times wt), suggesting both mutations cause similar dysfunctions. The transgenic Mut-KI/KI mice survived post-weaning, had increased blood propionylcarnitine levels and showed no obvious physical differences compared to littermate controls. Heteroallelic Mut-KO/KI mice showed even higher levels of propionylcarnitine and reduced body weight. Our novel MMAemia mouse models allow the study of long-term complications and pathomechanisms of MMAemia, and may be used to investigate novel treatment approaches.

12. Organic acidurias: others

O-032

Identification of pharmacological chaperones in monogenic diseases caused by misfolded proteins

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Misfolded proteins are the cause of many monogenic diseases. Pharmacological chaperones (PCs) are small molecules that bind to and stabilize the folded conformation of mutant proteins. PCs help to avoid the degradation of partially folded proteins and favour their correct sub-cellular localization and function. Here, we have developed a strategy for the identification of compounds that may act as PCs taking Glutaric Aciduria type I as a disease model. Recombinantly-expressed and purified GCDH protein was used in a high-throughput assay based on differential scanning fluorimetry to screen compounds from a commercial library. Eight compounds were identified that thermally stabilized purified recombinant wild-type GCDH protein. In silico studies (molecular docking), together with in vitro conformational and stabilization assays (Blue Native-PAGE and monitoring the steady-state of the protein by Western-Blot) corroborated the stabilizing activity and efficacy of one compound on recombinant wild-type and mutant GCDH proteins. Furthermore, enzymatic studies in fibroblasts derived from two patients with different missense mutations leading to misfolded proteins (p. Arg402Trp and p. Val400Met) have substantiated the efficacy of the compound in a cellular system. Therefore, our strategy is an effective approach to identify compounds that may act as PCs for monogenic diseases caused by partially misfolded proteins.

O-033

Health-related quality of life in children and adolescents with intoxication-type inborn errors of metabolism – patients' and caregivers' perspectives

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Background: The progress in diagnosis and treatment of patients with intoxication-type inborn errors of metabolism (IT-IEM) such as urea cycle disorders or organic acidurias results in a growing number of long-term survivors. IT-IEM require intense patient and caregiver efforts in terms of adherence to treatment; fear of metabolic crises is always present. Therefore outcome evaluation should include health-related quality of life (HrQoL) of patients and families. Data on HrQoL in IT-IEM are sparse. A disease-specific HrQoL assessment tool does not yet exist.
Aim: To develop a disease-specific HrQoL questionnaire for IT-IEM patients.
Methods: Ten focus group interviews with patients and caregivers were conducted in three metabolic centres in Austria, Germany and Switzerland. Based on systematic qualitative content analysis of the interviews a pilot-version of a disease-specific HrQoL questionnaire is developed.
Results: Focus groups revealed insight into topics and issues relevant for patients' and caregivers' everyday life: constraints exerted by diet and constant monitoring, fear of clinical deterioration and the experience that a rare disease is not familiar to medical professionals.
Conclusion: Focusing on stressors and resources perceived by patients and caregivers enables to assess HrQoL both precisely and meaningful thus allowing improved evaluation of treatment strategies and interventions in future.
Conflict of Interest declared.

13. Carbohydrate disorders

O-034

Structural basis of glycogen branching enzyme deficiency and potential for pharmacological chaperone therapy

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Branching enzyme (GBE1) plays an essential role in glycogen biosynthesis, by generating α-1,6-glucosidic branches from α-1,4-linked glucose chains, to increase solubility of the glycogen polymer. Mutations on the GBE1 gene lead to the heterogeneous early-onset glycogen storage disorder type IV (GSDIV) or the late-onset adult polyglucosan body disease (APBD). Using an insect cell expression system, we generated wild-type human GBE1 for structural studies, and demonstrate that three predominant APBD-causing mutations (p. Y329S, p. R515H, p. R524Q) result in drastically-reduced expression level and destabilized mutant protein. Crystal structures of GBE1 in complex with a series of maltosaccharides reveal a conserved amylase core that houses the active centre for the branching reaction and harbours almost all GSDIV and APBD mutations. An additional binding cleft, away from the active centre but proximal to the p. Y329S mutation site, was found to bind different maltosaccharides and may represent part of an extended channel required to bind and position the highly-complex glycogen substrate for the branching reaction. Our data provide the first evidence of mutation-induced protein destabilization for GBE1, opening up the possibility for pharmacological chaperone therapy whereby the secondary binding cleft of GBE1 could be targeted by small molecules to stabilize the mutation protein and promote its catalysis.

O-035

Assessing white matter microstructure in classic galactosemia using neurite orientation dispersion and density imaging

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Background: White matter (WM) abnormalities have been observed in patients with classic galactosemia, but have only been qualitatively described. Our objective is to obtain a quantitative overview of the abnormalities, and to find correlations with outcome and behaviour.

Methods: We used diffusion weighted imaging to investigate WM microstructure in 8 patients (aged 16-21y) and 8 matched controls (aged 15-20y). Besides standard diffusion tensor imaging (DTI) analyses, we applied neurite orientation dispersion and density imaging (NODDI). This novel analysis technique estimates indices of neurite density (NDI) and orientation dispersion (ODI), showing great correspondence to histology

Results: Extensive WM abnormalities were found: NDI was lower in the patient group in bilateral anterior areas, and ODI was increased in middle regions of the left hemisphere. The regional profiles are in general agreement with the cognitive profile observed in galactosemia showing higher order cognitive impairments, and language and motor impairments, respectively. Moreover, WM properties correlated with several variables (e.g., age, age at onset of diet, visual working memory)

Conclusion: We provide the first quantitative evidence of WM pathology showing lower axon density and increased orientation dispersion, which is in agreement with abnormal myelin in the galactosemia group.

14. Disorders of fatty acid oxidation and ketone body metabolism

O-036

Triheptanoin treatment: long-term effects in mice with a long-chain fatty acid β-oxidation defect

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A rather new therapeutical approach in the treatment of very-long-chain acyl-CoA dehydrogenase deficiency (VLCADD) is represented by the application of the odd-medium chain triglyceride triheptanoin (C7) because of its supposed anaplerotic effects. However, the long-term outcome with C7 supplementation is still unknown. Here, we investigated the consequences of the supplementation over one year with C7 in VLCAD-deficient ($VLCAD^{-/-}$) mice. $VLCAD^{-/-}$ mice developed hepatic steatosis accompanied by a significant increase of saturated and monounsaturated fatty acids as a consequence of the strongly upregulated de novo biosynthesis and elongation of fatty acids. However, blood lipids were not altered by diet. Analysis of cardiac morphology and function revealed a marked hypertrophy with a concomitant reduction of ejection fraction in C7 supplemented $VLCAD^{-/-}$ mice in contrast to wildtype littermates. Importantly, although significantly reduced, the cardiac function of supplemented $VLCAD^{-/-}$ mice was not improved as compared to mutants under control diet. C7 diet has been suggested as being effective in the treatment of fatty acid oxidation disorders, however our data demonstrate that long-term C7 supplementation in the $VLCAD^{-/-}$ mice results in a disturbed lipid metabolism as observed upon even-medium-chain triglycerides. Moreover, C7 diet was not able to prevent the development of cardiac dysfunction in $VLCAD^{-/-}$ mice.

O-037

Multi-scale biology approaches in mouse genetic reference populations for investigating pathophysiology of inborn errors of metabolism

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Background: Amino acids (AA) and acylcarnitines (AC) levels are classical markers for inborn errors of metabolism (IEM), however their associations are poorly characterized in the general population, information which could reveal novel insight into pathophysiology underlying IEMs such as fatty acid oxidation disorders.

Methods: We characterized the influence of genetics and diet on approximately 75 plasma metabolites in 40 BXD mouse genetic reference strains fed either a chow or high fat diet. Archived molecular, genetic and clinical

data were integrated with the current metabolite data through expression QTL mapping as well as coexpression network analysis.
Results: We identified five metabolite modules that could be summarized by their major metabolite-type including long-chain ACs, short-chain ACs, branched chain AAs, other AAs and lipids. Metabolite modules most impacted by diet included short-chain ACs and lipids. Interestingly, long-chain AC and other AA modules significantly correlated to fasting-induced weight loss (FIWL). Integration of molecular and metabolite module data revealed that liver lipid metabolism and muscle protein degradation mechanistically linked these metabolites to FIWL.
Conclusion: Multi-scale biology approaches in mouse genetic reference populations provide an experimental model for investigating IEM pathophysiology, and revealed novel molecular pathways likely to affect fatty acid oxidation disorder pathophysiology.

O-038

Aberrant protein acetylation in fatty acid oxidation disorders

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Background/objectives: Post-translational modification of mitochondrial proteins by lysine acetylation has emerged as a crucial regulatory mechanism of mitochondrial function. We and others have recently identified that acetyl-CoA levels drive mitochondrial protein acetylation. Since fatty acid oxidation (FAO) is a major source of acetyl-CoA, we hypothesized that protein acetylation is altered in human fatty acid oxidation disorders.
Materials/methods: We analyzed protein acetylation in patient cells and mouse models resembling human FAO disorders using novel LC-MS/MS methods and followed acetylation dynamics in organelles and tissues.
Results: Protein acetylation was abundant in mitochondria and peroxisomes in human liver and was significantly increased in conditions associated with elevated FAO. In long chain acyl-CoA dehydrogenase (LCAD) knockout mice and PPARα knockout mice, acetylation was unresponsive to nutrient deprivation, indicating that FAO drives mitochondrial protein acetylation in vivo. Importantly, in FAO deficient patient cells, we found that protein acetylation was greatly disturbed.
Discussion/conclusion: We identified that mitochondrial protein acetylation relies on functional fatty acid oxidation and demonstrated for the first time that protein acetylation dynamics is disturbed in patients with FAO disorders. Since protein acetylation controls mitochondrial function, aberrant protein acetylation could play a role in the pathophysiology of FAO disorders.

15. Disorders of pyruvate metabolism and the Krebs cycle

O-039

Protein succinylation contributes to the molecular pathogenesis of succinyl-CoA synthetase (SCS) deficiency

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Background/objectives: Succinyl-CoA synthetase (SCS) deficiency is a rare but severe inherited metabolic disease caused by mutations in succinyl-CoA ligase (SUCL) genes. Patients suffer from a mtDNA depletion syndrome which is incompletely understood. We aim to understand the molecular pathogenesis of SCS deficiency by studying the mitochondrial dysfunction and the role of protein acylation modifications.
Materials/methods: Succinylation proteomics and functional follow-up was used to identify molecular targets in SCS deficiency. Mitochondrial (dys) function and mitochondrial enzymatic activities were analyzed in SCS deficient patient cells.
Results: We identified a striking increase in protein succinylation of multiple mitochondrial proteins in SCS deficient cells, due to build-up of succinyl-CoA. Targeted analysis uncovered that nucleoside diphosphate kinase (NDK), which provides deoxynucleotides for mtDNA synthesis, was hypersuccinylated and less active in SCS deficient cells. Interestingly, the human desuccinylase SIRT5 decreased succinylation and partially rescued NDK activity. Furthermore, SIRT5 increased mitochondrial spare capacity and decreased lactic acidosis in SCS deficient patient cells.
Discussion/conclusion: We identified a role for aberrant protein succinylation in the pathogenesis of SCS deficiency. Our findings demonstrate the relevance of protein acylation modification in inborn errors of metabolism and open-up possibilities for therapeutic intervention using sirtuin targeting compounds.

O-040

Phenylbutyrate increases pyruvate dehydrogenase complex activity in cells harboring a variety of defects

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Deficiency of pyruvate dehydrogenase complex (PDHC) is the most common genetic disorder leading to lactic acidosis. Phenylbutyrate enhances PDHC activity by increasing the unphosphorylated enzyme through inhibition of pyruvate dehydrogenase kinase (PDK) isoforms 1-to-3 and has potential for therapy of PDHC deficiency. PDHC deficiency is genetically heterogenous: most patients have defects in X-linked PDHA1 gene and remaining patients harbor defects in other components of the complex encoded by PDHB, PDHX, DLAT, DLD genes or in the regulatory enzyme encoded by PDP1. We investigated the response to phenylbutyrate in cell lines harboring all known gene defects of PDHC deficiency. Large PDHA1 deletions resulting in lack of detectable protein were unresponsive whereas increased PDHC activity was detected in most fibroblasts with PDHA1 missense mutations. Mutations affecting R349-α residue were directed to proteasome degradation and were consistently unresponsive to short-time drug incubation but longer incubation resulted in increased enzyme activity and protein levels due to an additional effect of phenylbutyrate as molecular chaperone. PDHC activity was enhanced in cells with mutations in PDHB, PDHX, DLAT, DLD, and PDP1 genes. In the prospect of a clinical trial, these results may allow prediction of response in patients with PDHC deficiency harboring a wide spectrum of defects.

16. Mitochondrial disorders: nuclear encoded

O-041

Mitochondrial NADP (H) deficiency due to a mutation in NADK2 causes dienoyl-CoA reductase deficiency with hyperlysinemia

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Background and objectives: Dienoyl-CoA reductase (DECR) deficiency with hyperlysinemia is a rare disorder affecting the metabolism of polyunsaturated fatty acids and lysine. We aimed to resolve the molecular basis of this condition. Case report: We found elevated plasma C10:2-carnitine and lysine in a patient with failure to thrive, developmental delay, lactic acidosis and severe encephalopathy suggestive of a mitochondrial disorder. Materials and methods: Exome sequencing and biochemical validation in patient fibroblasts.
Results: A causal mutation was identified in NADK2 encoding the mitochondrial NAD kinase. NADK2 is crucial for NADP biosynthesis evidenced by decreased mitochondrial NADP (H) levels in patient fibroblasts. DECR and lysine degradation require NADPH explaining their in vivo deficiency. DECR activity was also deficient in lysates of patient fibroblasts and could only be rescued by transfecting patient cells with functional NADK2, which illustrates that NADPH can activate and stabilize enzymes. NADPH also plays a role in various other mitochondrial processes. We found decreased oxygen consumption and increased extracellular acidification in patient fibroblasts, which may explain why the disease course is consistent with clinical criteria for a mitochondrial disorder.
Conclusion: DECR deficiency with hyperlysinemia is caused by mitochondrial NADP (H) deficiency due to a mutation in NADK2.

O-042

Eyes on MEGDEL - distinctive basal ganglia involvement in dystonia deafness syndrome

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Background: MEGDEL syndrome is a highly distinctive disorder characterized by 3-methylglutaconic aciduria, severe developmental delay, deafness, progressive spasticity and dystonia. Mutations are found in SERAC1, encoding a phosphatidylglycerol remodeller essential for both mitochondrial function and intracellular cholesterol trafficking. We hypothesized a characteristic MRI pattern.
Methods: 35 complete MRI studies of 24 patients were systematically re-evaluated.
Results: In the neonatal period the basal ganglia appear normal on MRI (stage 0). After occurrence of the first neuroradiological signs during the first year of life, the brain images display a rapidly progressive course. Most patients show end stage disease by the age of three years. The pallidum is the first structure to be affected (stage 1). In stage 2, prominent swelling of caudate nucleus and putamen is seen with sparing of the mid-dorsal putamen; we suggest referring to as “putaminal eye”. This “eye” represents an apparently healthy region in the mid-dorsal putamen which gradually becomes smaller, leaving a small cystic remnant with gliotic borders (end stage 4).
Conclusion: This study adds a typical MRI pattern of basal ganglia involvement as another hallmark to MEGDEL syndrome. The pathognomic "putaminal eye" should easily allow the diagnosis of MEGDEL solely based on MRI pattern recognition.

O-043

Natural course of TMEM70 deficiency in 48 patients

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TMEM70 deficiency is the most common nuclearly encoded cause of ATP-synthase deficiency. We characterize the natural course in 48 patients with mutations in TMEM70 gene. The age of patients ranged between 1 month and 26.5 years. Neonatal onset was observed in 41 patients, infantile onset in 6; one child was symptomatic from 2 years. The most frequent symptoms were hypotonia, respiratory and heart failure accompanied by lactic acidosis, 3-methylglutaconic aciduria and hyperammonemia. In 40 children surviving the neonatal period, common symptoms included failure to thrive (94 %), short stature (89 %), microcephaly (71 %), developmental delay (98 %), hypotonia (95 %), non-progressive cardiomyopathy (89 %), pulmonary hypertension (22 %), WPW syndrome (13 %), facial dysmorphy (66 %) and hypospadias in boys (50 %). One or more acute metabolic crises developed in 41 children, often associated with regression in development. Attacks of hyperammonemia reacted well to infusion with glucose, lipid emulsion and ammonia scavengers. Ten-years survival was 63 %, no child died after the age of 5 years. We found 11 various mutations, mutation c.317-2A>G was the most common.
Conclusion: TMEM70 deficiency is a multiethnic multisystemic disease with variable outcomes depending mainly on adequate management of the hyperammonemic crises after birth or in childhood. Supported by IGA NT 14156/3, IGA NT 13114/4.

O-044

Diagnosis and molecular basis of mitochondrial respiratory chain disorders in Japan: exome sequencing for identification of disease genes

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Background: Mitochondrial respiratory chain disorders (MRCDs) are a group of the most common congenital metabolic disorders of energy production. We aimed to obtain a correct diagnosis of MRCDs and identify the causative genes.
Methods: MRCDs were basically diagnosed using enzyme analysis including in vitro enzyme assay and blue-native polyacrylamide gel electrophoresis. Next, the whole exome analysis was performed in those with no known mtDNA abnormalities, and the obtained candidate causal genes were confirmed by rescue experiments or other methods such as siRNA experiment.
Results: Of the 1033 candidate patients, 341 were diagnosed with MRCDs by enzyme analysis. Complex I deficiency was most frequent (41 %). Of the 204 patients analyzed, 56 had mitochondrial DNA pathogenic mutations. Of the 150 patients analyzed using exome sequencing, 33 known genes such as NDUFAF6, ACAD9, and BOLA3, and 13 unreported candidate genes for MRCDs were identified. Furthermore three cases with a BOLA3 mutation had the homozygous mutation previously identified as a common mutation in Japan.
Discussion: Using high-speed sequencers, we are currently identifying the causative genes and pathogenic mechanisms. Our study aims to illustrate how large-scale sequencing, coupled with functional prediction and experimental validation, can help in identifying disease mutations in MRCDs.

O-045

Two siblings with myopathy, mild mental retardation and combined OXPHOS deficiencies caused by a mutation in the gene encoding asparaginyl tRNA synthetase, NARS2

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We report a pathogenic mutation in NARS2, a gene coding for an asparaginyl tRNA synthetase, previously not associated with a disease. The two reported siblings were from consanguineous origin and presented with myopathy and mild mental retardation. Using homozygosity mapping we found two candidate genes and detected a pathogenic homozygous mutation (c.822G>C; p. Q274H) by conventional dideoxysequencing in NARS2. In both siblings, a combined OXPHOS deficiency involving complex I and IV was detected in skeletal muscle. Additional analysis by BN-PAGE followed by in-gel activity staining confirmed these findings and revealed the presence of complex V subcomplexes, the hallmark of defective intramitochondrial protein synthesis. The pathogenic character of the mutation was demonstrated by northern blotting experiments. The findings in these siblings add a new aminoacid tRNA synthetase (aaRS) to the list of nuclear defects causing OXPHOS deficiency and increase the likelihood that ultimately all mitochondrial aaRS will be associated with clinical phenotypes. During the last decade, an increasing number of new molecular defects residing within the nuclear genome have been recognized causing OXPHOS deficiencies. Most of these genes encode proteins related to mtDNA transcription or translation. A particular subgroup encodes the mitochondrial aaRS, responsible for linking the correct amino acid to its corresponding tRNA.

O-046

Protein misfolding in nuclear encoded mitochondrial proteins can lead to impaired mitochondrial import

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Background: Inherited metabolic mitochondrial diseases are predominantly caused by dysfunction of nuclear encoded proteins. Mitochondrial matrix proteins require translocation through the mitochondrial membrane with subsequent protein refolding to acquire functional states. It is well accepted that missense mutations can induce protein misfolding. However, it is not known whether missense mutations have impact on translocation of the affected proteins.
Methods: We cloned 30 different missense mutations of glutaryl-CoA dehydrogenase causing glutaric aciduria type I and medium-chain acyl-CoA dehydrogenase causing medium-chain acyl-CoA dehydrogenase deficiency. Variant proteins were expressed and the processing states of precursor and mature proteins were analyzed. Localization of precursors at the mitochondrial membrane was determined by a protease protection assay.
Results: Variant proteins displayed a shift in the ratio of precursor to mature protein towards precursor indicating impaired mitochondrial import. Only a minor share of variant proteins localized to the mitochondrial matrix. When compared to wild-type proteins, precursors of variant protein showed a higher sensitivity to proteinase K digestion pointing towards a misfolded protein structure.
Discussion: We demonstrated that protein misfolding has impact on mitochondrial trafficking. Thus, impaired translocation of variant proteins into mitochondria adds to the molecular mechanisms in inborn errors of nuclear encoded mitochondrial proteins.

O-047

Exome sequencing in pediatric mitochondrial disorders: lessons learned from 400 cases, clinical translation, and associated challenges

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Defects of the mitochondrial pyruvate oxidation route comprise a clinically and genetically heterogeneous group of disorders. Together with the fact that many disease genes are unknown, exome sequencing is considered an efficient tool to genetically diagnose these patients and is underway to be implemented at an early stage in the diagnostic algorithm.
Over the course of three years we investigated >400 pediatric cases with suspected mitochondrial disorders using exome sequencing. We established firm molecular diagnoses in about half of them with mutations being identified in almost 100 different genes. Clinically relevant mutations were identified in both known disease genes (≈25 %) and genes previously not associated with mitochondrial dysfunction (≈25 %). With larger numbers of individuals sequenced, joint analyses are increasingly powerful to identify promising candidates based on statistical evidence such as a new tRNA-modifying enzyme.
Emerging issues in routinely applied diagnostic exome sequencing are turnaround time, clearness of reports, and considerations related to accidental findings. Existing analysis pipelines need to be optimized for rapid prioritization of potentially treatable defects e.g. in cofactor metabolism and should enable queries for clinically relevant DNA variants in actionable disease genes. However, the latter require comprehensive counselling of the patients and their families, and adequate documentation.

O-048

Depressive behavior and mitochondrial dysfunction in a recessive Ndusf4 mouse model

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Decreased energy production in the brain has been hypothesized to play an important role in the pathophysiology of depression in both mitochondrial patients and in the general population. To test the hypothesis, whether suboptimal mitochondrial function contributes to the pathobiology of depression we evaluated whether genetically engineered mice with decreased mitochondrial function have an impaired ability to adapt to stressors and more prone to develop depression.
Our unique mouse model has a mutant Ndufs4 allele that consists of a gene trap insertion in an early intron of the locus, resulting in premature termination and deficiency of NDUFS4 protein. As a small amount of wild type protein is still produced, these animals are viable beyond 18 months of age, unlike constitutive Ndufs4 knockouts that die by 8 weeks of age. This specific mutation results in 70 % reduction in OXPHOS complex I activity in the brain.
We found that both acutely and chronically stressed NDUFS4 deficient animals exhibited depression-like phenotype as reflected by increased immobility time in the Prosolt swim test. No difference in anxiety-like behavior was detected in the marble burying and novelty suppressed feeding tests. These results support our hypothesis that impaired mitochondrial function increases the risk to develop depression.

19. Disorders of purines, pyrimidines and nucleic acids

O-049

Clinical, biochemical and molecular analysis of 13 Japanese patients with β-ureidopropionase deficiency demonstrates high prevalence of the c.977G>A (p. R326Q) mutation

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β-Ureidopropionase (βUP) deficiency is an autosomal recessive disease characterized by N-carbamyl-β-amino aciduria. To date, only 16 genetically confirmed patients with βUP deficiency have been reported. Here, we report on the clinical, biochemical and molecular findings of 13 Japanese βUP deficient patients. In this group of patients, three novel missense mutations (p. G31S, p. E271K, and p. I286T) and a recently described mutation (p. R326Q) were identified. The p. R326Q mutation was detected in all 13 patients with 8 patients homozygous for this mutation. Screening for the p. R326Q mutation in 110 Japanese individuals showed an allele frequency of 0.9 %. Transient expression of mutant βUP enzymes in HEK293 cells showed that the p. E271K and p. R326Q mutations cause profound decreases in activity. Conversely, the p. G31S and p. I286T mutations possess residual activities of 50 and 70 %, respectively, suggesting we cannot exclude the presence of additional mutations in the non-coding region of the UPB1 gene. Analysis of a human βUP homology model revealed that the effects of the mutations (p. G31S, p. E271K, and p. R326Q) on enzyme activity are most likely linked to improper oligomer assembly. Highly variable phenotypes ranging from neurological involvement to asymptomatic, were observed in diagnosed patients. Screening for βUP deficiency should be included in diagnosis of patients with unexplained neurological abnormalities.

20. Lipid and lipoprotein disorders, porphyrias

O-050

Molecular mechanism of Sjögren Larsson Syndrome causing mutations in fatty aldehyde dehydrogenase

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More than 80 mutations in the gene coding for membrane-bound fatty aldehyde dehydrogenase (FALDH) are known, that result in toxic accumulation of lipid metabolites and development of the Sjögren Larsson Syndrome (SLS), a rare disorder characterized by skin defects and mental retardation. In earlier studies, we tracked the intracellular metabolic fluxes with fluorescence labelled lipids and found abnormal levels of fatty alcohols in patient cells. However, the molecular mechanisms how many SLS causing mutations affect enzymatic activity are largely elusive.
Here, we present the first crystallographic structure of human FALDH, which displays a previously unrecognized element in its C-terminal region: A "gatekeeper" helix extends over the adjacent subunit and controls the access to the substrate cavity. At the same time this gatekeeper helix also helps to orientate both substrate tunnel entrances towards the membrane surface and ensures efficient substrate transit between membranes and catalytic site. Activity assays demonstrated that the gatekeeper helix is important for directing substrate specificity of FALDH towards long-chain fatty aldehydes. As a result of our analysis we found a series of SLS causing mutations that impair the correct orientation of the gatekeeper helix and thereby diminish the enzymatic capacity of FALDH to clear toxic fatty aldehydes.

21. Peroxisomal, sterol and bile acid disorders

O-051

A whole-organelle peroxisomal protein interaction screen identifies potential novel roles of ABCD1 in pathways beyond fatty acid import

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X-linked adrenoleukodystrophy (X-ALD) is caused by impaired function of ABCD1, a peroxisomal transporter involved in fatty acid import. X-ALD pathophysiology is also linked to dysregulation of metabolism of amino acids, carbohydrates, and lipids as well as oxidative phosphorylation. To map the links of ABCD1 with peroxisomal biogenesis and metabolism, we

performed binary protein-protein interaction assays with subsequent network and bioinformatics analyses. ABCD1 was screened against a library comprising 97 peroxisomal proteins (92 % of all) by means of bioluminescence resonance energy transfer (BRET). We confirmed homooligomerization of ABCD1 and known interactions with ABCD2, ABCD3, and PEX19. We found novel interactions with ALDH3A2, FAR1, PXMP2, DAO, PECI, PEX10, PEX13, PEX5, and PIPOX. Network analysis revealed that these proteins are involved in 5 distinct non-overlapping biological processes (lipid metabolism, matrix protein import, peroxisome organization, membrane assembly, protein/amino acid metabolism). The OMIM database lists 8 of the 12 genes encoding interaction partners as disease genes and X-ALD showed overlap in signs and symptoms with these disease genes. In summary, these data substantiated and expanded the role of ABCD1 in cellular processes beyond fatty acid transport. Novel interaction partners of ABCD1 identified in this study may provide targets for alternative approaches in X-ALD therapy.

O-052

Identification of a novel defect in bile acid biosynthesis due to peroxisomal ABCD3 deficiency

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Background: ABCD3 is one of three ATP-binding cassette (ABC) transporters present in the peroxisomal membrane catalyzing the ATP-dependent transport of substrates for metabolic pathways localized in peroxisomes. So far the precise function of ABCD3 is not known. Here we report the identification of the first patient with a defect of ABCD3 .
Case report: The patient presented with hepatosplenomegaly and severe liver disease, and showed a striking accumulation of the peroxisomal C27-bile acid intermediates. For this reason, peroxisomal parameters in fibroblasts were studied.
Results: Genetic analysis revealed a homozygous deletion at the DNA level of 1758 bp (predicted protein p. Y635NfsX1). Liver disease progressed and the patient required liver transplantation at 4 years of age, but expired shortly after transplantation. To corroborate our findings in the patient, we studied a previously generated Abcd3 knockout mouse. Analysis of bile acids revealed a reduction of C24-bile acids, whereas C27-bile acid intermediates were significantly increased in ABCD3 −/− mice.
Conclusion: Both in the patient and in the ABCD3 −/− mice there was a marked bile acid biosynthesis defect. Our studies show that ABCD3 is involved in the transport of C27-bile acids into the peroxisome and that this is a crucial step in bile acid biosynthesis.

O-053

PEX11β deficiency: a novel human peroxisome biogenesis disorder affecting peroxisome division

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Objective: We report the clinical, biochemical and genetic characterization of 7 patients with a defect in PEX11β.
Case report: All 7 patients, i.e. 3 males (including one sib pair) and 4 females (including 3 sibs), presented with congenital cataracts. The older patients all have mild intellectual disability, ataxia and sensorineural deafness. In addition, most of them presented with a short stature and convulsions.
Results: Biochemical parameters did not show clear peroxisomal abnormalities in plasma, except for (occasionally) mildly abnormal very long chain fatty acid levels. Subsequent studies of biochemical parameters in fibroblasts were normal to slightly abnormal. However, immunofluorescence microscopy analysis of fibroblasts from 2 patients showed peroxisomes that were often enlarged or elongated and frequently arranged in rows. All patients had 2 null mutations in the PEX11β gene.
Conclusion: PEX11β deficiency is a novel defect in peroxisome division that expands the clinical and genetic spectrum of peroxisomal disorders. Although it is difficult to diagnose by standard laboratory analysis, due to the absence of clear biochemical abnormalities reflecting peroxisome dysfunction, the clinical phenotype and biochemical presentation in plasma and fibroblasts is consistent in these 7 patients.

22. Lysosomal disorders: mucopolysaccharidoses, oligosaccharidoses

O-054 Withdrawn

23. Lysosomal disorders: sphingolipidoses

O-055

Resting cerebral blood flow is increased in white matter but not grey matter in adult Fabry disease: an MRI arterial spin labeling study

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Background: To understand mechanisms of brain injury in Fabry disease biomarkers are needed. Cerebral blood flow (CBF) measurement using MRI arterial spin labeling (ASL) is promising in assessing cerebral haemodynamics; we used ASL to test the hypothesis that CBF is altered in Fabry disease.
Methods: We included 21 adults with Fabry disease and matched controls. Resting CBF was quantified in anatomically-defined regions of interest (ROIs) in white and deep grey matter using an ASL.
Results: The mean whole brain CBF was higher in Fabry patients (29.70 vs 23.64 ml/100 ml/min (p=0.027)). The mean white matter CBF was significantly higher in the Fabry group (24.83 vs 18.34 ml/100 ml/min (p=0.043)). In contrast, there was no statistically significant increase in mean deep grey matter CBF in the Fabry group (39.73 vs 35.88 ml/100 ml/min (p=0.24)). Within white matter regions, statistically significant increases were only found in temporal white matter and corpus callosum splenium.
Conclusion: Resting CBF is increased in Fabry disease; the increase was statistically significant in white matter, but not deep grey matter. These results suggest that Fabry vasculopathy preferentially affects the small vessels supplying white matter, particularly posteriorly. ASL is a promising tool in cerebrovascular manifestations of Fabry disease.
Conflict of Interest declared.

O-056

Lipid raft abnormalities and protein trafficking in Niemann-Pick disease type C1

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Background: Niemann-Pick disease type C1 (NPC1) is a lysosomal storage disease characterized by entrapment of cholesterol and sphingolipids. Membrane microdomains, known as lipid rafts (LR), are involved in membrane transport and signaling. LR are enriched in cholesterol and sphingolipids and resistant to extraction with non-ionic detergents, such as Triton X-100. Accumulation of cholesterol and sphingolipids in NPC1 may alter LR composition.
Methods: LR status and trafficking of LR-associated dipeptidylpeptidase IV (DPPIV).were analyzed in skin fibroblasts from patients with NPC1.
Results: Sucrose-density gradients of Triton X-100 cellular lysates revealed a shift of the LR-marker flotillin 2. Cell surface expression of DPP IV increased 2-fold in NPC 1 cells, the overall protein level of DPP IV remained constant. FACS analysis corroborated the increase of DPP IV at the cell surface. This accumulation results from delayed lipid rafts-dependent endocytosis as assessed by reduction of internalized biotin-labeled DPP IV and reduced co-localization of DPP IV with the endosomal marker EEA-1 in NPC 1 cells. N-butyl-deoxynojirimycin restored LR levels and endocytosis of DPP IV. This showed that the accumulation of storage material is related to LR-abnormalities.
Conclusion: We conclude that alterations of LR constitute a potential pathophysiological mechanism in NPC 1, NB-DNJ restores biochemical anomalies.

24. Lysosomal disorders: others

O-057

Outcome of renal transplantation in adult cystinosis patients

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Introduction: Cystinosis is a rare lysosomal disorder leading to end stage renal disease (ESRD) in more than 90 % of patients before 20 years of age. We report outcome of renal transplantation in 30 adult cystinosis patients.
Material and methods: Data of 30 adults patients were retrospectively analysed in 5 French university centers.
Results: 31 transplantations in 30 patients were performed between 1980 and 2013. Median age at transplantation was 20.4 years (7–36.5). At transplantation, all patients had corneal deposits, 3 had diabetes and 7 hypothyroidism. All patients except one were treated by oral and ocular cysteamine at day 0. Median leukocyte cystin level was 1.4 nmol ½ cystin per mg protein (0.4-4.7) at day 0, and 1.9 (0.5-10) during follow up. Median age at last follow up was 32.7 years (18.7-54.5). Cystinosis complications occurred during follow up: diabetes mellitus (n=4), hypothyroidism (n=1), liver involvement (n=1), neurologic involvement (n=1). Six patients (19 %) reached ESRD during follow up, requiring dialysis, because of chronic graft dysfunction (n=5) or graft rejection (n=1).
Conclusion: Renal transplantation appears to be safe and efficient in adult cystinosis patient. Nevertheless, long-term evolution of these patients remains unknown.

O-058

Gene disruption of Mfsd8/Cln7 in mice provides the first animal model for CLN7 disease

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Background: Mutations in the major facilitator superfamily domain containing 8 (MFSD8) gene coding for the lysosomal CLN7 membrane protein result in CLN7 disease, a lysosomal storage disease of childhood characterized by autofluorescence, neuroinflammation, photoreceptor- and neurodegeneration.
Materials and Methods: We disrupted the Mfsd8 gene by insertion of a lacZ gene-trap cassette in mice and analyzed the impact of Cln7 depletion on neuronal and visceral tissues.
Results: Mfsd8 gene trap mice resembled biochemically the neuronal ceroid lipofuscinosis-phenotype of CLN7 patients including the accumulation of autofluorescent material in the brain and peripheral tissues and of subunit c of mitochondrial ATP synthase in different brain regions, and the degeneration of photoreceptor cells in the retina. Lysosomal storage was found in large neurons of the medulla, the hippocampus and the cerebellum. The ultrastructure of the storage material revealed dense lamellar bodies with irregular forms within cerebellar and hippocampal neurons. In the brain loss of Cln7 was accompanied by mild reactive microgliosis and subtle astrogliosis by 10 months of age, respectively.
In summary we have generated a mouse model which is partly valuable as some neuropathological features of human CLN7 disease are recapitulated thus representing an animal model to study CLN7-specific disease mechanisms.

25. Lysosomal disorders: treatment, enzyme replacement therapy

O-059

Levomepromazine as a treatment for non-epileptic movement disorder in advanced Sanfilippo disease (mucopolysaccharidosis type III, MPSIII)

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Objective: MPSIII leads to cognitive decline and epilepsy as the disease advances. Some patients additionally develop a distressing non-epileptic movement disorder (NEMD). Medical treatment for NEMD is unsatisfactory. Levomepromazine, a low-potency antipsychotic with multiple receptor effects, is widely used in Europe for treating anxiety, agitation and nausea in palliative care. We report our experience of using levomepromazine in the palliative care of MPSIII patients.
Methods: 30 MPSIII patients were treated at our hospital between 2004–2014. 16 were/are being treated through the end-stage of their disease. NEMD was judged to be present from case record review in 9 (56 %) end-stage patients but may have been historically under-recognised. Where considered suitable, levomepromazine was started at 6.25 mg twice daily,

increasing according to response. Efficacy was judged subjectively by carer report and objectively in some patients with an Actigraph recording upper limb movements.
Results: Of 9 advanced MPSIII patients with NEMD, 8 (89 %) received levomepromazine. Subjective improvement in NEMD severity/distress was reported in all 8 children. Where performed, actigraphy demonstrated reduced upper limb movement frequency. No patient stopped treatment due to side effects, the most frequent being sedation.
Conclusions: Levomepromazine appears to be well tolerated and effective for managing distressing NEMD in advanced MPSIII.

O-060

Treatment with Migalastat results in reduced levels of disease substrate and stable renal function in a Phase 3 study of Fabry disease

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Objectives: Assess effects of migalastat, a pharmacological chaperone for alpha-galactosidase A, on disease substrate and glomerular filtration rate (GFR) in the FACETS study (NCT00925301).
Methods: This study included a 6-month double-blind placebo-controlled period (Stage 1), a 6-month open-label period (Stage 2), and a 12-month extension. Sixty-seven subjects (24 male) were randomized. Stage 2 analyses of kidney interstitial capillary GL-3 inclusions per capillary (IPC), plasma lyso-GB_3, and GFR were performed in subjects with amenable GLA mutations.
Results: In the pre-specified primary analysis at month 12, subjects randomized to placebo and switched to migalastat at month 6 demonstrated statistically significant (±SEM) decreases in IPC (−0.31±0.10; p=0.013). Subjects randomized to migalastat demonstrated statistically significant decreases in IPC (−0.25±0.10) at month 6, compared to an increase (+0.07±0.13) on placebo (p=0.008). Statistically significant reductions in plasma lyso-Gb_3 were observed after 6 months of treatment with migalastat in both groups (p=0.0033, p<0.0001). Reductions in IPC and plasma lyso-GB_3 were maintained at month 12. GFR (eGFR: CKD-EPI, MDRD, and iohexol mGFR) remained stable after 18–24 months of treatment; mean annualized GFR changes were −0.30±0.66, +0.79±1.03 and −1.51±1.33 ml/min/m^2, respectively.
Conclusions. Treatment with migalastat resulted in statistically significant and durable reductions in disease substrate and stable renal function over 18–24 months.
Conflict of Interest declared.

O-061

Enzyme replacement therapy (ERT) for mucopolysaccharidosis VII (MPS VII; Sly disease) reduces lysosomal storage in a phase 1/2 clinical study

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Background: ERT has significantly improved the clinical outcomes of MPS I, II, VI and IVA. We evaluated the effects of rhGUS intended as an ERT for MPS VII, a disease caused by beta-glucuronidase deficiency.
Methods: We are conducting an open-label study to assess safety, efficacy and dose of rhGUS with interim data presented here. Three subjects with MPS VII (5.6 – 25.5 years of age) and urinary glycosaminoglycan (GAG) excretion at ≥2-fold over normal at baseline were infused every other week with 2 mg/kg rhGUS for 12weeks. To assess for optimal dosing, subjects are continued on 1 mg/kg and 4 mg/kg rhGUS for intervals of 8weeks.
Results: Following the first infusions of rhGUS at 2 mg/kg, the three subjects experienced a rapid decline in urinary GAG excretion of ~40 % vs. baseline. Ultrasound assessments confirmed significant reduction in hepatomegaly. Infusions of rhGUS were well tolerated, with no infusion-associated reactions or serious adverse events observed after up to 16weeks of treatment. The study is ongoing. Assessments include the six minute walk test, pulmonary function tests and PROs.
Conclusions: Interim results from the first clinical study of rhGUS provide evidence of lysosomal storage reduction in patients with MPS VII without infusion-associated reactions reported to date.
Conflict of Interest declared.

26. Glycosylation disorders/CDG, protein modification disorders

O-062

Biochemical role of GMPPA, the subunit of GDP-mannose pyrophosphorylase mutated in triple-A like syndrome

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Mammalian GDP-mannose pyrophosphorylase catalyzes the formation of GDP-mannose from mannose 1-phosphate and GTP. The enzyme comprises two types of subunits (GMPPA and GMPPB), which form an oligomeric complex. Patients with mutations in the GMPPA gene display a triple A-like syndrome whereas patients with mutations in the GMPPB gene show a dystroglycanopathy, indicating different roles for the two subunits. In lymphoblasts from patients with mutations of GMPPA, only GMPPB is present, whereas in healthy controls both subunits are present. Lymphoblasts from patients and controls showed similar enzymatic activity, but a significant increase of intracellular GDP-mannose was found for the patients, indicating that GMPPA may serve as a regulatory subunit. Purification of the GMPPA/B complex and of GMPPB from patients' lymphoblasts showed that the GMPPA/B complex was more strongly inhibited by GDP-mannose than GMPPB alone, with apparent Ki's of≈ 3 mM and 8 mM, respectively. Additionally, in HEK293 cells stably co-transfected with GMPPA and GMPPB, the intracellular GDP-mannose levels were decreased by 50 % compared to cells transfected with GMPPB alone. Taken together our results indicate that GMPPA is a feedback inhibitory subunit of the GDP-mannose pyrophosphorylase complex.

28. Disorders of vitamins, cofactors and trace elements

O-063

Characterization of functional domains of the cblD (MMADHC) gene product

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Vitamin B_{12} (cobalamin, Cbl) must be converted into two coenzyme forms, methylcobalamin (MeCbl) and adenosylcobalamin (AdoCbl), to maintain intracellular homeostasis of homocysteine and methylmalonic acid, respectively. In cblD patients three types of MMADHC mutations exist: 1) truncating mutations N-terminal to Met116 cause methylmalonic aciduria (cblD-MMA) due to AdoCbl deficiency; 2) null C-terminal mutations (p. Y140-R250) cause methylmalonic aciduria and homocystinuria (cblD-MMA/HC) due to AdoCbl and MeCbl deficiency; 3) missense mutations in a conserved region (p. D246-L259) cause homocystinuria (cblD-HC) due to MeCbl deficiency. To better understand MMADHC domain boundaries, we made 20 mutations (15 missense and 5 C-terminal truncations) across p. P154-S287 and tested rescue of MeCbl and AdoCbl synthesis in cblD-MMA/HC fibroblasts. Our results reveal a new region (p. R197-days) responsible for MeCbl synthesis, mutation of which gave a similar cellular phenotype as cblD-HC. Mutations in two other regions (p. D226-days and p. L259-R266) gave cellular phenotypes intermediate to those of cblD-HC and cblD-MMA/HC. C-terminal truncation of >20 amino acids resulted in a cblD-MMA/HC like cellular phenotype, while truncation of 10–20 amino acids resulted in a cblD-HC like cellular phenotype. These data suggest that specific regions of MMADHC are involved in differential regulation of AdoCbl and MeCbl synthesis and help better define their boundaries.

O-064

Riboflavin responsive multiple acyl-CoA dehydrogenation deficiency due to deletion of p. Ser495 in the FAD synthase

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Multiple acyl-CoA dehydrogenation deficiency (MADD) is an autosomal inherited disease characterized by dysfunction of multiple mitochondrial flavoprotein dehydrogenases. Riboflavin responsive forms of MADD have been explained mainly by gene variations in the electron transfer flavoprotein dehydrogenase (ETFDH) and more recently by variations in the riboflavin transporter (RFT) genes, the latter once with mostly progressive neurological symptoms. We present two siblings suffering from riboflavin responsive MADD, one present with cardiomyopathy at 2 months of age and the other in the neonatal period, but with no disease-causing variations in the ETFDH or RFT genes. Sequence analysis revealed that both siblings were homozygous for a deletion of c.1484_1486 in the FLAD1 gene, causing deletion of Serine-495 in the FAD synthase, which converts FMN into the obligate FAD cofactor for the dysfunctional flavoprotein dehydrogenases in MADD. Western blot analysis showed decreased levels of FAD synthase in the patient's fibroblast cells as compared to controls suggesting that deletion of Serine-495, which is located in the highly conserved PAPS reductase domain, gives rise to an unstable protein. This is the first report of a disease-causing FLAD1 variation and it expands the number of disease genes and symptoms in a heterogeneous treatable disease.

O-065

Update on novel treatments for pyridoxine-dependent epilepsy due to ATQ deficiency

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Background & Objectives: Seventy-five percent of patients with pyridoxine-dependent epilepsy (PDE) due to Antiquitin (ATQ) deficiency suffer developmental delay and/or intellectual disability (IQ <70) despite adequate seizure control with high dose pyridoxine. We aimed to assess the safety and efficacy of two novel therapeutic strategies to reduce accumulation of potentially toxic intermediates in this cerebral lysine degradation defect.
Methods: In two open-label observational studies, seven children with confirmed ATQ deficiency were started on dietary lysine restriction with regular nutritional monitoring. Biochemical outcomes were evaluated using pipecolic acid and AASA levels in body fluids; developmental/cognitive outcomes were evaluated using age-appropriate tests and parental observations. Two other patients received additional arginine supplementation to reduce cerebral lysine flux.
Results: Lysine-restriction was well tolerated and diet is safe, resulted in partial normalization of lysine intermediates in all body fluids in all patients (up to 80 % reduction AASA in cerebrospinal fluid), with beneficial effects on seizure control and psychomotor development. Additional arginine fortification resulted in dramatic improvement of psychomotor development in 2 patients. Early intervention seems most effective.
Discussion: To disseminate these novel strategies, and generate more evidence our PDE Consortium published Recommendations, developed a Digital Diet App and established a RedCap study database (www.pdeonline.org/).

01. Inborn errors of metabolism: general, adult

P-001

Consanguinity, endogamy and inborn errors of metabolism in Oman: a cross sectional study

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Background: Sultanate of Oman has relatively high rates of consanguinity. Reports suggest that the incidence of Inborn Errors of Metabolism (IEM) is also high.
Objectives & Methods: This retrospective cross-sectional study was designed to evaluate the number of patients with IEM being followed at the only two tertiary centers in Oman where these patients are managed, and to calculate the consanguinity rate among these families. The electronic medical records were reviewed.
Results: A total of 285 patients are being followed with IEM at the two centers involved. 162 (56.8 %) were males and 123 (43.2 %) were females. The history of consanguinity was documented or available for 241 patients, and 229 patients (95 %) were born to consanguineous parents related as

second cousins or closer. First cousin marriages were reported in 191 families (79.3 %), while 31 patients (12.9 %) were born to second cousins. The parents of 5 patients in this study (2 %) were related as double first cousins. The average coefficient of inbreeding (F) in this study was 0.081. 17 patients (6 %) had associated co-morbid conditions other then IEM.
Conclusion: The study highlights the clinical burden of IEM in Oman and emphasizes the high consanguinity rates among parents of patients with IEM in Oman.

P-002

Novel FAH mutations in Russian patients with tyrosinemia type 1

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Background and objectives: The history of NTBC treatment in Russia began in 2008. Since 2005, 18 patients were diagnosed with hereditary tyrosinemia 1a and 1b type. 3 patients died from sepsis before NTBC registration in Russia.
Patients and methods: Our experience includes 14 alive patients with HT1, both acute and chronic. That fact suggests that most patients with severe acute form of HT1 die misdiagnosed. We confirmed the disease not only by biochemical techniques (based on succinylacetone concentration in urine or serum and the concentration of tyrosine in serum) but also by molecular genetic diagnosis. Direct sequencing of all coding regions of FAH gene was performed in all patients.
Results: The 4 major mutations (c.1062+5G>A, c.554-1G>T, p. Pro342Leu, p. Arg174X) were found in 10 patients. The first novel homozygous mutation c.1090G>C (p. Glu364Gln) was found in two mongoloid children of Yakut's and Buryat's origin. The second novel homozygous mutation c.1025C>T (p. Pro342Leu) was found in girls from Dagestan. Another child of Armenian origin was the carrier of the third novel mutation c.998A>C (p. His333Pro) in compound-heterozygous state with c.497 T>G FAH mutation, previously described in patient with HT1.

P-003

Rhabdomyolysis in LPIN 1 deficiency is not limited to children

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Introduction: We present a case of severe rhabdomyolysis in a young adult whose only previous episode was at age 3.
Case: A 22 year old male presented to hospital with acute stiffness the day after significant alcohol ingestion, in the course of which he acquired a tattoo. Preceding this event he had evidence of a mild upper respiratory tract infection. Peak CK of 245,000 was associated with renal impairment (Cr 0.2 mmol/L). Family history was significant for a sister who had died in hospital aged 4 years following a muscle biopsy. Genetic testing had been performed several years prior to this event but the family was unaware of the results: the patient was homozygous for the common Exon 18–19 deletion in LPIN1.
Discussion: LPIN 1-related rhabdomyolysis is generally considered a disease of childhood. However, a mouse model of LPIN1 deficiency has demonstrated exacerbated hepatic steatosis on alcohol exposure, suggesting a link with this episode.
Conclusion: Patients with LPIN 1 deficiency should be warned that they remain at risk of life-threatening rhabdomyolysis episodes in adulthood and should avoid excessive alcohol intake.

P-004

Clinical manifestations of mitochondrial respiratory chain defects in an adult population during follow-up: broadening the spectrum

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Mitochondrial respiratory chain defects are associated with significant morbidity. The follow-up of 17 patients (mean age 38.6 years) is described. Eleven have MELAS (3 families diagnosed from 2 index cases in infancy and 1 in adulthood), two Kearn-Sayre Syndrome (KSS), one MNGIE and two PEO. The average time from first symptom to diagnosis was 15.9 years. Fatigue (9/17), myalgia (8/17), sensorineural deafness (8/17) and hyperlactacidemia (8/17) are the most prevalent manifestations. One MELAS family has a rarer T3271C mutation reported predominantly in Japanese. Four of them have migraine since adolescence and one had a stroke-like episode at 29 years. During 6 years of follow-up none developed hearing loss. The other two MELAS families have 3243A>G mutation and are more symptomatic (diabetes, neurossensorial deafness, terminal renal failure). Two KSS patients with 5 years of follow-up developed progressive neurological worsening, sensorineuraldeafness and intraventricular conduction defects. A PEO patient has cardiac noncompaction. Another PEO patient developed peripheral neuropathy and gastrointestinal dysmotility. MNGIE with a TYMP gene mutation (c.221 T>A/478 T>C) was diagnosed. In conclusion, this adult population has high phenotypic variability. The dominant manifestations are neurological and muscular, although clinical spectrum has expanded to other organs, such as the heart, gastrointestinal tract and kidney.

P-005

Vanishing white matter disease, experience of adult neurologic department

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Introduction: Vanishing white matter disease (VWMD) is a leukodystrophy, with great phenotypic variation. The MRI findings are diagnostic.
Case Reports: The medical records of 6 adult subjects with VWMD were reviewed.
1st: 45-year-old woman, with 3 tonic-clonic seizures who became hemiparetic and mute within a few days.
2nd: 60-year-old woman with a progressive clinical picture of urinary incontinence and paraparesis at 20, losing the ability to walk at 40. She currently has severe dysphagia and spastic tetraplegia.
3rd and 4th: 2 sisters. The younger, 32-year-old, started having neuropsychiatric symptoms and frequent falls at 27 years. She progressively worsened and now she has spastic tetraparesia. The older is 37-year-old and developed epilepsy and an acute neurological deterioration becoming totally dependent within a few days.
5th: 49 years old man, who started in twenties with asymmetrical tetraparesis and loss of walking capacity at 47.

6th: 45 years-old man with gonadal insufficiency, who had hyperacute neurologic symptoms at 42. Two women had premature menopause. All had mutation in EIF2B5 gene and fulfilled the MRI criteria.
Conclusion: Three cases showed hyperacute presentation and the remaining were progressive. VWM is the most frequent leukodystrophy in adults. This is the first report of gonadal insufficiency in a man with VWMD.

P-006

Epidemiology of inherited metabolic diseases in intensive care setting: Serbian experience

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Background: Inherited metabolic diseases (IMD) may present with acute deterioration during childhood. There are scarce reports of IMD epidemiology in intensive care units (ICUs).
Materials and methods: We assessed demographic and clinical parameters of IMD patients admitted to Neonatal and Pediatric ICUs of our Institution during 2004–2013.
Results: During study period, there were 81 admissions in ICUs due to IMD: 15 newborns, 22 infants 1–12 months of age and 44 older children. In total number of ICUs patients, IMD participated with 1.01 %. Main causes for admission were: encephalopathy (24.7 %), metabolic acidosis (23.5 %), respiratory insufficiency (19.7 %) and seizures (14.8 %). Most frequent diseases were mitochondrial disorders (27.1 %) and organic acidemias (23.5 %). Median length of hospitalization was 10 days. Mechanical ventilation (MV) was administered in 49.4 %, with median length of 5 days. Hemodiafiltration was used in 11 cases. Lethal outcome occurred in 23.5 %, without significant difference in mortality between newborns and older children. Death occurred in 45 % of patients requiring MV. Patients with mitochondrial disorders had significantly higher rate of MV (77.3 %, p 0.000).
Conclusions: Inherited metabolic diseases have important impact and high mortality in neonatal and pediatric ICUs. Mitochondrial disorders pose high risk for deterioration requiring ICU admission and mechanical ventilation.

P-007

Interlaboratory comparison of testing results for succinylacetone and nitisinone in dried blood spots

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Background and objectives: Succinylacetone (SA), the primary metabolite accumulated in tyrosinemia type 1 (HT1), is measured in dried blood spot (DBS) samples to detect HT1 in newborns. Nitisinone (NTBC) is the standard therapeutic drug for HT1. Monitoring of NTBC and SA concentrations is crucial for determining patient compliance and efficacy, respectively. The objective was to evaluate the current analytical performance of 6 different laboratories on the measurement of DBS SA and NTBC.
Methods: Two mixed sets of DBS materials enriched with predetermined SA (0–100 μM) and NTBC concentrations (0–150 μM were prepared and sent blinded to 6 participating laboratories for analysis. Each set consisted of 8 samples, either prepared with EDTA or Li-Heparin.
Results: For samples without NTBC, the reported median concentration was 0.0 μM. For the 6 samples containing no SA, a median of 0.0 μM was reported for 3 samples; for the 3 other samples, median values close to the assay detection limit were reported. At higher concentrations, the differences between reported and expected values were highly variable: up to 66.7 % (150 μM NTBC) and 471 % (100 μM SA). The anticoagulant used did not impact the results.
Conclusion: Efforts to harmonise analytical performance between laboratories seems justified.
Conflict of Interest declared.

P-008

Inborn errors of metabolism (IEM): Burden of disease in Indonesia

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Background and objective: IEM are rare disorders resulting from the absence or abnormality of an enzyme or its cofactor, expressing wide spectrum of clinical manifestations and often poor outcome or even death early in life. Indonesia is a country with high birth and infant mortality rate, as well as high prevalence of disability. We should therefore consider the possibility of high incidence of IEM. The aim of this study is to collect patients with IEM diagnoses to know the burden of the disease in Indonesia.
Method: Data concerning patients with IEM diagnoses, which were obtained from daily practices, were collected by interviewing or mailing the members of Nutrition and Metabolic Disease Working Group of Indonesian Pediatric Association.
Results: From 2002, forty-eight children were diagnosed as having IEM disorders. More than half were diagnosed since 2010. Based on metabolic pathways patients were classified as follows: metabolic disorders of amino acid and protein 14/48, carbohydrate 2/48, energy 12/48, lysosomes 13/48, familial hypercholesterolemia 3/48, neurotransmission 3/48, Wilson disease 1/48. The most common disease group was mucopolysaccharidoses 11/48.
Conclusion: IEM disorders seem high in Indonesia and still need more action to discover the diseases.

02. Novel diagnostic/laboratory methods

P-009

Amino acid profiling by tandem mass spectrometry: a good alternative to ion exchange chromatography

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Many inherited metabolic diseases are related to amino acids. In those disorders, metabolic pathways of amino acids can be directly or indirectly involved leading to accumulation of metabolites in physiological fluids. We have tested the a TRAQ™ Kits for Amino Acid Analysis of Physiological Fluids (AB Sciex). It enabled us to analyse about 45 amino acids in a single run. Analysis time remains quite short with 18 min per sample. Moreover, this method requires only 40 μL of sample. Each analyte has its own internal standard leading to accurate and precise identification and quantification. Lower (LLOQ) and upper (ULOQ) limits of quantitation for each amino acid are <1 μM and>10,000 μM, respectively, allowing a wide range.

Interestingly, we have extended the ability of the kit with the addition of 8 amino acids which are: sulfocysteine, pipecolic acid, aspartylglucosamine, homocarnosine, glutathione, saccharopine, cysteine-homocysteine mixed disulfides and iminodipeptides. Firstly, we describe the determination of iminodipeptides and arginosuccinic acid which can be analysed without previous hydrolysis. Secondly, we show the detection of pipecolic acid which is particularly important in diagnosis of peroxisomal disorders.

P-010

A capillary electrophoresis procedure for the screening of oligosaccharidoses and related diseases

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Background: The most widely used method for the biochemical screening of oligosaccharidoses is the analysis of the urinary oligosaccharide pattern by thin-layer chromatography. Our aim was to standardize the analysis of urine oligosaccharides by capillary electrophoresis with laser-induced fluorescence (CE-LIF).
Methods: CE-LIF (Beckman P/ACE MDQ) was equipped with a 488 nm argon ion laser module. All analyses were conducted using the Carbohydrate Labelling and Analysis Kit (Beckman-Coulter), which derivatizes samples with 8-aminopyrene-1,3,6-trisulfonate. Urine samples from 40 control subjects (age range: 1 week to 16 years) and from 10 patients diagnosed with eight different oligosaccharidoses (6 of them included in the Educational Oligosaccharide Kit from ERNDIM EQA schemes) were analysed.
Results: Two oligosaccharide excretion patterns were established in our control population according to age. Abnormal peaks with electrophoretic mobilities above the tetrasaccharide position were observed for fucosidosis, α-mannosidosis, GM1 gangliosidosis, GM2 gangliosidosis variant 0, Pompe disease and glycogen storage disease type 3. Urine from patients with aspartylglucosaminuria and Schindler disease displayed normal results.
Conclusions: In this study, the first CE-LIF method to screen for oligosaccharidoses and related diseases has been standardized. The method is simple, fast, automatable and allows the analysis of large series of samples.

P-011

High resolution nuclear magnetic resonance spectroscopy (NMRS): metabolic profiling in urine in healthy children

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Background: NMRS profiling in body fluids has become a powerful tool to investigate genetic metabolic diseases. Metabolic profiling using NMRS, however, needs reference profiles from healthy individuals since deviations from the "normal" state can only be detected using multivariate statistical analysis on base of reference profiles.
Patients and methods: 120 healthy children (age 0-15years) were recruited from an outpatient medical center to investigate NMRS profiles in spontaneous urines. Somatic, nutritional and clinical data were documented. The samples were measured with an Advance IVDr system at 600 MHz.
Results: Different metabolic profiles were found showing an age dependency with higher excretion (based on creatinine) of metabolites in younger children. Selected metabolites were quantified and respective distributions were compiled to obtain reference ranges. Preliminary results in PKU patients demonstrate possibility to monitor compliance of treatment by metabolic profiling in urine.
Conclusion: NMRS profiling and quantification in urine using high resolution NMRS offer new aspects in metabolic research and monitoring of treated patients with genetic metabolic diseases. Methodological advantages of NMRS over HPLC/MS/MS or GC/MS are short analysis time, simple sample preparation. Additionally, NMRS in body fluids is fully quantitative at a large linear range of up to 6 orders of magnitude.
Conflict of Interest declared.

P-012

Validation of oxygen consumption measurements in muscle and fibroblasts from patients with mitochondrial diseases

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Background: Diagnosis of mitochondrial disorders is mainly based on the analysis of OXPHOS complexes in muscle biopsies. However, normal enzyme activities do not exclude such diagnosis. Analysis of the intact mitochondrial energy generating system by oxygen consumption is frequently used.
Purpose: Comparing the diagnostic value of oxygen consumption versus OXPHOS analysis in fibroblasts and muscle from patients with a genetically confirmed mitochondrial disease.
Methods: A standardized substrate uncoupler inhibitor titration (SUIT) protocol was used for measuring respiration of permeabilised fibroblasts and single muscle fibers. OXPHOS activities were determined spectrophotometrically according to standard protocols.
Results: Only the combination of both measurements enable us to identify all of the patients: In fibroblasts 4/15 patients would have been missed by OXPHOS measurements, but could be identified with oxygen consumption. In muscle 1/6 patients was missed by oxygen consumption, but was identified by OXPHOS measurements. Moreover, specific flux control ratios showed higher diagnostic than complex specific O2 fluxes.
Conclusion: The established SUIT protocol is an important tool in the diagnostic process. Therefore we recommend oxygen consumption measurements in addition to OXPHOS analysis, to increase the number of identified patients.

P-013

A practical enzymatic assay for determination of propionyl-CoA carboxylase activity using high-performance liquid chromatography

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Background: Propionic acidemia (PA) is a life threatening inborn error of organic acid metabolism caused by a defect of propionyl-CoA carboxylase (PCC). Prompt diagnosis and appropriate dietary restriction may prevent acute metabolic decompensation and brain damage. The measurement of PCC activity by a radioisotopic method is still the gold standard for the diagnostic confirmation of the disease. To realize a practical test, we developed a simple and rapid enzymatic assay for PCC.
Methods: Crude PCC enzyme was prepared from frozen-thawed human lymphocytes. Aliquots were incubated with propionyl-CoA, sodium biocarbonate and adenosine triphosphate. The production of methylmalonyl-CoA in samples was separated by high performance liquid chromatography and detected using an ultraviolet spectrophotometer.

Results: The assay was applied to four patients with PA and demonstrated pathologically low levels of residual activity in all subjects. There were no significant differences of enzyme activity between the obligated carriers, the parents of PA patients (221–365 pmol/min/mg protein, n=5), and normal individuals (170–575 pmol/min/mg protein, n=20).
Conclusion: These results indicate that our method is a practical and sensitive assay for PCC and that it can be a useful adjunct in confirm diagnosis of PA.

P-014

Rapid and accurate determination of phenylalanine with advanced aptamers in a hand-held device

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Background: Aptamers are oligonucleotide based receptors that were discovered 1989. Aptamers have a high target specificity, provide a direct readout after binding, are temperature independent and cheap to manufacture. We developed advanced aptamers with an up to 1000-fold binding affinity that enables detection of amino acid and other small molecules for the first time. This platform technology is amenable to point-of-care (PoC) devices.
Objective: Evaluate advanced aptamers for phenylalanine determination in a PoC device
Method: 20 ul serum is diluted 1:600, incubated for 20 min with advanced aptamers (0.05 uM) and read in a hand-held fluorescent reader against a standard curve.
Results: Range: 30–2000 uM. Limit of detection: 20 uM. Linearity R^2= 0.99 (30–720 uM range). Linear least-squares regression analysis demonstrates a high degree of correlation between spiked plasma and serum over the clinically relevant range (30–720 uM): y=0.8001x +185.4, R^2= 0.97. (Whole range (0–2000 uM): y=0.9494x +53.63, R2=0.89)). 25 patient samples were analyzed with HPLC and aptamers. Mean phe 69.8 uM (Range 42–124), constant bias: −14.67 (95 % CI; −40 to 1.23), proportional bias: 1.29 (1.003 to 1.67 95 % CI) (Passing & Bablok fit).
Conclusion: Advanced aptamers enable accurate measurement of phe.
Conflict of Interest declared.

P-015

Analysis of bile acid profiles by liquid chromatography-mass spectrometry

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Background: Bile acids are the major products of cholesterol catabolism. They serve many important physiological functions such as cholesterol homeostasis and lipid digestion and absorption. In this work we focus on bile acid profiling by liquid chromatography-tandem mass spectrometry (LC-MS/MS) in patients with necrotizing enterocolitis (NEC) and possible peroxisomal disorders.
Methods: Bile acid extraction from serum was performed by protein precipitation with methanol, and then incubated for 20 min at room temperature. After centrifugation, the supernatant was obtained and dried under nitrogen. The residue was dissolved in methanol and 10 μl was injected for LC-MS/MS analysis in multiple reaction monitoring (MRM) and in negative ion mode.
Results and Conclusions: Nineteen bile acids and two internal standards were detected within a twelve minute analysis time. For the healthy participants, the main bile acids detected were taurocholic (TCA), glycocholic (GCA), taurochenodeoxycholic (TCDCA), glycochenodeoxycholic (GCDCA) and glycoursodeoxycholic (GUDCA) acids. Bile acids profiling of serial specimen from two NEC patients were analyzed over time during their treatment, which included surgery and antibiotic treatment. Serum specimens from a patient suspected to have a peroxisomal disorder were analyzed as well. Further, age-related reference intervals will be established.

P-016

Discovery of novel biomarkers for mucopolysaccharide disorders in patient urine samples using label free proteomics

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Background: Development of novel treatments such as enzyme replacement therapies for Mucopolysaccharidoses means that quicker, better and more specific tests for diagnoses are required for patients with MPS. Current diagnosis of the Mucopolysaccharidoses includes the measurement of glycosaminoglycans in urine as a screening test and then further analysis of white cell enzymes. Most urine tests are still performed using 2days electrophoresis methodology which is semi-quantative and labour intensive.
Method: Label free proteomics was performed on urine samples from MPS I, II, VI and control.
Results: A total of 305 proteins were detected. Interestingly, 74 of these proteins were observed to be changed significantly ($p<0.05$) in the MPS groups compared to controls. Many of these novel markers were found to be involved with proteoglycan binding and extracellular matrix function. At least 6 proteins have never been reported previously in urine. Seven, ten and eight proteins were able to distinguish MPS I, II and VI from the other MPS disorders respectively.
Conclusion: Label free proteomics on urine from patients with MPS I, MPS II, MPS VI and controls was used to find new biomarkers and to produce a new test that could be used for diagnosis and monitoring disease progression/treatment.

P-017

Liquid-liquid extraction and solid phase extraction for urinary organic acids - a comparative study

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Introduction: The qualitative and quantitative analyses of urinary organic acid are important diagnostic tools for organic acidurias. The composition of urine samples may vary considerably and require sample preparation prior to GCMS analyses.
Materials and methods: We performed comparative studies on the isolation of organic acids from urine using liquid-liquid extraction and solid-phase extraction, the extracted residue was air dried, converted into trimethylsilyl derivatives and analysed by GC-MS.
Results: Here we present the % recovery of 16 organic acids by solvent and solid phase extraction. Lactate – 72.75/67.8, oxalate – 113.25/78.9, pyruvate – 81.75/108.75, methylmalonate – 99/99.6, ethylmalonate – 83/87.25, fumarate – 75.25/96.25, lactate dimer – 98.5/60, glutarate – 100.5/99.6, 3-methylglutarate – 82.5/80.4, 3-phenylbutyrate – 84.75/64.6, adipate – 83.25/201, suberate – 105.75/211, azelate – 133.5/97.9, sebacic acid – 98.25/93.4, orotate – 56/85.75, succinylacetone - 67.75/123.4. In SPE phosphate and urea are the major unwanted peaks that mask the other metabolites in the

chromatogram. Metabolites of carbohydrates and its derivatives are also extracted through the SPE process but are absent in LLE.
Conclusion: The extraction efficiencies of the SPE were better for the analysis of urinary organic acids with mean analytical recoveries of 93.9 %. SPE could be an accurate and quick analytical test with optimum recoveries for the monitoring of organic acids concentrations in urine samples.

P-018

Lessons learned from the experience of NGS-based target gene analyses for clinical diagnosis

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Background: Target gene capture sequencing analysis of a group of genes for known diseases or metabolic pathways provides much higher diagnostic yields than 25 % of WES.
Methods: We havedeveloped ~30 panels using SeqCap EZ enrichment followed by next generation sequencing (NGS). Target genes are sequenced at ~1000X and validated by Sanger sequencing. Insufficiently covered regions are sequenced separately by PCR/Sanger.
Results: The diagnostic yields of pathway driven panels and diseases with defined phenotypes are high. These panels include cobalamin and related pathway, keto acids dehydrogenase complexes, fatty acid oxidation, glycogen storage disease (GSD), Usher syndrome, and retinitis pigmentosa (RP). GSD has a diagnostic yield of 64 %. Whereas for clinically defined Usher syndrome the diagnostic yield is close to 100 %. Analysis of 66 RP genes contributing to nonsyndromic RP makes confirmatory diagnosis in about 80 % patients. Nevertheless, a diagnostic yield of about 25 % is observed in the most genetically and clinically heterogeneous mitochondrial disorders.
Conclusion: Our experience in NGS based target gene analysis suggests that unbiased capture of target genes with consistently deep coverage is essential to high diagnostic yields, particularly for genetically heterogeneous but phenotypically distinct disorders. Consistently deep coverage of individual exons allows copy number variation (CNV) assessment.

P-019

Liquid chromatography-high resolution mass spectrometry based analysis of the cerebrospinal fluid metabolome enables the stratification of patients having unknown encephalopathies

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Background and objectives: We have developed a liquid chromatography coupled to high resolution mass spectrometry (LC/HRMS) method to investigate the cerebrospinal fluid (CSF) metabolome of patients having unknown encephalopathies, which may be caused by inborn errors of metabolism (IEM).
Patients and methods: 138 CSF samples were collected from adult patients including negative controls without progressive neurological disease, patients with IEM, and patients having unexplained encephalopathies. CSF samples were deproteinized and injected in two complementary LC/HRMS systems. Data were processed using XCMS peak detection software, SIMCA P12 (Umetrics, Sweden) and an internal spectral database.
Results: CSF metabolomic analyses enabled the grouping of 6 samples, based on a similar metabolic signature (increased concentrations of few tens of metabolites such as sugar derivatives, acetylated amino-acids and acylcarnitines). Four of these samples have already been grouped from a previous NMR study in which a significant elevation of free sialic acid was observed (i.e., CAFSA syndrome, Mochel et al., Brain, 2009), and interestingly, the two other samples correspond to Kearns-Sayre syndromes.
Conclusion: Our results indicate that CAFSA metabolic profile is close to that of Kearns-Sayre syndrome and show that LC/HRMS based metabolomics is relevant to stratify patients with unknown encephalopathies.

P-020

Diagnosis of glycine encephalopathy in a pediatric patient by detection of a GLDC gene mutation by next generation DNA sequencing

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Background and objectives: Early diagnosis for metabolic encephalopathy caused by inborn errors of metabolism is very important for early treatment and for prevention of sequelae. Metabolic encephalopathy can result from many inborn errors. Considering the large number of disorders causing metabolic encephalopathy, enzyme assays or conventional molecular tests are expensive and take considerably long period of time.
Patient and Methods: We have used next generation DNA sequencing technology as an initial diagnostic test to look for about 700 disorders at the same time for the etiology in a 4-month-old female infant with intractable seizures. Ion Ampliseq™ Inherited Disease Panel, which includes about 10,000 primer pairs for 328 genes accounting for about 700 disorders and Ion PGM™platform were used for the analysis.
Results: The patient was found to have homozygous c.2203-2A>G mutation in the GLDC gene which was previously shown to cause glycine encephalopathy. The assay and interpretation took considerably shorter compared to the conventional methods.
Conclusions: Up to our knowledge, this would be the first case with glycine encephalopathy in the literature who was approached by this novel panel method initially.

P-021

Austrian practice guidelines of molecular diagnosis of congenital adrenal hyperplasia (CAH) due to 21-OH-deficiency

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Background and objectives: Aim was to develop consensus guidelines regarding the molecular analysis of CYP21A2 gene in individuals suspected of 21-OH-deficiency.
Methods: CAH-Best Practice Meeting was held in Antwerp, Belgium in 2009 with 21 participants from 12 countries (CAH experts from each European country had been invited). On the basis of extensive practical experience and after discussions addressing aspects of relevance for patients, geneticists, clinicians and scientists we decided to draft practice guidelines for local conditions in Austria.
Results/Conclusion: Due to wide range of phenotypical differences in CAH, different legal obligations, and the complex molecular background, consensus was hard to achieve in all issues. Therefore the guidelines focus on consensus and controversies in best practice CYP21A2-genotyping. Main aspects covered by these guidelines are pretest requirements,

appropriate methodologies including detailed information on molecular organization of the chromosomal region containing the gene, mutations, polymorphisms and proposed primers as well as on interpretation and standardization of reporting. Since pathophysiology and therapeutic aspects are yet covered by recently published "Endocrine Society Clinical Practice Guidelines" (J Clin Endocrinol Metab. 2010;95 and 2013; 98), the present guidelines complete clinical diagnosis of CAH individuals.

P-022 Withdrawn

P-023

Development of a large next generation sequencing panel for cost-effective application to a range of inborn errors of metabolism

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Background: Next generation sequencing (NGS) represents a leap forward from traditional Sanger sequencing, in terms of the amount of genetic information that can be generated in a reasonable timescale and for a reasonable cost. Objective: The aim was to develop a large NGS panel for a range of inborn errors of metabolism (IEMs): glycogen storage diseases, fatty acid metabolism disorders, peroxisomal disorders, urea cycle disorders/hyperammonaemia, and rhabdomyolysis.
Methods: A panel of 96 genes was designed, using genome browsers and locus-specific mutation databases to select known, clinically relevant areas of each gene. Samples from 10 patients were selected to aid panel validation. SureSelect (Agilent) was used for target enrichment and HiSeq (Illumina) for sequencing. Data analysis used an optimised in-house bioinformatics pipeline based on open-source programs.
Results: NGS data showed complete concordance with previous Sanger sequencing and satisfactory depth of coverage/quality parameters for diagnostic use.
Conclusion: For IEMs, a large targeted NGS panel, with selective analysis of only clinically relevant genes, enables a) confirmation of biochemical diagnoses for more disorders allowing subsequent family testing; b) more diagnoses for genetically heterogeneous disorders; and c) reduced time to diagnosis and overall cost for non-specific presentations, if used use early in the diagnostic pathway.

P-024

Computational modelling of inborn errors of metabolism

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Genome-scale metabolic networks integrate genomic, physiological and biochemical knowledge of a target organism. Such a network has been assembled for human metabolism (Recon 2) and more specifically for small intestinal metabolism (sIEC). These human metabolic networks can be used to study the inborn errors of metabolism (IEMs) and to propose potential treatments. We compiled a compendium of 235 IEMs, whose responsive genes, and metabolic reactions, were captured in Recon 2. We then used Recon 2 to predict known and novel biomarkers, and also assessed the role of diets on metabolic functions of sIEC's when an enzymatic deficiency is present in the network. Recon2 predicted known changes of IEM-associated biomarkers with 77 % accuracy and predicted many novel ones that may enable differential diagnosis. The sIEC network captured 104 IEMs, 76 % of which affected its metabolic functions. Our predictions proposed a biochemical link in porphyrias between the heme availability in diet and its degradation. The model was also used to investigate adaptive mechanisms in citrullinemia and Smith–Lemli–Opitz syndrome. We demonstrate that this modeling approach can be used to predict novel biomarkers and the effect of diets, as well as to provide network-level understanding to IEMs.

P-025

Monoamine neurotransmitter analysis in mouse CSF: Collection and method development by LC-MS/MS for characterization of a hypomorphic tyrosine hydroxylase knock-in mouse model (Th-ki) of severe L-Dopa deficiency

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Tyrosine hydroxylase deficiency (THD) is a rare autosomal recessive neurometabolic disorder caused by a defect in TH, the rate-limiting enzyme in catecholamines biosynthesis and a marker for dopaminergic neurons. THD is classified in types A and B, being the later a more severe and complex encephalopathy with neonatal or early infancy onset and L-Dopa treatment occasionally effective. A constitutive knock-in mouse model mTH-p. R203H ("Th-k"), with the severe mutation p. R203H was generated aiming to aid in the elucidation of molecular mechanisms in THD, and to provide insights for evaluation of novel therapeutics. Until today, the common way to evaluate catecholamines and effectiveness of therapeutic options in mice is analyzing brains extracts, while in human is CSF. Goals of this work are to develop: 1) a method to obtain CSF from mice and 2) a highly sensitive method to determine catecholamine and their metabolites by LC-MS/MS (TripleQuad5500, ABSciex) in small sample volumes of CSF (3-7 mL). Mouse CSF, extracted from cisterna magna using glass capillary, was directly diluted in the mobile phase. Thus far, collected data are being used to analyze the correlation between catecholamines, serotonin and metabolites in THD patients and Th-ki mice.

P-026

The use of LC-MS QTOF based metabolomics to reveal the identity of metabolites contributing to false positive results in the acylcarnitine profile

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Background: Reliable MS quantitation of acylcarnitines is fundamental in laboratory diagnosis of many IEM's, both in NBS and in the biochemical diagnostic lab. Identification of different acylcarnitines is based on the m/z of molecular ions producing a fragment of m/z 85.
Objectives: To use chromatography combined with Q-TOF MS in a to separate and identify compounds in plasma and urine and by that reveal non-acylcarnitine compounds contributing to falsely elevated acylcarnitines in the standard acylcarnitine method.
Material and Methods: Plasma and urine from a VLCAD patient and from a newborn with initial symptoms indicating an urea cycle disorder, having acylcarnitine profile from NBS indicating glutaric aciduria type 2 and acylcarnitines from the metabolic lab indicating VLCAD. The baby soon recovered and laboratory parameters normalized raising the question of falsely elevated acylcarnitines in the earlier samples. All samples were analyzed using LC-QTOF extracting precursors of m/z 85.

Results: In the patient samples several significant peaks with molecular ions very close to the true diagnostic acylcarnitines, but with different chromatographic retention times, were found.
Conclusion: LC-QTOF is a valuable tool in identifying compounds contributing to falsely elevated acylcarnitine results.

03. Newborn screening

P-027

Mutations in BTD gene causing biotinidase deficiency: Regional Report

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Background: Biotinidase deficiency is an inherited autosomal recessive disorder characterized by neurological, cutaneous manifestations and metabolic abnormalities. Individuals with less than 10 % of mean normal serum biotinidase activity have profound biotinidase deficiency. While children with partial biotinidase deficiency have 10 %-30 % of mean serum biotinidase enzyme activity.
Objectives: This report summarizes the demographic features of patients identified as biotinidase deficiency from August of 2012 through August of 2013, and mutation analysis results for 20 cases in southeast region of Turkey. Methods: Twenty patients with biochemically proven biotinidase deficiency measured in DBS samples were included in this study. Mutation analysis was performed as part of the clinical work up.
Results: All children were the products of consanguineous mating, and all of the children were homozygous or compound heterozygous for specific mutations (c.235C>T in exon 2, and c.470G>A, c.557G>A, c.1330G>C, c1368A>C, c1489C>T, c1595C>T mutations in exon 4) known to cause profound biotinidase deficiency.
Discussion: Mutation analysis provides valuable support to the enzymatic analysis and should be considered as a supplement to the biochemical data by those performing newborn screening for biotinidase deficiency. Newborn screening allows early presymptomatic treatment that can prevent neurological impairment. In conclusion, we determined the mutation spectrum causing partial and profound biotinidase deficiency in patients from southeastern part of Turkey.

P-028

Detecting multiple lysosomal storage diseases by tandem mass spectrometry - A national newborn screening program in Taiwan

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Background: Interest in lysosomal storage diseases in newborn screening programs has increased in recent years. We report a pilot study of large scale newborn screening for Fabry, Pompe, Gaucher, and MPS I diseases by using the tandem mass spectrometry (MS/MS) method and compared the performance with fluorescence (4-MU) methods. Due to high incidence of MPS II in Asia region, we further validated the screening method for MPS II.
Methods: More than 100,000 dried blood spots (DBSs) were collected consecutively as part of the national Taiwan newborn screening programs. The enzyme activities were detected by the MS/MS method from DBS punch. Mutation analysis was performed for newborns with enzyme deficiency.
Results: The intra- and inter-assay coefficients of variation for 5 diseases are all less than 15 %. The DNA sequence analysis for suspected cases revealed 89 newborns with confirmed Fabry mutations, 22 were classified as infantile or late-onset Pompe disease, and 1 was characterized as Gaucher disease. The positive predict value increased from 3 % to 7 % in the Pompe study, and from 61 % to 89 % in the Fabry study by the MS/MS method compared to 4-MU assay.
Conclusions: The MS/MS method provides a multiplex solution of newborn screening for lysosomal storage diseases.

P-029

The inclusion of ADA SCID in expanded newborn screening by tandem mass spectrometry

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Adenosine deaminase (ADA)–severe combined immunodeficiency (SCID) is caused by adenosine deaminase deficiency. Early-onset form is rapidly fatal in infancy because of severe recurrent infections. When diagnosis is made, permanent damage caused by infections or metabolites is often present. Delayed/late-onset ADA-SCID is characterized by insidious progressive immunodeficiency that leads to permanent organ damage or death. Gene therapy, bone marrow transplantation, enzyme therapy might be effective if performed early. ADA-SCID complies with all the criteria for inclusion in a newborn screening program. However, screening methods are still expensive or provide a non-negligible number of indeterminate results. Quantification of T-cell receptor excision circles (TRECs) or tandem mass spectrometry (MS) analysis of dried blood spots (DBSs) collected at birth can identify newborns with early-onset ADA SCID. However, it was unclear whether these analyses identify newborns who will have delayed or late-onset ADA-SCID. We performed a retrospective study to evaluate whether MS and TREC analyses of DBSs could identify newborns presenting delayed-onset ADA-SCID later in life. Our results demonstrate that MS but not TREC identifies newborns with delayed/late-onset ADA deficiency. Moreover a comparative cost-effectiveness analysis between TREC and MS based method was performed. A new MS test for PNP SCID is also ready.
Conflict of Interest declared.

P-030

Implementing a pilot expanded newborn metabolic screening service in Hong Kong: A major step towards a territory-wide programme

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Background and objectives: Newborn screening programme for inborn errors of metabolism (IEM) by tandem mass spectrometry (MSMS) has never been implemented in Hong Kong. The Center of Inborn Errors of Metabolism (CIEM) of The Chinese University of Hong Kong has started a pilot service with an expanded newborn screening programme by MSMS using dried blood spots since July 2013. This study is to evaluate the result and implementation of the programme.
Patients and Methods: We retrieved the data from July 2013 to March 2014, a total of 3,529 samples collected from public and private hospitals

were screened. The demographic data, collection time, reporting time, and results were analysed.
Results: More than 99 % of the samples were collected within 7 days of life. The overall re-call rate of our screening program was 0.57 % (20/3529). Sixteen out of 20 call back cases had normal second dried blood spots result, with a false positive rate of 0.45 % (16/3529). The four positive cases including one medium-chain Acyl-coA dehydrogenase deficiency (MCAD), one suspected maternal carnitine uptake defect, and two milder conditions.
Conclusion: Our expanded newborn screening programme service to both the public and private sectors demonstrates that the expanded newborn screening could be implemented successfully in Hong Kong.

P-031

Newborn screening for inborn errors of metabolism using tandem mass spectrometry (tandem mass screening) on over 300,000 babies in Tokyo, Japan

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Background: Every year, about 1,000,000 newborns receive a mass screening for inborn errors of metabolism (IEM) in Japan and 10 % of them are screened in the Tokyo. From 2004 to 2012, a study group supported by the Ministry of Health, Labor and Welfare of Japan started a pilot study of NBS using tandem mass spectrometry method (TMS), and we joined this program in Tokyo. After April 2012, TMS became a standard method of NBS in Japan. Here we report the results of this screening in Tokyo.
Materials and Methods: We screened 102,059 and 199,689 babies during the pilot study and from April 2012 to March 2014, respectively. We used a Quattro micro and three TQ Detectors.
Results: We detected 16 and 21 patients with IEM (incidence 1/6,400 and 1/9,500) in the pilot study and after April 2012, respectively. All the patients we detected were healthy without any metabolic crisis.
Conclusion: A pilot study of TMS throughout Japan showed the incidence detected by this method was 1/9,100 for about 2 million babies. Our results were similar. These results showed that TMS in Japan is a useful screening method for IEM to diagnose patients without symptoms and subsequently start treatment before crisis.

P-032

Recommended guidelines for newborn screening in the homocystinurias

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Background: In 2013 the European network and registry for homocystinurias and methylation defects (E-HOD) was initiated. The present work focuses on methodological aspects of newborn screening (NBS) and the potential impact of early treatment on outcome in the homocystinurias.
Methods: A systematic literature search was performed using the terms newborn screening, clinical course, outcome, treatment in combination with all diseases. A total of 194 publications were graded according to level of evidence as defined by the SIGN method.
Results: Sensitivity and specificity of elevated/decreased methionine as primary marker for CBS deficiency/methylation defects is limited. Specificity may be improved by second tier homocysteine measurement. The primary markers for the CblC defect, C3 acylcarnitine and C3/C2 ratio, are complemented by second tier homocysteine and/or methylmalonic acid measurements. Early detection and treatment improves outcome in CBS deficiency and seems beneficial in MTHFR deficiency. In the infantile onset CblC defect, neurological disease progresses despite early treatment while other symptoms improve. In late-onset CblC patients the disease course is ameliorated by treatment.
Conclusion: Early treatment can improve clinical outcome in some homocystinurias. Therefore, new strategies to improved NBS like first and second tier total homocysteine measurement need to be studied.

P-033

A pilot study on an expanded newborn screening program in Palestine

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Objective: Until this day newborns in Palestine are being screened for phenylketonuria and congenital hypothyroidism only. However, a number of other metabolic diseases have been recognized amongst the population, including four main types of organic acidemias: MMA, PA, GA1, and IVA. The study was conducted in an effort to investigate the possibility of finding such cases in newborns throughout the West Bank of Palestine by screening newborns for a number of organic acid intermediates. In addition, reference ranges for these intermediates will be established.
Study Design: A cross-sectional observational study design was used. The study blood collection cards (4240) from all 12 districts were collected by Ministry of Health staff and the samples were analyzed by the Human Genetics Department/Liege University using tandem mass spectrometry.
Results: Significant differences were observed in mean newborn weight and organic acid levels and in mean organic acid levels and gender. The reference range for the main types of organic acids tested was calculated based on the non-parametric percentile method as indicated by CLSI C28-A3.
Conclusion: Based on our established reference ranges results showed that 10.7 % of the tested samples (455) had at least one organic acid level in the upper 2.5 % of the population.

P-034

Improvement of neonatal screening for Pompe disease using Ba/Zn method in Japan

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Background: High incidence (3.3-3.9 %) of acid α-glucosidase (AαGlu) pseudodeficiency, c.[1726G>A; 2065G>A] homozygote (AA homozygote), in Asian populations complicates newborn screening (NBS) for Pompe disease. Barium hydroxide/zinc sulfate method (Ba/Zn method) was developed to improve the enzymatic diagnosis of Pompe disease in dried blood spots (DBSs) by the hemoglobin elimination on 4-methylumbelliferone (4MU) detection. Purpose: In this study, we examined the effectiveness of Ba/Zn method on neonatal mass screening for Pompe disease in Japan.
Method: We screened 17,022 DBSs from the NBS program in Kumamoto, Japan from April 2013 to March 2014. We assayed AαGlu activity in DBS with two different methods; conventional 4-MU method and Ba/Zn method. Definitive diagnosis was done by the measurement of AαGlu activity in fibroblasts using glycogen as substrate.
Results: A total of 30 (0.18 %) newborns were recalled for second DBSs with low enzyme activity. The recall rate was significantly decreased from 1.59 % to 0.18 % using the Ba/Zn method instead of 4-MU method. AαGlu activity in fibroblasts was carried out for 6 samples with abnormal low enzyme activity, revealed 2 late-onset Pompe disease.
Conclusions: The Ba/Zn method has been validated as a more efficient tool than the 4-MU method in Asian populations because of high frequency of AA homozyqote.

P-035

Clinical, molecular and biochemical characteristics of patients with elevated tetradecenoylcarnitine (C14:1) detected by newborn screening in New Zealand: support for a common, likely benign mutation in the Pacific population

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Background: Very long chain acyl-CoA dehydrogenase deficiency (VLCADD) has been diagnosed more often than anticipated since expanded newborn screening (ENBS) began, broadening the phenotypic and genotypic spectrum of this disease. A common ACADVL mutation c.1226C>T (p. Thr409Met) is observed in the Pacific/Hawaiian population. This has relevance in NZ, where Maori and Pacific people comprise 22 % of the population.
Objectives: To define the clinical, biochemical and molecular characteristics of patients with elevated tetradecenoylcarnitine (C14:1) detected on ENBS in NZ from 2006–2014. Methods: Retrospective review of notes and laboratory data of 16 patients with C14:1>2.5 μmol/L on ENBS.
Results: Follow-up ranged from 1–26 months. 11/16 were asymptomatic, 2/16 mildly symptomatic and 1/16 died day 4 of life. 2/16, with affected siblings, were treated from birth. 79 % were of Maori/Pacific ethnicity. The c.1226C>T allele frequency was 61 %. Homozygosity for this mutation resulted in normal fatty acid oxidation flux. Compound heterozygosity, with a pathogenic mutation, resulted in mild impairment at 41 °C.
Conclusion: Despite a relatively high cut-off, a higher than anticipated number of newborns with elevated C14:1 have been detected by ENBS. The majority are asymptomatic. We hypothesise that this is due to the high prevalence of a possibly benign Pacific mutation.

P-036

Expansion of newborn screening program for inherited metabolic disorders in the Czech Republic

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Objectives: At present 10 inherited metabolic disorders (IMDs) are screened in the Czech Republic. The aim of our project was to evaluate the methodological options of screening 20 additional IMDs (urea cycle disorders, biotinidase deficiency, tyrosinemia type I and disorders of methionine and propionate metabolism) as a basis for decision on further expansion of newborn screening (NS) program.
Methods: 1. We increased the number of first-step markers by: a) modification of current LC-MS/MS method and b) using of diagnostic kits for biotinidase deficiency and tyrosinemia type I. 2. We developed new LC-MS/MS second-tier method for sulfur aminoacids.
Results: During 15 months we analysed 49,211 newborns samples and second-tier analysis in a 1 % subset. False positive rate and positive predictive value increased slightly from 0.12 % and 22 % for 10 IMDs to 0.19 % and 23 % for 30 IMDs, respectively.
Conclusions: Based on laboratory results from our study and clinical efficacy of treatment the National Coordination Centre for NS recommended the expanding the newborn screening program by 5 diseases (citrullinemia type I, argininemia, CBS/MTHFR deficiency, biotinidase deficiency). The detection rate would increase from 1:3,800 to 1:2,600 and all Region4Screening criteria would have been achieved. Supported by MH CZ-DRO-VFN64165, CZ.2.16/3.1.00/24012 from OPPC.

P-037

Mitochondrial trifunctional protein (MTP) deficiency in a patient identified by newborn screening with normal to mildly abnormal plasma acylcarnitines and only one heterozygous mutation in the HADHA gene

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Background: The enzyme activities of long-chain hydroxy acyl-CoA dehydrogenase (LCHAD) and long-chain 3-ketoacyl-CoA thiolase, both part of mitochondrial trifunctional protein (MTP), were determined in lymphocytes of a one-month-old patient, who was picked up by newborn screening with mild elevations of hydroxy-acylcarnitines in bloodspot.
Results: Both enzyme activities were reduced, strongly suggesting MTP deficiency. Plasma acylcarnitine analysis showed normal to (occasionally) mildly abnormal C16:x/C18:x-carnitine and hydroxy-C16:x/C18:x-carnitine levels. gDNA analysis revealed only one heterozygous mutation in the HADHA gene and no mutations in the HADHB gene. Subsequent studies in the patient's fibroblasts confirmed the enzymatic findings in lymphocytes: both LCHAD and long-chain 3-ketoacyl-CoA thiolase activities were reduced and immunoblot analysis revealed almost complete absence of MTP protein. Palmitate loading studies of the cells were abnormal, characterized by elevated hydroxy-C16 carnitine in the medium.
Conclusion: Enzyme activity measurements identified MTP deficiency in a patient with normal to mildly abnormal acylcarnitine profile in plasma. At the gDNA level, however, only one heterozygous mutation was detected in the HADHA gene; HADHA cDNA analysis is pending. This

case stresses the importance of enzyme activity measurements to confirm findings of newborn screening programs and for diagnostics of inherited metabolic disorders in general.

P-038

ELMA Lab four-years experience with selective screening for IEM by tandem MS in Slovakia

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In 2009, tandem MS was established for the diagnosis of IEM by the Experimental Laboratory for Metabolomic Analyses (ELMA Lab) in Slovakia. Our activities increased the interest in IEM among health and government representatives. In January 2013 expanded newborn screening (ENBS) was adopted by Newborn Screening Centre of SR. ELMA Lab is now involved in the selective screening for IEM in the country, in the monitoring of IEM patients on treatment and in follow-up examinations of positively identified newborns by regular ENBS. ELMA Lab successfully participates in ERNDIM acylcarnitines scheme. We examined >8100 healthy newborns and 2280 suspected IEM patients. Within selective screening we indicated 13 patients suspected for IEM, many of them in a severe clinical state, 5xSCADD, 3xMMA, 1xPA, 3xMCADD, 1 adult form GA-II. In two cases, ACC profiles were atypical and demanding to interpret: MCADD (11 m girl, in coma, with hypoglycemia, suspected CTD) and adult form GA-II (37y female, muscle pain, suspected CPT-II). ELMA Lab also makes R&D of new diagnostic methods for IEM by tandem MS. This work is supported by R&D OpProg, funded by ERDF, project ITMS26240220071.

P-039

Low carnitine level in Estonian sick neonates

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Endogenous synthesis of carnitine plays a minor role in early life due to lack of enzyme called γ-butyrobetaine hydroxylase. So neonates and infants may be at risk of developing carnitine deficiency. The aim of the study was to evaluate the plasma free carnitine (pFC) level in neonates hospitalized promptly after the birth and compare their results with randomly born newborns.
Methods: The first study group consists of 563 newborns hospitalized promptly after birth in two regional hospitals in Estonia during one year. The second study group consists of 980 newborns born randomly since 2014. pFC was measured from 3 mm in diameter blood spots by tandem mass-spectrometry.
Results: The median value of pFC in first group was only 8.81 μmol/l and in second group 30.26 μmol/l. First group's children with birth weight below 1500 g median pFC was 7.57 μmol/l (74 patients; range 0–59.5 μmol/l), and with birth weight 1500-2500 g it was 9.53 μmol/l (131 patients; range 1.24-45.5 μmol/l). Preterm babies in first group had more frequently pFC value below 5 μmol/l ($p<0.0001$). All had secondary cause of pFC deficiency.
Conclusion: This study results showed markedly decreased pFC value in children hospitalized promptly after birth.

P-040

Successful prospective treatment of patients with remethylation disorders detected by newborn screening – cobalamin E deficiency, cobalamin G deficiency, and methylenetetrahydrofolate deficiency

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Background: Disorders resulting from defective remethylation of homocysteine (Hcy) to methionine (Met) present early in life with failure to thrive, megaloblastic anemia and severe neurological symptoms.
Case reports: Four cases of remethylation defects were identified by newborn screening. Two patients (with cobalamin G and cobalamin E deficiency respectively), were detected by approach combining low/low normal Met with second-tier Hcy determination. Treatment resulted in better outcome than symptomatically diagnosed patients. Two additional patients with methylenetetrahydrofolate reductase (MTHFR) deficiency were detected by low Met and despite being symptomatic, both recovered after treatment. These patients are compared to a patient with MTHFR deficiency diagnosed clinically at 3 months of age with residual complications despite treatment.
Conclusion

1) Early diagnosis and treatment of remethylation disorders is important to avoid short- and long-term complications.
2) This can be achieved by newborn screening utilizing Met and second tier Hcy determination done in the initially submitted blood spot, facilitated further by the use of R4S post-analytical tools. This approach significantly shortens the analytical time and provides accurate results based on the specific markers for these diseases (low/low normal Met and elevated Hcy).
3) In suspected cases pathway-specific treatment could be started even prior to confirmatory testing.

P-041

Parents' information, knowledge and expectation about newborn screening: Results from an Italian survey

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Background and Objectives: The newborn metabolic screening is mandatory in Italy since the early 1990's, but several differences exist at the regional level as for the extended screening. In both cases,parent-oriented information and education are crucial. The main aim of this study is to evaluate whether the information on newborn screening issuccessfully conveyed to parents before they consent to have their baby screened.
Materials/Patients and Methods: An observational cross sectional study was conducted in March 2013 at Maternity Ward of the University Hospital "Santa Maria della Misericordia", Udine, Italy. A self-administered anonymous questionnaire was given to each of the 55 pairs, 12 mothers and 1 father who participated to the study.

Results: Only 23.9 % mothers and 48.2 % fathers reported having received specific informationbefore their baby's birth. Information was mostly provided by midwives and neonatologist and was sufficiently clear for 77 % and complete for 79 %. However, when specific questions were asked in order to check whether or not the correct information was acquired by parents, a poor result was obtained.
Discussion/Conclusion: Several points of the informative process to parents on standard newborn screening need to be revised. Timing should be anticipated and information should be provided by midwives during the third trimester prenatal visits.

P-042

Adenosylcobalamin deficiency (MMAB) diagnosed by slightly abnormal routine newborn screening

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Background: Adenosylcobalamin deficiency cbl B is a very rare autosomal recessive inherited disorder caused by mutations in the MMAB gene on chromosome 12q24. Less than 50 patients worldwide are published. Most reported patients presented with acidotic crisis in the neonatal period or in the first year of life in catabolic situations similar to the classical methylmalonic acidurias. No patient was identified by expanded newborn screening (NBS) using MS MS yet.
Case report: We present a now 18 months old, still asymptomatic patient, first child of consanguineous parents. NBS showed slightly elevated C3/C0 (0.36 - cut off<0.28). The other screening parameters C3/C2 (0.27 - cut off<0.28) and C3 (4.9 μmol/l - cut off <6.6) were below the recall range. Further investigations showed moderate methylmalonic aciduria (~400 mmol/mol Crea) and propionate incorporation in fibroblasts indicated cbl A/B defect. Finally, the diagnosis of Cbl B deficiency was proved by genetics (new, unpublished mutations: c.487C>T/c.562G>A).
Discussion: As known from literature propionylcarnitine (C3) as screening parameter for propionate metabolism has low specificity. Adding ratios like C3/C0 and C3/C2 can improve the diagnostic value for even mild forms of cobalamin disorders that can be missed using only C3.

P-043

Multiple mutations in the SLC22A5 gene in patients with OCTN2 carnitine transporter deficiency identified by newborn screening

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Carnitine transporter deficiency (CTD) is caused by mutations in the SLC22A5 gene encoding the OCTN2 carnitine transporter. Mothers and infants with CTD can be identified by newborn screening and the diagnosis is confirmed by identification of two mutations in the SLC22A5 gene or reduced carnitine transport in patients fibroblasts. Carnitine (C0) levels were always low in infants whose mother had CTD, but not in infant with CTD, with some patients detected only by the second newborn screening, routinely collected at 7–21 days of age. Sequencing of the SLC22A5 gene failed to identify causative mutations in about 15 % of affected alleles. Expression of missense variants in Chinese Hamster Ovary cells indicated that most reduced carnitine transport to <10 % of normal, with some exceptions. Carnitine transport in fibroblasts was reduced to <20 % of normal in CTD patients. Our results show that infants with CTD might be missed by newborn screening if samples are collected too soon after birth. DNA testing does not identify all mutations in the SLC22A5 gene causing CTD and some variants retain near normal carnitine transport. Functional studies in fibroblasts remain the most accurate system to confirm a diagnosis of CTD.

04. Dietetics and nutrition

P-044

Relationship of dietary regime, age, phenylalanine (Phe)-tolerance and metabolic control in PKU

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Background: A strict dietary management is required in PKU to maintain good metabolic control. Nevertheless, different models of dietary regimes are used. To investigate the effect of the dietary regime on metabolic control, a multicenter evaluation was performed.
Patients/Methods: Data of 108 patients (1-9y; Phe-intake/day: max. 800 mg) were categorized into groups of dietary regimes ("very strict", "strict", "moderate", "simplified", "loose") and analysed with respect to Phe-intake (mg/day) and metabolic control (median Phe-concentrations, % of Phe-concentrations above therapeutic range (%-Phe-a.th.r) over 6 months).
Results: Median Phe-concentrations did not differ between groups (p= 0.175). However, the group "loose diet" showed the poorest metabolic control (median phe: 288 μmol/l; range 156–529 μmol/l). Patients under "very strict" dietary regime showed significantly lower %-Phe-a.th.r (p= 0.035; Kruskal-Wallis; Mann–Whitney-U-test). A significant correlation between dietary regime and metabolic control could be revealed (Kendall's tau: median Phe-concentration p=0.046; %Phe-a.th.r. p= 0.014). Phe-intake was not related to median Phe-concentration (p= 0.804) or %-Phe-a.th.r (p=0.505).
Discussion: Metabolic control is directly related to dietary regime. Since peaks of Phe-concentrations seem to negatively impact cognitive development, these data favour strict over loose dietary regimes. Although it is more cumbersome, daily recording of Phe-intake remains advisable, at least until ten years of life.

P-045

Cross sectional study on the dietary management of patients with hepatorenal tyrosinaemia

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The treatment of hepatorenal tyrosinaemia (Type I) includes regular drug administration of NTBC, combined with a protein-restricted diet. While NTBC is administered whenever available, dietary treatment is heterogeneous among centres. We collected cross-sectional data on dietary management in 11 metabolic centres from Germany, Austria and Switzerland. They contributed individual patient data. Before the workshop, we sent questionnaires to the participating centres. Questionnaires addressed epidemiological data, diagnostics, dietary treatment/management and unmet needs in dietary management. Furthermore, we collected data on nutritional and laboratory monitoring, adherence/compliance of each single patient. Data from 42 patients were analyzed and subsequently discussed during a workshop in Hannover. The participating dietitians agreed on the intake of amino acid mixture (AAM) in at least three portions per day. Weighing out food and calculation of tyrosine and protein are not necessary if appropriate food is chosen and AAM is taken regularly. During dietary follow-up the amount of AAM is adjusted to body weight and monitoring by nutritional protocols is performed. New products are presented and patients and parents are trained regularly. A less restricted diet may increase compliance and might be an option in older children as long as tyrosine levels are kept in target range.

P-046

European practices in prescribing total protein equivalent amounts in PKU

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Introduction: In PKU little is known about prescription of total protein in Europe. Methods: A questionnaire completed by 66 dietitians/doctors from IMD centres in 18 countries collected data on total protein intake (natural protein and protein substitute [PS]).
Results: The amount of total protein (from PS and natural protein) varied according to the European region. Higher amounts of total protein were prescribed in infants and children in Northern Europe (n=25 centres) (infants <1y, >2-3 g/kg/day; 1-3y, >2-3 g/kg/day; 4-10y, >1.5-2 g/kg/day), followed by Southern Europe (n=12 centres) (infants <1y, >2.5-3 g/kg/day; 1-3y, 2 g/kg/day; 4-10y, 1.5-2 g/kg/day), then by Eastern Europe (n=4 centres) (infants <1y, 2.5 g/kg/day; 1-3y, >2-2.5 g/kg/day; 4-10y, >1.5-2 g/kg/day), with Western Europe (n=25 centres) giving the least (infants <1y, >2-2.5 g/kg/day; 1-3y, 1.5-2 g/kg/day; 4-10y, 1–1.5 g/kg/day). Total protein prescription was similar in patients aged >10y (1–1.5 g/kg/day) and maternal patients (1–1.5 g/kg/day).
Conclusions: The amounts of total protein prescribed were variable between European countries and appear to be influenced by geographical region. All give higher than the recommended 2007 WHO/FAO/UNU safe levels of protein intake in PKU.
Conflict of Interest declared.

P-047

Feeding patterns and practices in young children with PKU during the first 2 years of life: a longitudinal study

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Objectives: A longitudinal study that observed feeding practices and introduction of a spoonable phenylalanine-free protein substitute (SPS) in infants with PKU.
Methods: 11 subjects (6 male) were reviewed monthly from weaning to 12 m of age and at 15, 18 and 24 m. The low phenylalanine diet, intake of SPS, infant protein substitute (IPS), and weaning development were observed.
Results: Median weaning age was 4.2 m (range: 3.2-5.7), followed by introduction of SPS increasing in increments of 2 g/day of protein equivalent (PE), titrated with reduction in IPS. The median percentage of total L-amino acids taken as SPS increased, from age 4 m, 18 % (0.3 g/kg/day); 7 m, 50 % (1.3 g/kg/day); 12 m, 75 % (1.9 g/kg/day); 18 m, 94 % (2.5 g/kg/day); 24 m, 100 % (2.6 g/kg/day). Median intake of IPS gradually decreased from age 4 m, 560 ml/day (1.3 g/kg/day); 9 m, 360 ml/day (0.8 g/kg/day); 12 m, 300 ml/day (0.6 g/kg/day); and 24 m, 0 ml (0 g/kg/day). Median natural protein intake remained at 3-5 g/day throughout the study. Mean blood phenylalanine remained within target ranges. Negative behaviours (mouth closing, head turning, pushing away spoon) and least relaxed mealtimes peaked at age 7-15 m, coinciding with teething.
Conclusion: Gradual introduction of SPS throughout weaning successfully enables children with PKU to become accustomed to solid foods with less dependence on high intakes of IPS.
Conflict of Interest declared.

P-048

Dietary intake in young adults with long chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency

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Background and objectives: No data has been published on the dietary management of LCHAD deficiency and the impact on complications in adults. Dietary intake of young adults with LCHAD deficiency, treated from childhood, was assessed and compared to their clinical outcome. Dietary management involves avoiding fasting with overnight feeding or uncooked cornstarch (UCCS), emergency regimens, severe restriction of dietary fat and supplementation of medium chain triglycerides (MCT), essential fatty acids and ensuring adequacy.
Patients and Methods: Dietary information was collected by recall from five adults aged 18–24 and analysed with DietPlan6 software. Case notes provided clinical outcomes and bodyweight.
Results: Mean daily intake of dietary fat 21 g (4.8-38.7 g/day), percentage energy from; dietary fat 8.8 % (1.5-15.9 %), MCT 10 % (4-27 %), omega 6 1.1 %, omega 3 0.2 %, protein 14 % (7.8-21.7 %) and carbohydrate 66 % (60.9-73.1 %). One patient continues overnight feeding and 4 use UCCS (mean dose 0.8 g/kg (0.7-1 g/kg)). Mean BMI 24.5 kg/m^2 (23.2-28.1 kg/m^2). One patient has hospital admissions for rhabdomyolysis (<1/year). Two patients used their emergency regimens within the last year. Three patients have stable macular dystrophy, one patient has learning difficulties, one has moderate neuropathy and none have cardiomyopathy.
Conclusion: Current diet composition varies considerably without obvious correlation to clinical outcome.

P-049

Plasma chitotriosidase activity in obese children

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Introduction: Existence of low grade persistent inflammation in obese children may increase the risk of metabolic and cardiovascular events in their later life. Activated macrophages secrete several proteins like chitotriosidase which could influence the atherosclerotic process. Human chitotriosidase is a recently described fully active chitinase expressed by activated macrophages.
Design and Methods: The aim of the present study was to determine whether obesity in children has an influence on inflammatory markers. Thirty-one obese children (mean age: 11.8±2.8 years; mean BMI: 27.6± 3.8 kg/m2) and twenty healthy controls (mean age: 12.1±3.2 years; mean BMI 20.1±3.5 kg/m2) participated in the study. All of the obese children were recruited from the Pediatric Endocrinology and Metabolism outpatient clinic of Gazi University Hospital.
Results: No difference was found among two groups in terms of age (p= 0.7). Plasma chitotriosidase activity were found to be significantly higher in the obese children than healthy age-matched controls, 9.5 (12.2±8.6) vs 2.5 (2.7±1.9) nmol/h/ml
Conclusion: Elevated chitotriosidase activity has been described in lysosomal storage diseases, glycogen storage disease type I, IV and several other disorders including multiple sclerosis, diabetes mellitus, cerebral adrenoleukodystrophy, malaria, sarcoidosis, atherosclerosis, inflammatory bowel disease and prostate cancer. We report significant elevations of chitotriosidase activity in patients with obese children.

P-050

Protein and calorie intake among adult subjects with urea cycle disorders (UCDs) participating in the pivotal glycerol phenylbutyrate (GPB) clinical trial

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Objective: To analyze protein and caloric intake among adult UCD patients in the GPB (HPN-100, RAVICT®) pivotal trial.
Methods: Forty-four patients (mean age 32, 89 % OTC) completed a 28 day, randomized, double-blind, placebo-controlled cross-over comparison of 24-h ammonia exposure on GPB vs. sodium phenylbutyrate (NaPBA). Weekly 3-day diet histories were collected. Prescribed and actual protein and caloric intakes were compared to those for healthy adults (HA) derived from the National Health and Nutrition Examination Survey and current recommendations for UCD patients (UCD-REC).
Results: Mean protein intake averaged 53-56 % of that for HA and greater than UCD-REC with 10 % caloric intake was from protein compared with 15 % for the HA. Caloric intake was ~70 % of that for HA and 20-25 % less than UCD-REC. Protein and caloric intake as a percentage of the amount prescribed was, respectively, 93 % and 87 % on NaPBA vs. 101 % and 91 % on GPB. There was an inverse correlation between fasting ammonia and compliance with caloric intake ($r=-0.22$; $p=0.16$) and a positive correlation between blood urea nitrogen (BUN) and total protein intake ($r=0.55$; $p=0.0003$).
Conclusions: UCD subjects ingested about 50 % less protein and one-third fewer calories than HA and ingested more protein but fewer calories than UCD-REC.
Conflict of Interest declared.

P-051

Management and long-term outcomes of classical galactosaemia – a single centre experience in the UK

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Background: Galactosaemia is an autosomal recessive disorder of galactose metabolism. Internationally the degree of dietary galactose restriction varies. UK practice avoids lactose from dairy food, galactose from fruit and vegetables is allowed. Galactosaemia is not part of newborn screening in the UK. The long-term outcomes remain sub-optimal despite early intervention.
Method: A retrospective review of the management and outcome of patients seen in a tertiary metabolic centre. Results: Fifty-one patients were identified, 24 female. Five patients were sibling pairs. Diagnosis was confirmed by enzymology and/or mutation analysis (median age 12 days, range 0–365 days). All treated with a lactose-free diet; 21 % patients met calcium requirements by soya milk/food alone; the remaining from lactose-free cheese, calcium enriched foods and/or supplements. 63 % patients had normal BMI (WHO classification). 70 % patients (>10 years of age) had normal bone density.
Outcomes: Median age at last follow up was 8.8 years (range 0.8-41 years). 68 % reported learning difficulties and 59 % speech and language problems (>3 years of age). 83 % of female patients (>12 years of age) required hormone replacement therapy.
Conclusions: We present a large UK series with long-term follow up. Patients with galactosaemia continue to develop significant complications despite dietary intervention.

P-052

Dietary management and growth outcomes in children with propionic acidaemia (PA)

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Background: Dietary management of PA is a low protein diet with adequate energy, avoidance of extended fasting and an emergency regimen in illness. Such treatment puts patients at potential risk of compromised growth. Objectives: To retrospectively review dietary management and longitudinal growth in our cohort of PA patients.
Case Series: 10 patients aged 5 m to 18y (median 3y 4 m): n=6 early onset, n=4 late onset. Results: Eight patients were restricted in natural protein to safe levels (FAO/WHO/UNU 2007), two self-selected a low protein diet. None had precursor free amino acids. Energy intake was 100 % estimated average requirement (EAR) for n=6, 80 % EAR n=2, 115 % EAR n=2. Five patients had a gastrostomy/long-term nasogastric feeding tube, of whom 3 used it daily. On treatment n=8 increased or maintained height z-score from diagnosis, n=2 dropped z-score. Nine increased or maintained weight z-score from diagnosis, n=1 dropped z-score. Emergency regimen use varied between monthly to less than once/year.
Discussion: It has been possible with protein restriction to safe levels and adequate energy (some requiring tube-feeding support) to achieve consistent or improved height and weight growth in most of our PA cohort. This was observed even in those requiring most frequent use of emergency regimen.

P-053

Overweight/obesity and associated causative factors in UK children with PKU

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Introduction: There is little data about the prevalence and causes of overweight and obesity in UK children with PKU.
Aim: to examine the prevalence and potential causes of overweight and obesity in children with PKU in one UK centre.
Methods: The BMI z scores of 79 children aged 2-17y with PKU were calculated. A lifestyle and activity questionnaire was completed by 50 children aged between 5-17y.
Results: In PKU, data for overweight/obesity was as follows: 25 % were overweight and 6 % obese. In boys, 20 % were overweight and 2 % obese; in girls 32 % were overweight and 12 % obese. Compared with data from the normal healthy population (aged 2–15 years), 31 % boys were overweight and 17 % obese; and 29 % girls were overweight and 15 % obese. In PKU, 88 % of obese children had at least one obese parent. 61 % of mothers of overweight/obese PKU children had a low educational level compared with 22 % from the healthy weight group. In PKU children, the median number of fruit/vegetable portions was only 3/daily (range 1–9). 65 % consumed >3 sweetened beverages daily.
Conclusions: Although overweight/obesity prevalence is similar to the general population, education is needed on healthy food choices in children with PKU.

P-054

A protein-free formula designed for children >1 years improves nutritional intake in children with organic acidaemias

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Introduction: In the UK, there is no suitable protein-free tube feed [child PFTF] for children aged >1 year.
Aim: A longitudinal evaluation of a pre-measured, child PFTF [Basecal (Vitaflo International)] for use in organic acidaemia in children >1y.
Methods: 12 subjects (5 PA, 4 MMA, 1 GA1, 2 IVA) median age 5 y (2–12) were recruited. Child PFTF replaced infant PFTF. Anthropometry and nutrient intake were studied for 12 months on child PFTF. Caregivers prepared samples of the infant and child PFTF for nutrient analysis of feed. This was to assess accuracy of feed production.
Results: The nutrient intake improved on the child PFTF [% RNI (DH 1991) calcium intake: infant PFTF 159 %, child PFTF 167 %; zinc intake: infant PFTF 160 %, child PFTF 193 %]. Iron intake remained unchanged. Median height (−1.3) and weight (0.6) z score remained unchanged. The nutrient analysis of prepared feed was variable. In the infant PFTF, the median value compared with calculated amount for fat and zinc was 91 % for both nutrients. In the child PFTF it was 94 % and 96 % respectively in prepared feed.
Summary: The child PFTF maintained growth and provided age-related nutritional requirements. The pre-measured child PFTF simplified free preparation, although water measurement accuracy was still problematic.
Conflict of Interest declared.

P-055

Dietary management in fatty acid oxidation defects (VLCADD, MCADD, MADD, SCHADD) - Experience from a Portuguese centre

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Background and objectives: There are no scientific evidences on which to support dietary treatment recommendations for fatty acid oxidation defects (FAOD). The aim of this work is to share our experience as there is an increasing need for establishing consensual dietary management guidelines, since FAOD were included in Portuguese newborn screening program in 2004.
Case reports: We have treated one neonate with Very long chain fatty acid dehydrogenase deficiency (VLCADD), two neonates with Medium chain acyl-CoA dehydrogenase deficiency (MCADD), one neonate with Multiple acyl-CoA dehydrogenase deficiency (MADD) and one neonate with Short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency (SCHADD). All of them were asymptomatic. The nutritional management in all cases were avoidance of fasting, the prevention of hypoglycemia, and use of aggressive intervention with hypercaloric "per os", tube or intravenous fluids in case of illness.
Discussion/Conclusions: Clinical monitoring and continuous management of the diet are the most important issues. In all of our patients a restriction (20-30 % total caloric value) of dietary fat was given, with adequate other macro and micronutrient supply, in order to ensure correct growth, and minimize long term consequences. The most concerning disease is VLCADD, because of frequent episodes of rhabdomyolysis. MADD responded well to riboflavin supplementation. In SCHADD, although it is a FAOD enzyme deficiency, protein is also important in the diet.

P-056

Is prealbumin a valuable marker for protein energy malnutrition in patients with phenylketonuria in the metabolic centre of Ghent (Belgium)?

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Background: PKU patients run the risk of a degree of protein malnutrition. Prealbumin has been suggested as a marker for protein deficiency in critically or chronically ill patients. In our PKU population we investigated whether prealbumin was correlated to body length and weight and if prealbumin could be used as an early nutritional marker.
Patients: In 35 PKU patients, the z-score of the body length, weight and the intake of amino acid supplements were registered. Furthermore, prealbumin, zinc, phenylalanine and amino acid profile were measured. For age-correlated statistical analysis, the group was divided in age categories.
Results: In our population, 60 % had a prealbumin lower than 20 mg/dL. None of the patients were severely growth impaired (z-score −2,8 to 0,9). However, 68 % of the patients had a negative z-score of height. Prealbumin correlated with age, but not with the z-score of height or weight in the different age categories. No significant correlation was seen between prealbumin, zinc, amino acid mixture and profile or phenylalanine.
Discussion: Although prealbumin levels were decreased in 60 % of our PKU population, no correlation was found with height or weight. The clinical significance remains unclear. Further studies of how prealbumin is influenced by the diet are needed.

P-057

Breast feeding in pyridoxine non-responsive homocystinuria (HCU)

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Background: There are no published reports of breast feeding (BF) in HCU. We report experiences of BF management of three infants with pyridoxine non-responsive HCU, diagnosed by ENBS project (www.expandedscreening.org/).
Methods: A retrospective review of patient records from diagnosis up to 6 months of age was performed. Data was collected on methionine-free infant formula (MFF) intake, BF, growth, plasma tHcy, fHcy, methionine and cysteine. Breast milk intake was estimated to be 160 ml/kg bodyweight/day minus volume of MFF.
Results: All were BF and had MFF (commenced aged 26, 32 or 43 days). All gained weight normally. One breast fed well but struggled to take adequate MFF and despite Betaine treatment, tHcy remained>90 μmol/l (median 110 μmol/l), BF was stopped 12 weeks into treatment. Two successfully took MFF before breast feeds, once treatment stabilised plasma methionine continued in normal range, tHcy, case 1; median 48 μmol/L (range 21–114), case 2; median 37 μmol/L (range 11–76).
Conclusion: HCU infants can be breast fed and achieve a low plasma tHc, if able to take adequate MFF. Plasma tHcy was mostly<50 μmol/l and methionine in normal range, when supplementary MFF limited BF to provide estimated intake of around 100-110 mg methionine/day.

P-058

Protein substitutes and special low protein foods available in Europe for the treatment of phenylketonuria

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Background and Objectives: Phenylalanine-free protein substitutes (PS) and special low protein foods (SLPF) remain the main stay of treatment in PKU. The objective of this study was to identify the number and differences in nutritional composition of PS and SLPF available for use in eight European countries and Turkey.
Methods: European Nutritionist Expert Panel on PKU (ENEP) members (Portugal, Netherlands, Denmark, Spain, Germany, Turkey, Italy, UK and Belgium) provided data on PS and SPLF available in each country. The nutritional composition of all PS and SLPF available in Portugal was critically analyzed.
Results: The number of different PS available in each country varied widely; median 61 (range 30 in Turkey; 105 in Germany). Glycomacropeptide PS was available only in Portugal and large neutral amino acids in 4 countries (Portugal, Italy, Turkey and Denmark). In Portugal, the nutritional profile of PS differed widely while some SLPF have a significantly higher energy content compared to others, which impairs its classification of free.
Discussion/Conclusion: While equal access to all PS and SLPF is desirable, as in the example of Portuguese products, the widely variable nutritional composition requires careful examination of all products when prescribing them for individual patients during their lifespan.
Conflict of Interest declared.

05. New metabolic disease groups

P-059

LARS2 variations associated with a hydrops, lactic acidosis, sideroblastic anaemia and multisystem failure

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Background: Mutations in the LARS2 have previously been associated with Perrault syndrome (premature ovarian failure and hearing loss). LARS2 encodes mitochondrial leucyl-tRNA synthetase which is required for mitochondrial protein synthesis. In this study we report LARS2 variations associated with a severe multi-system disorder.
Patient & Methods: The proband was born prematurely (27 weeks) and had severe lactic acidosis, hydrops and sideroblastic anaemia. She developed multi-system disease and succumbed at 5 days of age. Whole exome sequencing (WES) and functional studies were undertaken in a bid to identify a genetic diagnosis.
Results & Discussion: WES revealed compound heterozygous variations in LARS2 (c.1289C>T; p. Ala430Val and c.1565C>A; p. Thr522Asn). The c.1565C>A (p. Thr522Asn) mutation has previously been associated with Perrault syndrome. Muscle samples displayed no clear respiratory chain enzyme deficiency. Western blotting of patient muscle, liver and fibroblasts did not reveal any deficiency in LARS2 or OXPHOS protein levels. Aminoacylation assays are currently being undertaken to determine the effect of each of these mutations on the catalytic efficiency of LARS2.
Conclusion: We speculate that LARS2 mutations may result in variable phenotypes. Further specific functional studies will clarify whether the LARS2 variations identified were responsible for the severe multisystem clinical phenotype seen in this baby.

P-060

Ethylmalonic aciduria and auditory neuropathy: a new clinical entity

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EMA is a non-specific finding in different metabolic disorders. We describe 3 cases with EMA associated with deafness. A 13-year old boy, from consanguineous parents, presents with microcephaly, deafness, EMA, mental retardation, autism spectrum disorder and hemophagocytic lymphohistiocytosis at 11 years. The karyotype in skin fibroblasts was normal. Micro-array showed two duplications, involving chr.5 p12 and chr. Xp22.33. Our second patient is a 9-year old boy, from consanguineous parents, presenting with deafness, mental retardation and EMA. We

found no ETHE1 mutations. Acylcarnitines were normal. A deletion in chr.10q11.22 was detected. His brother is also affected with deafness, delayed psychomotor development and behavioral problems. EMA was found in two samples. Our patients presented not only EMA but also hearing loss. The clinical impact of the duplications on chr.5 and chr. X in our first patient are unknown. Concerning our second patient, the deleted region contains 33 genes. Comparable deletions are described in patients with very variable phenotype and are possibly a factor of susceptibility for mental retardation but have also been detected in healthy parents. Neither deafness, nor EMA have been reported. We suggest that these children with EMA and auditory disorder belong to a same entity. SCAD-deficiency and ethylmalonic encephalopathy seem to be unlikely.

06. Phenylketonuria: general

P-061

Melatonin and dopamine as biomarkers to optimize treatment in phenylketonuria: Effects of tryptophan and tyrosine supplementation

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Objective: To determine if additional supplementation of Trp and Tyr improve serotonin and dopamine metabolism in the PKU individuals treated with large neutral amino acid (LNAA) tablets: LNAA therapy provides Trp 30 mg/kg and Tyr 100 mg/kg.
Methods: Ten adult individuals with PKU participated in a randomized double-blind placebo controlled crossover study consisting of three 3-week phases: washout, treatment with LNAA tablets plus supplementation with either Trp/Tyr tablets or placebo, and LNAA tablets plus the alternate supplementation: LNAA+Trp/Tyr provides Trp 100 mg/kg and Tyr 200 mg/kg. An overnight protocol to measure blood melatonin, a serotonin metabolite in the pinealocytes, and 6-sulatoxymelatonin and dopamine in first void urine specimens was conducted after each phase.
Results: Serum and urine melatonin and urine dopamine levels were increased in the LNAA phase compared to the washout phase. Serum and urine melatonin did not increase in the LNAA+Trp/Tyr phase compared to the LNAA phase. A negative correlation between urine melatonin and blood Phe levels was observed.
Conclusion: Melatonin levels did not increase with the larger dose of Trp supplementation although dopamine levels were increased corresponding to the larger dose of Tyr supplementation. Serotonin synthesis appears to be suppressed by high Phe levels at the Trp hydroxylase level.
Conflict of Interest declared.

P-062

The high dietary acid load provided by an amino acid diet increases renal net acid and calcium excretion and reduces femoral cross sectional area in wild type and phenylketonuria Pah^{enu2} mice

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Background: Human phenylketonuria (PKU) utilizes amino acid (AA) formula and is associated with skeletal fragility of unknown etiology.
Objective: We determined if the high dietary acid load provided by an AA diet increases calcium and renal net acid excretion (NAE), and reduces bone size and bone mineral density (BMD) compared with the low-acid glycomacropeptide (GMP) diet.
Methods: PKU (Pah^{enu2}) and wild type (WT) mice were fed low-phe AA, low-phe GMP or high-phe casein diets from 3 to 20 weeks of age. Urine pH, calcium excretion, NAE, and femoral size and BMD were determined.
Results: In both WT and PKU mice, the AA diet significantly decreased urine pH (from 7.4 to 5.5), and increased renal mass, 24 h urine calcium excretion, and NAE compared with the GMP diet. The AA diet significantly reduced femoral BMD and length in WT mice. Femoral cross sectional area was significantly reduced with the AA diet compared to the casein and GMP diets.
Conclusions: The high-acid AA diet increases renal net acid and calcium excretion and reduces femoral size compared with the low-acid GMP diet in both WT and PKU mice. Current usage of AA formula may contribute to skeletal fragility in human PKU.
Conflict of Interest declared.

P-063

A systematic review of bone mineral density and fractures in phenylketonuria

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Objective: Skeletal fragility is a common but poorly understood complication of phenylketonuria (PKU). We analyzed published data on bone mineral density (BMD) and fracture rates in patients with PKU.
Methodology: We searched PubMed, CINAHL and Cochrane databases from January 1966 to November 2013 for studies of spine BMD or fracture in PKU and control subjects. Sixteen of 52 studies met eligibility criteria.
Results: Meta-analysis of 3 eligible studies found that spine BMD was 0.100 g/cm^2 lower (95 % CI, −0.110, −0.090 g/cm^2) in 67 subjects with PKU, compared to 161 controls. Among 6 studies, 20 % of PKU subjects (53 of 263 subjects) experienced clinical fractures. In the single study with controls, the fracture rate was 2.6 fold higher (95 % CI, 1.1-6.1) after age 8 in PKU subjects, compared to healthy sibling controls. Among 12 studies in 412 subjects, there was no consistent relationship between phenylalanine levels and BMD in 71 % of the subjects studied.
Summary: Spine BMD is lower in PKU than control subjects and 20 % experience clinical fractures. However, there is no consistent relationship between phenylalanine levels and BMD. Future studies are needed to clarify the etiology and health consequences of low BMD in PKU.
Conflict of Interest declared.

P-064

Assessment of the quality of life in Russian children with phenylketonuria

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Background: Phenylketonuria (PKU) is one of the most common inherited disorders of amino acid metabolism. In accordance with the results of neonatal screening the PKU incidence in Russia is 1:7145.
Objective: Evaluation of the quality of life in PKU children.
Patients and Methods: We examined 64 PKU children with age 5 to 12 years. The questionnaire PedsQLtm4.0 (Varni et al.,USA, 2001) and SPSS (USA,version 14) were used for analysis.

Results: We revealed physical and psychosocial health level decreases in PKU patients compared with healthy children. The most significant differences ($p<0.001$) were observed in social and role functioning in nursery or at school. Psychosocial health levels in 8–12 years aged PKU patients were lower compared to 5–7 years aged patients ($p<0.001$). There was an inverse correlation identified between the social ($r=-0.336$; $p<0.001$) and role functioning ($r=-0.205$; $p<0.001$) in PKU patients and the age of the dietary treatment start. Inverse correlation was detected between the role functioning and the age of patients ($r=-0.251$; $p<0.001$).
Conclusion: The results confirm the need for early detection of phenylketonuria, efficiency of timely beginning of dietary treatment and psychological support for PKU patients families.

P-065

Development and validation of disease-specific quality of life questionnaires for individuals with phenylketonuria and their parents

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Introduction: Because generic quality-of-life (QoL) questionnaires fail to detect impaired QoL in phenylketonuria (PKU), this study developed and validated PKU-specific QoL questionnaires for individuals with PKU and their parents.
Methods: Observational study conducted in seven European countries to finalize and validate four PKU-QoL questionnaires in individuals with treated PKU aged 9–11, 12–17 and ≥18 years, and in parents of individuals aged
Results: Overall, 559 participants (306 individuals with PKU aged 9–45 years; 253 parents aged 24–66 years) were included. Return rate and quality of completion of the questionnaires were good, indicating good acceptability. Scores were defined to assess all relevant aspects of QoL experiences: impact of PKU (symptoms, emotional, practical, social, financial and overall), impact of PKU treatment (dietary protein restriction and supplementation). Reliability and validity (concurrent and clinical) were satisfactory overall for the adolescent, adult and parent PKU-QoL questionnaires, and poorer but acceptable for the child version.
Conclusion: The PKU-QoL questionnaires demonstrated satisfactory measurement properties for use in clinical research. Its use in the management of PKU in clinical practice is being defined.
Conflict of Interest declared.

P-066

The effect of blood Phe levels on plasma concentrations of biogenic amine in PKU patients

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Background: High levels of Phe may influence the activity of Tyrosine Hydroxylase (TH) and Tryptophan Hydroxylase (TRH), resulting in a defect of biogenic amine homeostasis.
Aim of the study was to explore the effect of blood phenylalanine (Phe) on the plasmatic levels of monoamine neurotransmitters (and related metabolites) in PKU patients.
Patients and methods: Homovanillic acid (HVA), serotonin (5-HT) and 5-hydroxyindolacetic acid (5-hiaa) were measured in plasma of 43 phenylalanine hydroxylase deficient patients (age range: 1,7-49,9 years) and 16 control subjects (age range: 1,9-55 years). In PKU patients concomitant blood Phe, Tyrosine (Tyr), Phe/Tyr, urinary pterins, and IDC of the last 6 months were also measured.
Results: PKU patients showed significantly lower levels of monoamine metabolites compared to controls (5-HIAA $p<.0001$; HVA $p<.0001$). Significant negative correlations were found between IDC in the last 6 months and: HVA ($r=-.59$, $p=.0006$) and 5-HIAA ($r=-.56$, $p=.0012$); and between the concomitant level of Phe and 5-HIAA ($r=-.62$, $p<.0001$).
Conclusion: These results confirm the negative effect of high Phe levels on monoamine metabolism in PKU patients involving the dopamine (DA) and the 5-HT metabolic pathways. The relationship between peripheral and central levels of these metabolites deserves further investigations.

P-067

IgE mediated allergies' prevalence and dietetic treatment's impact on sensibilization in PKU affected patients

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Background and objectives: Hyperphenylalaninemia (HPA) is a genetic disorder caused by defect in phenylalanine hydroxylase enzyme activity or in synthesis/regeneration of its cofactor, leading to plasmatic Phe increase and consequent brain damage. Main available therapy to date consists in diet-therapy, low in protein and controlled in Phe intakes. We evaluated food and inhalant allergic sensibilization prevalence and compared diet-therapy effects on food allergies among HPA patients on (OD) or without diet (NOD).
Patients and methods: 659 patients were studied (49 % OD, 51 % NOD), evaluating Skin Prick Tests (SPT) and IgE levels.
Results: No difference between food and inhalant allergic sensibilization prevalence among HPA patients (17.4 %) and general population (17.4 %) was shown. Diet therapy has no influence on inhalant allergic sensibilization prevalence, nor on its age of onset, symptoms or allergens to which there is sensibilization. Food allergies prevalence was found to be significantly different between OD and NOD patients: 17.4 % vs 27.8 %, respectively.
Conclusions: Diet therapy has a protective effect regarding food allergies, being free of animal derivatives. In particular, as previously shown in literature, OD patients demonstrate lower IgE levels then NOD, both show lower IgE levels than the general population. Risk of developing food allergy sensibilization however is not eliminated.

P-068

Occupational issues and compliance to dietary treatment in a population of adults affected by classic phenylketonuria

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Background and objectives: Phenylketonuria (PKU) is a metabolic disorder caused by mutation of the phenylalanine hydroxylase (PAH) gene leading to accumulation of phenylalanine (Phe) in the body and developmental delay. To date, main available treatment is a diet-therapy to be followed longlife. With improvements in clinical and metabolic follow up, an increasing number of PKU patients reach adulthood entering work

world. We tried to evaluate possible issues having and keeping a job in this population with daily scheduled dietary regimen to be merged with work time programming.
Patients and methods: 25 PKU patients on diet were enrolled, analyzing their history and dietary compliance. Data regarding work activities were collected from a questionnaire expressively built for this study.
Results: 76 % of patients declare that their pathology influences working activity needing permissions for periodic examinations or daily breaks to follow dietary regimen. Having full or part-time activity seems to influence Phe levels, with a mean Phe increase of 407 micromol/l for full-time activity and a mean Phe decrease of 186.79 micromol/l for part-time.
Conclusions: Clinicians have to pay attention dealing with working PKU patients, in order to best fit their needs facing work time schedules and guarantee adequate dietary compliance.

P-069

A systematic review (SR) of the effectiveness of reducing blood phenylalanine (Phe) levels in adults with phenylketonuria (PKU) on neuropsychiatric symptoms

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Background and Objectives: Treatment guidelines recommending blood Phe maintenance levels in PKU are based on studies in children. A SR was conducted to assess evidence that reducing blood Phe levels in individuals with PKU ≥16 years of age results in decreased neuropsychiatric symptoms.
Methods: The SR of published literature included searches in MEDLINE, Embase, and Cochrane Collection databases from January 1980 through June 2013 supplemented by manual searches. Accepted studies and case reports described the effects of changing blood Phe levels in adults with PKU and neuropsychiatric symptoms.
Results: The SR identified 10 studies that assessed changing blood Phe levels for at least 3 weeks in adults with PKU and psychiatric symptoms. With the exception of one small case series (5 subjects), all studies showed a marked reduction in symptoms when mean blood Phe was reduced from 1160–1676 μmol/L to 479–758 μmol/L. Similar findings were reported in 20 cases of PKU adults with late-onset neurologic symptoms and 7 cases of PKU adults with intellectual disability and severely disruptive behaviors.
Discussion/Conclusion: Results from published interventional studies and case reports suggest a reduction of blood Phe to at least ‹800 μmol/L to reduce neuropsychiatric symptoms in adults with PKU.
Conflict of Interest declared.

P-070

Scanning cognitive dysfunctions in Hungarian adult PKU patients

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Objectives: We conducted a pilot study for scanning cognitive dysfunctions in adult PKU patients. CANTAB (Cambridge Cognition) research tests are sensivite methods to show tiny cognitive deficits in diseases involving the central nervous system. Question evoked, whether average Phe-level would influence these test results.
Patients and Methods: In a monocentric study 45 Hungarian adult patients with PKU (mean age: 29,9 years) were enrolled. Patients were divided in two groups whether the mean Phe-level was under (Strict diet group: SDG) or above (Moderate diet group: MDG) 600 micromol/L. Different CANTAB tests were used to detect sensorimotor (MOT), executive function (SOC) and spatial working memory (SWM) deficits.
Results: The mean Phe level was 631±201 micromol/L. We did not find any correlation with serum Phe, Tyr or Phe/Tyr ratio and the different tests results (p-value: Phe-MOT:0.217; Phe-SOC: 0.312; Phe-SWM: 0.198). Comparing the SDG and MDG group, there was not any significant difference in tests' result (p-value: MOT: 0.15; SOC: 0.145; SWM: 0.32).
Conclusion: In this small sample size pilot study there was not any significant correlation in Phe level with CANTAB cognitive tests' results. There is an ongoing larger population study allowing us to have more results in adult patients with PKU.

P-071

Impact of family structure on dietary adherence in patients with phenylketonuria

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Background and objectives: Individuals with phenylketonuria (PKU) treated with a phenylalanine-restricted diet from the first month of life tend to decrease dietary adherence during adolescence. This study evaluates the impact of family structure on dietary management of PKU patients.
Patients and methods: Forty-four families of children with PKU aged 4–16 years were evaluated for physician/family relationship, for individual psychological assessment and for family structure. These parameters were evaluated through a semi-structured interview for psychological evaluation of the family and its interaction with physicians and by tests for cognitive, psychological and social estimation of the patients.
Results: The physician/family relationship was not a factor involved in reducing adherence dietary treatment. However there was a relationship between family structural features and the reaction to PKU diagnosis. In particular the first family reaction to PKU diagnosis was predictive of the psychological process of acceptance or rejection of the disease and consequent adherence to the treatment. Moreover most patients with low dietary adherence had psychological distress symptoms; in fact 78 % of preadolescents and adolescents patients had poor emotional and social level of autonomy and they were unable to manage by themselves the diet.
Conclusions: Our study suggests that an better initial communication could improve adherence treatment.

P-072

Age–related psychophysiological vulnerability to phenylalanine in young-adult phenylketonuric subjects

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Background and objectives: An increased risk of neurocognitive and psychiatric problems in adulthood remains a challenging aspect in phenylketonuric (PKU) patients. In order to assess the neurocognitive vulnerability to Phe in adolescent and young adult PKUs, we explored: a) the effect of a rapid increase in blood Phe on event-related potentials (ERP); b) the association between psychophysiological and neurocognitive features.
Patients and methods: Seventeen early-treated PKU subjects, aged 10 to 20, underwent ERP (Mismatch Negativity, auditory P300, Contingent Negative Variation (CNV), and Intensity Dependence of Auditory Evoked Potentials) recording before and 2 h after an oral loading of Phe. Neurocognitive functioning, historical and concurrent biochemical control (blood Phe, Tyr, Phe/Tyr) were included in the statistical analysis.
Results: ERP components were normal in all the subjects. In those younger than 13 CNV amplitude, W2-CNV areas, P3b latency, and Reaction Times in motor responses were negatively influenced by Phe loading. Moreover, also neurocognitive skills were more impaired in younger patients. Biochemical control and neurocognitive and psychophysiological findings were not correlated.
Discussion/Conclusion: The vulnerability of the emerging neurocognitive functions to Phe suggests a strict metabolic control in adolescents affected by PKU and a neurodevelopmental approach in the study of neurocognitive outcome in PKU.

P-073

Neuropsychological profile and relations to phe levels in early-treated adults with PKU

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Aims: To establish which cognitive functions remain impaired in early-treated PKU patients and associations with Phe levels.
Method: 36 early-treated adults (18–41 years) and 26 matched controls were administered a large number of tasks (N=28) across different areas.
Results: PKU participants performed worse in all tasks requiring speed, but also in non-speeded tasks probing: phonological processing (digit-span, spoonerisms, phoneme-deletions, nonword repetition); planning (WCST, Tower of Hanoi) and sustained attention (RPV). NO group impairment was found in visuo-spatial memory; lexical processing (learning new words, word recall/recognition and picture naming) and orthographic processing (word and nonword spelling, word and nonword reading accuracy). A combination of five tasks discriminate patients from controls in 81.8 % of cases. Performance correlated with current and past levels of Phe only for a few tasks, but correlations with Phe variation were more pervasive. The best discriminating tasks were different from those showing a Phe association.
Conclusions: Cognitive impairments are selective involving especially planning, attention, speed, and motor coordination. The relation between Phe-levels is not linear. While certain functions remain mildly impaired across the range of phe levels in our sample (resulting in no correlation, but a group impairment), others appear impaired only when a certain threshold is reached.

P-074

Dendritic anomalies in disorders associated with mental retardation: evidence from phenylketonuria

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Background and objectives: Kaufmann and Moser (2000) reported that dendritic abnormalities are the most common anatomical correlates of Mental Retardation (MR) in genetic disorders, suggesting that a primary genetic defect leads to alterations of the protein expression pivotal for dendritic protein expression. This work aims to investigate molecular dendritic mechanisms underlying MR in phenylketonuria (PKU), the typical human Mendelian disease, caused by a mutation in the gene coding for the enzyme phenylalanine hydroxylase, and characterized by MR if untreated.
Materials and Methods: ENU2 mice represents a valid tool to identify molecular mechanisms underlying MR in PKU. Since our previous data showed a crucial role for serotonin in the PHE-induced cognitive dysfunctions, we firstly have analyzed the expression of serotonergic receptors, and then neuroligins, a family of synaptic cell adhesion molecules playing a role in the recruitment of neurotransmitter receptors at the synapse.
Results: We observed higher messenger levels of cortical 5-HT2A receptors in ENU2 compared with WT mice. Analysis of protein and messenger levels for the Neuroligins revealed significant increased levels of Neuroligin1 in ENU2 compared with WT.
Conclusion: Our data reveal pieces of a puzzle that strongly support dendritic abnormalities towards higher overall cortical excitation in PKU mice.

P-075

Three girls with microcephaly and hyperphenylalaninemia

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Case series: Here, we present three Turkish girls with microcephaly and hyperphenylalaninemia, who shared another comorbid condition.
Patient A was a 22-day-old premature girl born at 32 weeks of gestation who was found to have a blood phenylalanine level (BPL) of 1698 μmol/l and put on phenylalanine-restricted diet. Despite good metabolic control, she had delayed developmental milestones. Microcephaly became evident as she thrived and her mother's BPL was found to be 1056 μmol/l.
Patient B presented at two months of age due to Guthrie test positivity. Upon examination she was found to have microcephaly and bilateral pes equinovarus. Her and her mother's BPLs were 906 and 984 μmol/l, respectively.
Patient C presented with a BPL of 144 μmol/l and microcephaly. At 14 months of age, while her developmental milestones were within normal limits, her mother's BPL was 364 μmol/l. Patient C and her mother were both compound heterozygotes for R241H/A403V mutations on the PAH gene.
Conclusion: These three cases demonstrate the necessity for screening for maternal phenylketonuria and mild hyperphenylalaninemia if the offspring has early-onset microcephaly not attributable to phenylketonuria, especially if the mother's date of birth precedes the onset of national newborn screening in a country with high phenylketonuria prevalence like Turkey.

P-076

A case series of maternal phenylketonuria: Hacettepe experience

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Case series: Here, we present the largest series of maternal phenylketonuria (MPKU) cases from Turkey.
Methods: PKU patients with at least one pregnancy were included. Their offspring were re-evaluated.
Results: There were 17 patients with a total of 39 pregnancies, 11 treated and 28 untreated. One of the pregnancies was being treated with sapropterin and was terminated due to partial hydatidiform mole. 9/10 treated pregnancies carried to term yielded offspring currently having normal mental development and 3 cases of mild intrauterine growth retardation (IUGR). The other treated pregnancy had only a mild deviation from metabolic control during the seventh week of gestation and the infant was found to have IUGR and esophageal atresia with tracheoesophageal fistula. 10/28 untreated pregnancies were terminated, 5 resulted with spontaneous abortion and 18 with live births, all of which had IUGR, microcephaly and mental retardation. There were 2 cases of congenital heart disease, 1 developmental hip dysplasia, 1 unilateral renal agenesis, 1 bilateral pes equinovarus and 1 iridial atrophy. 4 of these children had hyperphenylalaninemia themselves.
Conclusion: The fetal risks of MPKU and the benefits of therapy are well demonstrated in our series.

P-077

A boy with dyslexia and attention-deficit hyperactivity disorder

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Case report: A thirteen-day-old boy was referred to our clinic upon detection of positive screening test for phenylketonuria (PKU) through nationwide screening programme. He was found to have a blood phenylalanine level of 669 μmol/l, diagnosed with mild phenylketonuria and started on a phenylalanine-restricted diet. Family history revealed an eight-year-old brother with dyslexia and attention-deficit hyperactivity disorder currently on methylphenidate and a distant relative with classical phenylketonuria. The elder brother was summoned to our clinic and was discovered to have mild phenylketonuria with a blood phenylalanine level of 860 μmol/l. He was found to be tetrahydrobiopterin (BH_4)-responsive based on BH4 loading test and started on sapropterin. Their genetic analyses revealed compound heterozygous mutations on the PAH gene, one of which was known to be responsive to BH_4. As of now, both brothers have good metabolic control with sapropterin alone on an unrestricted diet.
Conclusions: This case underlines the importance of investigating phenylketonuria in patients with mild cognitive and behavioural problems, especially in countries where nationwide neonatal screening was started realtively late or has poor coverage rate.

P-078

Dihydropteridine reductase (DHPR) deficiency in Ireland: Long-term follow-up and outcome of three patients

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Dihydropteridine reductase (DHPR) deficiency, an autosomal-recessive disorder of tetrahydrobiopterin (BH4) regeneration, causes hyperphenylalaninaemia and a profound deficiency in neurotransmitters. If left untreated, this can cause severe neurological symptoms. Treatment consists of a phenylalanine-restricted diet along with supplementation of L-dopa, 5-hydroxytryptophan and folinic acid. In this study, we report on the long-term clinical outcome of all DHPR deficient-patients in Ireland (n=3) diagnosed in Ireland since 1966 following the introduction of Newborn Screening for PKU. All patients are being treated at the National Centre for Inherited Metabolic Disorders in Temple Street Children's University Hospital, Dublin. These 3 patients represent 0.4 % of our total cohort of 716 patients with PKU/HPA. We present three cases in two families; all are members of the Irish Traveling Community. Two patients are adults (26 and 28 years., respectively, brother and sister of consanguineous parents); the third patient is currently 8 months of age. In both affected families, we have identified the mutation c.353C>T in the QDPR gene in a homozygous state which, to the best of our knowledge, has not been previously described. The 28 year-old woman has two unaffected children and had a miscarriage. We here describe the patients' biochemical profiles and clinical course.

P-079

Identification and characterization of critical postnatal period in a murine model of mental retardation: focus on PND14 in phenylketonuric mice

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Background and objectives: Phenylketonuria (PKU) is a typical human Mendelian disease caused by a mutation in the gene coding for the enzyme phenylalanine hydroxylase, and characterized by cognitive delay if untreated. To be effective, PKU treatments need to be administered from early infancy to adult life. Although the adult phenotype of ENU2 mice, the murine model of PKU, is well described, little is known about their neurobehavioral development. The aim of our study is to characterize early neurobehavioral development and to identify the critical postnatal period for neurochemical, functional and morphological brain characterization, in PKU mice.
Materials and Methods: WT, heterozygous and ENU2 BTBR mice are tested from PND4 to 28 on Developmental Reflex, Open Field, Homing Test, ultrasonic vocalization (USVs), body growth.
Results: ENU2 mice show deficits in the acquisition of all tested reflexes (particularly evident between PND14-17), in the USVs and in the Homing Test; their size is smaller than controls'; and they don't show motor deficits until PND28.
Conclusion: Our data describe neurobehavioral alterations occurring earlier then motor deficits in untreated PKU mice, consistently with the human pathology. The results also identify PND14 as a critical period for neurochemical, functional and morphological brain characterization. Follow-up experiments are currently in progress.

P-080

A possible role for neuroinflammation in the pathophysiology of brain damage in phenylketonuria

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Phenylketonuria is a disease caused by the deficiency of phenylalanine hydroxylase activity, leading to accumulation of phenylalanine in tissues and biological fluids of affected patients. Considering that the patients present a severe intellectual disability and that the pathophysiology is still uncertain, the aim of this study was to investigate neuroinflammatory parameters in brain of rats submitted to an experimental model of hyperphenylalaninemia (HPA). It was observed that cytokines interleukin 1 betta (IL-1β), interleukin 6, interleukin 10 (IL-10), and tumoral necrosis factor alpha levels were increased in cerebral cortex of HPA animals (5.2 μmol/g phenylalanine plus 0.9 μmol/g p-chlorophenylalanine) compared to control group. Increased IL-1β and IL-10 levels were also found in striatum. Phosphorylated inhibitor of kappa B kinase (pIKK) and nuclear factor kappa B (NFκB) levels were increased only in cerebral cortex of HPA animals. Furthermore, HPA increased myeloperoxidase activity in striatum and hippocampus, without altering the blood–brain barrier integrity. The present results showed that animals submitted to HPA experimental model present increased levels of cytokines, which could lead to phosphorylation of IKK and further activation of NFκB in the brain. These results suggest a possible role for neuroinflammation in the pathophysiology of brain damage characteristic of phenylketonuria.

P-081

Presentation and management of hyperthyroidism during pregnancy in a classical PKU patient

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Case Report: A 28 year old female with phenylketonuria (PKU) known to our service for excellent compliance and good metabolic control was duly and successfully started on a pre-conception diet. At week 5 of pregnancy, clinically well, she attended the clinic for confirmation of her pregnancy and was incidentally found to have a plasma phenylalanine level outside the desired range. Intensification of dietary therapy lowered plasma phenylalanine levels, but this trend was reversed by week 6. Re-assessment at this point led to the diagnosis of hyperthyroidism due to Graves Disease coinciding with the start of her pregnancy. Treatment with propylthiouracil instigated at week 6 swiftly resolved the hyperthyroidism. Restriction of natural protein and caloric supplementation was gradually lifted in tune with normalisation of thyroid function.
Conclusion:
1. Treatment with propylthiouracil for hyperthyroidism during pregnancy in has been effective and well tolerated in this individual with classical PKU
2. Hyperthyroidism overrode the capability of dietary interventions to control hyperphenylalaninemia in this case.
3. Plasma phenylalanine increase pre-empting clinical signs of hyperthyroidism and mirroring of control of hyperthyroidism by plasma phenylalanine levels are consistent with experimental evidence that whole-body amino acid and protein metabolism are early primary targets for thyroid hormone action.

P-082

Disturbed synaptic connectivity in the Pah^{enu2} mouse, a model of human phenylketonuria

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Phenylketonuria (PKU) is an autosomal-recessive inherited metabolic disorder, which is characterized by severe mental retardation unless the patients are treated with a specialized diet. The underlying mechanisms for the development of mental retardation are not known yet. In this study, we evaluated a mouse model of PKU (Pah^{enu2}) during postnatal development regarding synapse density and long-term potentiation (LTP) in the hippocampus, a brain area closely associated with learning and memory. In adult mutants, we found reduced synapse density and impaired LTP, a cellular parameter for memory. Synapse pruning, however, the physiological loss of synapses during early postnatal development up to 12 weeks, was clearly delayed, resulting in increased synapse density as compared to controls during this developmental period. Since physiological synaptic pruning depends on microglia activity, we found a consistent reduced number of microglia cells in the hippocampus of the mutants. Our data provide evidence for microglia as a target in PKU, which may account for disturbed synaptic connectivity in the hippocampus in response to elevated levels of phenylalanine and could contribute to mental retardation in PKU.

P-083

Novel transcription silencers in non-coding regions of PAH gene

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Phenylketonuria (PKU) is a metabolic disease caused by mutations in phenylalanine hydroxylase gene (PAH). Although PAHgenotype remains the main determinant, phenotype cannot always be predicted precisely. Previously, we found a transcription enhancer in PAHintron 8 that could affect genotype-phenotype correlation. In this study, we analyzed additional non-coding PAH gene alterations. In silico prediction for transcription factor binding sites pointed to a population-specific promoter alteration (PAH: c.-170delC) and VNTR alterations in 3' region. We transiently transfected HepG2 cell line with various CAT reporter constructs to determine the effect of PAH gene non-coding sequences on transcription. We found thatthe construct with additional binding site in promoter and constructs with VNTR3, VNTR7 and VNTR8 alterations had a 50-60 % reduction of CAT activity in comparison to pBLCAT5. EMSA supershift showed binding of KLF1 transcription factor to the analyzed promoter sequence, and also binding of C/EBPalpha to VNTR3. Our study pointed to new elements in promoter and 3' region of PAH gene that could act as transcription silencers and thus influence genotype-based prediction of PKU severity. New transcription regulators in non-coding regions will contribute to better understanding of PKU phenotype complexity and may become important for optimization of PKU treatment.

P-084

Phenylalanine hydroxylase genotypes in Europe and the Middle East

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Background: A comprehensive dataset on the frequency and geographical distribution of full phenylalanine hydroxylase (PAH) genotypes is not available.
Materials and Methods: A PubMed research resulted in 24 publications without pre-selection constraints reporting PAH genotypes in 19 countries from Europe and the Middle East spanning the region from Portugal to Iran. We evaluated 3,207 PKU patients that displayed 734 different genotypes based on 269 mutations.
Results: 10 % of PKU patients in the study population showed homozygous PAH genotypes, while 90 % were compound heterozygotes and 46 % of patients carried missense mutations on both alleles. The 30 most frequent genotypes were carried by 53 % of the patients with highest frequency for R408W/R408W (20 %), followed by IVS10-11G>A/IVS10-11G>A (5 %). These two common genotypes showed a different geographical distribution, R408W/R408W was predominant in Central Europe (Lithuania, Poland, Slovakia, Sweden) and IVS10-11G>A/IVS10-11G>A in the Middle East (Iran, Turkey, Israel).
Discussion: The work provides a comprehensive overview on which genotypes have to be expected in different geographical regions and sets the basis for functional analyses. A related web application provides detailed country- and region-specific information on PAH genotypes and their association with PAH function and clinical phenotypes.
Conflict of Interest declared.

P-085

Modular training programme for phenylketonuria with a multiprofessional approach: Benefit for affected paediatric patients and families assessed by questionnaires – a pilot study

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Background: Phenylketonuria (PKU) is a rare disorder of phenylalanine catabolism. 'Diet for life' is recommended in this chronic disorder. As in many other chronic diseases, treatment of PKU is a big challenge requiring training commensurate with age. The German ministry of health has solicited a modular training programme for children with chronic diseases (MODUS).
Methods: Within the framework of MODUS we created a modular training programme for PKU with 4 generic (common in all indications tested) and 3 PKU-specific modules (pathophysiology, inheritance, diagnosis, treatment; routine management; management during crises). An interdisciplinary approach (paediatrician, dietician, psychologist and paediatric nurse) was used. This intervention was tested in 4 groups (2 separate parent groups of children aged 2–12 years, 1 group of children aged 8–12 years and 1 adolescent group aged 13–17 years).
Results: Evaluation of the intervention was an integral part. There was a sustained gain of knowledge in all 4 groups as judged by questionnaires pre, post and 6 weeks after the intervention. Quality of life remained unchanged. Satisfaction with the intervention was very good.
Conclusion: We have developed and successfully tested a modular group training programme for families with PKU. This forms the basis for negotiations with health insurance companies.

P-086

Plasma phenylalanine levels and offspring outcome in maternal hyperphenylalaninemia: an Italian experience

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Background and objectives: Women with hyperphenylalaninemia (HPA) should pay careful attention to their phenylalanine (Phe) levels when planning or going through pregnancy. We report a retrospective analysis of plasma Phe levels preconceptionally and during pregnancy and the offspring outcome.
Materials and methods: Twelve pregnancies in ten HPA women were enrolled. Eight were classical PKU on diet from birth and two were mild HPA. Median age at time of pregnancy was 23 years (range: 30–18). Preconceptionally, in classical PKU, 6 had poor compliance (plasma Phe: 658±280 umol/L) while in mild PKU (Phe: 219±47 umol/L). Phe-restricted diet was initiated in classical PKU within 8 weeks of gestation, while in the mild HPA later (20 weeks) . No abortion was reported during the study.
Results: Except for one (plasma Phe 650±48 umol/L), all patients strictly followed the dietary regimen (plasma Phe: 190±34 umol/L). All pregnancies result in normal offspring.
Conclusions: These data indicate the importance of a Phe-resticted diet during pregnancy although women treated preconceptionally and by 8 weeks of gestation showed no difference. Surprisingly, a normal offspring was achived in one non-compliant patient. Mild HPA showed a normal fetal outcome even if the diet was not initiated preconceptionally.

P-087

Phenylalanine toxicity induces methylome repatterning in PAH deficient PKU

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Background and Objectives: PKU is an intoxicating metabolic defect. A plethora of intoxications induce methylome repatterning. We hypothesized PKU associated PHE toxicity will alter methylome patterning and tested this in mouse and human.
Materials and Methods: DNA was prepared from brain tissue of $PAH^{enu2/enu2}$ mice (hyperphenylalaninemic, PHE controlled), brain tissue of $PAH^{enu2/WT}$ mice, or human blood (PKU, control). Methylated DNA immunoprecipitation prepared sequencing template (Illumina) and informatic assessment identified differential methylation. Microarrays were used to codify methylation and expression data.
Results: Hyperphenylalaninemia causes massive aberrant methylation which is attenuated when PHE is restricted. An intriguing aspect involves methylome modification of gene coding regions as non-coding RNA genes were frequently targeted. Several orthologous non-coding RNA genes were differentially modified in human and mouse. Differential

methylation of gene promoters identified genes recognized as causal in neurologic disease. Expression studies demonstrated relationships between methylation status and mRNA abundance.
Discussion: Methylome repatterning occurs in human PKU (blood) and the $PAH^{enu2/enu2}$ mouse (brain). Aberrant methylation of noncoding RNA genes may result in an overabundance or paucity of non-coding RNA species to impact gene expression and contribute to disease. Promoter methylation of genes recognized as causal in neural dysfunction may indicate involvement in PKU neurological phenotypes.

P-088

Phenylalanine hydroxylase gene mutation spectrum in patients with PKU in Khabarovsk territory, Russian Federation

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Phenylketonuria (PKU) is the most common inborn error of amino acid metabolism in Europeans which is caused by a deficiency of hepatic phenylalanine hydroxylase (PAH). There are a high variety of mutations in the PAH gene (up to date more than 550 mutations is known). PKU is a genetic disease with a great allelic heterogeneity and a wide variability in the common mutations between ethnic groups and geographical areas. We have studied 27 PKU-patients from Khabarovsk Territory, Russian Federation. For screening of 8 PAH gene mutations (p. R408W, p. R261Q, p. P281L, p. R252W, p. R158Q, IVS10-11G>A IVS12+1G>A, IVS4+5G>T) the multiplex system for MLPA PCR-analysis was created. Using this method we detected mutation p. R408W on 37 (68.5 %) chromosomes. The frequencies for other detected mutations were as follows: p. R261Q - 7.4 % (4 chromosomes), p. P281L – 5.6 % (3 chromosomes), IVS12+1G>A – 3.7 % (2 chromosomes). PAH mutations have been identified in all PKU patients including 19 (70.4 %) with two revealed mutations and 8 (29.6 %) with one revealed mutation. The p. R408W, p. R261Q, p. P281L and IVS12+1G>A mutations account 85.2 % of PAH mutations detected in PKU patients from Khabarovsk Territory.

P-089

Disease perception among the mothers of PKU children

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Background/Objective: There is a general misunderstanding in society when it comes to the perception of metabolic disorders. Research aim is to highlight mothers' understanding of their children's condition.
Patients/Methods: Participant-observation and focus study were carried out in Istanbul, along with observations from the formal and informal interviews, 30 in-depth interviews were carried out with mothers of PKU patients from various cities.
Results: Study problematizes and analyzes mothers' view towards disease and their response in order to fashion meaning, directing attention to significance of first-response of mothers at diagnosis and its determinant potential of various aspects, pointing to importance and its content. Presence of supportive social environment or lack thereof, social exclusion as determinants of disease perception are discussed. Number of various strategies may prevent from being pushed out of culture.
Conclusion: Positive approach toward treatment, demedicalization, harm reduction in relation to meaning-making and a nuance in life-style may not be undermined. Minimization of fatalistic views in disease perception is emphasized. Changes in views of medical care and its quality over time are significant, which impact healing and disease perception. Positively experienced clinical relationships determined by various factors help maintain a relatively more positive outlook towards life.

P-090

Supporting patients with a congenital metabolic disease and their social environment: the development of an educational book for children and adolescents

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Background and objectives: Increasing numbers of treated individuals with congenital metabolic diseases are surviving childhood and have a better medical outcome. Several problems are associated to the management of patients with PKU. There is an important need for clear information about PKU for pediatric patients and their network. Developing an informative book could play a crucial role in enforcing self-esteem and social integration.
Methods: Based on interviews with patients and their families we selected the most common aspects of growing up with this metabolic disease. Their experiences were integrated in the book by creating a main character with PKU, so the reader is able to witness this child's daily life. This recognition can be meaningful in accepting their diagnosis and feeling more confident. By adding medical and diet related information, this book aims to be helpful in explaining PKU to their environment. Also, earlier research assessed the importance of psycho-education to enhance therapy compliance.
Conclusion: Developing an educational book for children and adolescents with PKU and their social environment could not only stimulate their knowledge, it could also have a positive effect on therapy compliance, self-esteem and social integration.

P-091

Anthropometric characteristics in patients with phenylketonuria: A cross-sectional study

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Background and objectives: Treating phenylketonuria based upon strict vegetarian diets with very low phenylalanine intake and supplemented by phenylalanine-free formula has sometimes been found to hamper physical development, some patients presenting with growth reterdation and malnutrition. The present study aimed to evaluate anthropometric characteristics of patients with PKU who follow a strict dietary treatment.
Patients and methods: Anthropometric measurements of 34 PKU patients (18 males, 16 females),followed-up at Gaziantep Children's Hospital over a one year period were included. Their median age was 66.1±60.9 months (range 1 to 207 months). We investigated the anthropometric characteristic (weight, height, BMI) in our patients and compared it to age matched 40 healthy subjects (20 males, 20 females), with the measurements expressed as z-scores.
Results: No significant differences were found between patients and healthy subjects on height z-scores, but differences were found on BMI z-scores. The patients who are between 0–5 years old had a lesser

tendency to obesity than healthy population. Whereas the patients between 6–18 years old were more prone to obesity than the healthy controls ($p<0.001$).
Conclusion: Patients and controls were similar in terms of height, but there was a tendancy to overweight and obesity with increasing age. There is a need to educate and follow-up PKU patients on low-phe diet to prevent further rises in BMI.

07. Phenylketonuria: treatment, BH4

P-092

Use of sapropterin dihydrochloride to stabilize phenylalanine levels without challenging phenylalanine intake

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Some patients have difficulty maintaining stable levels within treatment range while following a phenylalanine restricted diet. A report that stability of phenylalanine levels may improve cognitive function in Phenylketonuria motivated us to trial three patients on Sapropterin Dihydrochloride. Their unstable phenylalanine levels were consistently above treatment range. Our goal was to assess if levels could be stabilized and decreased to within treatment range on a Phe restricted diet and Sapropterin therapy. Baseline, Patient 1 had a mean phenylalanine level of 7.4 mg/dl +/− 2.9 (448 mmol/L +/−176). After initiation of therapy, phenylalanine levels dropped to a mean of 4.5 mg/dl +/− 0.4 (273 mmol/L +/− 24). Baseline, Patient 2 had a mean phenylalanine level of 7.3 mg/dl +/− 1.0 (442 mmol/L +/− 61). After initiation and response to therapy, phenylalanine levels dropped to a mean of 3.4 mg/dl +/− 0.2 (206 mmol/L +/− 12). Baseline, Patient 3 had a mean phenylalanine level of 7.2 mg/dl +/− 1.2 (436 mmol/L +/− 73). After initiation of therapy, phenylalanine levels dropped to a mean of 3.6 mg/dl +/− 0.6 (218 mmol/L +/− 36). No dietary changes were initiated during this assessment period. These findings confirm stabilization in phenylalanine levels with Sapropterin Dihydrochloride.

P-093

A semi-mechanistically-based, non-linear mixed-effect modelling approach for tetrahydrobiopterin (BH_4) responsiveness in neonates with hyperphenylalaninemia

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Introduction: Tetrahydrobiopterin (BH_4) loading tests are based primarily on an observed fractional decrease of blood phenylalanine concentrations. Pharmacodynamic modelling may yield a more objective description of responsiveness.
Methods: We investigated BH_4 loading test data (0–24 h) from 375 neonates with phenylalanine hydroxylase (PAH) deficiency and 194 BH4-deficient neonates. Modelling of blood phenylalanine concentrations was performed using non-linear mixed-effects modelling (NONMEM) software.
Results: In a subset of 340 neonates (0- and 24-h data available) with PAH deficiency, 129 (37.9 %) had a blood phenylalanine response of >30 %. Neonates with dihydropteridine reductase deficiency (n=53) could not be differentiated from BH_4-responsive PAH-deficient neonates. Using a phenylalanine turnover model with BH_4-driven "stimulation of loss" which provided the best data fit, 193/194 (99.5 %) neonates with deficiency of BH_4 synthesis and 216/375 (57.6 %) with PAH deficiency were classified as responders. The new modelling approach was validated using a set of published data and, using only 0- to 24-h blood phenylalanine data, demonstrated good correspondence with the 48-h prognostic test.
Conclusion: Phenylalanine pharmacodynamic modelling has the ability to characterize responsiveness to BH_4 loading and its large intersubject variability. Further studies should evaluate the clinical significance of slow or late responses.
Conflict of Interest declared.

P-094

Management and outcome of late treatment of 6-pyruvoyl-tetrahydropterin synthase deficiency in Indonesia

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Background: The 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency is a tetrahydrobiopterin (BH4) deficiency disorder presenting with developmental delay, seizures and abnormal movements associated with hyperphenylalaninemia. This report describes outcomes after late treatment.
Cases: Three cases of PTPS deficiency were diagnosed at 9–12 months and treated with sapropterin, L-DOPA/Carbi-DOPA and 5-hydroxytryptophan. The first case was a 20-year-old female who had been self-managed by parents with insufficient BH4 dosage before coming to our hospital. Recent monitoring demonstrated increased phenylalanine (81 μmol/L, normal 39–74) and prolactin (1165.75 mIU/L, normal 108–557). Patient has impaired cognitive performance and abnormal gait. Adjusting dosage becomes problematic since she experienced adverse effects from sapropterin and L-DOPA. In addition, phenylalanine and neurotransmitter metabolite measurements could not be performed regularly due to unavailability of laboratory. The other two cases were 2-year-old boy and 20-month-old girl showing marked improvement after institution of therapy, however they still have not reached normal milestones. Monitoring of phenylalanine and prolactin showed normal levels for both.
Conclusions: Early diagnosis and treatment of PTPS deficiency determines outcome. Late treatment causes irreversible brain damage. Long-term management of hyperphenylalaninemia remains a challenge in developing countries. Access to orphan medicine and metabolic laboratory should be ensured to support a successful management.

P-095

The first molecular study of Egyptian patients with 6 -pyruvoyl-tetrahydropterin synthase (PTPS) deficiency

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Background: 6 -Pyruvoyl-tetrahydropterin synthase deficiency (PTPSD) is one of the tetrahydrobiopterin (BH4) deficiencies. It results in BH4 deficiency, hyperphenylalaninemia, brain serotonin and dopamine depletion. Deficiency of BH4 may lead to phenotype of phenylketonuria with mental retardation and severe neurological disorder. The PTPSD is the most common form of BH4-deficient hyperphenylalaninemia.

Aim: The study aims to report the progress of the clinical picture and mutational data with the diagnosis of PTPSD in order to add more PTPSD reported cases to the BH4-database of Dr Blau for a better understanding of the metabolic pathways.
Patients and methods: Six patients were clinically evaluated and history of development and management were recorded. Extended metabolic work-up was pursued. Neopterin and biopterin levels in urine were analysed. BH4 loading test and mutation analysis of PTPS gene were performed.
Results and Conclusion: Six patients were diagnosed to have BH4 deficiency based on the BH4 loading test, abnormal pterin levels in urine and mutational analysis. Mutations were found in exons 4 and 5, which might be a hotspot for Egyptians with PTPSD. The experience of treating late BH4 deficient patients is a challenging task and attaining improvement is very gratifying.

P-096

Efficacy and safety of sapropterin dihydrochloride in patients with phenylketonuria aged less than 4 years old: results from the SPARK study, a phase IIIb, multicentre, open-label, randomized, controlled study

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Background: Sapropterin dihydrochloride (Kuvan®) is not approved in Europe for phenylketonuria (PKU) patients ‹4 years.
Objectives: To evaluate efficacy and safety of sapropterin in increasing phenylalanine tolerance in PKU patients Methods: Phase IIIb, multicentre, open-label, randomized controlled study. Patients received phenylalanine-restricted diet alone (PheRD) or in combination with sapropterin (S+PheRD). Phenylalanine tolerance was defined as prescribed dietary phenylalanine (mg/kg/day) at blood phenylalanine concentrations 120–360 μmol/L.
Results: In the intent-to-treat population, mean±SD baseline Phe tolerance was 37.1±17.3 mg/kg/day in S+PheRD group (n=27, 21.1±12.3 months, 16 males) and 35.8±20.9 mg/kg/day in PheRD group (n=29, 21.2±12.0 months, 14 males). At 26 weeks, adjusted Phe tolerance was 80.6±4.2 (S+PheRD) versus 50.1±4.3 mg/kg/day (PheRD); significant difference=30.5 mg/kg/day ($p<0.001$). Sapropterin was well tolerated, and safety profile comparable in both groups. Most frequent sapropterin-related adverse events were rhinitis and vomiting.
Conclusion: This is the first controlled study on sapropterin therapy in PKU patients ‹4 years in Europe. Addition of sapropterin (10–20 mg/kg body weight) to phenylalanine-restricted diet significantly improves phenylalanine tolerance in PKU patients ‹4 years and shows a good safety profile. This demonstrates a favourable benefit-risk profile for sapropterin in the very young PKU population, supporting potential early pharmacological treatment in responsive patients.
Conflict of Interest declared.

P-097

Fourth interim analysis of the Kuvan® Adult Maternal Paediatric European Registry (KAMPER)

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Objectives: The primary objective of KAMPER is to assess long-term safety in patients treated with sapropterin dihydrochloride.
Methods: Observational, multicentre drug registry, including a maternal sub-registry.
Results: Data for 411 patients (379 phenylketonuria [PKU], 32 BH_4 deficiency) are included; median (range) age at enrolment: 10.2 (3.2–39.7) and 10.6 (0.1–34.9) years, respectively. Safety: 197 adverse events (AEs; 174 PKU, 23 BH4 deficiency) were reported in 109 patients. No deaths were reported. Seventeen AEs in the PKU and none in the BH_4-deficient groups were considered related to sapropterin treatment. Nine serious AEs (SAEs) were reported (tachycardia, drop attacks, unresponsiveness to stimuli, nephrolithiasis, headache, abnormal behaviour, suicidal behaviour, seminoma, upper limb fracture) in the PKU group and six SAEs (grand mal convulsion [2], injury, skin laceration, epistaxis, laryngitis) were reported in the BH_4-deficient group. Except for headache, none of the SAEs were considered related to sapropterin. Efficacy: Data on blood phenylalanine concentration, daily natural protein and phenylalanine intake, and auxological parameters (height, weight and BMI standard deviation score) in the PKU and BH_4-deficient groups will be presented.
Conclusion: The interim results from this registry show that sapropterin has an acceptable safety profile.
Conflict of Interest declared.

P-098

Long term treatment of phenylketonuria with a new medical food containing LNAA

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Background and objectives: Phenylketonuria is an autosomal recessive disease caused by deficient activity of phenylalanine hydroxylase. A low phenylalanine diet is used to treat PKU. The diet is very restrictive and dietary adherence erodes as patients get older. Methods to improve dietary adherence and blood Phe control are continuously under investigation. A new formula (PheLNAA) has been tested in this study with the purpose to improve compliance and lower blood phenylalanine.
Patients and Methods: The formula has been tested for nitrogen balance and is nutritionally complete. It is fortified with more nutritional additives that can be deficient in PKU diet, such as B12, biotin, DHA, lutein and increased levels of large neutral amino acids to help lower blood Phe. The new formula has been tested on 12 classic PKU patients with a loading test of 4 weeks and the patients who initially responded to treatment continued the PheLNAA administration for an additional five months.
Results: Fifty-eight percent of patients had a significant decline in blood Phe concentration from baseline throughout the study. The PheLNAA was well tolerated with excellent compliance and without illnesses or side effect.
Conclusion: The new formula is suitable for PKU treatment and offers the PKU clinic a new choice for treatment.

P-099

Effect of genotype on sapropterin responsiveness in ENDURE: a phase IV, prospective, open-label, uncontrolled trial to assess the responsiveness of patients with phenylketonuria to treatment with sapropterin 20 mg/kg/day for 28 days

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Background: Tetrahydrobiopterin (BH4) reduces blood phenylalanine concentration in a subset of patients with phenylketonuria (PKU).
Objectives: To evaluate the response (≥30 % reduction in phenylalanine) to synthetic BH4 (sapropterin) 20 mg/kg/day and compare the response by phenotype and genotype classification. Phenylalanine concentrations at first confirmatory testing are usually expected to match phenotype predicted from mutations.
Methods: Open-label, single-arm cohort study in Norway and Denmark. PKU patients with known, suspected or unknown BH_4-responder mutations in phenylalanine hydroxylase (PAH) gene and ≥2 historical blood phenylalanine concentrations >400 μmol/L were included. Patients were classified as classic PKU (phenylalanine >1200 μmol/L), mild PKU (600–1200 μmol/L) or mild hyperphenylalaninemia (‹600 μmol/L) at first or second confirmatory test or screening.
Results: 44/59 (75 %) patients responded to sapropterin. Most frequent PAH gene mutations were Y414C (c.1241A>G; 27/59 patients), R408W (c.1222C>T; 16/59) and IVS-12 (c.1315+1G>A; 12/59). PKU phenotype classification varied with method used, affecting response interpretation. Some mutations (Y414C, G46S) were associated with clear response to sapropterin. Some patients had high phenylalanine levels at first confirmatory test but mild genotype-based phenotype.
Conclusion: Phenotype classification varies with method used, affecting interpretation of classic PKU patients' response to sapropterin.
Conflict of Interest declared.

P-100

Molecular epidemiology, genotype-phenotype correlation and BH4 responsiveness in phenylketonuria patients from Bulgaria

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Background: The goal of our study was to identify the most common genotypes in the phenylketonuria (PKU) population of Bulgaria, assessing the correlation with the phenotype and the possible sapropterin responsiveness.
Methods: We conducted a combined retrospective study of phenotype and genotype of 34 Bulgarian PKU patients. PAH deficient patients passed the 48-h BH4 loading test. Those with ≥30 % phenylalanine (Phe) reduction during the sapropterin loading test (20 mg/kg/day) were regarded potential responders and were invited to start BH4 intake to determine long term responsiveness.
Results: Our patient group exhibited 18 different mutations, in a total of 23 different genotypes. The most common mutations were found to be: p.R408W (29.41 %); p.R261Q (19.11 %); IVS10-11G>A (8.82 %); p.L48S (8.82 %). In 20 PKU subjects BH4 challenge was carried out. They all successfully completed the 48-h BH4 loading test. Although generally there is a good genotype-phenotype correlation, for the most of the repeated genotypes a slightly different phenotype was observed. One patient with previously reported unresponsive mutation on one of the alleles showed a positive response.
Conclusions: Our data reveal a limited genetic heterogeneity in the Bulgarian population. Genotype is quite a good predictor of the phenotype and of the responsiveness to tetrahydrobiopterin.

P-101

Does diet liberalization impact anthropometric measures in PKU patients on relatively long-term BH4 treatment

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Background and objectives: Overweight has been reported in some studies and little is known about how BH4 treatment affects the physical development in PKU patients. The aim of this single center retrospective study was to evaluate anthropometric characteristics in a cohort of PKU patients receiving relatively long-term BH_4 treatment.
Materials/patients and methods: Fifty five (30 boys, 25 girls) PKU patients aged between 1.04 and 14.1 year on BH_4 for more than 1 year (14.8 to 36 months) were recruited. Follow-up data of height for age (HAZ) and body mass index (BMIZ) z scores were collected yearly over a period of 3 years and calculated using WHO Anthro programmes.
Results: Median age at the start of BH4 was 4.5 years. Diet was liberalized in 46/55 patients (83.4 %). After the diet liberalization, median HAZ significantly increased ($p=0.000$), BMIZ did not change during 3 years follow-up. In patients on partially-restricted diet, HAZ and BMIZ did not change.
Discussion/conclusion: Growth, in terms of height, improved after the diet liberalization possibly due to consumption of higher amounts of natural protein in the study group. Further longitidunal studies are needed to assess the effect of BH4 therapy on anthropometric measures.

P-102

Clinical and laboratory features of phenylalanine hydroxylase deficient phenylketonuria patients developing secondary unreponsiveness to sapropterin treatment

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Background: BH_4 loading tests and molecular genetic analyses would be useful to detect sapropterin responsive patients. However, there is no accurate tool for the prediction of unresponsiveness in the long run. The aim of this study was to determine clinical and laboratory features of the patients who developed secondary unresponsiveness while under BH4 treatment.
Material and Methods: 150 patients with PKU were treated with sapropterin. 48-h-BH_4 loading was used to identify responsive patients.
Patients: Blood Phe levels could not be controlled in 11 patients (5 males). These patients' BH_4 loading test results revealed 43.6 % decrease (mean) in blood Phe levels. Mean age of the patients at the start of sapropterin and the time of evaluation of the results was 3.5±0.6 and 5.8±0.6 years, respectively. Nine patients had mild-moderate PKU. Treatment duration ranged from 13 to 38 months. Dietary Phe tolerance increased 1.6 folds (mean) but treatment was stopped because of unsatisfactory metabolic control. Mutation analyses were done in 6 patients. Two patients had at least one responsive allele.

Conclusion: BH4 loading test and genotyping are useful for the detection of BH_4 responsive patients but there is still need for the development of tests to predict the responsiveness in long run.

P-103

BH4 treatment in BH4-responsive PKU patients: blood prolactin concentrations suggest increased cerebral dopamine synthesis

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Background and aim: In PKU patients, cerebral neurotransmitter deficiencies are hypothesized to contribute to impaired neuropsychological functioning. To investigate whether BH4 could improve cerebral neurotransmitter synthesis, this study compared blood prolactin –as parameter of cerebral dopamine– in early-treated PKU males with and without BH4.
Patients and methods: We compared blood prolactin concentrations in 30 samples of 10 BH4-treated and 110 samples of 27 BH4-untreated PKU males at different phenylalanine, tyrosine, and phenylalanine:tyrosine levels. Females were excluded because of too much possible variation by other factors.
Results: In BH4-untreated males, blood prolactin positively correlated to phenylalanine ($p=0.000$), while negatively to tyrosine concentrations ($p=0.046$), at blood phenylalanine >600 μmol/l. In BH4-treated males, blood prolactin concentrations were lower than in BH4-untreated males at low phenylalanine:tyrosine ($p=0.019$) and high tyrosine levels ($p=0.035$). In BH4-treated males, blood prolactin positively correlated to phenylalanine:tyrosine ratios ($p=0.000$), while negatively to BH4 dose ($p=0.022$). Discussion: BH4 may increase cerebral dopamine synthesis in PKU patients, possibly in a dose-dependent manner. Moreover, it may require adequate tyrosine availability and not too high phenylalanine and phenylalanine:tyrosine levels.
Conclusion: BH4 seems to directly improve cerebral dopamine synthesis in BH4-responsive PKU patients beyond its effect through lowering blood phenylalanine.
Conflict of Interest declared.

P-104

Genetic analysis of hyperphenylalaninemic patients from Chile

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Here, we report the genetic analysis of a cohort of 40 unrelated patients with hyperphenylalaninemia (HPA) from Chile by conventional Sanger analysis and by next generation sequencing (NGS) of whole genes causing HPA (PAH, PTS, GCH1 and QDPR). We detected one PTPS-deficient patient, who responded to BH4, but without mutations on a PAH gene, by pterins analysis in DBS. Twenty-two differentmutations, including two partial gene deletions, have been found. The majority of the mutations, (≈70 %) were located in exons 7 and 11 and the allele frequency (AF) is ranging from 1 to 21 %, p.V388M being the most common gene variant, followed by a new deletion of exon 5 detected in eight alleles (12 %). Overall the genomic rearrangement is unusually relevant in this cohort of Chilean patients; other common variant was the Mediterranean splicing variant c.1066-11G>A with an AF of 11 %. The majority are missense, splicing or severe deletion variants, although there are a number of possible BH4-responsive variants (www.biopku.org/), such as p.V388M, p.R261Q, p.Y414C, p.E390G. In summary, genetic analysis of HPA can be used in the emerging countries for differential diagnosis of HPA and additionally to target the BH4 therapy more rationally.

P-105

The comparison of the effect of tetrahydrobiopterin and phenylalanine deficient diet on oxidative stress in hyperphenylalaninemia

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In recent years, the antioxidant effects of tetrahydrobiopterin, which is used in treatment of phenylketonuria, has been defined. In this prospective study, the effects of tetrahydrobiopterin and phenylalanine deficient diet treatment on oxidative stress and antioxidant parameters in hyperphenylalaninemic children were investigated.
The study was carried out on 40 hyperphenylalaninemic children with a mean age of 4.2 years (14 with hyperphenylalaninemia, 13 with mild phenylketonuria and 13 with classical phenylketonuria). The first group consisted of tetrahydrobiopterin treated, the second group consisted of tetrahydrobiopterin responsive diet-treated, the third group consisted of tetrahydrobiopterin unresponsive diet treated patients. Parameters were measured in plasma before tetrahydrobiopterin loading test (INIT) and in the sixth month.
As compared to the INIT, while the values of antioxidant markers rose (Glutathione peroxidase, paraoxonase, paraoxonase arylesterase), the oxidant marker values dropped (catalase, thiobarbituric acid reactive substances (TBARS), 8-hydroxy-2'-deoxyguanosine) in the first group in sixth month. In second and third groups, the results were compatible with the oxidative process continuation (drop in: Glutathione peroxidase, paraoxonase, paraoxonase arylesterase, rise of: Catalase, TBARS, 8-hydroxy-2'-deoxyguanosine). Nitrite/Nitrate value rose and asymmetric dimethylarginine value dropped in all groups in sixth month. Our study revealed the protective effect of tetrahyrobiopterin against oxidative stress compared to phenylalanine restricted diet.

P-106

Effect of a specific nutrient combination on synaptic markers in a mouse model of phenylketonuria

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Background: Research in PKU models showed reduced synapse formation and aberrant synaptic morphology. The specific nutrient combination (SNC), a novel nutritional intervention in the field of synaptic dysfunctions including Alzheimer's disease, has shown beneficial effects on synapse formation, morphology and function. This makes SNC an interesting new nutritional approach in PKU.
Methods: In this proof-of-concept study, the open field and the immunohistochemical marker PSD-95 were examined for 6 groups of C57Bl/6 PKU mice on a 12 week diet with one of three different Phe-contents (8.8, 6.4 or 4.0 g/kg diet) either with SNC or an isocaloric control, starting on

postnatal day 31. Wild type (WT) mice on Phe 8.8 g/kg without SNC were used as control.
Results: PKU mice on control diet showed a reduced PSD-95 expression in hippocampal proximal synapses of the CA1 in comparison with WT mice ($p=0.004$). This difference was no longer present in PKU mice on diets with SNC ($p=0.332$). Secondly, SNC restored the observed behavioral phenotype of PKU mice to WT level. Finally, SNC together with Phe content improved the growth of PKU mice.
Conclusion/Discussion: This proof-of-concept study revealed a positive effect of SNC in PKU mice on growth, behavior and the post-synaptic marker PSD-95.
Conflict of Interest declared.

08. Sulphur amino acid disorders

P-107

Bloodspot total homocysteine for diagnosis and monitoring of homocystinuria

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Objectives: Development and validation of a LC-MS/MS method for quantification of bloodspot total homocysteine as a second tier test for newborn screening plus comparison to plasma total homocysteine for monitoring treatment of patients with homocystinuria.
Method: Bloodspots were analysed using a modification of our plasma total homocysteine method. Briefly, homocystine-d8 was added to a 3 mm diameter bloodspot punch and reduced with dithiothreitol. Analyte and internal standard were eluted on a LC-CN 3 μm 3.3 cm x 4.6 mm column using isocratic HPLC. Detection and quantification were by electrospray ionisation tandem mass spectrometry using multiple reaction monitoring. Aqueous calibrators were used and method performance was evaluated with spiked-haemolysate bloodspot internal quality control (IQC) produced in-house, and with commercial plasma IQC and EQA materials.
Results: The lower limit of quantification was 1 μM and the assay was linear to 200 μM. Between 4-150 μM the coefficient of variation was <6 % within batch and <10 % between batch. The population 99th centile was 6.8 μmol/L. Bloodspot total homocysteine concentrations correlated linearly to plasma values at 37 % (95 % confidence interval 26-48 %); bloodspot IQC recovery was 39 %.
Conclusion: Bloodspot total homocysteine measurement demonstrates utility in the diagnosis of homocystinuria and enables home collection of specimens for monitoring patient treatment.

P-108

Mitochondrial permeability transition is induced by sulfite in rat brain mitochondria via thiol group alteration

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Background: Sulfite oxidase (SO) deficiency is a disorder biochemically characterized by increased plasma levels of sulfite, thiosulfate and S-sulfocysteine. The symptoms include severe neurological dysfunction and seizures, whose pathophysiology remains to be elucidated.
Objective: We investigated the in vitro effects of sulfite on mitochondrial function in rat brain. Material and Methods: Mitochondrial preparations from brain of thirty-day-old Wistar rats were used to assess mitochondrial homeostasis parameters in the presence or absence of Ca^{2+}.
Results: We observed that sulfite dissipated the $\Delta\Psi m$ only in the presence of Ca^{2+} in a sulfite and Ca^{2+} dose-dependent manner. Furthermore, in the presence of Ca^{2+}, sulfite induced swelling and decreased matrix NAD (P) H pool, Ca^{2+} retention capacity and cytochrome c immunocontent in mitochondria. These effects were prevented by ruthenium red, cyclosporine A and ADP, indicating the induction of mitochondrial permeability transition (MPT). We also found that N-ethylmaleimide prevented the mitochondrial swelling provoked by sulfite. Finally, sulfite decreased membrane protein thiol group content in mitochondria.
Discussion and Conclusion: Our findings indicate that sulfite acts directly on thiol groups of MPT pore proteins. It is presumed that the induction of MPT may be involved in the neurological symptoms observed in SO deficiency.
Support: CNPq, Propesq-UFRGS, PRONEX, FINEP, INCT-EN.

P-109

Monitoring of reduced glutathione in Indian children with homocystinuria and response to treatment

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Background: Classical homocystinuria caused by mutations in the CBS gene is associated with elevations of plasma and tissue homocysteine and methionine, which lead to a range of vascular, neurological and connective tissue disturbances.
Objective: To analyze levels of glutathione as a measure of oxidative stress in patients with homocystinuria and to determine relationship between levels of homocysteine and methionine in these patients with oxidative stress in response to medication.
Materials and methods: We analyzed glutathione levels in over 600 patients with suspected IEMs including 4 patients with homocystinuria. These patients were followed up for their glutathione levels along with other parameters such as homocysteine and methionine. Blood samples of 54 healthy individuals were analyzed for reference ranges of glutathione.
Result: We found reduced levels of glutathione in patients with homocystinuria (27.32 ± 19.65 nmol/mg Hb, $n=4$) as compared to healthy individuals (46.64 ± 17.51 nmol/mg Hb, $n=54$). Glutathione levels were found to vary inversely with levels of homocysteine and methionine indicating increased oxidative stress. Improvement in GSH, homocysteine and methionine levels were seen in these patients after starting n-acetylcysteine supplements and Vitamin E.
Conclusion: Reduced glutathione level monitoring and anti-oxidant supplementation has beneficial effects in patients with homocystinuria.

P-110

Binding of NO·and CO to the heme sensor in cystathionine β-synthase and regulation of H_2S production

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Cystathionine beta-synthase (CBS) is a key enzyme in the transsulfuration pathway. Besides the condensation of homocysteine

and serine into cystathionine, CBS catalyzes alternative reactions yielding hydrogen sulphide (H_2S), making CBS a major source of H_2S. H_2S signalling is related to regulation of vascular tone, neuronal activity, mitochondrial energy production and oxidative stress response, and CBS-catalysed H_2S production has been associated with colorectal tumour proliferation. The N-terminal domain of CBS harbours a heme that acts as redox sensor, being able to bind carbon monoxide (CO) and nitric oxide (NO·), which is communicated to the PLP active site, impairing enzymatic activity. Herein, we evaluated the CO and NO·binding to the human CBS ferrous heme by static and stopped-flow spectroscopy. CBS binds NO·much more quickly (kon≈8x103 M-1 s-1) and tightly (Kd≤ 0.23 μM) than previously thought, in line with the in vivo role of NO in CBS modulation. Subsequently, we have investigated the NO·and CO inhibition of CBS-catalysed H2S production with a H_2S-specific electrode. Our findings provide new mechanistic clues for CBS regulation and a link between the 'gasotransmitters' NO·, CO and H2S, therefore opening new ways for the modulation of CBS activity for therapeutic purposes.

P-111

A novel mutation in the methylenetetrahydrofolate reductase (MTHFR) gene in a Turkish child

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Background: Severe methylenetetrahydrofolate reductase (MTHFR) deficiency caused by homozygous or compund heterozygous mutations in the MTHFR gene is an autosomal recessive disorder of folate metabolism with elevated levels of homocysteine and decreased levels of methionine. The phenotypic spectrum ranges from severe neurologic deterioration and early death in infancy to asymptomatic adults. We here describe a novel homozygous mutation in MTHFR gene in patient with non-specific signs.
Case report: A 12-year-old boy was presented with the signs of inability to walk, muscular weakness, speech disorder, confusion, and visual hallucinations in last two weeks. His medical history showed surgery for strabismus, febrile convulsions at the age of 2 and 4 years, mild developmntal delay and learning disabilities. Laboratory investigations revealed low methionine (15 and 25 μmol/L) and elevated homocysteine (148 and 116 μmol/L) levels in plasma. Severe MTHFR deficiency was suspected and he was given vitamin B12, folate, betaine, vitamin B6 with subsequent improvement of his symptoms and reduction in his serum homocysteine levels. Molecular genetic analysis identified homozygous p. F564V (c.1690 T>G) MTHFR mutation in the patient.
Conclusions: Measurement of plasma homocysteine levels should be included in the metabolic investigation of the patients with febrile convulsions, learning disabilities and other non-specific neurologic findings.

P-112

The one-carbon metabolism: evaluation of B-vitamin status and genetic factors on plasma homocysteine and methylmalonic acid levels in children and adolescents

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Hyperhomocysteinemia is a risk factor for vascular disease. Inadequate B-vitamin status and/or polymorphisms in key enzymes of one-carbon metabolism may elevate plasma total homocysteine (tHcy) and methylmalonic acid (MMA) concentrations. Limited data are available in pediatric ages.
We investigated the influence of several SNPs and B-vitamin status on tHcy and MMA concentrations in 324 Portuguese children (195) and adolescents (129). Results were stratified according to age (9 versus 17 years-old, respectively): tHcy (3.78±1.83; 8.72±3.41 μmol/L); folates (or Vit. B9) (22.87±9.25; 11.65±4.17 nmol/L); holotranscobalamin (holo-TC, biologically active form of Vit. B12) (100.60±31.63; 66.20± 25.10pmol/L); total Cbl (514.74±165.53; 302.51±118.32pmol/L) and MMA (0.275±0.262; 0.180±0.105 μmol/L). In the 17-year-old group we observed significant differences between tHcy and MTHFR677 genotypes (p=0.027), folates and TCNII776 genotypes (p=0.048) and total Cbl and MTR2756 genotypes (p=0.041). Our results also showed that folate, holo-TC and total Cbl concentrations significantly decreased with age in the first two decades of life, which was associated with a two-fold increase in tHcy levels. Moreover, MMA levels also decreased with age and, in the adolescent group, they were significantly correlated with holo-TC concentrations. In conclusion, this observation agrees with the notion that plasma MMA levels are the most sensitive indicator of early Vit. B_{12} deficiency.

P-113

Cranial MRI findings in classical homocystinuria

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Homocystinuria is an inborn error of methionine metabolism caused by deficiency or low activity of cytathionine-β-synthase leading to increase in plasma and urine homocysteine levels. Brain imaging in HCYU may show cerebral infarction, atrophy and venous occlusion. Diffuse white matter changes consistent with leucoencephalopathy have also been reported. Here we report the cranial magnetic resonance imaging (MRI) findings of a series of five patients with classical homocystinuria; two with subcortical and deep white matter changes, one with increased intensity on T2 due to infarct in the left corona radiata and nucleus caudatus because of occlusion of lenticulostriate arteries of left middle cerebral artery, and two with normal findings.

P-114

Perturbation of mitochondria-associated endoplasmic reticulum membranes and mitophagy in homocystinuria patients with genetic remethylation defects

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Remethylation disorders are inherited metabolic diseases of enzymes involved in the remethylation of homocysteine to methionine causing homocystinuria. Previously, we have shown an increased expression of IP_3R1 and HERP proteins involved in endoplasmic reticulum (ER) Ca^{2+} homeostasis and the activation of autophagy in homocystinuria patients-derived fibroblasts. Mitochondria-associated ER membranes (MAMs) are lipid raft-like regions of ER playing an important role in Ca2+ homeostasis, among others. The goal of this work was to investigate ER-mitochondria contacts, calcium levels and mitophagy activation in five patients-derived fibroblasts with defects in MTRR, MTR and MTHFR genes. The expression of Grp75, Mfn2 and σ1 receptor, three MAM-associated proteins, was significantly increased in patients' cells compared to controls. Several different agents including thapsigargin and bradykinin disrupting intracellular Ca^{2+}

homeostasis showed no differences in the basal calcium content between patients and control fibroblasts. Staining immunofluorescence of cathepsin and cytochrome c showed their colocalization suggesting that mitochondria were being degraded within autophagosomes. Electron microscopy studies in patients-derived fibroblasts exhibited a reduced number of mitochondria and distinct mitochondria morphology compared to controls and confirmed a massive degradation of the altered mitochondria by mitophagy. Our data suggests a possible role of ER-mitochondria contacts and mitophagy in homocystinuria pathophysiology.

P-115

Treatment of homocystinuria in adult and paediatric patients with betaine anhydrous: results from the RoCH study

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Objective: RoCH (Registry of Cystadane® - Homocystinuria) is a multicentre, non-interventional safety study which aims to identify adverse drug reactions for patients treated with betaine anhydrous (BA, Cystadane®), and to establish a better clinical knowledge on the use of this therapy in patients with homocystinurias and methylation defects.
Methods: Clinical data were collected retrospectively from 2007 to end February 2013, and the clinical and biological status of patients was monitored at least once a year.
Results: A total of 125 patients were enrolled in 29 centres: CBS deficient B6-non responsive or B6-responsive, MTHFR deficient or Cbl deficient. Patients were treated for a mean duration of 7.5 ±5.4 years. After treatment with BA, the vast majority of clinical symptoms either improved (32.7 %) or remained stable (64.5 %). Treatment with BA led to a decrease in homocysteine levels in all diagnostic groups overall by 25.8 % between the first and the last visits. No particular safety concerns related to the product were reported by the investigators.
Conclusion: The present registry provides real-life information on the use of BA in homocystinurias and related disorders. BA has a good safety profile and is effective in the management of this group of disorders.
Conflict of Interest declared.

P-116

Dietary practices and betaine administration in pyridoxine non-responsive homocystinuria: a Polish experience

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Homocystinuria (HCU) is a disorder of methionine metabolism due to cystathionine beta synthase deficiency that leads to increased methionine and its main metabolite homocysteine concentration in body fluids. The management of pyridoxine non-responsive homocystinuria bases on administration of low methionine diet, betaine or both. The aim of the study was to evaluate the results of various treatment options of Polish patients with pyridoxine non-responsive HCU. Eleven pediatric patients from nine families were identified with classical homocystinuria. In 6 patients diagnosis was suspected on the basis of on clinical symptoms and was confirmed by measurement of tHCY level in serum. Five patients were diagnosed through newborn screening. In all patients, low methionine diet was introduced immediately after diagnosis. In 8 patients, betaine was added later. In 2 presymtomatic patients, diet and betaine was introduced together. Only one patient stays solely on diet. No clear relationships between treatment options and the levels of tHCY and methionine was observed. In most patients, diet lowers tHCY and methionine levels, betaine supplementation slightly decreased homocysteine levels but increased levels of methionine. This highlights the need for frequent biochemical controls and individual planning of treatment method.

P-117

In vitro model of mild hyperhomocysteinemia causes modulation in redox state in cerebral cortex and heart

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In the present study we verify the effect of different homocysteine on status redox in slices of brain and heart of adult rats, an in vitro model. Male Wistar rats were sacrificed by decapitation and heart and cerebral were isolated and sliced. Homocysteine (0 μM, 15 μM, 30 μM and 50 μM) was added to incubation medium for subsequent analysis of oxidative stress (TBARS, DCFH oxidation, sulfhydryl groups, carbonyl content, SOD and GPx). In cerebral cortex, we observed that homocysteine increased lipid peroxidation, DCFH oxidation and SOD activity in all concentrations and times studied. But at 50 μM, carbonyl content was increased after 1 h of incubation; GPx activity was decreased in a concentration-dependent manner. In heart, homocysteine at 30 and 50 μM increased lipid peroxidation and decreased the sulfhydryl content after 1 h of incubation. SOD activity was increased by homocysteine (30 and 50 μM) when incubated for 30 min, but its activity was decreased after 1 h of incubation. These results showed that this model mimics the effects of the redox state promoted by hyperhomocysteinemia in vivo, demonstrating the potential of this model to be used for screening of different therapeutic substances. Supported by CNPq, PROPESQ/UFRGS.

P-118

Development of an animal model for gestational hypermethioninemia in rat and its effect on brain Na^+,K^+-ATPase/Mg^{2+}-ATPase activity and oxidative status of the offspring

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In this study we developed a chemically induced experimental model for gestational hypermethioninemia in rats and evaluated in the offspring the activities of Na^+,K^+-ATPase, Mg^{2+}-ATPase and oxidative stress parameters, namely sulfhydryl content, TBARS and superoxide dismutase and catalase in encephalon. Serum and encephalon levels of methionine and total homocysteine were also evaluated in mother rats and in the offspring. Pregnant Wistar rats received methionine throughout the gestational period (21 days). Pregnant rats received dose 1 (1.34 μmol methionine/g body weight), dose 2 (2.68 μmol methionine/g bw) or saline (control). Pups were decapitated at the 7th day of life or at the 21st day of life. Mother rats were decapitated at the 21st day postpartum. Both doses 1 and 2 increased methionine levels in encephalon of the mother rats and dose 2 increased methionine levels in encephalon of the offspring. Maternal hypermethioninemia decreased the activities of Na^+,K^+-ATPase, Mg^{2+}-ATPase and catalase, as well as reduced total sulfhydryl content in the encephalon of the pups. This chemical model seems to be appropriate for studies aiming to investigate the effect of maternal hypermethioninemia on the developing brain during gestation in order to clarify possible neurochemical changes in the offspring. Supported by CNPq, CAPES, PROPESQ.

P-119

Creatine prevents the imbalance of redox homeostasis caused by homocysteine in skeletal muscle of rats

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Homocystinuria is a neurometabolic disease caused by severe deficiency of cystathionine β-synthase activity, resulting in severe hyperhomocysteinemia. Affected patients present cognitive and motor dysfunctions. In the present study we investigated the effect of hyperhomocysteinemia on reactive species, levels of thiobarbituric acid-reactive substances (TBARS), antioxidant enzymes activities (SOD, CAT and GPx), reduced glutathione (GSH), total sulfhydryl and carbonyl content and nitrite levels in skeletal muscle of young rats subjected to a model of severe hyperhomocysteinemia. We also evaluated the effect of creatine. Wistar rats received daily injections of homocysteine (0.3-0.6 μmol/g body weight), and/or creatine (50 mg/g body weight) from their 6th to the 28th days age. Rats were decapitated at 12 h after the last injection. Chronic homocysteine administration increased 2'7'dichlorofluorescein (DCFH) oxidation and TBARS levels. SOD and CAT were increased, but GPx activity was not altered. The content of GSH, sulfhydril and carbonyl were decreased, as well as levels of nitrite. Creatine concurrent administration prevented some homocysteine effects. Our data suggest that the oxidative stress caused by chronic hyperhomocystinemia may provide insights into the mechanisms by which homocysteine exerts its effects on skeletal muscle function. Creatine prevents some alterations caused by homocysteine. Supported by CNPq.

P-120

Effect of methionine exposure on purinergic system in zebrafish (Danio rerio) brain

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Neurological dysfunction is observed in patients with hypermethioninemia, whose pathophysiology remains still poorly understood. ATP exerts important roles in synaptic transmission while adenosine acts as a neuroprotective agent. Ectonucleotidases are responsible for controlling extracellular nucleotides and nucleosides levels. Therefore, we investigated the effects of methionine exposure on nucleotide catabolism, E-NTPDases and adenosine receptors gene expression in zebrafish brain. Animals were exposed to 7 days of methionine treatment at two different concentrations (1.5 and 3.0 mM). For enzyme assays, brain membranes were prepared daily. For molecular experiments, brains were removed for total RNA extraction and cDNA synthesized. We observed a significant decrease for ATP and ADP hydrolysis in both concentrations tested. However, methionine treatment significantly increased ntpd1 ntpd2mg, ntpd2mv, ntpd3, A1, and A2A1 transcript levels. Methionine exposure decreased ATP and ADP hydrolysis, leading to ATP accumulation on synaptic cleft, which can be potentially toxic for nervous system. Conversely, ntpd gene expression was increase after methionine treatment, which could be acting as a compensatory effect, and changes on adenosine receptors expression can modulate the effects of hypermethioninemia. These results may contribute to better understanding of pathophysiological mechanisms that increase the susceptibility to neurological symptoms observed in hypermethioninemic patients. Support by CNPq.

P-121

Diagnosis and management of homocystinuria in Moroccan patients

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Homocystinuria, due to cystathionine β synthase deficiency is the most severe disorder of homocysteine metabolism. In this study, we report our laboratory's experience in diagnosis and survey of Moroccan patients with homocystinuria. Screening was performed by aminoacids thin layer chromatography and nitroprusside test. Diagnosis was confirmed by ion exchange chromatography and/or immunoenzymatic assay. Since 1996, 40 cases were diagnosed representing the third amino-acid disease screened in our service, after phenylketonuria and tyrosinemia I. Mean patients age was 8 years. Clinical signs were ectopia lentis, vascular disease with thromboembolisms, skeletal abnormalities and mental retardation. High levels of homocysteine (often >150 μmol/L) associated with elevated plasma methionine suggested a defect in cystathionine β synthase. Only 9 cases have been treated with vitamins B6+B12 and diet, betaine was used in few patients. Reduced homocysteine levels were reached in 4 patients. Betaine led to improved effect on homocysteine levels in non responsive patients. Clinical outcomes showed variation depending on the age at diagnosis and management. Precocious diagnosis and management of homocystinuria may prevent

deleterious complications too difficult to correct when the treatment is introduced late.

P-122

Impact of CPS1 gene rs7422339 polymorphism in Argentinian patients with hyperhomocysteinemia

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Carbamoyl phosphate synthetase I (CPSI) is a key gene in the first step of urea cycle and has been correlated with nitric oxide level and vascular smooth muscle activity. CPS1 rs7422339 (T1405N) was associated with high homocysteine (Hcy) plasma values in European descents and Filipinos. High plasma Hcy level is related to cardiovascular disease and may play an etiologic role in vascular damage. While genetic variants of MTHFR and CBS are known to influence Hcy concentration, other genetic determinants of Hcy remain largely unknown. The association between the CPSI T1405N SNP and the risk of hyperhomocysteinemia in Latin American populations is currently unknown. Here, we correlated rs7422339 and hyperhomocysteinemia state in Argentinian patients.
Subjects: 100 hyperhomocysteinemic patients without MTHFR C677T polymorphism and 100 healthy subjects.
Methods: CPSI rs7422339 was studied using PCR-RFLP. Statistical analysis was made with INFOSTAT. Results: Comparisons of the CPS1 rs7422339 genotype distributions revealed a significant difference between groups (P=2,3 x 10–3), without gender specific differences (P=0.857). Patients carrying polymorphic allele (A) achieve almost three times higher risk (OR=2,47) of hyperhomocysteinemia than wt allele (C).
Conclusion: Our data suggest that rs7422339 SNP should be connected to high Hcy levels in the Argentinian population.

09. Other amino acid disorders

P-123

Cerebral visual impairment in a patient with serine deficiency

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Background: Clinical features of serine deficiency disorders include congenital microcephaly and development of psychomotor retardation and intractable seizures. Cataracts have been reported in some cases. We believe this is the first reported case of Serine deficiency presenting with Cerebral visual impairment.
Case Report: A 1 month old boy was seen in clinic due to maternal retroviral infection. Results of tests showed no evidence of active infection. On examination he was noted to have severe microcephaly (Chemistry investigations included plasma and CSF amino acids. Plasma serine and glycine concentrations were reported to be at the low end of their respective reference range. CSF serine and glycine concentrations were low (10 μmol/L ref. 35 – 80 and ‹5 μmol/L ref. 0–10) respectively. He has been prescribed Serine 1000 mg TDS and Glycine 500 mg TDS.
Conclusion: We present a case of serine deficiency presenting with microcephaly, visual impairment and dystonia.

P-124

Dexamethasone administration prevents blood–brain barrier breakdown and cerebral edema in an animal model of maple syrup urine disease

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The mechanisms of brain damage in Maple Syrup Urine Disease (MSUD) are still poorly understood, and drugs for attenuating acute cerebral edema in this disorder remain unclear. Given that vasogenic edema is widely considered to be detrimental for patients with MSUD, in this study we evaluated whether dexamethasone can be used for alleviating cerebral edema. Wistar rats (10 days) received three injections of a branched-chain amino acids (BCAA) pool containing leucine, isoleucine and valine or saline (control group), at intervals of one hour between injections, subcutaneously. One hour after the first administration, the animals received dexamethasone (0.7 mg/kg i.p.). One hour after the last BCAA administration the blood–brain barrier (BBB) integrity and cerebral edema were investigated. Our results showed that acute exposure of BCAA to 10-day-old rat increased the BBB permeability to sodium fluorescein in cerebral cortex and hippocampus, but not to Evan's Blue dye. Furthermore, we also observed that BCAA increased brain water content, suggesting cerebral edema. Interestingly, we showed that administration of dexamethasone successfully reduced BBB breakdown and cerebral edema caused by BCAA. In conclusion, these findings suggest that dexamethasone can improve acute cerebral edema and brain injury associated with high levels of BCAA observed in MSUD patients.

P-125

In vivo administration of glycine to neonatal rats provokes cerebral cortex damage through a dual mechanism

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Background: Glycine encephalopathy (GE) is an inborn error of glycine (GLY) metabolism biochemically characterized byaccumulation of GLY in brain of affected individuals. The main clinical symptoms include mental retardation, hypotonia and seizures.
Objective: We investigated the ex vivo effects of a single intracerebroventricular GLY administration (0.2 μmol/g) on redox homeostasis and energy metabolism in cerebral cortex of 1- or 5-day-old neonatal rats.
Materials and Methods: The biochemical parameters were analysed in cerebral cortex homogenates from rats euthanized 1, 5 or 10 days after GLY injection.
Results: GLY increased reactive species generation, decreased glutathione concentrations and modulated glutathione peroxidase activity (GPx) at all periods after GLY injection in 1-day-old rats. Lipid peroxidation was induced 5 days after GLY exposure, whereas catalase activity was altered 1 and 5 days after the administration of the amino acid. The activities of creatine kinase and respiratory chain complex IV were decreased 1, 5 or 10 days after injection. The only alteration observed in 5-day-old animals was a decrease in GPx activity 1 day after GLY administration. Discussion and
Conclusion: Our findings clearly demonstrate that GLY elicits bioenergetics dysfunction and oxidative stress in newborn rats, which may contribute to the brain injury found in GE.

P-126

Absence of urinary succinylacetone in an infant with hereditary tyrosinaemia type 1

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Background: Diagnosis of hereditary tyrosinaemia type 1 (HT1) is usually made by detection of urinary succinylacetone.
Case report: A 4 month old girl presented with abdominal distention and failure to thrive. Investigations showed synthetic liver dysfunction, elevated alphafetoprotein and a liver mass. Plasma amino acids showed elevated tyrosine (333 μmol/L) and methionine (835 μmol/L). Urinary organic acids were non-specific for liver dysfunction. Succinylacetone was not detected. She was transferred to a tertiary hepatology unit for further investigation. Repeat urinary organic acids showed a trace of succinylacetone and small peaks of dihydroxyhepatanoate and hydroxyketoheptanoate. Bloodspot PBG synthase screen was equivocal. Bloodspot succinylacetone was slightly increased (3.41 μmol/L). Urine 5-aminolevulinic acid was increased (29.8 μmol/mmol creatinine). DNA analysis showed two pathogenic mutations of the FAH gene. Liver dysfunction improved immediately following commencement of Nitisinone therapy. The patient went on to curative liver transplant. The explanted liver showed hepatocellular carcinoma with underlying abnormal architecture. Subsequent enzyme analysis in liver and cultured fibroblasts showed deficient fumaryl acetoacetate hydrolase activity.
Conclusion: Absence of urinary succinylacetone does not exclude the diagnosis of HT1. It is important to look for the related metabolites dihydroxyhepatanoate and hydroxyketoheptanoate when performing urinary organic acid analysis.

P-127

Clinical and biochemical spectrum of tyrosinemia type 1 and outcome in patients from India and Pakistan

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Background: Tyrosinemia type I (HT1) is autosomal recessive disorder caused by deficiency of fumarylacetoacetate. It is characterized by progressive liver failure, renal tubular dysfunction and coagulopathy.
Objective: To review clinical spectrum of the disease at the time of diagnosis and outcome in developing countries with limited ressources.
Materials and methods: This is a retrospective study of 31 patients. Clinical details and tyrosine, phenylalanine, methionine, urine succinylacetone, PBG synthase, AFP, USG and MRI of abdomen were analyzed.
Results: We diagnosed 25 (F=8, M=17) cases with HT1. Additional 6 patients were referred to us after initiation of treatment. In 25 patients we found tyrosine levels of 528.46±199.59 uM/L and methionine 204.91±165.95 uM/L. Succinylacetone in urine was 425.35±606.56 uM/mMCr, PBG synthase activity was 0.14± 0.13 nkat/gHb, AFP was 49,439.73±89,892.59 ugm/L. Of 31 patients, 9 lost for follow up, 16 were on NTBC and 6 remained untreated and expired. Of 16 follow up patients, 2 patients with cirrhosis received liver transplant. 2 patients who were on NTBC developed HCC of which one was inoperable and lethal. The other transplanted one was doing well immediately after transplant and stopped NTBC with slight increase in urinary succinylacetone.
Discussion: 38.70 % (12/31) are well controlled with NTBC. Overall mortality is 22.58 % (7/31).

P-128

Hyperprolinaemia – a case series from Ireland

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Background & Objectives: Hyperprolinaemia (HP) is characterised by elevated levels of proline in plasma and urine. There are two inherited forms of hyperprolinemia: type I & type II. We report on 7 cases of hyperprolinaemia in Ireland, describing quantitative proline levels; urinalysis; co-morbidities, and current clinical status.
Results: Seven patients attending NCIMD have hyperprolinaemia. Two have type I and had plasma proline levels around 1000 umol/l on diagnosis, never had seizures, and are developmentally normal. Four patients have type II HP with plasma proline levels of 2700–3100 umol/l; all had pyrroline-5-carboxylic acid (P5C) detected in urine. Of these four: 3 are from the Irish Traveller population, 3 have developmental delay, 2 have seizures, and 1 has schizophrenia. The seventh patient has severe developmental delay, seizures and has proline levels in the region of 1,500-2,200umol/l. There is no P5C detectable in his urine and he is heterozygous for the common Irish Traveller mutation in the ALDH4A1 gene. His underlying diagnosis remains unknown.
Discussion: As expected type I HP was not associated with symptoms. Type II HP was associated with seizures, developmental delay & behavioural issues. Type II carriers may have very elevated proline levels and no P5C in urine.

P-129

Compound heterozygous mutations $^{c.1A>G}$/c.784 T>A in a patient with tyrosinemia type 1 from Macedonia

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Background: Mutations in fumarylacetoacetate hydrolase (FAH) gene cause tyrosinemia type 1 (HT1), a rare autosomal recessive disorder. No molecular analysis of HT1 from Macedonia has been previously described. We investigated a child with HT1 and the parents for mutations in FAH gene.
Methods: 14 coding exons and adjacent intronic regions of the FAH gene were amplified by PCR. The fragments were purified (Wizard SV Gel and PCR Clean-Up System, Promega) and sequenced.
Results: A 3.5 year old girl was diagnosed with HT1 at age of 3 months based on clinical manifestations and biochemical abnormalities. The child is successfully treated with nitisinone. Analysis of FAH gene revealed two heterozygous mutations c.1A>G and c.784T>A transmitted from her father and mother respectively. The c.1A>G is a previously described mutation which changes the initial Met into Val and probably negatively affects initiation of FAH protein translation. The other c.784T>A in exon 10 is a novel mutation which results in amino acid substitution p.

Trp262Arg. By in silico analysis this mutation was predicted to be disease causing.
Conclusion: This is the first Macedonian patient in whom the diagnosis of HT1 was confirmed by molecular analysis. Detection of underlying mutations in HT1 patients is helpful for genetic counseling and further research.

P-130

Ornithine aminotransferase deficiency presenting with vomiting and encephalopathy due to hyperammonaemia in neonatal period

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Hyperornithinaemia due to ornithine aminotransferase (OAT) deficiency results in gyrate atrophy of choroid and retina in childhood. Rarely, OAT deficient neonates can present with hyperammonaemic encephalopathy. Diagnosis of neonatal presentation of OAT deficiency could be difficult because of the absence of hyperornithinemia. In this case report, a patient with a history of encephalopathy and vomiting attacks due to hyperammonaemia who was diagnosed as OAT deficiency in infancy is reported. An 18 days old female newborn was admitted to emergency department with complaints of projectile vomiting and encephalopathy. The laboratory findings revealed increased plasma ammonia level accompanied by low levels of ornithine, arginine and citrulline in plasma aminoacid analysis whereas urinary aminoacid profile was normal. No orotic aicd excretion was detected in urine. Within 24 h of treatment with ammonia scavenging agents with arginine and citrulline supplementation blood ammonia levels decreased to normal ranges. By 3 months, a significant elevation in plasma ornithine level was detected. Urinary homocitrulline excretion was found normal. These laboratory findings along with the clinical findings were found to be consistent with OAT deficiency. Her ophthalmological examination was performed biannually and no pathological findings were found. Mutation analysis is pending.

P-131

Efficacy of a MCT supplementation with galactose restricted diet in an infant with citrin deficiency and a novel variant in the SLC25A13 gene

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Citrin deficiency (CD) is a disorder prevalent in the Asian population, as a cause of neonatal intrahepatic cholestasis. We describe a case of a male newborn of Chinese descent, exclusively breastfed, who presented at 7 weeks of age with jaundice, scleral icterus, conjugated and unconjugated hyperbilirubinemia, hypoalbuminemia, elevated ferritin, AFP, abnormal coagulation factors, poor weight gain and positive urine reducing substance. Plasma amino acids revealed a mild elevation in citrulline with elevated threonine/serine ratio, raising suspicion of CD. Given this, breastfeeding was restricted by a high MCT complete formula (Lipistart) and aqueous form of lipid soluble vitamins, with minimal improvement. Subsequently we discontinued breastfeeding completely and he received only Lipistart with aqueous form of lipid soluble vitamins. Biochemical parameters dramatically corrected and the scleral icterus and jaundice resolved rapidly. The diagnosis of CD was confirmed by molecular analysis, which revealed 3 deleterious mutations in the SLC25A13 gene. He was homozygous for the common c.852_855delTATG mutation as well as heterozygous for a novel splice site mutation c.1452+1G>A. This case emphasizes the importance of considering CD in infants with cholestasis and the efficacy of galactose restricted/MCT supplemented diet in CD; avoidance of breast milk was crucial in fast recovery of liver function.

P-132

To what extent are biological and imaging findings reliable in the diagnosis of liver nodules in tyrosinemia type 1?

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Background: Tyrosinemia type 1 (TT1) is associated with an increased risk of liver nodules. A careful monitoring of AFP levels and liver imaging is necessary to a precocious and precise diagnosis of these nodules. Their treatment varies depending on their benign or malignant nature.
Aim: To study the reliability of AFP levels and liver MRI findings in predicting the nature of liver nodules in TT 1. Methods: We collected the data of 8 Tunisian patients with TT1 and liver nodules. We studied the correlation between the initial diagnosis made on biological and MRI findings and the final diagnosis revealed by the clinical course or histological data.
Results: An initial diagnosis of liver cancer was made in 4 patients, but confirmed in only 2. In one patient the diagnosis was rectified only after liver transplantation. In the last one, a preoperative biopsy allowed to rectify the diagnosis by showing no histological signs of malignancy.
Conclusion: AFP levels and MRI findings are important in the diagnosis of liver nodules in TT1, but in doubtful cases, a preoperative biopsy can precise the diagnosis and permit to avoid an unnecessary liver transplantation.

P-133

Co-administration of branched-chain amino acids and lipopolysaccharide causes matrix metalloproteinase activation and blood–brain barrier breakdown

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Infections play a significant role in precipitating acute metabolic decompensation in patients with Maple Syrup Urine Disease (MSUD). However, the mechanisms underlying the neurotoxicity in this disorder are poorly understood. Thus, we evaluate the effects of the co-administration of lipopolysaccharide and high concentrations of branched-chain amino acids (H-BCAA) on the permeability of the blood–brain barrier (BBB) and on the levels of matrix metalloproteinases. Wistar rats (10 and 30 days) received three injections (1 h interval) of a pool of BCAA (leucine, isoleucine and valine) or saline (control group), subcutaneously. Immediately after the first administration, the animals receive a single intraperitoneal injection of lipopolysaccharide (3 mg/kg). One hour after the last administration of BCAA the BBB integrity was investigated using Evan's blue and MMP-2 and MMP-9 were evaluated.

Our results demonstrated that co-administration of H-BCAA and lipopolysaccharide causes breakdown of the BBB in cerebral cortex and hippocampus of the 10 and 30-day-old rats. Moreover, LPS plus H-BCAA increased levels of MMP-2 and MMP-9 in hippocampus of 10 and 30-day-old rats, whereas in the cerebral cortex only MMP-9 was increased in 10-day-old rats. These results suggest that the inflammatory process associated with high levels of BCAA causes BBB breakdown and increased matrix metalloproteinases.

P-134

Congenital microcephaly and psychomotor retardation due to inborn error ofserine biosynthesis

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Serine has important functions besides its role in protein synthesis as it is a precursor of a number of compounds including glycine, serine phospholipids, sphingomyelin and cerebroside. Serine biosynthesis disorders are severe but potentially treatable inborn errors of metabolism. This disorder should be mentioned in differential diagnosis in patients with microcephaly, psychomotor retardation and encephalopathy. In this case report, a patient with a history of encephalopathy who was diagnosed as serine biosynthesis disorder is reported. A 15 months old male patient was admitted to emergency department with complaints of altered level of consciousness and hypotonia. Physical examination revealed microcephaly, profound psychomotor retardation and hypotonia. Biochemical and microbiological examination of cerebrospinal fluid (CSF) were normal. He had no seizures and his electroencephalography was normal. Brain magnetic resonance showed pathological changes of dysmyelination, frontal atrophy and volume loss in white matter. In comparison to plasma, serine and glycine concentrations were found markedly decreased in CSF sample that was consistent with serine biosynthesis disorder. Oral L-serine and glycine treatment was started. Aminoacid supplementation resulted in dramatic improvement in his consciousness and neurological findings.

P-135

BCKDK deficiency: Understanding the impact of an unrestrained branched-chained amino acid metabolism on the mitochondrial function

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A defective branched-chain α-ketoacid dehydrogenase kinase (BCKDK) activity, resulting in the hyper-activation of the BCKDH complex, was recently reported as responsible for the unrestrained branched-chain amino acid (BCAA) metabolism observed in several patients showing dietary-treatable autistic behavior. Since impairments in physiological processes as energy generation or redox homeostasis have been reported underling the etiology of autism, we have assessed the mitochondrial function on both, a subset of BCKDK-deficient patients' fibroblasts [p. R174Gfs1* and p. L389P] and BCKDK-silenced control fibroblasts. We have appraised the respiratory capacity of the cells through the measure of the Oligomycin Sensitive Respiration (OSR), i.e., the mitochondria ATP turnover capacity [Seahorse Bioscience]. In addition, we have measured the ATP pools present in the patients' derived fibroblasts [ATP bioluminescence assay] and the mitochondrial morphology through Electron Microscopy. BCKDK-deficiency results in a decrease of the OSR in both patients' fibroblasts and in BCKDK-interfered control fibroblasts, confirming that this reduction is the result of BCAA abnormal metabolism. However, not all the cells showed a decreased ATP concentration, which might be fueling through different compensatory metabolic pathways. These results show an alteration in mitochondrial function that could be contributing to the autism disorder due to a metabolic disease observed in the described patients.

P-136

Streptococcus pneumoniae septicemia in two tyrosinemia type 1 patients

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Hereditary tyrosinaemia type 1 is characterized by progressive liver disease and renal tubular dysfunction. Although the frequency of sepsis is higher in tyrosinaemia type 1, predisposition to any special microorganism is not defined. We report, streptococcus pneumoniae septicemia in two patients with tyrosinemia type 1. First patient was admitted to hospital with respiratory distress. His physical examination revealed abdominal distension, hepatosplenomegaly and crackles in both lungs. Pancytopenia, hypoalbuminemia, hypophosphatemia and glucosuria were detected and coagulation profile was significantly disturbed. High methionine and tyrosine levels in plasma aminoacid profile along with elevated α-fetoprotein and urinary succinylacetone was consistent with tyrosinemia type 1. NTBC treatment was started. Blood culture taken on admission was found positive for streptococcus pneumonia. Second patient, a 8 months of age female patient, was diagnosed as tyrosinemia type 1 at 6 months of age and was under NTBC treatment. At 8 months of age she was admitted to hospital with complaints of fever and abdominal distension. Blood culture was found positive for streptococcus pneumonia. Both patients responded to antibiotherapy with complete recovery. This report highlights the probability of increased predisposition in tyrosinaemia type 1 to streptococcus pneumonia septicemia in infancy.

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Ketogenic diet in nonketotic hyperglycinemia: Can there be some hope?

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Nonketotic hyperglycinemia (NKHG) is a rare inborn error of aminoacid metabolism due to deficient activity of glycine cleavage system, a multienzyme complex with four protein subunits. The classical neonatal form after a short period of normal appearance, presents in the first days of life with lethargy, encephalopathy, hiccups, multifocal myoclonic seizures rapidly progressing to intractable seizures, coma and respiratory failure. The typical burst-suppression pattern in EEG can be detected even after 30 min after birth. There is no effective treatment of the disease and most children surviving after the first few days are severely retarded. Efforts in lowering the plasma glycine levels with sodium benzoate, imipramine and dietary restriction or in reducing NMDA receptors excitatory properties with ketamine and dextromethorphan have been disappointing in long-term follow-up. The ketogenic diet (KD) is a medically supervised high-fat, low-carbohydrate diet found useful in patients with refractory

epilepsy. Here we represent the effects of KD in a case series of three patients ages of 1 $^{2/12}$years, 27 months and 2$^{10/12}$ years with classical NKHG with refractory myoclonic seizures and severe neurodevelopmental delay. KD led to dramatic reduction of seizures in all three patients and improved neurocognitive development in one with increase in quality of life.

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Maple Syrup Urine Disease (MSUD): Clinical phenotypes, genotypes and treatment outcome after four decades of newborn screening in the Republic of Ireland

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Objective: To study phenotypes, genotypes and treatment outcome in MSUD patients diagnosed on NBS and treated at the NCIMD, Dublin (1972–2011), and to stratify into clusters according to 1) leucine levels and 2) clinical findings.
Methods: 17 patients (10 females, 7 males; 15 alive, 2 deaths). Individual yearly median values of leucine, isoleucine, and valine (BCAAs), maximum leucine levels and area under the curve (AUC) were determined. Leucine results were grouped to differentiate 3 clusters according to metabolic control.
Results: Mean age 20.2 years. (±11.4, range 0.6-33.7); 17,834 complete data sets for BCAAs, mean 58/pt/year, total sum of patient-years 314.2 years. Determinants of outcome were early diagnosis, peak leucine and AUC, reflecting the actual body exposure. Three clinical clusters were identified for live patients according to outcome, neuroimaging and IQ results. Both sets of clusters were congruent. 14 patients had a novel mutation, 9 x BCKDHB gene, 3 x DBT, 2 x BCKDHA.
Conclusions: This study illuminates the beneficial effects of NBS and the impact of metabolic control on treatment outcome. It provides the opportunity to give an Irish perspective to the international experience with MSUD.
Acknowledgement: Alexander Kürwitz, Berlin - Statistical consulting. Children's Fund for Health, Vitaflo - Financial assistance.
Conflict of Interest declared.

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The first related living donor liver transplantation in a patient with MSUD in Poland

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Background: Maple syrup urine disease (MSUD) is an autosomal recessive disorder characterized by branched-chain ketoacid dehydrogenase complex deficiency, which results in accumulation of branched-chain amino acids and branched-chain alpha-ketoacids. The acute metabolic crises in MSUD cause irreversible damage to the CNS. Patients are exposed to metabolic decompensation during increased catabolism. The treatment is based on dietary protein restriction. Nonetheless, the prognosis is uncertain. One of alternative methods of treatment is liver transplantation (Ltx).
Case report: A 2 year old girl with MSUD from neonatal period presented signs of metabolic intoxication. Hemodiafiltration and diet treatment was introduced immediately. Later the patient only had one metabolic decompensation. When she was two, her psychomotor development was normal. The MRI of CNS revealed hyperintense T2 dependent changes in the nuclei gear, midbrain, and hypothalamus. The related living donor liver transplantation was carried out at 2 years.
Results: Typical for age protein content diet is well tolerated. Leucine after transplantation significantly decreased. Average levels of leucine before and after transplantation were 566 μmol/L vs 191 μmol/L, 66.3 % range of decrease. The alloisoleucine is still detectable in very low average levels 3.5 μmol/L. Further course of disease needs to be observed.

P-140

Blood measures of cerebral and peripheral neurotransmitter homeostasis in tyrosinemia type 1 patients compared to PKU patients

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Background and aim: In Tyrosinemia type 1 (HT1), lower IQ is found. In PKU, cerebral neurotransmitter deficiencies are hypothesized to contribute to impaired neuropsychological functioning and IQ. This study aimed to investigate possible relationships between blood amino acid concentrations and parameters of cerebral and peripheral neurotransmitters in HT1 compared to PKU patients.
Patients and methods: We retrospectively compared blood phenylalanine, tyrosine, and tryptophan concentrations in relation to blood prolactin and platelet serotonin concentrations in 73 samples of 6 HT1 patients compared to 300 samples of 62 PKU patients. Blood prolactin was investigated in males only.
Results: In HT1 patients, blood prolactin concentrations did not correlate with phenylalanine nor tyrosine concentrations. In PKU patients, blood prolactin concentrations positively correlated to phenylalanine concentrations (p=0,000), while negatively to tyrosine concentrations (p=0.026), at blood phenylalanine >600 μmol/l. In HT1 patients, platelet serotonin concentrations positively correlated to tyrosine (p=0.012), but not to phenylalanine nor tryptophan concentrations. In PKU patients, platelet serotonin negatively correlated to phenylalanine (p=0.001), while positively to tryptophan (p=0,000) but not to tyrosine concentrations.
Discussion: In HT1, a different pattern is seen compared to PKU that needs further studies to learn the importance and complexity of phenylalanine and tyrosine concentrations for brain functioning.
Conflict of Interest declared.

P-141

Effect of proline on cell death, cell cycle and oxidative stress in C6 glioma cell line

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Hyperprolinemias are metabolic diseases caused by enzyme deficiencies in proline catabolism. This study aimed to investigate the proline effects at concentrations similar to those found in hyperprolinemic patients, on cell

death and oxidative stress in glioma cells. C6 cells were incubated with proline (0–5 mM) by 1 h, 24 h, 48 h, 72 h or 7 days. Then, we examined the cell death (MTT, LDH and Annexin-PI kit), cell cycle (flow cytometry), acetylcholinesterase (AChE), superoxide dismutase (SOD) and catalase (CAT) activity. Proline (3 and 5 mM) induced cell death (48 and 72 h) detected by MTT and LDH methods, but not by Annexin-PI. The cell cycle progression was not altered. AChE activity was inhibited by proline at 1, 3 and 5 mM (48 and 72 h). SOD and CAT activities were increased by proline (1 mM) after 72 h, suggesting an increase in reactive species levels. Proline induces oxidative stress, but it appears not to be sufficient to induce cell death or cell cycle alterations. AChE that may act as tumor suppressor, is inhibited by proline favoring cell proliferation. These data may explain, at least in part, the increased susceptibility to tumors development in hyperprolinemic individuals. Supported by CNPq, PROPESQ/UFRGS.

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Executive functioning and social information processing in tyrosinemia type 1 and phenylketonuria

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Background: IQ abnormalities may be due to impairments in executive (EF) and social (SF) functioning. In PKU, impairments in EF are related to suboptimal phenylalanine and tyrosine levels, while impairments in SF are unclear. In Hereditary Tyrosinemia Type 1 (HT1) IQ is decreased, but EF and SF have not been studied. Both PKU and HTI have abnormalities in phenylalanine and tyrosine metabolism, but concentrations are discordant.
Objective: To compare EF and SF between HT1-patients, PKU-patients and controls, to learn more about the influence of abnormal phenylalanine and tyrosine on brain function. Patients and methods: Twelve HT1-patients (7.9-25.4 years), 12 matched PKU-patients, and 12 healthy controls completed tasks measuring three EF (inhibition, cognitive flexibility, sustained attention) and the ability to identify emotions. Non-parametric tests were used to compare groups.
Results: No differences in inhibitory control and sustained attention were observed. However, HT1-patients performed worse on cognitive flexibility than controls ($p=.048$), and they were poorer than both controls and PKU-patients at identifying emotions.
Conclusion: HT1-patients had more difficulty with cognitive flexibility than controls, and more difficulty with identifying emotions compared to PKU-patients and controls. Cognitive functioning of these patient groups should be examined with reference to treatment and metabolic control.

P-143

Clinical features in two cases of hyperprolinaemia type-II

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Background and objectives: Hyperprolinaemia type-II is a rare inborn error of proline catabolism. It is caused by an inactivity of P5C-dehydrogenase and is characterised by highly elevated plasma proline and urinary N-pyrrole-2-carboxyglycine levels. The phenotype is associated with neurological involvement. However, the variability of the condition makes monitoring outcomes a significant challenge. We describe the features of two probands with unusual genotypes and compare them with previously known cases.
Case reports: Child A presented at 16 months of age with a polymorphic seizure disorder involving motor seizures, atypical absences and febrile-related episodes, poorly controlled with anticonvulsants. He has complex behavioural problems and obsessive-compulsive traits. Child B had sensorineural deafness from birth and was diagnosed with 15q13.3 microduplication syndrome. She presented with recurrent absences from 2 years of age, well controlled with valproate, and was diagnosed with HP-II by urinary organic acid analysis. We have requested DNA mutation analysis (results pending).
Discussion: HP-II is rare. DNA analysis of ALDH4A1 confirmed child A is a compound heterozygote for two mutations in trans-, inherited from heterozygote parents who are unrelated. This is unusual for a disease of such low penetrance. Child B demonstrates the potential for HP-II to superimpose other genetic syndromes.

10. Urea cycle disorders

P-144

Results from a nationwide cohort temporary utilization authorization (ATU) survey of patients in France treated with Pheburane (sodium phenylbutyrate) taste-masked granules

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In October 2012 a cohort ATU was established in France to monitor the use of Pheburane on named-patient basis in urea cycle diseases (UCD). All UCD treated patients were included in a follow-up protocol developed by Lucane Pharma and ANSM which recorded demographics, dosing characteristics of NaPB, concomitant medications, adverse events and clinical outcome during the period of treatment. Following the granting of the Marketing Authorization in Europe, the cohort ATU was terminated approximately one year after its initiation, as the product was launched on the French market.
Results: The ease of administration and acceptability were much better with the new taste-masked formulation than with the previous treatment. The number of episodes of metabolic decompensation was decreased to none over a treatment period ranging from 3 to 11 months with Pheburane and the range of ammonia and glutamine levels shrank and remained within normal limits. In all, no adverse events were reported with Pheburane treatment.
Conclusions: The recently developed taste-masked formulation of NaPB granules improved the quality of life for UCD patients. This may translate into improved compliance, efficacy and safety, which may be demonstrated either in further studies or in the post marketing use of the product.
Conflict of Interest declared.

P-145

Clinical, laboratory features and outcome of argininosuccinic aciduria - an Iranian experience

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Background: Argininosuccinic aciduria (ASA) is an autosomal recessive urea cycle disorder, due to argininosuccinate lyase (ASL) deficiency. ASL catalyzes the reversible cleavage of argininosuccinate to arginine and fumarate within urea cycle and is required for nitric oxide synthesis. It is caused by mutation of ASL gene located on chr.7 $q^{11.21}$. It manifests as either a severe neonatal form with hyperammonemia within the first days of life or as a late onset type with episodic hyperammonemia and/or long term complications including liver disease, trichorrhexis nodosa, hypertension and hypokalemia. Results: 2 male and 1 female, consaguinity: 3/3, death in siblings: 2/3. Case 1 developed convulsions on the 4th day of life and died. Urinary argininosuccinic acid: 170 μmol/L (nl<1 μmol/L). Case 2 and 3 were referred with 24 days and 15 months for delayed development and showed high ammonia, glutamine and citrulline, low arginine and high excretion of ASA in urine (case 1) and plasma (case 3: 32.6 μmol/L (nl< 0.7)). Case 2 developed trichorrhexis nodosa at 15 months responsive to high dose arginine, also hypokalemia of 3 weeks duration at 34 months. Both responded well. The mutation of c. 1273delTA was discovered in homozygous state in ASL gene, resulting in p. 1425 L.
Conclusion: One severe neonatal and two responsive chronic forms were reported.

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Perinatal diagnosis and neonatal treatment for a large family with ornithine transcarbamylase deficiency

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Ornithine transcarbamylase (OTC) deficiency is the most common inborn error of the urea cycle. OTCD results from deficiency of the mitochondrial enzyme and shows extensive phenotypic heterogeneity influenced by allelic heterogeneity and modifying environmental influences such as protein intake, infection, starvation etc. Clinical presentation of OTCD is complex because male hemizygotes usually present in infancy, whereas female heterozygotes may be totally asymptomatic. On the other hand, hemizygous males may also present at any age without any precedent symptoms or effects, whereas heterozygous females may be severely affected in childhood. Although many symptomatic females may present because of skewed distribution of the mutant gene in hepatocytes due to lyonization, reasons for late-onset male presentations remain obscure; however, some males clearly have residual enzyme activity. We have a large family with a splicing mutation of OTC gene. We try to appropriately diagnose each newborn. Their family members received appropriate genetic counseling, leading to successful treatment.

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A case of carbamoylphosphate synthetase 1 deficiency presenting at second day of life

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Carbamoylphosphate synthetase 1 (CPS1) deficiency is a rare autosomal-recessive disorder with severe hyperammonemia and early or late-onset. The early-onset form presents as severe neonatal hyperammonemia and often has a poor prognosis. We present a 2-days-old term infant with feeding intolerance, weight loss and encephalopathy. She was first child of consanguineous Turkish parents born after an uneventful pregnancy. Symptoms started at 24 h of life leading to hospitalization in a neonatal ICU with suspected hypernatremic dehydration and sepsis. On day 3, she became lethargic and hypothermic. Laboratory data, echocardiography and brain ultrasound were normal apart from respiratory alkalosis. She deteriorated rapidly with severe hyperammonemia (2300micromol/L; normal <60). Mechanical ventilation and peritoneal dialysis were started. Increased plasma glutamine and alanine but undetectable urinary orotic acid suggested CPS1 or N-acetylglutamate synthase deficiency. Protein-free formula and carglumic acid via nasogastric tube, intravenous lipids, sodium benzoate, and L-arginine were given. Peritoneal dialysis could be stopped on day 5 and oral feeding was started. She was discharged on day 27. CPS1 transcript sequencing revealed homozygous skipping of exon 7 (r.622_711delExon7; p. Asp208_Lys237del) but no classic splice mutation was identified. With immediate treatment, even early-onset CPS1 deficient patients may survive. At the age of 3 months she has been neurologically normal, receiving protein restricted diet, citrulline, L-arginine, Na benzoate and carglumic acid with good metabolic control.

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Medium-chain triglyceride supplementation under a low-carbohydrate formula is a promising therapy for adult-onset type II citrullinemia

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Background: Citrin, encoded by SLC25A13, is a component of the malate-aspartate shuttle, which is the main NADH-transporting system in the liver. Citrin deficiency causes neonatal intrahepatic cholestasis (NICCD), and small numbers of adults develop hyperammonemic encephalopathy, adult-onset type II citrullinemia (CTLN2). Liver transplantation is the only definitive therapy for patients with CTLN2. We previously reported that a lactose (galactose)-restricted and medium-chain triglyceride (MCT)-supplemented formula is notably effective for patients with NICCD. Citrin deficiency may impair the glycolysis in hepatocytes because of an increase in the cytosolic $NADH/NAD^+$ ratio.
Methods: An MCT supplementation therapy under a low-carbohydrate formula was administered to five patients with CTLN2. Four of the patients had episodes of hyperammonemic encephalopathy, and one patient had postprandial hyperammonemia with no symptoms.
Results: All patients notably improved in terms of clinical findings. One of the patients displaying hyperammonemic encephalopathy completely recovered with all normal laboratory findings. Others displayed persistent mild citrullinemia and occasionally had post-prandial mild hyperammonemia.
Conclusions: An MCT supplement can provide energy to hepatocytes and promote hepatic lipogenesis, leading to a reduction in the cytosolic $NADH/NAD^+$ ratio. MCT supplementation under a low-carbohydrate formula is a promising therapy for CTLN2 and should also be used to prevent CTLN2.

P-149

Experimental evidence that the metabolites accumulated in HHH-syndrome provokes impairment on redox homeostasis in cerebellum of young rats

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Hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome is an inherited metabolic disorder biochemically characterized by ornithine (Orn), homocitrulline (Hcit) and ammonia accumulation. Besides liver dysfunction and delayed psychomotor development, affected patients usually present cerebellar ataxia, whose pathophysiology is practically unknown. Therefore, the aim of the present work was to investigate the ex vivo effects of Orn and Hcit on important parameters of redox homeostasis in cerebellum from 30-day-old rats. Animals received a single intracerebellar injection of Orn, Hcit or NaCl (control) and were sacrificed 4 h after injection. We observed that Orn significantly increased thiobarbituric acid-reactive substances (TBA-RS) levels and the activities of catalase, glutathione reductase, glutathione peroxidase, superoxide dismutase and glucose-6-phospate dehydrogenase, whereas Hcit did not modify these parameters. Furthermore, GSH concentrations and carbonyl formation and sulfhydryl content were not changed by Orn or Hcit. We also found that Orn significantly reduced Na^+, K^+ - ATPase activity. The present data show that Orn, but not Hcit, disrupted cerebellum cellular redox homeostasis in vivo, a pathomechanism that may contribute to the pathophysiology of the cerebellar ataxia characteristic of the patients affected by HHH syndrome. Financial support: CNPq, PROPESq/UFRGS, FAPERGS, PRONEX, FINEP IBN-Net and INCT-EN.

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Blood ammonia and glutamine as predictors of hyperammonemic crises (HACs) in patients with Urea Cycle Disorders (UCDs)

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Limited information is available regarding blood ammonia vs. glutamine as predictors of HACs in UCD patients.
Methods: Over 1000 timed blood samples from 114 UCD patients who participated in switchover studies from sodium phenylbutyrate (NaPBA) to glycerol phenylbutyrate (GPB) and/or long-term GPB dosing were analyzed for relationships among fasting ammonia, glutamine and HACs.
Results: Thirty of 100 patients reported 54 HACs during the 12 months pre-enrollment on NaPBA and 19 experienced 27 HACs after switching to GPB. Patients with fasting ammonia≥1.0 ULN vs. < 0.5 ULN had a shorter time to first HAC ($p=0.008$) and a higher rate of HACs (~5x, $p=0.006$ for all patients; 20x; $p=0.009$ for patients≥6 years). Every 10 or 25 umol/L increase in 12-month ammonia exposure increased the relative risk of a HAC by ~50 % or ~250 % ($p<0.0001$), respectively. Baseline ammonia correlated weakly with glutamine ($r=0.27$; $p=0.008$) and was significantly lower ($p=0.0013$) in patients without a crisis; whereas glutamine was not ($p=0.150$). HAC risk appeared independent of UCD severity as reflected by age, UCD subtype, or dietary protein.
Conclusions: As compared with glutamine, fasting ammonia correlates strongly with the risk and frequency of HAC.
Conflict of Interest declared.

P-151

A first Iranian case of N-acetyl-glutamate synthase (NAGS) deficiency treated by carbamyl-glutamate

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Background: N-acethyl glutamate synthase (NAGS) deficiency is a rare cause of severe neonatal hyperammonia.
Method: An 8-day old boy, who was born of non-consanguineous Iranian parents by C/S, was admitted in NICU due to poor feeding, unconsciousness, and seizure. High Ammonia (920 μmol/l), high plasma Glutamine and Alanine, low plasma Citrulline and Arginine, without Orotic aciduria was revealed in metabolic workup. Regarding to these results CPS or NAGS deficiency was suspected. The infant was treated by peritoneal dialysis, intravenous Na-Benzoate, Na-Phenyl butyrate, L-arginine, and the Ammonia declined to140 μmol/l.
Results: The genetic analysis confirmed the NAGS deficiency with a heterozygous missense mutation in exon 5 c.1172 T>G (p. Leu391Arg) and exon 6c.1450 T>C (p. Trp484Arg) on 17q21.31. The analysis of the parents for carrier status indicated the mutation c.1172 T>G in exon 5 in mother and c.1450 T>C in exon 6 in father. Carglumic acid (Carbaglu) was started and Ammonia declined to normal level (55 μmol/l) after 24 h. Unfortunately the patient died at 4 months of age after 5 days discontinuation of Carbaglu.
Discussion: In cases of hyperammonemia without Orotic aciduria which plasma citrulline is low, NAGS deficiency should be rule out.

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A new case of late onset OTC presenting with severe coagulopathy

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OTC is life threating disorder presenting usually with acute hyperammonaemia, encephalopathy and liver disease. There are few cases reported with severe coagulopathy as the main symptom.
We describe a 5 years old boy [R40H (c.119G>A) mutation on OTC gene]. Diagnosis established at the age of 1 year because of hyperammonaemia. He was well until 3 years old, when he presented with mild fever, vomiting, acute liver failure (SGOT: 2304 U/L, SGPT 2168 U/L), coagulopathy (PT 44,4 s, aPTT 48,7 s, Factor I 210 mg%,V 31 %, VII 4 %,IX 11 %, proC 13), without encephalopathy and hyperammonaemia. Treatment was given with FFP transfusions for 9 days, sodium benzoate, arginine and fluids (iv). He was discharged asymptomatic on a special diet. No particular trigger or infection were identified. Four months later, the patient had another metabolic decompensation during a routine check-up, where it was found that he had again coagulopathy. The boy was feeling well. He was treated with FFP, iv fluids, sodium benzoate and vitamin K and recovered quickly.
The boy had frequent follow up after these 2 episodes, over a period of nearly 2 years and is doing well. Pathogenesis is not clear for the attacks of the severe coagulopathy.

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In vivo redox imbalance in Hyperammonemia, Hyperornithinemia, Homocitrullinuria

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Hyperornithinemia-hyperammonemia-homocitrullinuria syndrome (HHHs) is an autosomal recessive disorder caused by impaired ornithine transport across the inner mitochondrial membrane. Although the metabolic alterations respond well to low-protein therapy and citrulline/arginine supplements, the disease course is characterized by neurological dysfunction with pyramidal signs. Although we cannot exclude that some uncommon biochemical abnormalities, such as those related to polyamine metabolism and/or to creatine metabolism, might be involved in pyramidal dysfunction, the pathomechanism of this picture still remains unknown. A recent report suggested the contribution of redox changes in the development of neurological dysfunction in HHHs, as often seen in mitochondrial disorders (Zanatta 2013). HHHs can be view either as primary urea cycle defect or as a mitochondrial disorder, therefore we investigated the redox status in HHHs patients. We assessed in vivo by HPLC the glutathione (GSH) status in lymphocytes from 2 HHHS patients. Our results showed an impaired glutathione homeostasis as indicated by the significant (p=.005) increase of protein glutathionylation, of the oxidized/total GSH ratio and by the reduction of total-GSH. Our study confirms in vivo a significant redox imbalance in HHHs, that may involved in the pathophysiology of pyramidal dysfunction and suggests the potential attempt of antioxidant drugs as new therapeutic strategy.

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Role of the allosteric domain of carbamoyl phosphate synthetase 1 (CPS1) in CPS1 deficiency (CPS1D)

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Background: CPS1 is a large multidomain urea cycle enzyme. Its C-terminal domain ("ASD"), representing 10 % of the protein, lacks substrate binding/catalytic machinery but hosts the site for N-acetylglutamate (NAG), an on/off type essential allosteric activator. The NAG binding mode and site require corroboration, and clarification of the activation mechanism. NAG site-bypassing CPS1 activation might have therapeutic potential in NAG site mutations.
Objective. To understand the impact on recombinantly expressed CPS1 of the reported CPS1D-associated mutations mapping in the ASD domain, and of additional mutations not found in patients but envisioned to be involved in NAG binding/activation.
Materials and methods. Ten mutations were investigated using site-directed mutagenesis, affinity-isolation, and kinetic and thermostability characterization. Data on previous expression studies with other ASD mutations were summarized and reviewed.
Results. In contrast with mutations affecting an equivalent domain of the enzyme N-half (UFSD or Integrating domain), few mutations compromised enzyme folding/expression. Few caused inactivation, but most reduced activity by impairing NAG binding or signal transmission.
Conclusions. A disease-causing role of the CPS1D mutations is supported, and the NAG site and parts of the transmission route are delineated.
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P-155

Outcome and Clinical-Biochemical Spectrum of Argininosuccinic aciduria in patients from India

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Background: Argininosuccinic aciduria (ASA), a type of Urea cycle defect, is an autosomal recessive disorder caused by deficiency of Argininosuccinate lyase (ASL) enzyme.
Objective: To evaluate the clinical and biochemical spectrum of patients with argininosuccinic aciduria and their outcome in India. Materials and method: This is a retrospective study of 5 patients with ASA over a period of 10 yrs. various biochemical parameters- ammonia, urinary orotic acid, plasma and urine amino acids and argininosuccinic acid levels were studied.
Results: We diagnosed 5 patients (F=1, M=4) with ASA. These patients showed altered sensorium, lethargy, convulsion and feeding difficulties. 3 patients presented in the neonatal period, had markedly elevated blood ammonia (993±485 μmol/L) and 2 patients presenting late (2 months and 2 yr) had mildly elevated blood ammonia level (92±2 μmol/L). Blood ASA levels have been available only recently. One patient had plasma-ASA 2188.0 μmol/L and urine ASA 7,427±4,933 μmol/mmol Cr, (n=4).
Discussion: 3 patients at neonatal stage remained untreated and expired. Of 2 late presenters one female child of 2 yrs has hepatomegaly, abnormal LFT and delayed milestones. Another male child of 12 years has convulsions, mild mental retardation and abnormal LFT. Special Diets are still not easily available in India

P-156

Diagnosis and management of urea cycle disorders in Tunisia

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Aim: to study clinical, biological characteristics of urea cycle disorders (UCD) and their outcome in Tunisian patients.
Methods: we report cases of UCD diagnosed since 1992 in one Tunisian pediatric department.
Results: 11 patients were diagnosed as UCD: 3 cases of Ornitine Transcarbamylase deficiency (OTC), 5 cases of argininosuccinate lyase deficiency (ASL), 2 cases of citrullinemia type 1 (CT1) and one case of carbamyl phosphate synthetase deficiency (CPS). 4 patients presented in the neonatal period (2 CT1, 1 OTC, 1CPS, 1ASL). Three of them survived to the first episode, but died during the first year of life because of poor metabolic control and severe failure to thrive. The 6 late onset cases (4 ASL, 2OTC) revealed between 8 months and 12,5 years. All of them had a normal growth but only 3 of them have a normal psychomotor development. One ASL patient died at 3,5 years old of acute decompensation. The molecular diagnosis made in 8 families permitted a prenatal diagnosis in 8 pregnancies.
Conclusion: A better knowledge of these diseases will allow an early diagnosis and thus a better management. Molecular study in family members of index cases can detect asymptomatic subjects and permit prenatal diagnosis.

P-157

Plasma methionine, threonine, and glycine in urea cycle disorders : connecting ammonia, polyamines and aging?

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Background/objective: We re-addressed the question how well plasma amino acids reflect ammonia levels in a large cohort of patients with urea cycle defects (UCD) followed-up in a single hospital.
Material/methods: We included 1027 samples from 68 patients with mutated carbamylphosphate synthetase (CPSD), ornithine transcarbamylase (OTCD) or argininosuccinate synthetase (ASSD/citrullinemia type I). Data (raw or loess-normalized on our database currently containing 53387 complete plasma amino acid profiles) were analyzed with age-adjustment and stratification by clinical form.
Results: In CPSD/OTCD, the strongest ammonia correlate was plasma glutamine, though with modest effect (r-squared=0.22). In ASSD, the strongest ammonia correlates (r-squared 0.30-0.32) were citrulline, methionine (positive) and threonine (negative). ASSD tended to increase levels of methionine to a greater extent than glutamine, and the trend was opposite for CPSD/OTCD. Glycine levels negatively correlated with ammonia in older ASSD patients.
Conclusions: Variations involving citrulline, threonine and glycine can be rationalized in terms of enzyme function, diet, and therapy, respectively, yet they may deserve greater attention relative to more usual follow-up biomarkers, such as glutamine. Methionine may feed into the polyamine pathway, thereby possibly assisting in ammonia disposal. The data suggest novel points of intervention to optimize the long-term personalized management of UCDs.

P-158

Rapid analysis of L-argininosuccinic acid (ASA) by using a short program of total homocysteine

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Argininosuccinic acid (ASA) is strongly hygroscopic and only stable in its anhydrous form. Arginosuccinic aciduria is an autosomal recessive inherited deficiency of arginosuccinate lyase (AL; OMIM 207900), an enzyme involved in the urea cycle. In AL deficiency, a huge excretion of ASA and two-ninhydrine positive anhydrides (anhydride I and anhydride II) are formed. The plasma sample of a argininosuccinic aciduria patient was additionally analysed using aminoethylcysteine (AEC) as an internal standard with total homocysteine short program. Coincidentally, the ASA peak was seen just before norvaline, the internal standard of short program of total homocysteine. This study presents a rapid quantitation of ASA by the modified method of short program of total homocysteine. The separation is achieved with 20 x 4.6 mm physiological high resolution column using predominantly buffer CII (pH: 3.15, Biochrom 30 aminoacid analyser). The rapid analysis of total homocysteine program allows the ASA peak to be easily seen just before norvaline internal standard and the quantification can be made less than 30 minutes.

P-159

Treatment of arginase deficiency revisited: guanidinoacetate as therapeutic target and biomarker for therapeutic monitoring

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Background: Hyperargininaemia is a disorder of the last step of the urea cycle. It is an autosomal-recessive disease caused by deficiency of liver arginase1 and usually presents later in childhood with progressive neurological symptoms including marked spasticity. In contrast to other urea cycle disorders, hyperammonaemia is not usually present but can be a feature. A number of guanidino compounds may accumulate in blood and CSF of these patients which could play an important pathophysiological role. Guanidinoacetate is of particular interest as a well known potent epileptogenic compound in guanidinoacetate methyltransferase (GAMT) deficiency.
Case report: We present a 9 year old male patient with arginase deficiency showing severe, progressive mental retardation and epilepsy who never suffered hyperammonaemia. We found markedly elevated guanidinoacetate levels which dropped significantly in response to dietary and medical treatment (similar to GAMT-deficiency). Homoarginine was normal. We observed significant clinical improvement as fits became shorter and less frequent, alertness improved.
Conclusion: Measurement of guanidinoacetate and other guanidino compounds may be of pathophysiological significance in arginase deficiency. They can be used for therapeutic monitoring. Elevated guanidino compounds are not only a biochemical hallmark of GAMT deficiency but can be elevated in arginase deficiency too.

P-160

The natural course of 63 patients with neonatal-onset UCD in the years 2001–2013

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Background: The natural course of newborn urea cycle defect (UCD) patients was only reported for few cohorts and conclusions are problematic because of treatment differences. We describe the outcome of neonatal-onset UCD patients (OTC/CPS1/ASS deficiencies) studied between 2001 and 2013.
Methods: A retrospective, multicenter, non-interventional case-series-study collected data from leading European metabolic centers. Study sites identified records of patients with an observation period of up to 5 years or until death.
Results: After screening 190 patients, 63 patients (27 ASSD, 23 OTCD, 12 CPS1D, 1 unknown) were enrolled. From these, 19 (30.2 %) died and 11 (17.5 %) underwent liver transplantation. The mean number of hyperammonemic events (HE) was 3.6 and the mean peak ammonia levels were 539±391 μmol/L corresponding to a mean of 2.7 hyperglutaminemic events and mean peak levels of glutamine of 1400± 662 μmol/L. The incidence of HEs (ammonia ≥250 μmol/L) was greater for OTCD and CPS1D versus ASSD patients.
Conclusions: This study provides data that markedly differ from published reports concerning the number of deaths caused by the first HE possibly explained by underreporting of severe cases elsewhere. The high risk of neonatal-onset UCD patients for continued hyperammonemia and repeated HE is confirmed and underlines the urgent need for novel therapies.
Conflict of Interest declared.

P-161

Molecular findings of Malaysian patients with neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD)

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Introduction: Citrin the aspartate-glutamate carrier of the mitochondrial inner membrane plays an important function in the synthesis of urea. Citrin is encoded by SLC25A13; mutations in this gene cause sdult-onset citrullinaemia Type 2 (CTLN2) and neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD). 81 sequence variations in SLC25A13 have been reported worldwide. We report the mutation spectrum of SLC25A13 in Malaysian patients with NICCD.
Methods: DNA of 30 paediatric patients with NICCD were amplified with touchdown PCR before being subjected to bidirectional sequencing. In-silico analyses were used to predict the pathological effects of new mutations.
Results: Common mutations identified were: IVS16ins3kb (20 alleles), 851del4 (13 alleles), G139R (5 alleles), IVS6+5G>A (2 alleles) and 1638ins23 (1 allele). In addition, 4 novel pathogenic mutations were uncovered. These were: missense mutations c.1475G>A (1 allele) and c.874C>T (1 allele), splice-site c.212+1G>T (2 alleles) and a large deletion of exon 14 (2 alleles). No mutation was found in the remainder 13 alleles. This cohort was made up of a multi-ethnic background of Malay, Chinese and indigenous Malaysian aborigines. In accordant with current literature, there was no genotype-phenotype correlation.
Conclusion: This data contributes to the knowledge of the complexity of the genotype and phenotype of patients with NICCD.

P-162

Folding defects of argininosuccinate lyase cause variant forms of the urea cycle disorder argininosuccinic aciduria

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Background: Mutations in the argininosuccinate lyase (ASL) gene cause argininosuccinic aciduria (ASA). ASA displays broad clinical spectrum from life-threatening neonatal to asymptomatic forms. To define the molecular characteristics underlying the phenotypic variability, we investigated all known ASL mutants identified in patients with late-onset or mild clinical and biochemical courses.
Materials and Methods: We overexpressed eleven ASL mutants associated with variant ASA (p. R12Q, p. D31N, p. R95C, p. I100T, p. V178M, p. E189G, p. R193W, p. V335L, p. R379C, p. R385C, p. R445P) and two severe ASL mutants (p. Q286R, p. R385H) in 293 T cells. ASL activities, kinetic properties, thermal stability, and computational structural analysis of ASL variants were addressed.
Results: Nine variant ASL mutants had >3 % of residual ASL activities. Six mutants had ≥18 % of WT activity and less than 2-fold reduced Km values displaying thermal instability. Structural modelling revealed protein instability, disruption of ionic interactions and hydrogen bonds between residues.
Conclusion: Variations in residual activity and folding defects of mutant ASL proteins contribute to the variant ASA forms. Folding mutations affect at least 25 % of known ASA genotypes and may be candidates for chaperone treatment to improve mutant protein stability.
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P-163

5 years favorable outcome, following a severe neonatal hyperammonemic coma in a patient with citrullinemia treated with intravenous combination of phenylacetate and benzoate

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Objective: We report the normal development after 5-years follow-up of a girl patient with neonatal citrullinemia who was rescued from severe hyperammonemic coma. Intravenous administration of a combination of sodium phenylacetate and sodium benzoate was used.
Methods: On day 3, two loading doses were administrated intravenously (250 mg/kg/dose). This was followed by a double maintenance dose of 500 mg/kg during 13 hours and 250 mg/kg/day during 3 days which enabled normalization of the ammonia (which was initially 2633 μmol/l) in 60 hours (97 μmol/l). Oral treatment with sodium phenylbutyrate, sodium benzoate and arginine was initiated together with dietary protein restriction.
Results: A 5 year regular monitoring of the child showed a normal psychomotor development: walking at 16 months, normal language, regular schooling in kindergarten, regular and harmonious height-weight growth (as the sibling). Long term treatment includes sodium phenylbutyrate, sodium benzoate, arginine and dietary protein restriction. The child never showed hyperammonemic decompensation. Metabolic and nutritional balance remained satisfactory.
Conclusion: This observation highlights the importance of early treatment, at birth, with sodium phenylacetate and sodium benzoate, for citrullinemia patient with severe hyperammonemia.

P-164

Characterization of the only frequently recurrent mutation in carbamoyl phosphate synthetase 1 deficiency

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Background: Mutations in carbamoyl phosphate synthetase 1 (CPS1) cause CPS1 deficiency (CPS1D) with hyperammonemia and death or severe neurological impairment. CPS1 catalyzes carbamoyl phosphate formation from ammonia, bicarbonate and two ATP molecules, and requires the essential allosteric activator N-acetyl-L-glutamate. Mutations in CPS1D are usually identified in only single families, with little recurrence. We characterize here the only frequently recurrent CPS1 mutation, p. Val1013del.
Materials and methods: The mutation was found in ten unrelated Turkish neonatal-onset CPS1D patients. Recombinant His-tagged CPS1 was expressed in baculovirus/insect cells and purified. The CPS1 global and partial reactions and protein thermal stability were assayed. Structural modelling was performed to rationalize the p. Val1013del effects.
Results: Wild type (WT) and mutant CPS1 showed comparable levels of protein expression and purity. The mutant p. Val1013del had no significant residual activities and displayed decreased temperature of unfolding than WT (thermofluor assays). CPS1 structural modelling suggested that Val1013 belongs to a hydrophobic β-strand of the carbamate phosphorylation domain close to the predicted carbamate tunnel.
Conclusion: The mutation p. Val1013del inactivates the enzyme but does not render the enzyme grossly unstable or insoluble. The deletion, by shortening the β-strand, may affect carbamate tunnel formation and possibly hamper the connection between both phosphorylation steps.

P-165

Lysinuric protein intolerance: keep hyperammonemia in mind

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Lysinuric protein intolerance (LPI) is an inherited aminoaciduria caused by defective cationic amino acid transport. Hepatosplenomegaly, haematological abnormalities, neurological involvement, hyperammonemia are basic clinical features. Here we report four patients with LPI. Mean age is 8.4 years (6–15.5 years). Two of them are girls. The mean age at the diagnosis was 7.6 years. Hepatosplenomegaly (three patients), hepatomegaly (one patient), growth retardation (one patient) were main clinical findings. Recurrent pulmonary infections were detected in one patient with interstitial lung involvement. This patient had hypogamaglobulinemia and was treated with IVIG. Hyperammonemia was detected in all patients. One of them had serious hyperammonemia episodes. All patients' laboratory investigations revealed high ferritin and high LDH levels. Bone involvement as osteoporosis has been detected all of them. SCL7A7 gene analysis was performed in three patients. The mutations detected were c.1098_1099 insT (p.1367Yfs*17) (novel mutation), homozygote p. L363P and homozygote IVD3+1G, respectively. On follow up (mean 6.8 years), one patient developed secondary hemophagocytic lymphohistiocytosis (HLH). LPI has long been considered a relatively benign urea cycle disease. However, the severe clinical course of this disorder suggests that LPI should be considered as severe multisystem disease.

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Retrospective analysis of 38 patients with neonatal urea cycle disorders: comparison between biochemical parameters at birth and neurological development

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Background: Severe urea cycle disorders lead to hyperammonemic comas during the first few days of life, which may cause subsequent severe brain damage.
Patients & Methods: 38 patients (median 7.4 year old) with neonatal-onset urea cycle deficiencies managed after year 2000. Charts review ofmetabolic parameters and EEGduring the neonatal hyperammonemic coma and of the neurological long-term outcome (IQ, schooling).
Results: Intra-mitochondrial enzyme deficiencies were symptomatic earlier (3.2d vs. 5.2d; p=0.003), had longer hyperammonemia periods (4.2d vs 2.3d, p=0,189), and had a higher rate of mortality (92 % vs 8 %; p<0,001). Deceased patients suffered from a longer period of hyperammonemia (4.7d vs 2.4d, p=0.064). But levels of Ammonemia or glutamine were not correlated to either survival or neurological outcome. Argininosuccinic aciduria was correlated to an impaired neurological development (57 % vs. 28 %; p=0.04). An abnormal neonatal EEG was correlated to high level of ammonemia during the neonatal coma (p=0,001) but not to the long-term neurological outcome.
Conclusion: Intra-mitochondrial urea cycle defects had lower survival rates, whereas an impaired neurological prognosis was associated with argininosuccinic aciduria. Ammonemia, plasma glutamine or EEG during the neonatal coma are not predictive of the long-term neurological outcome.

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Case Report: First reported case of Lysinuric Protein Intolerance in South Africa

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Background: Lysinuric protein intolerance (LPI), an autosomal recessive condition caused by defective cationic amino acid transport in the kidney and intestine, typically presents with the introduction of protein-rich meals at weaning. It is characterised by post-prandial episodes of vomiting, diarrhoea and coma (with hyperammonaemia); failure to thrive, hepatosplenomegaly, muscular hypotonia, progressive osteoporosis and pulmonary alveolar proteinosis. Plasma levels of lysine, arginine and ornithine are decreased while urinary excretion of these amino acids and orotic acid are increased. LPI is caused by mutations of the SLC7A7 cationic amino acid transporter.
Case report: We present the case of a two year old boy, presenting at six months following weaning, with depressed level of consciousness, post

prandial (protein–rich meal) vomiting with hyperammonaemia (highest 340umol/L), failure to thrive, hypotonia and hepatosplenomegaly. Biochemical analysis revealed massive lysinuria at 46398 nmol/mg creatinine (<850), with elevated urine orotic acid, ornithine and citrulline levels; and decreased plasma lysine and ornithine (below reference intervals) levels. Molecular studies of the SLC7A7 gene revealed three homozygous synonymous variants, c.498G>A; c.837 T>C (splice site variant) and c.1866A>G; with a strongly suspected large deletion. This is the first known case of LPI reported in South Africa.

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Cognitive outcome in patients with urea cycle disorders in a center of Northern Italy

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Urea cycle disorders (UCDs) are a heterogeneous group of diseases characterized by hyperammonemic crisis leading to coma, death or gray-white matter injury and cognitive function impairment. Neurological and cognitive involvement is much severe in relation to the earliest onset of disease, the highest levels of ammonia and long-lasting of hyperammonemic crisis. We report the neurological follow-up of 40 patients affected by UCDs. We analyzed plasma aminoacids and ammonia levels, psychometric scales, EEG and neuroimaging. Although they had few episodes of metabolic decompensation and normal values of glutamine levels during follow-up, the early onset patients had a progressive decrease of Z-score-IQ during the time, while the late onset patients were stable (in this groupreduction of Z-score was evident only if brain MRI was abnormal). There was a correlation between epilepsy and poor cognitive outcome in all groups. Argininsuccinate lyase deficient patients, especially those with neonatal onset, had more severe neurogical outcome than the other UCDs.
Conclusions: despite good compliance to therapy and few episodes of hyperammonemia many patients affected by UCDs have cognitive performance lower than 50° centile. Z-score-IQ decreases during time in early onset patients in a statistically significant way, while it is almost constant in late onset patients.

P-169

Recurrent coma episodes during treatment of nephrotic syndrome in a patient with argininosuccinic aciduria

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In a clinically non-affected boy, newborn screening suggested argininosuccinic aciduria (ASL), confirmed biochemically. Protein restriction and supply of essential amino acids and arginine was started. At 11 months, a mild hyperammonemic crisis occurred, treated successfully by detoxification and protein omission. At 25 months, he developed severe proteinuria. Idiopathic NS was diagnosed. Standard steroid therapy was applied. At 28 months, cyclosporine A (CSA) was added. Kidneybiopsy showed focal segmental glomerulosclerosis. WT-1, NPHS1 and HPSH2 mutations were not detected. At 35 months, the boy received a single intravenous steroid pulse during severe nephrotic relapse. He became irritable, aggressive, and lost consciousness for 4 days, while remaining in a stable cardiorespiratory state. Laboratory including plasma ammonia and CSA showed no derangement; C-MRI was normal. Over the next 6 years, several nephrotic relapses occurred and steroid-pulses again led to transiently altered mental state. Renal hypertension developed; kidney function declined. At 92 months, CSA was replaced by mycophenolate mofetil. No further NS relapses occurred and kidney function recovered. He suffers from persistent psychomotor retardation, but never lost earlier acquired abilities. Increased cerebral concentrations of argininosuccinic acid combined with arginine depletion may have led to an increase in free radicals and enhanced steroid toxicity.

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Argininemia patients diagnosed by newborn screening in Portugal

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INTRODUCTION: Argininemia is a rare disorder due to arginase 1 deficiency. Clinically, it differs from other urea cycle defects, since hyperammonemia is episodic and patients are usually diagnosed after the onset of neurological symptoms. Newborn screen by MS-MS allows an early intervention, preventing neurological insults.
AIM: characterize patients wth argininemia diagnosed by neonatal screening in Portugal
RESULTS: Five patients were diagnosed since 2004. At first visit, all patiens were asymptomatic but two had colestasis. Arginine values varied from 105 to 1111 mmol/L. Diagnosis was confirmed by molecular stud and three patients are homozygous for the mutation R21X. Four patients started treatment during the first month of live, one at 4.5 month. All were treated with diet and essential aminoacids and two with sodium benzoate. At the end of first year all patients were on ammonia chelants therapy. No hyperammonemic episodes were reported. Nowadays with ages between 1 and 7 years, one patient still has elevated transaminases. All present a normal growth, nutricional status and normal neurodevelopment evolution.
CONCLUSIONS: Argininemia is still a difficult disease with a not well known prognosis, particularly in patients diagnosed prior neurologic symptoms. Although a short follow up, our patients shows the promising results of newborn screening.

11. Organic acidurias: branched-chain

P-171

Identification of two novel BCKDHB mutations in Korean siblings with maple syrup urine disease showing mild clinical presentation

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Background: Maple syrup urine disease (MSUD) is a disorder that involves the metabolism of branched chain amino acids, resulting from a defect in branched-chain α-keto acid dehydrogenase complex. Mutations have been identified in the BCKDHA, BCKDHB, or DBT genes, which encode each subunit of the BCKDH complex. Although encephalopathy and progressive neurodegeneration are its major manifestations,

the severity of the disease may range from the severe classical type to the mild variant type.
Case report: We report two Korean siblings with the milder variant MSUD, who were diagnosed via a newborn screening test using tandem mass spectrometry and family screening for MSUD, respectively. BCKDHA, BCKDHB, and DBT analysis was performed, and two novel mutations (p. R170C and p. L225V) were identified in BCKDHB.
Conclusion: Although MSUD is a typical metabolic disease with poor prognosis, better outcomes can be expected if early diagnosis and prompt management are provided, particularly for milder forms of the disease.

P-172

Role of cardiac monitoring in patients with Propionic Acidaemia following liver transplantation: a retrospective review

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Background and Objectives: A well recognised complication of propionic acidaemia (PA) is cardiomyopathy (CM), which can be suggested by findings of impaired systolic function (ISF) on echocardiography. In patients receiving liver transplantation (LT) the role for echocardiography post-LT is unclear. This study aimed to review the cardiac findings and management of PA patients in a tertiary referral centre and to determine the role of cardiac follow up in patients with PA post-LT.
Methods: All patients with a diagnosis of PA who received LT were identified and case notes retrospectively reviewed. The diagnosis of ISF suggestive of CM was defined from echocardiography data and any documentation of associated clinical signs of impaired cardiac function.
Results: Five patients (3 male) were identified with a median age at LT of 1.8 yrs (0.75-7 years). 2/5 patients had ISF suggestive of CM. Post-LT 4/5 patients received echocardiography, one patient was receiving regular annual cardiac follow up post-LT. The median follow up was 4.25 years (0–10.5 years).
Discussion: The finding of ISF in one asymptomatic patient post-operatively may highlight the need for regular cardiac follow up. Patients with PA should undergo cardiac assessment including echocardiography, those with impaired systolic function requiring closer monitoring.

P-173

Whole genome expression profiling of 3-methylcrotonyl-CoA carboxylase deficient human skin fibroblasts

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Isolated 3-methylcrotonyl-CoA carboxylase (MCC) deficiency is an autosomal recessive inherited metabolic disease of leucine catabolism presenting with a highly variable phenotype. The clinical, biochemical, and enzymatic characterisation of MCC deficiency are well documented. However, apart from extensive mutation analyses of the MCCC1 and MCCC2 genes encoding 3-methylcrotonyl-CoA carboxylase (EC 6.4.1.4), no other molecular studies have been reported on MCC deficiency. In this study the first whole genome expression profile of immortalised cultured skin fibroblast cells of two clinically affected MCC deficient patients and two normal healthy individuals were generated using Affymetrix®HuExST1.0 arrays. There were 16191 significantly differentially expressed transcript IDs of which 3555 transcript IDs were well annotated and used for downstream functional analyses. The whole genome expression profile showed that both MCCC1 and MCCC2 transcripts were significantly differentially down regulated even though the MCCC2 gene does not have any deleterious mutations. The underlying molecular interactions and functional relationships underscore the complexity of MCC deficiency. The whole genome expression profile of MCCA impaired skin fibroblasts provides the first genetic evidence that MCC deficiency results in mitochondrial dysfunction, disruption of energy homeostasis as well as a considerable number of transcripts associated with the xenobiotic metabolism suggesting an increased demand for detoxification.

P-174

The first case in Asia of 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency (HSD10 disease) without intellectual disability.

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Background: The HSD10 disease (2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency) is a rare inborn error of metabolism. Typical cases of this disease have severe intellectual disability.
Case report: A 6-year-old Japanese boy, who had been well, was presented with severe ketoacidosis following 5-day history of gastroenteritis. Urinary analysis showed elevated excretion of 2-methyl-3-hydroxybutyrate and tiglylglycine. He was, initially, diagnosed with β-ketothiolase (T2) deficiency.
Materials and Methods : His fibroblasts were used for enzyme assays, mutation analysis, immunoblotting, and MitoTracker staining. Results: Enzyme assays showed normal T2 activity and no T2 mutation was found. Instead, a hemizygous c.460G>A (p. A154T) mutation was identified in HSD17B10 gene. This mutation was not found in 258 alleles from Janpanese subjects. The activity of 2M3HBD enzyme was almost deficient. These data confirmed that this patient was affected with HSD10 disease. His fibroblasts also showed punctate and fragmented mitochondrial organization by MitoTracker staining and had relatively low respiratory chain complex IV activity to those of other complexes.
Conclusion: Our patient had a novel mutation in HSD17B10 gene that causes HSD10 disease. Although he had no neurological regression until now, our data suggest mitochondrial dysfunction in his fibroblasts.

P-175

Ammonia levels control during an episode of septic shock in a patient with propionic acidemia

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Carglumic acid (CA), an N-acetylglutamate analog, helps control hyperammonemia secondary to inhibition of N-acetylglutamate synthase by propionic acid.

Objective: To report the experience of CA use to treat a propionic academia patient during a septic shock episode.
Case report: 22-year-old patient diagnosed with neonatal-onset propionic acidemia. Since puberty, the patient had had occasional episodes of hyperammonemia, successfully treated with diet and CA. Current decompensation episode was caused by an intestinal infection with diarrhea, with moderate hyperammonemia (180 mmol/L). CA and diet were used to control the levels of ammonia. While in hospital, the patient developed sepsis due to Klebsiella pneumoniae; Candida parapsilosis was found in the blood, seriously affecting his health condition and causing PICU admission. Treatment with previously initiated CA, which was being decreased (3,500 mg/day), was maintained; blood ammonia levels did no increase despite severe symptoms. Dosage increase was not necessary.
Discussion: This case confirms CA efficacy in preventing increases in blood ammonia, in spite of the occurrence of a serious clinical complication during treatment of a metabolic decompensation. This is the first report on CA efficacy in keeping low ammonia levels in a patient with propionic acidemia who was receiving CA and developed a septic shock.

P-176

Genetic, enzymatic and whole genome expression characterisation of a possible new X-linked associated disease with a metabolite profile indicative of atypical isolated 3-methylcrotonyl-CoA carboxylase deficiency

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Four males of a non-consanguineous family presented with elevated levels of urinary 3-hydroxyisovaleric acid and trace amounts of 3-methylcrotonylglycine acid which usually suggests atypical isolated 3-methylcrotonyl-CoA carboxylase (MCC) deficiency. Metabolic screening of the family suggested an X-linked associated inheritance pattern. In vivo L-leucine loading confirmed a bottleneck in leucine catabolism. The MCC activity in primary skin fibroblast homogenates were>70 % of the controls. Sequencing showed no deleterious mutations in the coding regions of MCCC1 and MCCC2. Whole genome expression profiling of primary skin fibroblasts from two MCC-like patients and two controls using Affymetrix GeneChip® Human Exon ST 1.0 (HuExST1.0) arrays showed 14237 significantly differentially expressed transcripts IDs. Only 1277 transcripts IDs had known annotations and were used for functional analyses. The whole genome expression profile confirmed that both MCC transcripts, MCCC1 and MCCC2, were intact and not significantly differentially expressed. Since the bottleneck in leucine catabolism and atypical MCC metabolic profile could not be explained by impaired MCC, we suspect that these individuals have an X-linked associated defect originating from another locus. Functional analyses revealed aberrant pathways, mitochondrial dysfunction and disruption of energy homeostasis which could contribute at least to some extent to the development of clinical symptoms.

P-177

An assay of methylmalonyl-CoA mutase activity by UPLC/MS/MS in patients with methylmalonic acidemia and carriers

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Background: An assay of the methylmalonyl-CoA mutase has been carried out by measurement of succinyl-CoA generated by reaction of methylmalonyl-CoA with enzyme obtained from patient. We established succinyl-CoA and methylmalonyl-CoA determination by UPLC/MS/MS, and examined the enzyme activity of patients with methylmalonic acidemia and carriers.
Method: Lymphocyte suspension from three patients with methylmalonic acidemia and their parents was lysed by sonic disruption, then mixed with 2.5 mM adenosylcobalamin and 40 μM methylmalonyl-CoA. This solution was incubated for 15 minutes. Succinyl-CoA in these solution was measured by UPLC/MS/MS using Acquity UPLC BEH C18 culumn (2.0 x 150 mm, Waters) and mobile phase (300 mM $HCOONH_4$ and acetonitrile).
Results: Succinyl-CoA eluted at a retention time of about 1.6 minutes and distinctly resolved from methylmalonyl-CoA. Succinyl-CoA was not produced in the reaction mixture from three patients, whereas the activity of methylmalonyl-CoA mutase from their parents was approximately 30–70 %.
Discussion: No succinyl-CoA was generated in the patients with methylmalonyl-CoA mutase deficiency. Enzyme activity of carriers varied from one individual to another. We determined the useful method for the methylmalonyl-CoA mutase activity measurement using UPLC/MS/MS.

P-178

Axonal peripheral neuropathy in patients with propionic acidaemia: a severe side effect of metronidazole treatment

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Introduction: Different guidelines for treatment of propionic acidaemia (PA) are utilized even if there is no full consensus on how to manage patients. Metronidazole is used to reduce production of propionate by gut flora.
Case reports: A teenage patient on good metabolic control, receiving metronidazole courses (500 mg/day, three weeks a month), presented with lower limbs hyporeflexia, paresthesias/hyperaesthesias and loss of deambulation within a few days. Nerve conduction studies displayed a sensory-motor peripheral polyneuropathy of lower limbs. CSF studies, autoimmune and virological profile, and serum vitamins were normal. Suspecting metronidazole-induced neuropathy, the drug was discontinued along with administration of vitamin (B1, B12, E) and coenzyme Q10 supplementation. Skin biopsy showed reduction of intraepidermal small fibers, consistent with diagnosis of axonal neuropathy. Following metronidazole discontinuation, deambulation recovery was observed within six months. Retrospectively, we observed another PA patient receiving the same metronidazole schedule with an identical course but in this case further investigations were not performed because the patient died for sudden metabolic decompensation.
Conclusions: Axonal neuropathy is a side-effect of metronidazole and has never been reported in PA. Our aim is to suggest a more careful use of potentially harmful therapies when their efficacy still need to be fully demonstrated.

P-179

Outcome and clinical spectrum in patients with isovaleric acidemia from India and Pakistan

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Background: Isovaleric acidemia (IVA) is an autosomal recessive disorder caused by deficient isovalery-CoA-dehydrogenase.
Objective: To investigate the outcome and clinical spectrum of IVA patients in India and Pakistan.
Materials and methods: Retrospective analysis of 8 IVA patients over 10 years.
Results: We studied 8 IVA patients (7 from India, 1 from Pakistan). 4 patients presented within one week of life with severe fatal course. All of them were born out of consanguineous marriage. 2 were identified by newborn screening and are doing well under therapy. 2 children presented after 1 year of age, of which one is lost for follow-up and another one is doing well under therapy with some scholastic backwardness. Both of them presented with lethargy, vomiting, and convulsions. Patients with severe form of the disease had low free carnitine (11.67±6.4 μmol/L) and elevated isovalerylcarnitine (6.40±2.92 μmol/L). Patients with milder form had normal free carnitine (27.63±3.33 μmol/L) and elevated isovalerylcarnitine (8.25±5.42 μmol/L).
Discussion: All patients presenting in neonatal period had fatal outcome due to probably late recognition and unavailability of the treatment, especially diet. Children detected by NBS had better outcome. Child presenting late had a better outcome due to milder form despite no dietary therapy.

P-180

10 years follow up of 2 Iranian patients affected of 3-hydroxy 3-methylglutaryl CoA lyase deficiency

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Background: 3-Hydroxy 3-methyl glutaric aciduria is a rare autosomal recessive inborn metabolic disorder due to defect of 3-hydroxy 3-methylglutaryl Co A lyase (3HMGCL), which is required for ketogenesis but also performs the final step in leucine catabolism. Patients present in neonatal or infantile period with severe hypoketotic hypoglycemia, metabolic acidosis, hyperammonemia and liver disease, with Reye like crises. If untreated it is often fatal or may result in permanent neurological damage. It is quite rare with estimated prevalence 1/100,000 live births.
Methods: Retrospective review of two 3HMGCLD cases diagnosed by increased blood level of 3-methyl glutarylcarnitine (C6DC) and 3-hydroxy isovalerylcarnitine (C5OH) by MS/MS or/and their metabolites in urine (2003–2013).
Cases: 1male, 1female, consanguinity: 2/2; both presented with seizure at 3rd day of life, age at diagnosis: 46 days and 18 months respectively, recurrent attacks of hypoketotic hypoglycemia, acidosis and hyperammonemia 2/2, hepatomegaly 1/2, hypotonia 2/2, delayed development 1/2. C6DC 0.57, 0.32 (NL<0.15), C5OH: 4.01, 2.36 (NL<0.8). After 10 years treatment with carnitine, protein and fat restricted diet and fasting avoidance, one with earlier diagnosis by neonatal screening despite three metabolic crises showed normal development and growth, but the other with 7 attacks of metabolic decompensation and recurrent seizures although better in tone, did not develop normally.
Conclusion: Early diagnosis and treatment was important in control of HMG-CoA lyase deficiency.

P-181

The first Japanese case of isolated methylmalonic acidemia caused by cblD defect

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Background: The prevalence of methylmalonic acidemia (MMA) in Japan has been estimated as 1/90,000, based on our neonatal-screening pilot study. In regard to MMA caused by adenosylcobalamin deficiency, only cblA defects were identified. Here, we report the first Japanese case of a cblD-MMA patient.
Case report: A one-year-old boy developed disturbance of consciousness accompanied by severe metabolic acidosis and mild hyperammonemia during noroviral gastroenteritis. Analysis of acute-phase specimens revealed huge excretion of methylmalonate in urine. Total plasma homocysteine concentration was normal. Methylmalonyl CoA mutase activity in lymphocytes measured under coexistence of adenosylcobalamin was not impaired, indicating adenosylcobalamin deficiency. Genomic DNA was extracted from the patient and his parents. Direct sequencing of MMAA,MMAB and MMADHC was carried out. In exon 3 and exon8 of MMADHC, 18 T>A (C6X) and 697_702 insT were found in the patient, respectively. His father showed C6X heterozygously, but his mother showed no mutation.
Discussion: According to previous reports, it is supposed that C6X allele should activate alternative start codon at and contribute to methylcobalamin production, while mRNA of 697_702insT allele should decay by losing stop codon, resulting in isolated MMA in our patient.

P-182

The management of methylmalonic aciduria by NMR spectroscopy

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Methylmalonic aciduria (MMA) comprises a group of diverse inherited disorders causing methylmalonic acid (MMAc) accumulation in body fluids.
Material: We report on 4 Moldavian children diagnosed with MMA (2 newborns, 11mo, 30mo old), last two due to frequent vomiting and coma. Amino acids by liquid chromatography (blood/urine), NMR spectroscopy (urine), tandem MS/MS were used for diagnosis.
Results: In all patients high urinary level of MMAc [1631.4-46614.0 mmol/molCrn] by NMR spectroscopy and slight high blood homocysteine were found. All of them showed positive results for MMA through tandem MS/MS. Two children with late diagnoses were B12 responsive. One of them (11mo) having the lowest urinary MMAc level [1631.4 mmol/molCrea] followed low-protein diet with B_{12} supplementation and achieved urinary MMAc decrease [570 mmol/molCrea] with a good neurologic and health development. Another one had initial MMAc level 20414.3 mmol/molCrea and neurological sequelae required diet including special formula, B_{12} and specific drugs with following significant decreasing of urinary MMAc. Two children with clinical signs from the birth showed severe forms of MMA with very high urinary MMAc required special diet and kept their high MMAc levels long time during treatment.
Conclusion: The neurological development and somatic health in MMA patients is in direct relationship with urinary MMAc level and NMR spectroscopy helps monitoring these patients.

P-183

One single dose of 1 mM 2-methylcitrate is sufficient to generate ammonium accumulation and secondary apoptosis in an in vitro model for brain damage in methylmalonic aciduria

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Background: We previously showed that exposure of 3D rat reaggregated brain cell cultures to repeated application of 2-methylcitrate (2-MCA) every 12 h over three days (DIV11-DIV14) resulted in ammonium accumulation and cell death by apoptosis. The aim of this study was to define the time-course of the observed effects, and to see whether a single dose of 1 mM 2-MCA given at DIV11, corresponding to the neonatal period, leads to the same effects.
Results: Ammonium already increased 3 hours after the first 2-MCA exposure on DIV11, continued to increase until DIV13 and then started to drop. Immunoblotting and immunofluorescence with cell death specific markers (caspase-3, alpha-fodrin, LC3) revealed significant increase of apoptosis at DIV14. Ammonium increase could not be prevented by application of the caspase inhibitor Z-VAD. Biochemical and immunohistological findings were similar after a single dose of 1 mM 2-MCA compared to the already described effects observed after repeated metabolite administration.
Conclusions: Ammonium increased rapidly after 2-MCA administration and could not be inhibited by administration of Z-VAD. These observations suggest that ammonium increase is rather the cause and not a consequence of apoptosis. A single dose of 1 mM 2-MCA was sufficient to cause the same deleterious effects in this in vitro model.

P-184

Secondary hemophagocytosis in propionic acidemia

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Propionic acidemia is one of the intoxication type organic acidemias, which often present in the neonatal period with lethargy, feeding difficulties, hypotonia, vomiting and coma if not identified and treated appropriately. Patients with propionic acidemia can decompensate during periods of increased metabolic demand. Hemophagocytic lymphohistiocytosis (HLH) is a life threatening disorder that can rapidly deteriorate and lead to multiple organ failure and death. It can be classified as primary (familial) or secondary (acquired). Secondary HLH is associated with infections especially viral, malignant disorders, inborn errors of metabolism such as multiple sulphatase deficiency, lysinuric protein intolerance, biotinidase deficiency, gaucher disease and galactosialidosis. In this report, we present a case of a 3 year old boy with propionic acidemia who experienced secondary HLH during his metabolic attack successfully treated with intravenous gammaglobulin, broad spectrum antibiotics and dexamethasone therapy. Now he is on regular outpatient clinic visits for propionic acidemia and has not relapsed during follow up. In conclusion this experience suggests that steroid and immune globulin could be considered as a first line therapy in patients with secondary HLH associated by metabolic diseases. Of course, awareness of the clinical symptoms and diagnostic criteria for hemophagocytic syndrome is crucial to start timely life saving therapy.

P-185

Isobutyryl-CoA dehydrogenase deficiency diagnosed following an episode of ketotic hypoglycaemia

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Background: Isobutyryl-CoA Dehydrogenase Deficiency (IBD) is an inherited disorder of valine metabolism caused by mutations in ACAD8. Most reported patients have been diagnosed through newborn screening programmes due to elevated C4-carnitine levels and appear clinically asymptomatic. A non-screened patient had dilated cardiomyopathy and anaemia. We report a child with IBD, diagnosed following investigation for an episode of ketotic hypoglycaemia.
Patient: A 13-month-old girl with rotavirus-induced gastroenteritis presented with hypoglycaemic encephalopathy (blood glucose 1.9 mmol/L). Metabolic investigations demonstrated an appropriate ketotic response (free fatty acids 2594 μmol/l, 3-hydroxybutyrate 3415 μmol/l), mildly elevated lactate (3.4 mmol/l), increased C4-carnitine on blood spot and plasma acylcarnitine analysis and other metabolic abnormalities secondary to ketosis. After recovery, C4-carnitine remained increased and isobutyrylglycine was detected on urine organic acid analysis. IBD was confirmed by finding a homozygous c.845C>T substitution in ACAD8. The patient was given a glucose polymer emergency regimen and has had no further episodes of hypoglycaemia. Neither cardiomyopathy nor anaemia have developed during 8 yrs of follow-up.
Conclusions: The clinical significance of IBD is uncertain. We cannot be certain if IBD contributed to hypoglycaemia in this patient. Patients should be followed-up carefully and glucose polymer emergency regimens may be indicated if episodes of hypoglycaemia occur.

P-186

Late-onset propionic acidaemia: cholestasis as only wake-up call in the first 3 months of life

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Male, born at 38 weeks by elective CS, from consanguineous Moroccan parents. The mother, HIV+, assumed antiretroviral therapy during pregnancy. Appropriate auxological parameters at birth, Apgar score 5/1' and 8/5'. Oral prophylaxis with zidovudine was started. During the first day, he presented tension pneumothorax and, subsequently, a congenital lobar overinflation (CLO) was diagnosed. At the age of 8 days, the baby was subjected to left upper lobectomy, without any complication. Afterwards, the child presented hypertransaminasaemia and hyperammonaemia, attributed at first to ZDV toxicity. High blood levels of glycine and C3 with low C0 were detected, but urine organic acids were not significantly altered. Hereafter, blood values normalized, except for increasing indices of cholestasis. At the age of 3 months, the child presented lack of appetite, vomiting and important loss of weight associated to pancytopenia. Moreover, he presented persistent axial hypotonia and critical episodes characterized by left clonus with concomitant hyperammonaemia and hyperlactacidaemia. The dosage of plasma aminoacids, acylcarnitine and urine organic acids showed typical alterations for propionic acidaemia and the brain MRI was characteristic for organic acidosis, as

well. The diagnosed was confirmed by molecular analysis. Treatment with biotin and carnitine associated to hypoproteic diet was started, successfully.

P-187

A patient with evidence of methylmalonyl-coA epimerase (MCE) deficiency, presenting with severe metabolic acidosis and biochemical profiles initially interpreted as propionic acidemia

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Background: Methylmalonyl-coA epimerase (MCE) follows proprionyl-coA carboxylase, and precedes methylmalonyl-coA mutase in the pathway converting propionyl-coA to succinyl-coA. MCE deficiency is described in five patients, one presenting with metabolic acidosis, others with nonspecific neurological symptoms or asymptomatic. Clinical significance and biochemical characteristics of this rare condition remain incompletely defined.
Case report: A 5-year-old girl presented acutely with vomiting, dehydration, confusion, severe metabolic acidosis and mild hyperammonemia. Organic acid profiles were dominated by increased ketones and 3-hydroxypropionate, with moderately elevated methylcitrate and propionylglycine. Acylcarnitines showed marked C3 elevation with normal C4DC. Propionic acidemia was suspected, but excluded by enzyme, DNA and RNA analysis. It was subsequently noted that methylmalonic acid was mildly but persistently elevated in urine, and clearly elevated in plasma and CSF. Cobalamin and homocysteine were normal. The overall biochemical profile prompted consideration of MCE deficiency. Fibroblasts showed moderately decreased propionate incorporation. Results of complementation analysis were compatible with assignment to the MCEE group. A heterozygous p. R47X mutation in MCEE was identified. RNA studies, seeking a second mutation, are planned.
Conclusion: We present a putative new case of MCE deficiency, contributing to clinical and biochemical characterisation of this disorder and highlighting potential causes of diagnostic confusion.

P-188

Efficient generation and characterization of iPS cells from methylmalonic aciduria cblB type fibroblasts

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Methylmalonic aciduria (MMA) cblB type is caused by mutations in the MMAB gene which encodes ATP:cob (I) alamin adenosyltransferase (ATR) enzyme. We recently described mitochondrial dysfunction in patients-derived fibroblasts and identified a potential chaperone that in vitro stabilizes p. Ile96Thr mutant ATR. Due to the absence of suitable cellular models of MMA disease, the goal of this work was the generation of induced pluripotent stem cells (iPSCs) and the later differentiation into neurons for modeling MMA disease and investigate the efficacy of pharmacological chaperones. Fibroblasts from one pair of cblB siblings with p. Ile96Thr/p. Ser174fs mutations were reprogrammed using CytoTune Sendai vectors which include the four Yamanaka factors (Oct4, Sox2, Klf4 and c-Myc). A total of two iPSC lines of each patient were generated and presented the hallmarks of iPS cells, including: (1) typical iPSC-like morphology and growth characteristics, (2) positive staining for alkaline phosphatase activity, (3) expression of pluripotency-associated markers (OCT4, NANOG and SOX2) and surface markers (SSEA3, SSEA4, TRA1-60, and TRA1-81) and (4) demethylation of the OCT4 and NANOG promoters. Our findings provide a basis for an experimental neuronal MMA model allowing the investigation of the cellular pathogenesis and the therapeutic application of the described pharmacological chaperone.

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A rapid and irreversible optic neuropathy in methylmalonic aciduria

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Background: Patients with MMA may develop late-onset optic neuropathy (ON) with visual dysfunction. An effective treatment with CoQ10 and Vit E has been recently reported.
Case report: A 14-year-old girl, affected by a severe form of MMA (mut0/mut0), caused by a compound heterozygosity: c.330 T>G; c.630delA metilmalonic aciduria (MMA) reported an unexplained bilateral acute visual loss. Her past medical history was consistent for subnormal psychomotor development. No kidney involvement was present at this age. At examination, horizontal nystagmus, mydriatic and poorly reagent pupils were present. On neuro-ophtalmic examination, VA (visual acuity) with myopic correction was 30/300 in both eyes. On ophtalmoscopic examination, bilateral temporal optic nerve pallor with normal cup-to-disc ratio was noted. Visual Evocated Potentials and Electroretinography showed an altered retinal function and abnormal retino-cortical transmission. A treatment was started with CoQ10 (200 mg/day) and vitamin E (200 mg/day). After theree months of therapy, the neuro-ophtalmic examination showed a further deterioration with VA of 10/300 in both eyes.
Conclusion: This case suggests that ON can develop despite good metabolic control and rapidly precipitate causing a severe loss of VA. We would recommend an early neuroprotective therapy with CoQ10 and VitE in patients with MMA to attenuate this complication.

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Severe visual loss regressive after intensive hemodialysis in a 18-y-old methylmalonic aciduria patient

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Background: Optic neuropathy has been previously reported in several methylmalonic aciduria (MMA) patients, but its physiopathology and treatement remained unclear.
Case report: A 18-y-old girl with severe B12-unresponsive MMA diagnosed at 5-m-old, was admitted for a subacute bilateral visual loss without acute metabolic crisis. She had a chronic impairment of renal function (estimated glomerular filtration rate 44 ml/min/1.73 m^2) with a project of renal transplantation. Ophtamologic exam confirmed severe visual loss (RE-20/100-P4, LE-20/200-P8) and visual evoked potentials suggested an optic neuropathy. Brain MRI was unspecific. Laboratory testing

disclosed elevated MMA in plasma (2296 mmol/L) and urine (8137 mmol/mmol creat) and increased lactate level in plasma (3,7 mmol/L) and CSF (7,22 mmol/L), suggesting that optic neuropathy was related to oxydative-phosphorylation defect due to MMA toxicity, as described in renal failure.
Results: With an intensive hemodialysis (6 days/week during 3 weeks, followed by 3 times per week), plasma MMA level rapidly decreased (ranging between 23 and 1015 mmol/L after and before dialysis). At day 21, CSF lactate level was reduced (3,8 mmol/L). and visual function progressively improved (RE-20/50-P2, LE-20/63-P2) and was normalized after 5 months.
Conclusion: Optic neuropathy was suspected to be due to chronic MMA toxicity and may be reversed by intensive hemodiaysis.

12. Organic acidurias: others

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Is glutaric aciduria type 3 really a benign disorder?

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Glutatic aciduria type 3 (GA3) was first described 23 years ago. GA3 has only been reported in a few individuals and data from over 20 years follow up led to the presumption that GA3 was a benign disorder. Our patient was the first ever case of GA3 to be described. She was diagnosed at 1 year old after being investigated for failure to thrive. During childhood riboflavin was commenced and no episodes of metabolic decompensation were documented. Subsequently the patient was lost to follow up. However, at the age of 24 years, following a brief illness she was admitted to the ITU with a GCS of 3 requiring ventilation. Confounding conditions including beta-thalassemia and coeliac disease did not account for her poor clinical condition. MRI demonstrated bilateral thalamic changes possibly secondary to metabolic disease and urine organic acid analysis demonstrated significant glutaric aciduria without increased acylglyines. Riboflavin was re-commenced followed by a rapid clinical recovery. The mechanism for riboflavin responsiveness is unclear. A further metabolic decompensation occurred 12 months later again requiring ITU admission.
Conclusion: We present a case of GA3 requiring ITU support secondary to metabolic decomposition following brief illness, suggesting that GA3 may not be a benign condition.

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Phenotypic and genotypic spectrum of Turkish patients with isovaleric acidemia

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Background and objectives: Isovaleric acidemia (IVA) is an autosomal recessive inborn error of leucine metabolism, caused by genetic alterations in the human mitochondrial enzyme isovaleryl-CoA dehydrogenase (IVD), which results in the accumulation of isovaleryl-CoA derivatives.
Patients and Methods: In this study, 27 patients with IVA were screened to investigate the genetic basis of IVD gene mutations and genotype-phenotype correlations in Turkish patients.
Results: We identified 9 novel and 6 previously reported pathogenic mutations. Two of the novel mutations created a premature stop codon (c.145delC, c.506_507insT), six were altering the coding sequences (p. E85Q, p. M147V, p. A268V, p. I287M, p. G346D, p. R382W) and one was affecting the consensus of the splice site (c.234+3G>C). No genotype-phenotype correlation has been established in our group of patients. Mortality rate was 10 % and were seen in acute neonatal form patients. Mild mental retardation was detected nearly half of the patients. It is not related to clinical subtype or genotype. It seems to be related with late diagnosis and treatment.
Conclusion: Early diagnosis and treatment are important to prevent complications. This study is the first comprehensive report from Turkey related to IVA genetics and provides information about heterogenious mutation spectrum, alongside with high number of disease causing novel mutations.

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Glutaric aciduria type 1 in a 23 year old mother while newborn screening revealed carnitine deficiency in her baby

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Glutaric aciduria type 1 (GA-1) is an autosomal recessive disorder caused by deficiency of glutaryl-coenzyme A dehydrogenase (GCDH). GCDH gene mutations lead to defective enzyme activity and during a catabolic state like infection, fever or dehydration illness clinically manifests. Most patients have their first symptoms during infancy or childhood, but some have a less severe form of the disease and some may even remain asymptomatic throughout their lives. A 23 year old female and her newborn child were directed to our clinic in order to investigate for metabolic disorders for the recent diagnosis of carnitine deficiency in her newborn child during neonatal screening. Mother was the sixth child of a consanguineous she did not have any complaints or symptoms. Her physical examination and neurological examination was normal. Plasma acylcarnitine analysis revealed elevated plasma glutarylcarnitine (C5DC) levels (0,59 μM/L) and low free carnitine levels (5,36 μM/L) and urine organic acid analysis revealed elevated levels of 3-hydroxyglutaric acid and glutaric acid. Magnetic resonance imaging demonstrated common, bilateral and symmetrical signal changes in the periventricular deep white matter, dentate nuclei and subthalamic nucleus. Glutaric aciduria type 1 was considered as the diagnosis and a homozygous mutation was defined R88H (c.263G>A).

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Unusual presentation of glutaric aciduria Type 1 (GA1) in a Family

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Cases: First patient was a 3 years and 3 months-old girl. Her developmental milestones were normal. She had frequent falls since the beginning of walking at 12 months. She had a wide based gait, mild ataxia and extensor plantar reflexes. Her head circumference was 48 cm. Tandem MS and urine organic acid (UOA) analyses performed in two reference laboratory were normal at presentation, and Brain MRI were normal. The patient and her aunt (7 years old) with similar clinical findings were evaluated by direct DNA sequencing for GCDH gene. Repeated analysis revealed borderline elevation of plasma C5DC and urine 3OH glutaric acid. Mutation analysis for GCDH gene showed homozygous c.1249C> T;p. His417Tyr mutation in both patients.
Conclusion: Glutaric aciduria type 1 may present without classical clinical, biochemical, and brain MRI signs. Severity of clinical and metabolic signs are not always correlated. We suggest repeated metabolic work-up and genetic analysis when there is clinical suspicion. The study was supported by TÜBITAK (Project No: 111S217)

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Diagnosis and molecular study in two novel cases with D-2-hydroxyglutaric aciduria (DHGA)

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The DHGAs are rare neurometabolic disorders characterized by isolated accumulation of D-2-hydroxyglutarate (D2HG) in body fluids. Two main different types have been documented: D2HGA type I caused by loss-of-function mutations in the D2HGDH gene, D2HGA type II caused by specific gain-of-function mutations in the IDH2 gene. We report two patients with D2HGA type I and II, respectively. Pt1 presented at 2,5 m with cyanotic crisis, hypotonia, facial dysmorphism, and vesicoureteral reflux. Cerebral MRI showed delayed mielinization and cysts. Urinary 2HG and 2HGlactone were highly elevated (2386 and 945 mmol/mol creat, respectively). Chiral differentiation performed with HPLC-MS/MS showed that 2HG was mainly (>99 %) D2HG. Mutation analysis revealed two novel mutations in heterozygous fashion: p. His229Tyr and a genomic rearrangement of 3,48 Mb including the D2HGDH gene. Pt2 presented at 5 m with developmental delay, hypotonia and mild facial dysmorphism. Cerebral MRI showed white matter abnormalities. At 17 m, urinary 2HG and 2HGlactone were highly elevated (4003 and 431 mmol/mol creat, respectively). D2HG was also confirmed by HPLC-MS/MS. Mutation analysis revealed the previously described de novo gain-of- function mutation p. Arg140Gln in IDH2 gene in one chromosome.
Conclusion: None of the patients have epilepsy or cardiomyopathy and both are now on therapeutic trial with riboflavin.

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Lysine overload provokes protein oxidative damage and reduction of antioxidant defenses in brain of infant glutaryl-CoA dehydrogenase deficient mice

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We evaluated the antioxidant defense system and protein oxidative damage in brain and liver of 15-day-old GCDH deficient knock out ($Gcdh^{-/-}$) mice following an intraperitoneal lysine (Lys) injection (8 μmol/g). We determined reduced glutathione (GSH) concentrations, sulfhydryl content, carbonyl formation and the activities of the antioxidant enzymes glutathione peroxidase, superoxide dismutase (SOD), catalase (CAT) and glutathione reductase, as well a 2',7'-dichlorofluorescein (DCFH) oxidation in brain and liver of these animals. The parameters altered in $Gcdh^{-/-}$ compared to wild type ($Gcdh^{+/+}$) mice were a reduction of liver GSH concentrations and of brain sulfhydryl content. Lys injection provoked a decrease of GSH concentration in the brain and sulfhydryl content in the liver, as well as an increase of carbonyl formation in brain and liver of $Gcdh^{-/-}$ mice. Lys also provoked a decrease of all antioxidant enzyme activities evaluated in the brain and an augment of the activities of SOD and CAT in the liver of $Gcdh^{-/-}$ mice. Finally, DCFH oxidation was caused by Lys in both tissues. It is concluded that Lys overload compromises the brain antioxidant defenses and induces protein oxidation probably secondary to reactive species generation in infant $Gcdh^{-/-}$ mice. Financial support: CNPq, HCPA/FIPE

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ACY1 deficiency presenting with isolated mild intellectual disability

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Background: Aminoacylase 1 (ACY1) deficiency is a rare inborn error of metabolism presenting with heterogeneous neurological symptoms such as psychomotor delay, seizures, intellectual disability, autistic features, and characterized by increased urinary excretion of N-acetylated amino acids.
Case report: A 10 year-old-girl presenting with mild intellectual disability was admitted in our Department for language and learning disabilities evaluation. The metabolic work-up, with the exception of organic acids analysis, was normal as well as the brain Magnetic Resonance Imaging (MRI) with proton spectroscopy, awake and sleep EEG and fundus oculi evaluations. Organic acids analysis disclosed the characteristic pattern of ACY1 deficiency, with the presence of numerous peaks corresponding to N-acetylated amino acids. Sequencing of the coding region of ACY1 revealed compound heterozygosity for the missense mutation c. 699A>C (p. Glu233Asp) in exon 10 and the c.574_575insG insertion in exon 8. The enzyme activity in the patient fibroblasts was undetectable, while in the controls cells (n=7) it was 0.91+ 0.30 nmol/mg protein/min (mean+SD).
Conclusions: Our patient expands the phenotypic spectrum of the ACY1 deficiency and the number of mutations in the responsible gene and stresses the importance to perform metabolic workup in patients with mild and unspecific developmental delay.

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Disruption of redox homeostasis caused by in vivo intracerebellar administration of L-2-hydroxyglutaric acid to young rats

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L-2-hydroxyglutaric aciduria is an inborn error of metabolism biochemically characterized by tissue accumulation and elevated urinary excretion of L-2-hydroxyglutaric acid (L-2-HG). Affected patients usually present neurological symptoms. However, the mechanisms of tissue damage in this disorder are poorly known. In the present work, we evaluated the effects of an acute intracerebellar injection of L-2-HG on parameters of oxidative stress in cerebellum of young rats. Thirty-day-old Wistar rats received a single intracerebellar injection of L-2-HG or NaCl (2,5 μmol) and sacrificed 30 min after the injection. Homogenates of cerebellum were used to assess thiobarbituric acid-reactive substances (TBA-RS, lipid peroxidation), carbonyl formation (protein oxidative damage), nitrate plus nitrite content (reactive nitrogen species), reduced glutathione (GSH) levels and the activities of the antioxidant enzymes glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT). L-2-HG induced lipid peroxidation (TBA-RS increase) and protein oxidation (increased carbonyl formation). L-2-HG also decreased GSH levels, the most important brain antioxidant, and the activities of GPx and GR, but did not alter SOD and CAT activities and nitrate plus nitrite content. Our results suggest that disruption of redox homeostasis induced by L-2-HG administration may be involved in the pathophysiology of the cerebellar injury observed in LHGA.

P-199

Astrocytes pretreated with lysine or glutaric acid provoke neurotoxic effects in striatum and cerebral cortex from a Gcdh−/− mouse model

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Glutaric acidemia I (GAI) is a neurometabolic disease characterized by brain accumulation of glutaric acid (GA) and related metabolites and acute degeneration of striatal neurons through still unknown mechanisms. We investigated whether astrocytes from the Gcdh−/− mice submitted to lysine (Lys) or GA overload may affect neuronal survival in a degree that could explain at least in part the typical neurodegeneration in GAI. Primary astrocyte cultures from Gcdh−/− and wild type (WT) mice were pre-treated with 10 mM Lys or 5 mM GA. Then striatal, cortical or hippocampal neurons were co-cultured on top of astrocytes. Isolated neuronal cultures were also treated with Lys or GA. Neither Lys nor GA affected morphology or survival of isolated neurons from all cerebral structures of WT mice, but Lys moderately decreased neuronal viability in striatum from $Gcdh^{-/-}$ mice. Furthermore, increased proliferation and induction of oxidative stress were observed in Lys- and GA-pre-treated $Gcdh^{-/-}$ and WT astrocytes and these astrocytes caused significant decreases in the survival of especially striatal neurons from $Gcdh^{-/-}$ as compared to WT cells. These results indicate that Lys and GA induce astrocytes to cause marked striatal neuronal death that may participate in the onset and progression of GAI neurodegeneration.

P-200

Ethylmalonic acid induces permeability transition in isolated brain mitochondria

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Short chain acyl-CoA dehydrogenase deficiency and ethylmalonic encephalopathy are metabolic disorders characterized by accumulation of ethylmalonic acid (EMA) in tissues and biological fluids of the affected patients. Clinically, they frequently present neurological abnormalities whose pathomechanisms are still obscure. We evaluated the in vitro and ex vivo effects of EMA in the presence of Ca^{2+} on mitochondrial homeostasis in succinate-supported brain mitochondria from adolescent rats. We verified that various parameters of mitochondrial homeostasis were disturbed only when EMA was associated with exogenous Ca^{2+}. Thus, EMA plus Ca^{2+} dissipated membrane potential and provoked mitochondrial swelling, as well as impaired mitochondrial Ca^{2+} retention capacity and decreased NAD (P) H matrix content. All effects were prevented by the mitochondrial Ca^{2+} uptake inhibitor ruthenium red and the mitochondrial permeability transition inhibitors cyclosporine A (CsA) and ADP. Furthermore, mitochondria isolated from rat striatum after intrastriatal administration of EMA were more susceptible to Ca^{2+}-induced swelling, which was fully prevented by CsA/ADP. Finally, EMA significantly decreased striatal slice viability, which was attenuated by CsA. It is therefore presumed that EMA compromises brain mitochondrial homeostasis in the presence of Ca^{2+} that may explain at least in part the neurologic alterations presented by patients in which this organic acid accumulates.

P-201

Mild disruption of bioenergetics associated with marked histological alterations in striatum of glutaryl-CoA dehydrogenase deficient mice submitted to a lysine overload

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Bioenergetics dysfunction has been postulated as an important pathomechanism of brain damage in glutaric aciduria type I, but this is still under debate. We investigated bioenergetics parameters in mitochondrial preparations from cerebral cortex and striatum of 30-day-old glutaryl-CoA dehydrogenase deficient ($Gcdh^{-/-}$) and wild type mice fed a normal or high lysine (4.7 % Lys) chow for 60 hours. Brain histological analyses were also performed. A moderate reduction of citrate synthase and isocitrate dehydrogenase activities and a mild increase of lactate release were observed in striatum from $Gcdh^{-/-}$ animals submitted to a high Lys chow. In contrast, respiration and $\Delta\Psi m$ were not affected. Histological analyses revealed intense vacuolation in cerebral cortex of 60 and 90-day-old $Gcdh^{-/-}$ mice fed a baseline chow and in striatum of 90-day-old Gcdh−/− mice submitted to Lys overload for 30 days. Our data demonstrate mild bioenergetics impairment in striatum from adolescent $Gcdh^{-/-}$ mice under a short exposition to a high Lys chow and important histological alterations in cerebral cortex from adult $Gcdh^{-/-}$ mice under a basal chow and in the striatum of these animals exposed to Lys overload for a long period.

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Routine use of a secondary ion transition to facilitate identification of glutarylcarnitine in plasma and bloodspot acylcarnitine profiling by tandem mass spectrometry

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Objectives: To improve the identification of glutarylcarnitine at low concentrations in routine profiling of acylcarnitines by LC-MS/MS. This is important for detecting low-excretor variants of glutaryl-CoA dedrogenase deficiency (GA-1) and in multiple acyl-CoA dehydrogenase deficiency (MADD), but can be problematic due to isobaric interferences.
Method: Alongside flow-profile scanning of acylcarnitine butyl-esters (precursor ions of m/z 85) by electrospray ionisation tandem mass spectrometry, multiple reaction monitoring of glutarylcarnitine (m/z 388–85 and m/z 388–115, the latter specific to glutarylcarnitine) and octanoylcarnitine-d3 (m/z 347–85, as internal standard) was employed.
Results: The 99th centile from a selectively screened population was chosen as the cut-off for the m/z 388–115 transition. Plasma and bloodspot specimens from patients with confirmed GA-1 or MADD, QC materials spiked with glutarylcarnitine, urine specimens and blood collected post-mortem, were reliably found to have increased glutarylcarnitine. The number of specimens requiring further investigation (those with glutarylcarnitine assumed to be falsely increased by 3-hydroxydecanoylcarnitine, which shares the m/z 388–85 transition) was significantly reduced.
Conclusion: Analysing a secondary ion transition specific for glutarylcarnitine during routine acylcarnitine profiling has increased confidence in our interpretation by reducing the number of false elevations and improving the identification of low concentrations of glutarylcarnitine.

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Molecular analysis of Cypriot patients with glutaric aciduria type I: Identification of two novel mutations

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Background: Glutaric aciduria type I (GAI) is an autosomal recessive metabolic disorder caused by a deficiency of glutaryl-CoA dehydrogenase, an enzyme involved in the catabolic pathway of lysine, hydroxylysine and tryptophan. More than 100 disease causing mutations have been identified so far and certain mutations show predominance in specific populations.
Objectives: The purpose of this study was to identify the mutations in the glutaryl-CoA dehydrogenase (GCDH) gene in Cypriot patients with GAI.
Methods: Molecular analysis of the GCDH gene was performed by direct sequencing of the patients' genomic DNA. In silico tools were applied to predict the effect of the novel variants on the structure and function of the protein.
Results: All disease alleles were characterized (mutation detection rate 100 %). Five missense mutations were identified: c.192G>T (p.Glu64Asp) and c.803G>T (p.Gly268Val), which are novel, and three previously described mutations, c.1123 T>C (p.Cys375Arg), c.1204C>T (p.Arg402Trp) and c.1286C>T (p.Thr429Met).
Conclusions: Marked genetic heterogeneity was observed in Cypriot patients with GAI. Two novel mutations, p. Glu64Asp and p.Gly268Val, account for the majority of the disease alleles (76.5 %) and are postulated to have arisen through founder effects. Identification of the GAI mutations in the Cypriot population has facilitated carrier detection.

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Glutaric acidemia type 3: is there a distinctive phenotype?

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Glutaric acidemia (GA) type 3 is a rare disorder, with no distinctive phenotype. We present two previously unreported children. The first is a 10 months old girl born to French-Canadian consanguineous parents, who presented with cyclic vomiting, gross motor delay and axial hypotonia. Physical exam revealed macrocephaly with frontal bossing. MRI of the brain showed delayed myelination and axial CSF enlargement. Plasma acylcarnitine and amino acids were normal. Subsequent metabolic work-up showed increased glutaric acid on urine organic acid analysis (UOA). Sequence analysis of the C7orf10 gene revealed homozygosity for a known pathogenic sequence variant (c.1006C>T: p. Arg336Trp), consistent with GA type 3. The second child presented at age 22 months with global developmental delay and recurrent admissions for lethargy and ketonuria without hypoglycemia. His OFC was 75th centile, weight and height 25-50th centile. MRI showed nonspecific periventricular white matter changes. Plasma acylcarnitine and amino acids were normal. UOA showed isolated elevation of glutaric acid. C7orf10 sequencing showed compound heterozygosity in trans of c.715G>A: p. Val239Ile and c.895C>T: Arg299Trp. Our cases demonstrate the nonspecific features of GA type 3 and the need to workup children with delay with urine organic acids and detailed investigation if isolated glutaric aciduria is found.

P-205

Glutaric aciduria type 1, before and after expanded newborn screening: the experience of a Portuguese Metabolic Diseases Unit

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Glutaric aciduria type 1 is a cerebral organic acid disorder caused by glutaryl-CoA dehydrogenase deficiency. Early detection by Expanded Newborn Screening Program (NBS) combined with guidelines for treatment and follow-up, have significantly improved neurological outcome.
Methods: Retrospective cohort of GA-1 patients followed in a Portuguese Metabolic Diseases Centre, from 1979 to 2014:clinical, biochemical and molecular data was analysed.
Results: Sixteen patients (12male, 4female) were included:9 diagnosed by NBS. Diagnostic median age: 5y [10 m-18y] in symptomatic group (SG) and 11d [6d-15d] in NBS group (NBSG). Metabolic decompensation occurred in 8/16 patients (7/7SG and 1/9NBSG) at median age of 10 m [6 m-12 m]. With a median follow-up of 5y [2 m-34y], severe phenotype was observed in 2/7SG and 1/9NBSG. Occurrence of neurological adverse outcomes was on average reduced by 78 % (RR 0.20-0.25) in NBSG. Molecular analysis revealed 8/14 homozygosity for R402W mutation. Regarding excretion of GA: 6/7SG were high excretors vs. 8/9NBSG. Levels of lysine in plasma showed a direct correlation with urinary excretion of GA.We found no correlation between GA (plasma and urine) and clinical outcome.
Conclusion: In our cohort, neither molecular nor biochemical phenotype seems to predict overall neurological outcome. Innovative models mimicking GA-1 metabolic derangement are required for a better understanding of neuropathogenesis and outcome biomarkers research. NBS coupled with prompt and proper treatment are effective strategies in GA-1 complications prevention.

P-206

Macrocephaly, slowly progressive dystonia and history of febrile seizures in siblings with L-2-hydroxy-glutaric aciduria

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Background: L-2-hydroxy-glutaric aciduria (L-2-HGA) is a rare neurometabolic disorder. Since the first description of L-2-HGA in 1980, less than 100 cases have been reported. This disorder is characterized by slow progressive neurological dysfunction with cerebellar ataxia, pyramidal and extrapyramidal signs, intellectual decline and seizures.
Case report: The boy was evaluated at 10 years of age for ataxia, macrocephaly and dystonia in our institution. Development during the first year of life was normal. At the age of 2 years, he presented with two episodes of typical febrile seizures. His adult sister also had history of febrile seizures and similar clinical picture of slowly progressive dystonia associated with macrocephaly. In both patients routine laboratory findings were unremarkable while brain MRI indicated leukodystrophy. Urinary organic acids analysis by using gas chromatography with mass spectrometry (GC/MS) revealed markedly increased excretion of L-2-hydroxyglutarate in both patients (538.16 mmol/mol creatinine and 2697.2 mmol/mol creatinine, respectively; normal range<52), indicating deficiency of L-2-hydroxy-glutarate dehydrogenase. Molecular analysis in L2HGDH gene revealed the same homozygous mutation c530_533delinsATT in both siblings.
Conclusion: Urine organic acids analysis by GC/MS is reliable test to diagnose L-2-HGA aciduria and should be performed in patients with cerebellar signs, macrocephaly and MRI findings suggestive of leukodystrophy.

P-207

Qualitative urinary organic acid analysis for detection of organic acidurias in high-risk population - a colombian experience

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Background/objectives: In Colombia, in our experience, organic acidurias (OA) are the most common group of inborn errors of metabolism (IEM) diagnosed. In our context, OA are detected after clinical onset using qualitative urinary organic acids analysis (qUOAA).
Aim: To present our experience regarding OA diagnosed and some limitations of qUOAA interpretation in high-risk population. Materials: Retrospective analysis of samples submitted for qUOAA between 2011 and 2013 to the Institute for the Study of Inborn Errors of Metabolism.
Results: Near to 1000 samples were analyzed. 17 % showed abnormal profiles, from which 15 % were confirmed as IEM including OA, urea cycle disorders and mitochondrial diseases. The most frequent OA was glutaric aciduria type I (GAI, 28 %). In 11 % of samples the urine profile could not be interpreted due to presence of overlapping drug metabolites.
Discussion/Conclusion: High rate of non-conclusive abnormal profiles may result mainly from lack of clinical information from patients making the profile interpretation difficult, since medication, diet, and clinical condition acquire special importance in high-risk population. It is outstanding that worldwide frequent OA were not detected. This could be related to late diagnoses that miss neonatal fatal presentations. Furthermore, the most frequent diagnosis is a chronic-progressive neurological disease (GAI).

P-208

Differential immunolocalization of glutaryl-CoA dehydrogenase (GCDH) in embryonic and adult rats

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Glutaryl-CoA-dehydrogenase (GCDH), a mitochondrial matrix protein, is the key enzyme of the catabolic pathway of tryptophan, lysine and hydroxylysine. Deficient GCDH activity leads to glutaric aciduria type I (GA-I). Little is known so far about the differential expression of GCDH during embryonic development and in adulthood. Here, we studied the localization of GCDH by immunofluorescence microscopy in adult and embryonic rats at E13, E15 and E17. In adult rat, we observed widespread neuronal expression in the brain with the strongest signal in cerebral cortex and Purkinje cells. Strong GCDH expression was also observed in liver and intestinal mucosa and with lower intensity in muscles, convoluted renal tubules and renal collecting tubes. In embryos, brain was the major expressing organ followed by spinal cord, dorsal ganglions and peripheral axonal bundles. A widespread expression of GCDH was found in peripheral organs of all studied embryonic stages. At E17, a strikingly strong expression was found in intestinal mucosa. Our results suggest an important function for GCDH during embryonic brain development. Its physiological role and pathophysiological implications in kidney and intestinal muscosa still remain to be determined. They might explain the low excretor phenotype or the frequent gastrointestinal problems in patients with GA-I.

P-209

Combined D2-/L2-hydroxyglutaric aciduria (SLC25A1 deficiency): clinical course and effects of citrate treatment

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Combined D,l-2-hydroxyglutaric aciduria (DL-2HGA) is a rare neurometabolic disorder characterized by muscular hypotonia, neurodevelopmental dysfunction, and intractable seizures. DL-2HGA patients excrete increased amounts of D- and L-2-hydroxyglutarate (D2HG and L2HG, respectively) and α-ketoglutarate, and show a decrease of urinary citrate. Impaired function of the mitochondrial citrate carrier (CIC) due to SLC25A1 mutations has been identified as the underlying cause. CIC mediates efflux of mitochondrial tricarboxylic acid cycle intermediates citrate and isocitrate in exchange for cytosolic malate. Depletion of cytosolic citrate and accumulation of citrate inside mitochondria have been considered to play a pathophysiological role in DL-2HGA. We report for the first time on a patient with genetically confirmed DL-2HGA and treatment with either malate or citrate. During malate treatment, urinary malate concentration increased, but beyond that, neither biochemical nor clinical alterations were observed. Treatment with citrate led to an increased urinary excretion of malate and succinate, and by trend to an increased concentration of citrate. Excretion of D2HG and L2HG was reduced and subnormal plasma cholesterol concentrations increased. Clinically, the patient showed a stabilization regarding frequency and severity of seizures. Treatment of DL-2HGA

with citrate should be considered in other DL-2HGA patients, and its effects should be studied systematically.

P-210

Mitochondrial dysfunction and oxidative damage studies in a hypomorph murine model of propionic acidemia

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Propionic acidemia (PA) is caused by a deficiency of the nuclear encoded mitochondrial enzyme propionyl-CoA carboxylase, composed of alpha and beta subunits encoded by the pccA and pccB genes. Clinically, it presents as a toxic encephalopathy exhibiting high mortality and morbidity. A secondary mitochondrial dysfunction mainly related to the accumulation of toxic metabolites has been proposed to contribute to the pathophysiology of PA. Recently we have shown the presence of increased reactive oxygen species (ROS) in PA patient cells, which can be reversed using antioxidant treatment. Now, using the hypomorph murine model pccA−/−(A138T) we have analyzed the mitochondrial bioenergetic signature in relevant tissues by reverse phase protein microarray and measurement of OXPHOS activities, determined mtDNA copy number and analyzed ROS and oxidative damage by detection of protein carbonylation, lipid peroxidation, DNA fragmentation (TUNEL assay) and superoxide production (dihydroethidium staining). The results show energetic deficiency, increased oxidative stress and cellular response resulting in increased mitochondrial mass. These data further support the role of mitochondrial dysfunction and oxidative stress in the pathophysiology of the disease and pave the way for identification of disease progression biomarkers along with and for the analysis of the therapeutic potential of antioxidants and compounds potentiating mitochondrial function.

P-211

Remarkable therapeutic response in an early treated patient with methylmalonic acidemia (MMA) associated with CblC deficiency

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CblC deficiency is one of the most frequent causes of MMA. Only a few data on biochemical and clinical outcome of early diagnosed (by the extended neonatal screening) and treated patients have been reported so far. We report on the remarkable and unusual hydroxocobalamin responsiveness in an early detected patient. This 6 month-old-girl was born at term after a normal pregnancy and delivery (weight 2620 gr, length 49 cm, head 31 cm) from normal unrelated parents. High propionyl-carnitine (C3) (6.39 μmol/L; n.v. 0.40-3.68) was detected at neonatal screening and prompted a diagnostic work-up which included: plasma homocysteine (64.1 μmol/L; n.v. 3.3-8.3), methionine (10.4 μmol/L; n.v. 15–57), and vitamin B12 (1089 pg/ml; n.v. 220–1130); urine methylmalonic acid (3893.1 mmol/mol creatinine; n.v. 0.4-2.4). The analysis of MMACHC gene revealed two variants already described in association to the disease: c.270_271insA; c.374 T>C. Under intramuscular hydroxocobalamin (1000 μg/die) and oral carnitine (100 mg/kg/die) treatment C3, homocysteine and methionine normalized in a couple of weeks and urine methylmalonic acid lowered to 384.5 mmol/mol creatinine. The reduction of the frequency of hydroxocobalamin administration (twice a week) didn't alter the quality of metabolic control. Clinical status and neurological development remained completely normal.

P-212

Multiple stable isotope labeled internal standards for gas chromatography/mass spectrometry in the clinical setting

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Background/objectives: First-line urinary organic acid analysis traditionally relies on a single internal standard (often 4-phenylbutyrate). This weakness reflects limited availability of stable isotope labeled organic acids. We investigated the added value of 24 labeled internal standards.
Material/methods: Before BSTFA/TMCS derivation and GC-MS analysis, we added the standards' cocktail to samples from urine, plasma and tissues of patients with relevant metabolic disorders, various tumors or from the ERNDIM quality program (N=100). Stable isotope labeled standards included custom syntheses (2-hydroxyglutaric, 2-ketoisocaproic, 3-methylglutaconic, isovalerylglycine, methylmalonic, mevalonolactone, N-acetylaspartic, propionylglycine).
Results: Correlated (artefactual) changes of labeled organic acid levels as assessed by principal component analysis were sensitive to matrix and sample effects. Nevertheless, labeled internal standards outperformed 4-phenylbutyrate for the quantification of structurally related analytes (e.g., labeled 2-hydroxyglutaric for glycolic and malic 2-hydroxyacids). In addition, by quantifying single labeled organic acids relative to the normalized mean of the others, the coefficients of variation tended to be lower than the best single internal standards.
Conclusion: The robust averaging effect and the consistency between chemical structure and quantification performance suggest that a limited selection of labeled internal standards may help to improve GC-MS analysis of organic acids.

P-213

The small molecule IDH2 (R140Q) inhibitor AGI-12026 rescues D-2-hydroxyglutaric aciduria in a knock-in mouse model

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Isocitrate dehydrogenase 2 (IDH2) R140Q mutation confers a novel enzymatic activity that converts a-ketoglutarate (aKG) to D-2-hydroxyglutarate (D-2-HG). This gain-of-function mutation has been identified in patients with type II D-2-hydroxyglutaric aciduria (D-2-HGA), an ultra-rare and severe neurometabolic disorder that presents with a range of clinical findings, including seizures, developmental delay, cardiomyopathy, dysmorphic features and early death. An IDH2 (R140Q) knock-in (KI) mouse model is generated by classic homologous recombination. The KI mice produce elevated D-2-HG level, and exhibit multiple defects consistent with symptoms from D-2-HGA patients, including facial dysmorphism, and early mortality. The potent and selective inhibitor AGI-12026 completely

suppresses 2HG production in all organs, dramatically ameliorates disease progression (including brain and cardiovascular defects), and provides profound survival benefit in IDH2 (R140Q) KI mice. Most strikingly, AGI-12026 not only prevents disease onset when administered to young KI animals (prophylactically) but also reverses disease progression in adult KI mice (therapeutically), and withdrawal of AGI-12026 treatment results in reappearance of disease features. The KI mouse model provides a valuable tool to elucidate the underlying disease mechanism of D-2-HGA. The potent and selective small molecule inhibitor of IDH2 (R140Q), AGI12026, rescues D-2-HGA disease phenotypes and normalizes overall survival.
Conflict of Interest declared.

13. Carbohydrate disorders

P-214

Molecular analysis of GALT gene in patients with galactosemia

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Background and objectives: Type I galactosemia results from mutations on both alleles of GALT gene which leads to absence or deficiency of galactose−1-phosphate uridyltransferase and is further classified into severe "classical" and mild "Duarte" galactosemia. Classical galactosemia is frequently associated with Q188R, S135L and K285N mutations and N314D is associated with Duarte galactosemia. The objectives of this study are to identify most common mutations Q188R, S135L, K285N and N314D for patients with classical and Duarte galactosemia and correlate genotype with its phenotype.
Materials and Methods: The present study aims at detecting Q188R, S135L, K285N mutations and N314D variant in the GALT gene by using amplification refractory mutation system (ARMS). ARMS assays were established using standard DNA samples and were used for eight galactosemia patients and 190 unrelated normal subjects all of Pakistani origin.
Results: S135L and K285N mutations were present neither in galactosemia patients nor in normal subjects. Only one galactosemia patient carried Q188R mutation that was in homozygous state. However, N314D variant was frequently found in affected 7 out of 16 alleles and normal subjects (55 out of 380 alleles).
Conclusion: This finding indicates that Duarte allele D314 might be far more common in Pakistani population than in European and North American ones.

P-215

Revised proposal for the prevention of low bone mass in patients with classic galactosemia

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Decreased bone mass is frequently encountered in classic galactosemia, an inborn error of galactose metabolism. This decrease is most prominent in adults, but is already seen in prepubertal children with increased risk of osteoporosis and fractures later in life. Therefore, bone health in patients with classic galactosemia is increasingly monitored. Although the pathophysiological mechanism is still not fully understood, several factors could affect bone metabolism in this disease. Patients are at risk of nutritional deficiencies due to the diet. Primary ovarian insufficiency in female patients also contributes to decreased bone mass. Furthermore, patients with classic galactosemia might be less physically active due to motor or neurological impairments. A disease specific intrinsic abnormality has been suggested as well. This revised proposal is an update of the previous one (2007). In this current approach we advise all-round dietary evaluation, optimization of calcium intake if needed, monitoring and if necessary supplementation of 25 (OH) D, hormonal status evaluation and HRT consideration, as well as a regular exercise, assessment of skeletal deformities and clinically significant fractures. We propose bone mineral density assessment by serial DXA scans of lumbar spine, femoral neck and total hip in adults and lumbar spine and total body less head in children.

P-216

The Galactosemia Network

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Three main determinants for a significant research activity level in rare diseases are the existence of patient organizations, patient registries and international networks (Orphanet RDR study, 2010). In 2012, a European-American Galactosemias reference network was established with involvement of clinicians, researchers and dieticians, and facilitated by the patient organizations. Seventeen European countries participate in the European arm.
Main objectives are creation and implementation of a patient registry, elaboration of guidelines for diagnosis, treatment and follow up, and collaborative research.
So far, the patient registry has been developed; implementation and first data entry are progressing. This database will provide information on complications prevalence in thousands of patients that can be correlated with many disease parameters to better understand these disorders.
Guidelines are being elaborated by review of existing evidence and by Delphi method and should call a halt to differences in patient care between countries (expected to be available end 2015).
Existing research collaborations are being strengthened and new collaborations emerge through this network, crucial to successful improvement of patient outcome. This international network is of outmost importance for improving patient care, education, advancement of research, and better treatment of patients and enriches the list of existing networks in rare diseases.

P-217

Transaldolase deficiency caused by a founder missense mutation and evidence of phenotypic variability in four Emirati cases

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Background and Aims: Transaldolase deficiency is a clinically heterogeneous condition of carbohydrate metabolism, characterized by dysmorphic features, cutis laxa, hepatosplenomegaly, hepatic fibrosis, pancytopenia, renal and cardiac abnormalities. In this review, we reported four

Emirati cases with transaldolase deficiency caused by a founder missense mutation showing evidence of phenotypic variability.
Methods and Results: We recruited 4 Emirati cases with transaldolase deficiency. Genetic analysis revealed the presence of homozygous missense mutation affecting arginine residue at position 192 (Arginine to cysteine, p. R192C) of transaldolase protein in all studied patients. This mutation was previously identified in cases from Saudi Arabia suggesting a founder effect in Arab population. The substitution of this amino acid is well known to affect the catalytic activity of transaldolase protein, thus the loss of protein function is the major cause of this condition. Genotype-phenotype correlation showed a wide clinical variability between all the cases along with highly variable severity.
Conclusion: This study's finding supports the premise that biallelic mutations in the TALDO1 gene are responsible for transaldolase deficiency and gives evidence that this condition showed a broadly phenotypic variability even for the same mutation.

P-218

Fanconi-Bickel syndrome - A new case from Turkey

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The Fanconi Bickel syndrome (FBS) is a rare, well defined clinical entity that is characterized by hepatorenal glycogen accumulation, fasting hypoglycemia, hypergalactosemia, proximal renal tubular dysfunction, rickets and marked developmental delay. In this report, we describe a patient with FBS who was initially diagnosed as galactosemia. The 18 months old male child, first born of a consanguineous marriage presented with failure to thrive, mental and motor retardation and abdominal distension. Growth retardation, clinical manifestations of rickets, genu valgum, doll like face and hepatomegaly were observed. He has sparse subcutaneous fat, thin extremities, distended abdomen and hepatomegaly. Investigations revealed reduced serum calcium (8.2 mg/dl) and serum phosphorus (1.8 mg/dl, normal 2.7-4.5) and mildly elevated serum alkaline phosphatase (504 U/L, normal 145–420 U/L) levels. Serum concentrations of liver aminotransferases were also high: AST, 132 IU/L; ALT 57 IU/L. Blood glucose levels three hours after feed were reduced (28 mg/dl, normal 60–100). Urinary pH was 6.5, specific gravity 1028, with 4+ glucosuria, 1+ proteinuria and generalized aminoaciduria. X-ray of the wrist showed active rickets. Blood gas analysis detected; pH: 7.28, HCO3 15, BE −7. The activity of enzymes involved in galactose metabolism has been found to be normal. On the basis of these findings, Fanconi-Bickel syndrome was finally confirmed by molecular analysis of SLC2A2 gene, showing homozygosity for the mutation p. G162Rfs*17 (c482_483insC).

P-219

Towards a fully comprehensive galactosemia proteins database

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Objectives: We present a Web-accessible database that collects data about structural and functional properties of the three enzymes linked to the different types of galactosemia (galactose-1-phosphate uridylyltransferase, galactokinase and UDP-galactose-4-epimerase) and of their published missense variants, to render them available in particular to clinicians and researchers.
Methods: The database has been implemented using PostgreSQL, integrated by dedicated SQL scripts developed in house. The web application has been developed using Java as the coding language. Analyses have been made on structures of proteins obtained from PDB database, when available, or modelled with a comparative modeling strategy, using well known bioinformatics tools and web servers.
Results: For each wild type and variant protein included in the Galactosemia Proteins Database, we provide data about structural and functional features, and their comparison in order to understand the impact of variations at a protein level. Additional sections include an introduction to galactosemia, several links to external resources, and a form to submit information about missense mutations not currently included in the database.
Conclusions: Galactosemia Proteins Database represents a general platform to collect structural data for the analysis and the storage of all mutations related to the three proteins involved in all forms of galactosemia.
Link: http://bioinformatica.isa.cnr.it/galactosemia-proteins-db/

P-220

Effects of temporary low-dose galactose supplements in children of over 5 years with Classical Galactosaemia

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Long-term complications occur in patients with Classical Galactosaemia despite life-long galactose restriction. Both intoxication by galactose (and its metabolites) as well as over-restriction of galactose may contribute to the pathophysiology. We investigated the effects of temporary, low-dose galactose supplements (300 mg of galactose orally followed by 500 mg for 2 weeks each) to affected children (n=13, 5–12 yrs.), assessed tolerance with measurement of biochemical and IgG N-glycosylation profiles, and compared data with patient controls on a strict diet. We observed no clinical changes with the intervention. Renal, liver, and bone biochemistry remained normal. Temporary mild increase in galactose-1-phosphate occurred following intake of 300 or 500 mg of galactose ($P<0.05$ and $P<0.01$, respectively). Patients in the galactose supplementation group had relatively higher leptin levels at the end of the study than patient controls ($P<0.05$). Soluble leptin receptor levels at 500 mg of galactose were higher than at 300 mg ($P<0.05$). We identified 6 patients (46 %) as 'responders' to this short-term intervention with a slightly improved IgG N-glycosylation pattern. We conclude that in a clinical setting 1) transient, low-dose galactose supplementation in children over 5 yrs. is well tolerated and 2) IgG N-glycan monitoring is suitable for determining individual optimum galactose intake.

P-221

Nutritional monitoring of children with glycogen storage disorders: a tertiary centre experience

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Introduction: Avoiding nutritional deficiencies and promoting normal growth are important objectives when managing children with Glycogen Storage Disorders (GSD). We report findings from a single-centre, year-long review of nutritional monitoring in children with GSD.
Aims: To assess the adequacy of dietary management of our GSD patients with emphasis on growth and nutritional parameters.
Methods: Retrospective chart review between January 2013 and January 2014.
Results: 26 children had adequate outpatient follow up and growth monitoring. Many patients were overweight (mean BMI z-score 1.65). 24 patients (92 %) had good glycemic control with no recorded hypoglycaemic events. Lipid profiles revealed mean cholesterol and triglyceride levels of 5.29 mmol/L and 3.32 mmol/L respectively. Protein malnutrition was suggested in some patients by low levels of serum prealbumin (3/11 patients) and urinary retinol binding protein (7/14 patients) where these were measured. Deficiencies of vitamin D and zinc were noted in 11/20 (55 %) and 5/18 (27 %) patients respectively. Most (14/15) patients studied had low Insulin-like Growth Factor 1 levels (mean 13.19units).
Conclusions: This review highlighted frequent dietary complications in children with GSD including obesity, growth failure and micronutrient deficiencies. Adequate monitoring, with close multidisciplinary liaison, is essential to identify and manage these, thereby optimising outcomes in children with GSD.

P-222

Normoglycemic (hyper) ketonemia in ketotic glycogen storage disease (GSD): implications for diagnosis and treatment

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Glycogen Storage Diseases (GSDs) are inborn errors of glycogen synthesis or breakdown. According to the textbooks, the hepatic GSD types O, III, VI, IX and XI are associated with fasting ketotic hypoglycemia and considered milder as gluconeogenesis is intact. The role of ketone bodies (KB) in these ketotic GSD types is underappreciated and has implications for diagnosis and treatment. We hypothesized that ketotic normoglycemia is a common biochemical phenotype in ketotic GSD patients.
Laboratory data were collected from all clinically supervised fasting tests performed in these patients in our metabolic center since 1993. For data analysis, hypoglycemia was defined as blood glucose concentrations.
Data could be collected from 13 tests in 12 patients, with GSD III (n=4), GSD VI (n=3) and GSD IX (n=5). In 6 patients with normoglycemia the median blood glucose concentration was 3.9 mmol/L [range: 2.8-4.6] with median total KB concentrations 1.9 mmol/L [range: 0.6-5.1]. Interestingly, in 5 patients the biochemical profile suggested a ketolysis defect. Patients with ketotic GSD can display normoglycemic (hyper) ketonemia upon fasting. In the diagnostic workup of fasting intolerance, these patients therefore may remain undiagnosed. This observation also emphasizes the importance to determine KB concentrations in monitoring dietary treatment.

P-223

Phosphoglycerate kinase-1 (PGK-1) deficiency presenting as neonatal onset hemolytic anemia, rhabdomyolysis, and mild developmental delay

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Background: Phosphoglycerate kinase-1 (PGK-1) deficiency is an X-linked recessive condition and caused by mutation in the PGK1 gene. Highly variable clinical symptoms including hemolytic anemia, myopathy, developmental delay and seizures, as well as variable age of onset (infancy to adult) are known in this condition.
Case report: The patient was a 2nd child born to a non-consanguineous Japanese couple (38 wks, 3410 g). Apgar scores were 81 and 95. He developed jaundice on the 1st day of his life (TB12.1 mg/dl) requiring phototherapy and exchange transfusion. At age 9 months, he was referred to us for a chronic indirect hyperbilirubinemia and hemolytic anemia. At age 15 months, he was hospitalized for worsening hemolytic anemia, and myoglobinuria which were preceded by a fever. He received RBC transfusion and antibiotics for 8 days. PGK deficiency was identified biochemically in erythrocytes.
Discussion: Neurologic involvement is associated in approximately 50 % of patients with PGK-1 deficiency. The presented case has motor and speech delay. The mechanism of neurologic involvement is unclear although glutaminergic synaptic metabolism is thought to be disrupted by PGK-1 deficiency. The patient has been followed with MCT supplementation and has not developed another severe episode of hemolysis or rhabdomyolysis for six months.

P-224

Increased propensity for protein aggregation of GALT variants resulting from the most prevalent missense mutations in classic galactosemia

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Classic galactosemia (CG; OMIM #230400) is an autosomal recessive disorder caused by GALT gene, resulting in deficient activity of galactose-1-phosphate uridylyltransferase (GALT, EC 2.7.7.12), a key enzyme in galactose metabolism. CG develops in the neonatal period, due to the ingestion of galactose-containing milk, and is a potentially lethal disorder. A galactose-restricted diet is essential to treat the acute illness; yet, it is insufficient to prevent long-term complications, namely cognitive and neurologic disabilities. Herein, we studied the structural-functional properties of nine GALT variants, by activity and thermal inactivation assays, and by several biophysical methodologies, namely circular dichroism, differential scanning fluorimetry and dynamic light scattering. Functional analyses showed that the variants with detectable enzymatic activity were more sensitive than wild-type GALT to thermal inactivation, indicating impaired functional and/or conformational stability. Structural analyses of all variants showed disturbed aggregation profiles, with limited impact on the secondary or tertiary structures. Aggregation proneness was particularly enhanced for p. Q188R, resulting from the most prevalent mutation (~60 % of mutant alleles worldwide). This study reports for the first time increased aggregation propensity of missense variants in CG, suggesting that chemical/pharmacological chaperones and proteostasis modulators could be highly beneficial in this inborn error of carbohydrate metabolism.

P-225

Cadaveric liver transplantation for glycogen storage disease type 1 non-A: A single centre experience

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Background: Glycogen Storage Disease Type 1 Non-A (GSD1nA) due to glucose-6-phosphate-translocase deficiency is associated with severely limited fasting tolerance, hypoglycaemia, hepatomegaly, neutropenia and inflammatory bowel disease (IBD). Liver transplantation (LT) has been indicated for fasting intolerance and reported to improve neutropenia. We report our experience of LT in five cases with metabolic correction at up to 5 yrs post-cadaveric-LT but with persisting neutropenia and gastrointestinal symptoms.
Methods: Five children with GSD1nA, each homozygous for one of three mutations in SLC37A4, underwent LT at our centre aged 2-8 yrs with follow-up between 7mths and 5 yrs.
Results: All patients are alive with 100 % graft survival . Metabolic correction was consistently achieved with a minimum overnight fasting tolerance reported of 8 hrs compared to 1.5-2 hrs pre-LT. Patients continue to require G-CSF with little change in neutrophil counts following LT although the frequency of Emergency Department attendance has reduced in most patients. Four patients had some form of gastrointestinal symptoms pre-LT which continue post-LT. Most patients displayed short stature and raised body mass indices (BMI) pre-LT and showed a sustained reduction in weight and BMI z-scores after LT.
Conclusions: LT results in excellent correction of GSD1nA fasting intolerance but in our experience neutropenia and IBD symptoms persist.

P-226

Cellular immunity characteristics and succinate dehydrogenese activity in children with hepatic form of glycogen storage disease

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The aim: To determine characteristics of cellular immunity and succinate dehydrogenase (SDH)-activity in lymphocytes subsets in children with the hepatic form of GSD. Patients and methods: We examined 53 children with GSD (types I,III,VI,IX) at the age from 1 to 17 years; control group consisted of 34 healthy children. We investigated SDH-activity in lymphocytes subsets by quantitative cytochemical method, based on the n-nitrotetrazolium violet ability to form insoluble formazan granules during enzymatic reduction with a help of flow cytometry.
Results: It was revealed that 40 % of children with GSD had the increase ?- lymphocytes absolute number. B-cell number was decreased in 35 % cases. The SDH-activity in T-cells was decreased by 18±4 %, in B-cells by 40±5 % compared with control group. Found a significant increase of activated T-helpers and regulatory T-cells (T-reg) numbers in children with GSD, SDH-activity in activated T-helpers was not differ from control group, SDH- activity in T-reg cells was significant decreased. The numbers of NK-cells was not differ from control group in 90 % cases, SDH-activity in NK-cells was increased on 20±5 % compared with control group.
Conclusion: We revealed changes of the lymphocytes subsets number and their functional activity, this findings correspond to increased infectious diseases rate in children with GSD.

P-227

Genetic diagnosis of glycogen storage disorders

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Glycogen storage disorder (GSD) is a locus heterogeneous disorder. The diagnosis of GSD depends largely on invasive liver and muscle biopsies and time consuming biochemical assays. Therefore, in some cases the DNA-based methods allow accurate diagnosis for GSD. Here, we report a genetic analysis of a cohort of 16 patients referred to our Lab for genetics diagnosis of GSD. We have done gene-by-gene conventional Sanger sequencing of genes related to the clinical history in 11 patients. In the last year next generation sequencing has been applied using either an Haloplex customized targeted gene panel including 111 genes related to metabolic disorders or a large gene panel including all known disease genes (CES: clinical exome sequencing) from Illumina. Overall we have detected eight AGL, five PHKA2, one SLC37A4 and one GAA affected patients. In this cohort we have detected 13 new mutations, 10 frame shift mutations and three likely missense changes. We have detected the most common mutation in ALDOB gene, p. Ala150Pro, in a patient sent for GSD genetic diagnosis. Taking into account all results we can conclude that for therapeutic and genetic counselling, the use of a NGS panel is more effective and accurate in the diagnosis of GSD.

P-228

Long-term follow up in a cohort of galactosemic patients detected by newborn screening

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Background: Classic galactosemia is a carbohydrate metabolism disorder characterized by a high frequency of long-term complications, even in early diagnosed/treated patients.
Materials and methods: Since 1980, fourteen classic galactosemic patients were therefore diagnosed by newborn screening and confirmed by GALT enzyme activity assay and molecular analysis. All patients received a galactose-restricted diet. Long term follow-up (33 years) consisted in bone, neurological (IQs, clinical, language and neuroradiological assessment) and, in women, endocrinological evaluations.
Results: Despite newborn screening, all patients, except one, showed hepatic failure (71,4 %), cataract (14,3 %), sepsis (7 %). Long-term complications included speech (50 %) and behaviour disturbances (25 %), intellectual disability (25 %) and cerebral white matter involvement (75 %) that were observed in 6 of 8 patients older than 9 years. One female presented ovarian insufficiency.6 of 14 patients showed a novel GALT gene variant and another variant was identified in a classic galactosemic patient diagnosed at 8 years old. Gene variants were studied using in silico analysis (SIFT, Polyphen-2). The new mutations effect was further investigated concerning protein stability and subunit localization/effect.
Discussion: Despite newborn screening and early diet intervention, the majority of patients showed long-term complications. Interestingly, a high rate of novel GALT gene mutations was found without genotype/phenotype correlation.

P-229

Glycogen storage disease XII complicated with hemizygous Duchenne muscular dystrophy mutation

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Background and objectives: Glycogen storage disease XII (GSD12) is a multisystem disease with exercise intolerance, rhabdomyolysis, and hemolysis and is caused by mutations in the ALDOA gene. Duchene muscular dystrophy (DMD) is an X-linked disorder caused by the mutations of dystrophin gene.
Case report: A 18-months-old boy of a consanguineous couple presented with ichthyosis, muscle weakness-fatigue, mild anemia aggravated with infections, and hepatosplenomegaly. He had normal mental development and had no dysmorphic features. Transaminases were mildly and creatine kinase was markedly elevated. He had myoglobinuria. Episodes of rhabdomyolysis with creatine kinase elevations of up to 17,800 U/L were observed during illness.
Results: Muscle biopsy revealed glycogen infiltrations in the muscle fibers with positive dystrophin. Whole Exome Sequencing (WES) presented two unrelated disorders. A novel homozygote missense mutation of ALDOA gene, c. C971T (p. A324V) was detected. This mutation was given in COSMIC database but not previously reported in GSD12. He had a hemizygous stop codon mutation on DMD gene, c. C571T (p. R191*).
Conclusion: This patient has two unrelated disorders by chance. As these disorders have both overlapping and unoverlapping findings, clinical features of the patient was very complex. WES is an effective method for diagnosing complex cases, and will be helpful for prenatal diagnosis.

P-230

Friedreich's ataxia in classical galactosaemia

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Background: Ataxia is a recognised complication of classical galactosaemia, although its pathogenesis is poorly understood. We report the co-existence of Friedreich's ataxia (FA) and classical galactosaemia in a cohort of patients from the Irish Traveller population.
Patients: Seven of the 114 Irish and Northern Irish paediatric galactosaemia patients have been diagnosed with FA. These seven patients are from five families which, while not related, are all members of the Irish Travelling Community. Ataxia began as early as five, and patients were aged between six and fifteen years at FA diagnosis. All are homozygous for the Q188R mutation (GALT) and the common pathogenic GAA expansion in frataxin (FXN).
Discussion: GALT and FXN genes are located on either side of the centromere of chromosome 9, at positions 9p13.3 and 9q21.11 respectively. Karyotyping excluded a pericentric inversion. The co-segregation of the two disorders in these families suggests that crossover during meiosis is occurring telomeric to both genes. The coexistence of these conditions occurs only in a subset of the Traveller population, as other families are affected by galactosaemia or FA alone.
Conclusion: Early onset progressive ataxia in Irish Traveller galactosaemia patients should prompt investigation for co-existing Freidreich's ataxia.

P-231

Characterization of a large deletion of the FBP1 gene in Turkish and Armenian patients with fructose-1,6-biphosphatase deficiency

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Background: Fructose-1,6-Biphosphatase deficiency (FBP-D) is caused by mutations of the FBP1 gene. The overall number of reported sequences variations is rather limited. Characteristic mutations for specific populations, to our knowledge, have only been reported for Japan (FBP1 c.960insG) and Pakistan (FBP1 c.841G>A). Here, we report the characterization of a large deletion of the FBP1 gene in Turkish and Armenian patients with fructose-1,6-biphosphatase deficiency. This mutation has probably been previously mentioned (Herzog et al. JIMD 2001) but has never been reported in detail.
Methods: Clinical description of three patients (2 with Turkish background, one from Armenia) with typical clinical and laboratory signs of FBP-D. Conventional Sanger sequencing of all 7 exons of the FBP1 gene and sequencing of a junction fragment including the deletion.
Results: All 3 patients were found to be homozygous for a 5412-bp deletion including exon 1 of FBP1 (−50_c.170+5192del)
Conclusion: This result confirms earlier assumptions that exon 1 deletions are relatively common in the Turkish (and Armenian) population. The detection of the deletion breakpoints allows the exact diagnosis particularly in compound heterozygous individuals.

14. Disorders of fatty acid oxidation and ketone body metabolism

P-232

Metabolic anomalies identified in the newborn period: Unusual acylcarnitine, and organic acid profiles suggesting possible mild multiple acyl-CoA dehydrogenase deficiency (MADD)

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We present 4 cases strongly suggestive of MADD, but with no clear confirmed diagnosis.
Case 1, Male: Hypoglycaemic at day 3. Plasma acylcarnitines (μmol/L) C8=0.90 (ref. <0.22), C14:1=9.09 (<0.18), C16=4.04 (<0.24), with gross hypoketotic dicarboxylic aciduria (C6 – C12) and dimethylglycinuria. Day 5 acylcarnitines and organic acids had normalised. Patient clinically well.
Case 2, Female: Increased C8 (0.54 cut-off <0.50) on newborn screening. Subsequent plasma C8=1.72 (ref.<0.22), C10=3.45 (ref.<0.30) C14:1=1.17 (ref<0.18), organic acids were unremarkable. Normal fibroblast fat oxidation. Clinically well, at 18 months with C8=1.65 and C10=2.32.
Case 3, Male: Newborn bloodspot C8 (0.62). Day 9 plasma C4=3.55, C8=1.09, C10=2.48, C14:1=1.04. Organic acids showed marked ethylmalonic aciduria (normal acylglycines) persistent at 7 years. Fat oxidation normal. Acylcarnitines remain abnormal. Patient has learning difficulties.

Case 4, Male: Newborn bloodspot C8 (2.61). Day 8 plasma C8=2.51, C10=4.66, C14:1=2.01. Organic acid profile showed increased hexanoylglycine and suberylglycine with hypoketotic dicarboxylic aciduria. Patient clinically well. These cases highlight the difficulty in accurately diagnosing and subsequent management of these 'types' of patients.

P-233

A first case of short-chain acyl-CoA dehydrogenase deficiency (SCADD) in Slovenia

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Background: Short-chain acyl-CoA dehydrogenase deficiency (SCADD) is a rare inherited mitohondrial fatty acid oxidation disorder associated with variations in the ACADS gene. SCADD has highly variable biochemical, genetic and clinical characteristics. Phenotypes vary from fatal metabolic decompensation to asymptomatic individuals.
Case report: A Romani boy presented at 3 days after birth with hypoglycemia, hypotonia and respiratory pauses with brief generalized seizures. Afterwards the failure to thrive and developmental delay were present. Metabolic investigation revealed highly elevated concentration of urinary ethylmalonic acid. Acylcarnitines analysis in dried blood spot showed elevation of butyrylcarnitine, C4. C4 was 3.5 times above the reference range (ACADS gene revealed 3-bp deletion at position 310–312 in homozygous state (c.310_312delGAG). Mutation was previously described as pathogenic.
Conclusion: An abnormal metabolic profile and a pathogenic mutation in the ACADS gene confirmed the diagnosis of SCADD. However, there is critical need for long-term follow-up data on SCADD patients diagnosed by selective screening based on clinical presentations versus those diagnosed by newborn screening.

P-234

Alu element insertions into intron 9 affect exon 10 recognition with a suboptimal splice acceptor site in human ACAT1 gene

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Background and objective: ACAT1 gene mutations result in beta-ketothiolase deficiency. Alu elements are the most abundant repetitive sequences in the human genome and are commonly located in introns. We hypothesized that Alu insertion in intron 9 affected exon 10 recognition with suboptimal splice acceptor site in ACAT1 gene.
Methods: We previously made several minigene splicing constructs to analyze the effects of exonic splicing enhancer (ESE) mutations on exon 10. We inserted tandem Alu elements which were originally located in intron 5 of ACAT1 gene into intron 9 in either sense or antisense directions. Then minigene splicing experiments were performed.
Results: Tandem Alu insertion into intron 9 of ACAT1 in an antisense direction induced exon 10 skipping. The effect disappeared if the splice acceptor site was strengthened at the first nucleotide of exon 10. These effects are similar with those of ESE mutations on exon 10, which we reported previously.
Conclusion: Alu element insertion in antisense direction acted as intronic splicing silencer in case of ACAT1 intron 9.

P-235

Ethylmalonic acid compromises in vivo energy and redox homeostasis in striatum of young rats

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High concentrations of ethylmalonic acid (EMA) have been observed in various metabolic disorders with neurological manifestations, such as ethylmalonic encephalopathy (EE) and short-chain acyl-CoA dehydrogenase deficiency activity (SCADD). The pathophysiological mechanisms responsible for the clinical manifestations of these diseases are unknown. Therefore, we investigated the in vivo effects of intrastriatal administration of EMA on important parameters of energy metabolism in rat striatum, including CO2 production from glucose, enzyme activities of the citric acid cycle and of the electron transfer chain (ETC.) complexes II-IV, creatine kinase and synaptic Na^+,K^+-ATPase. We also evaluated the effects of EMA administration on reduced glutatione (GSH) concentrations (non-enzymatic antioxidant defense) and TBA-RS levels (marker of lipid peroxidation). EMA significantly reduced CO2 production from glucose, diminished the activities of complex II and II-III of the ETC., citrate synthase and synaptic membrane Na^+, K^+-ATPase. EMA also decreased the concentrations of GSH and increased TBA-RS levels. Our present data indicate that EMA compromises in vivo energy and redox homeostasis, as well as neurotransmission in striatum. We presume that these pathomechanisms are involved in the neurological damage found in patients affected by EE and SCADD.
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P-236

A probable case of a symptomatic simple heterozygote VLCADD

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Case Report. We report the case of a girl, whose father has VLCADD. Her DBS acylcarnitine profile on day 3 gave a C14:1 of 0.58 (ref <0.17), plasma acylcarnitine profile gave free carnitine 13 μmol/L (15–53) and C14:1 0.88 (ref <0.18). At 2 months repeat acylcarnitine, liver function and CK were normal. Full sequencing of ACADVL gene showed her to be heterozygous for c.1375C>T only. She presented at 9/12 and 16/12 with gastroenteritis, hypoglycaemia and encephalopathy. She was also found at 1 year to be unresponsive after a prolonged fast. She proceeded to have fatty acid oxidation flux studies. Fat oxidation was reduced with oleate giving 51 % (n=3). Her father and paternal uncle have symptomatic VLCADD and both have two mutations (c.1360G>A and c.1375 C>T) and low oleate flux at 11 %. We describe a symptomatic patient with 1 mutation in the ACADVL gene, with reduced fat oxidation. Her father and uncle have confirmed VLCADD. Her paternal grandparents are known to each carry 1 mutation in the ACADVL gene but are asymptomatic and both have completely normal flux studies. The patient appears to be a symptomatic heterozygote with moderately reduced fat oxidation flux - possibly as a result of other genetic/epigenetic factors.

P-237

Improvement of fatty acid oxidation capacity of cells from fatty acid oxidation defects at low temperature: evaluation by in vitro probe assay

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Background: Patients with mitochondrial fatty acid oxidation (FAO) disorders often show myopathy-like symptoms, acute encephalopathy, or even sudden death following long fasting or infection. In vitro probe (IVP) assay is a tool to evaluate FAO capacity and defective enzymes, using culture cells and tandem mass spectrometry (MS/MS). We previously reported that hyperthermia enhanced accumulation of long-chain (LC) acylcarnitines (ACs), indicating that hyperthermia may inhibit FAO in LC-FAO disorders like deficiencies of VLCAD, or CPT2. In this study, we examined the FAO capacity at low temperature in various FAO disorders by IVP assay.
Methods: Fibroblasts from patients with deficiencies of CPT2, VLCAD, and glutaric acidemia type 2 (GA2) were cultured at 33 °C, as well as 37 °C and 41 °C.
Results: In LC-FAO disorders like deficiencies of CPT2 and VLCAD, accumulated C16 was reduced at 33 °C, although LC-ACs was increased at high temperature (41 °C). In GA2 cells, at 41 °C, LC-ACs were increased, and medium (MC)- and short-chain (SC)-ACs decreased, whereas at 33, accumulated LC-ACs as well as MC-ACs and SC-ACs were decreased.
Conclusion: Hypothermia possibly improves FAO capacity in FAO disorders. These findings suggest that hypothermia therapys may be beneficial in acute condition of FAO disorder.

P-238

In vivo cardiac magnetic resonance spectroscopy in the long-chain acyl-CoA dehydrogenase knockout mouse

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Background and Objectives: The etiology of cardiomyopathy in patients with inborn errors in long-chain fatty acid β-oxidation (lcFAO) is unclear, preventing the design of rational therapeutic strategies. Here, we employed magnetic resonance imaging (MRI) and spectroscopy (MRS) for in vivo investigations of the long-chain acyl-CoA dehydrogenase knockout (LCAD KO) mouse heart.
Materials and Methods: We investigated fed and overnight fasted male LCAD KO mice and wild-type controls (n=7–8). Heart function was quantified with MRI. Triglyceride levels (with proton MRS), energy status (with phosphorus MRS), and pyruvate dehydrogenase (PDH) flux (with carbon-13 MRS after hyperpolarized [1-^{13}C] pyruvate administration) were measured in the in vivo heart, and corroborated with ex vivo assays.
Results: Fasting led to reduced cardiac performance in LCAD KO mice, associated with prominent elevation of myocardial triglyceride content and high ceramide levels. PDH flux remained higher in fasted LCAD KO hearts than in controls, along with enhanced activity of anaplerotic pathways. Importantly, myocardial phosphocreatine-to-ATP ratio was lower in fasted LCAD KO mice, revealing a disturbed energy homeostasis.
Conclusion: Lipotoxicity and energy shortage may contribute to the development of cardiomyopathy in lcFAO disorders. With cardiac MRS methods, non-invasive longitudinal mouse studies to explore treatment options are now feasible.
Conflict of Interest declared.

P-239

Non diagnostic bloodspot acylcarnitine profile during metabolic crisis in a patient presenting with myopathic CPT2 deficiency

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Background: Carnitine palmitoyl transferase 2 (CPT2) is the most common disorder of lipid metabolism affecting skeletal muscle and the most frequent cause of hereditary myoglobinuria. Diagnosis is suspected with elevation of long chain acylcarnitine species, most prominently C16 and C18:1.
Case Report: A 7 year old girl presented with rhabdomyolysis; CK 85000, ALT 580 and normal renal function. She had a history of recurrent myalgia triggered by infections, fasting and exercise. CK between episodes was normal. There was no history of second wind phenomenon. Clinical suspicion was of a disorder of fatty acid oxidation. Metabolic tests were taken at the time of crisis with rhabdomyolysis. Urine organic acid analysis was normal. Bloodspot acylcarnitine profile showed C16 1.80 (ref 0.33- 4.01) and C18:1 2.13 (ref 0.43-2.06). However plasma acylcarnitines demonstrated significant elevation of C16 and C18:1 at 0.95 (ref<0.24) and 1.48 (ref <0.28) respectively. Mutational analysis confirmed CPT2 deficiency.
Conclusion: Blood spot acylcarnitine profile may be non-diagnostic at the time of metabolic crisis in patients with myopathic CPT2 deficiency. A plasma acylcarnitine profile is superior to a bloodspot sample in the evaluation of a patient suspected of CPT2 deficiency during metabolic crisis.

P-240

Romani origin of first Slovak patients with short-chain acyl-CoA dehydrogenase deficiency (SCADD)

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Background: SCADD is an inborn error of mitochondrial fatty acid oxidation. Here we report on the first two patients with SCADD in Slovakia, both of Romani origin.
Case 1: This girl was born as premature product of her mother's second pregnancy, which was complicated by preeclampsia. The patient developed a devastating clinical course with dysmorphic features, feeding difficulties, failure to thrive, axial hypotonia, acral hypertonia, severe developmental delay, strabismus, nystagmus, recurrent infections, basal ganglia and white matter damage. Biochemical testing disclosed lactic acidosis, low serum free carnitine, increased plasma C4-carnitine, ethylmalonic aciduria (66–1301 mmol/mol creatinine). Analysis of the

ACADS gene revealed homozygosity for the c.310_312delGAG mutation. At 7 years of age the girl is severely handicapped.
Case 2: This girl with 7 healthy siblings was born at term. During respiratory infection at age 8 months she presented with coma, extreme hypoglycemia, rhabdomyolysis. Metabolic investigations showed ethylmalonic aciduria (172 mmol/mol creatinine), normal plasma C4-carnitine, and increased C4-carnitine/C3-carnitine ratio. The patient was compound heterozygous for mutation c.1138C>T and variation c.625G>A in the ACADS gene. At present the 4-year-old girl is clinically asymptomatic.
Conclusion: Our results demonstrate that clinical heterogeneity in SCADD is also present in the Romani ethnic group in Slovakia.

P-241

Approaches to deciphering the balance between survival and death mechanisms in cells with mitochondrial dysfunction

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Short-chain acyl-CoA dehydrogenase (SCAD) deficiency is a fatty acid oxidation disorder. Since it is important to delineate if individuals carrying SCAD (ACADS) gene variations are at risk of developing disease during acute stress we hypothesized that starvation and/or fever in symptomatic patients may shift the homeostatic balance towards cell death and treatment by N-acetylcysteine (NAC) can prevent this.
Fibroblasts from a selected SCAD deficient patient and a control were cultured for 0, 6, 12, 24, and 48 hours at normal and stressed conditions. We determined the expression rate of oxidative stress markers (SOD2 and TRAP1) at protein level and measured superoxide generated in the mitochondria using MitoSOX assay.
SOD2 and TRAP1 expressions and also MitoSOX assay showed SCAD deficient cells responded significantly to the stress exposure after 12 hours. The procedure is repeated at 12 hours with 3 of each SCAD deficient, MCAD (Medium-chain acyl-CoA dehydrogenase) deficient and control cells while they are also treated with NAC.
SOD2 and TRAP1 upregulation in SCAD and MCAD deficient cells indicates significantly more oxidative stress, but less damage in MCAD deficient cells than SCAD. NAC, inducer of survival mechanisms, increases SOD2 and TRAP1 expression more in MCAD than in SCAD deficient cells.

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Molecular mechanisms underlying medium-chain acyl-CoA dehydrogenase (MCAD) deficiency: an in silico assessment

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MCAD deficiency (MCADD) is the most frequent genetic disorder of the mitochondrial fatty acid β-oxidation (mFAO) pathway. The commonest mutation found in MCADD patients is the substitution of lysine 304 residue by a glutamic acid (p. K304E) in the mature protein (396 aminoacids), being associated with conformational changes. To better understand the molecular mechanisms underlying MCADD an in silico assessment of structural features of the wild-type MCAD and p. K304E mutant form were performed through Molecular Dynamics simulations. The enzyme's coordinates were obtained from the crystal structure of Sus scrofa (pig) MCAD complexed with FAD and 3-thiaoctanoyl-CoA as substrate. Our results show that upon substrate binding the conformation of E99 and Y375 residues' side-chains change significantly to accommodate the substrate. The side-chain of the catalytic residue E376, switches towards the opposite side of the lipid's C2-C3 bond suggesting an indirect interaction between them. Our simulations also reveal that R256 residue may stabilize the intermediate enolate form. To our best knowledge, this is the first in silico study of MCAD dynamics behavior. The MCAD structural data gathered are currently being applied in the study of other MCAD mutants aiming the discovery of potential therapeutic drugs for the treatment of MCADD patients.

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HMG-CoA lyase Deficiency: One disease three pictures

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3-hydroxy-3-methylglutaric aciduria is a rare autosomal recessive inborn of metabolism due to the deficiency of 3-hydroxy-3-methylglutaryl-coenzyme A lyase (HL), an enzyme involved both in the ketogenic pathway and leucine catabolism. Acute decompensations present with lethargy, vomiting and metabolic acidosis with hypoketotic hypoglycemia. Here, we report clinical, laboratory features of 3 Turkish patients and a novel mutation identified in the HMGCL gene. All patients were male. Median age of initial symptoms and diagnosis was 6 months and 7 months respectively. Interestingly, one of our patients has situs inversus totalis and gastroschisis. All cases developed Reye like clinical picture, metabolic acidosis and hypoketotic hypoglycemia. Two patients had peritoneal dialysis due to hyperammonaemia and metabolic acidosis. In plasma elevated acylcarnitines C5:OH was found. In urinary organic acid analysis excessive amounts of 3-methylglutaric acid, 3-OH 3-methyl glutaric acid, 3-methyl glutoconic acid were detected. In molecular analysis of HMGCL gene, homozygote c.374_375delTC mutation in a patient and de novo mutation of c.476C>A was detected in the other patient which to our knowledge is the first case described with this mutation. These cases underline the need of suspecting such inborn metabolic disorder in cases with hypoketotic hypoglycemia, metabolic acidosis and especially Reye-like illness.

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Heterozygous carriers of VLCAD deficiency detected by newborn screening may have latent risk of symptomatic hypoglycemia

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BACKGROUND: In newborn screening of VLCAD deficiency, simple and accurate enzymatic study is essential to differentiate affected patients from false positive subjects, where heterozygous carriers can be revealed. However, it is not established how to manage such carriers.
METHODS: Newborns positive for both C14:1 and C14:1/C2 in dried blood spots were evaluated by our enzymatic assay method, in which C16-CoA dehydrogenase activities in lymphocytes were quantified by measuring C16:1-CoA. Patients with various symptoms screened by serum C14:1 were also evaluated. Further confirmation of abnormal results was attempted by genetic analysis.

RESULTS: Among 28 screening-positive newborns, 15 were diagnosed to be affected patients. There were 9 false positive cases, 8 of whom showed marked weight loss due to poor breastfeeding without using formula milk. The other 4 newborns showed enzymatic activities equivalent to those of obligate heterozygotes; similar results were observed in 3 hypoglycemic children, including one case of severe hypoketotic hypoglycemia.
CONCLUSION: It was suggested that heterozygous carriers of VLCAD deficiency could present with symptomatic hypoglycemia in early childhood. Newborns with carrier-levels of enzymatic activities should be advised to avoid too long starvation, especially caused by infectious gastroenteritis.

P-245

Neurodevelopmental profiles of children with very long chain acyl-CoA dehydrogenase deficiency (VLCADD) diagnosed by newborn screening

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Background and objectives: Very long chain acyl-CoA dehydrogenase deficiency (VLCADD) is a disorder of fatty acid oxidation that can cause hypoketotic-hypoglycaemia, cardiomyopathy, encephalopathy, and potentially impact on brain development. Newborn screening (NBS) and early treatment are intended to prevent these complications. We aimed to characterise the cognitive, behavioural and social abilities of children with VLCADD diagnosed by NBS in Victoria, Australia.
Method: Seven/eight children with VLCADD aged 4–8 completed a standardised neurodevelopmental assessment including measures of intelligence, attention, executive functioning, memory, language, motor skills, social perception and behaviour (one child had a previous assessment elsewhere).
Results: IQ scores in three children were >120. Language was below average in one child (GLI=77; M=100, SD=15). Parents' reports regarding all eight patients indicated that one child had social skills below average and peer problems, and two children had behavioural problems such as hyperactivity. One child rated high on an autism spectrum subscale; another was formally diagnosed with autism spectrum disorder. There were no motor deficits in any of the children.
Conclusions: Some children with VLCADD may have language deficits and may be more vulnerable to social and behavioural issues. Research in larger cohorts is required before any definitive conclusions can be made.

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Early diagnosis and treatment of the fatty acid oxidation disorder Very Long-chain Acyl-CoA Dehydrogenase deficiency in patients identified by newborn screening

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Introduction: From 2008 we added VLCADD to our newborn screening panel.
Methods: MS/MS Xevo (Waters); Neogram kits (derivatized method; PerkinElmer). Blood sampling at 3–4 days postpartum. Screening parameter C14:1 acylcarnitine and C14:1/C2 ratio.
Results: Between 2012–2013, three patients affected by VLCADD were found in our population with a C14:1 acylcarnitine concentration of 1.33, 3.59 and 4.10 μmol/L, respectively. The residual enzyme activity of VLCADD was 0.61, 0.24 and 0 nmol/min/mg protein, respectively (controls: 1.84-4.80 nmol/min/mg protein; 10 % enzyme activity= 0.66). The diagnosis was confirmed by DNA analysis. The first two girls found were asymptomatic, including normal cardiac findings at the age of diagnosis (4–6 weeks). The last girl presented a severe dilated cardiomyopathy at the early age of 16 days postpartum: high glucose infusion and inotropic medication stabilized the clinical condition and cardiac parameters normalized within 4–6 weeks. The two patients with the lowest enzyme activity were put on a diet restricted in long chain fatty acids and tube feeding overnight; the other patient was carefully followed-up.
Conclusion: Early detection by newborn screening is life-saving in VLCADD.

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Beta-ketothiolase deficiency: a case report

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Background: Beta-ketothiolase deficiency is a rare autosomal recessive disorder of isoleucine and ketone body metabolism. The disease is characterized by recurrent episodes of ketoacidosis.
Case report: We presented a twenty two months old boy who was admitted to the hospital with severe acidosis and dehydration because of vomiting induced by protein intake. He had three episodes of severe acidosis and required admission to hospital and parenteral alkaline fluid therapy. He was lethargic and ketoacidosis was suspected because of the acetone odor. Urine ketone was 4+. Blood glucose, ammonia, lactate and pyruvate levels were normal. He was treated with intravenous alkaline fluid therapy and then oral Scholl solution was given. Branched chain amino acid levels were elevated in his blood sample. 3-OH butyric acid, acetoacetic acid and 3-OH isovaleric acid levels were eleveted in the urine organic acid analysis. The result of the enzyme assays in lymphoblast homogenates provided no indication for the ketolysis defect of succinyl–CoA transferase deficiency. Genetic analysis for ACAT1 gene showed compound heterogenous mutation as p. D317N (c.949G>A)/p.D317D (c.951C>T).
Result: p. D317N (c.949G>A) mutation was not identified previously in the literature.
Conclusion: Beta-ketothiolase deficiency is a life-threatening treatable illness and death has been reported. It should be considered in the differential diagnosis of ketoacidosis.

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Challenges in the diagnosis of methylacetoacetyl-coenzyme A thiolase (beta-ketothiolase) deficiency

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Methylacetoacetyl-coenzyme A thiolase deficiency (MATD) is a disorder of ketone body utilization and isoleucine catabolism. We report a 7-month-old Croatian girl who - following half a day with little food intake - presented with weakness, hypotonia, tachypnea, tachycardia and dehydration. Due to life-threatening respiratory failure mechanical ventilation was required. A metabolic acidosis (pH 7.1, BE −22.2) with low blood glucose (2.5 mmol/l and elevated 3-OH-butyrate (6.3 mmol/l) was noted, while lactate and ammonia levels were normal. Urinary organic acids revealed massive ketonuria including a pronounced signal of 2-methyl-3-hydroxybutyrate, but only small/trace amounts of 2-methylacetoacetate and tiglylglycine. After stabilization, 8 hours of fasting resulted in normal

bicarbonate and 3-OH-butyrate. Although somewhat decreased, enzyme activity determined in fibroblasts under standard conditions was not considered strongly indicative for a ketolysis defect. However, ACAT1 gene analysis yielded compound-heterozygosity for mutations c.135_138delAAGT (predicted to result in a frameshift and an early stop of translation) and c.890C>T (p. Thr297Met; known to yield some residual activity). Our results support the view that a mild biochemical phenotype with residual MAT activity can nevertheless result in severe ketoacidotic crises. Even if not all characteristic metabolite abnormalities are identified, ketolysis defects should be included in the differential diagnosis of unexplained ketoacidosis.

P-249

The long-chain acyl-CoA dehydrogenase KO mouse has a fasting-induced defect in anaplerosis: Therapeutic implications

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Background and objectives: It has been hypothesized that the cardiac and muscle pathology in long-chain fatty acid oxidation (FAO) disorders is a result of leakage of catalytic intermediates from the citric acid cycle (CAC). This has instigated the development anaplerotic triheptanoin therapy. We aimed to provide formal proof that there is depletion of CAC intermediates in a mouse model for long-chain FAO disorders, with the goal to develop a preclinical model to test the effectiveness of triheptanoin.
Materials and Methods: Fasting tolerance tests, and levels of CAC intermediates and circulating amino acids in the long-chain acyl-CoA dehydrogenase (LCAD) KO mouse.
Results: LCAD KO mice have a rapid onset of hypoglycemia upon fasting. This is caused by an inability of the liver to increase gluconeogenesis due to a shortage in the supply of amino acids. During fasting, amino acids serve not only as gluconeogenic precursors, but also as the major source of anaplerosis as evidenced by depleted cardiac CAC cycle intermediates in the LCAD KO mouse.
Conclusion: The LCAD KO mouse displays a fasting-induced defect in anaplerosis, which may be caused be decreased protein mobilization. The LCAD KO mouse is a suitable model to test the effectiveness of anaplerotic triheptanoin therapy.
Conflict of Interest declared.

P-250

Lipotoxicity in fatty acid oxidation disorders

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Inborn fatty acid oxidation (FAO) disorders are characterized by specific deleterious gene variants, which may lead to the production of dysfunctional and unstable proteins with a resultant reduction of enzymatic activity. Alternatively nonsense mRNA is degraded by nonsense-mediated decay or leads to production of truncated proteins. This gives rise to three plausible disease causing factors in FAO disorders namely: insufficient energy production, cellular disturbance resulting from stress induced by missfolded proteins or nonsense mRNA and toxic effects from accumulation of excessive concentration of specific lipid species and their metabolites (lipotoxicity). In this work we show how the presence of long chain fatty acids sensitizes Very Long Chain Acyl-CoA Dehydrogenase (VLCAD) deficiency patient fibroblast to metabolic stress factors. The presence of excessive lipids during metabolic stress results in ROS production, disruption of mitochondrial function and reduced viability. This emphasizes the requirement for effective therapeutic methods for metabolizing or detoxifying particular fatty acids, as well as supplying energy producing substrate to the patient in sufficient quantities. In addition it opens the possibility of screening specific FAO genotypes in cases where there is still uncertainty as to which of the three above mentioned factors should constitute targets for development of novel therapies.

P-251

Favourable outcome after physiological dose of sodium-d,l-3-hydroxybutyrate in severe multiple acyl-CoA dehydrogenase deficiency

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Background: So far, the cornerstone of the treatment of Multiple Acyl-Coenzyme A Dehydrogenase Deficiency (MADD) consists of dietary fat and protein restriction, along with supplementation of riboflavin, glycine and L-carnitine. Experiences with sodium-d,l-3-hydroxybutyrate (3-HB) treatment are limited and the general outcome of severely affected MADD patients is relatively pessimistic.
Case report: We present an infant with MADD in whom the previously reported dose of 900 mg/kg/day 3-HB did not prevent an acute severe, metabolic decompensation or the development of a progressive cardiomyopathy in the subsequent months. Median (range) concentrations of acetoacetate and beta-hydroxybutyrate increased to measurable concentrations of 0.10 mmol/L (0.02-0.29) and 0.15 mmol/L (0.02-0.46), respectively, only after a physiological dose of 2600 mg/kg/day of 3-HB. The clinical course, as well as NT-proBNP and echocardiographic parameters subsequently improved. Long-term studies are warranted on 3-HB treatment in MADD patients.

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Pediatric reference values of plasma acylcarnitines in supervised clinical fasting studies

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Background: Childhood fasting intolerance is a common problem and can be a presenting symptom of Inborn Errors of Metabolism (IEM). Obtaining critical plasma samples for acylcarnitine analysis can be helpful to determine the etiology of fasting intolerance. To date, there are no pediatric reference values of individual plasma acylcarnitines upon fasting.
Methods: Retrospective investigation of individual plasma acylcarnitines during supervised clinical fasting studies in the Beatrix Children's Hospital, University Medical Center Groningen between January 2005 and September 2012 (n=139). Exclusion criteria were: established diagnosis of a metabolic or endocrine disorder, incomplete data of plasma acylcarnitines, 18 years of age or older, having undergone multiple clinical fasting studies. Data analysis was performed using Graphpad (n=75). Paired t-tests and Wilcoxon matched pairs tests were performed.
Results: The following acylcarnitines increased significantly upon controlled fasting: C2-, C6-, C12-, C14-, C16-, C12:1-, C14:1-, C16:1-,

C18:2-, C18:1-acylcarnitine. In contrast, C0- and C3-acylcarnitine decreased significantly.
Conclusion: Determined reference values are essential for correct interpretation of supervised clinical fasting studies. Moreover, they are useful to interpret plasma acylcarnitine profiles from a critical sample of a patient with fasting intolerance of unknown etiology, to prevent planning of an invasive clinical fasting study.

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Short-term follow-up in Polish patients with VLCAD deficiency detected by MS/MS screening

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Background: Very-long-chain acyl-coenzyme A dehydrogenase deficiency (VLCADD) manifests in 3 phenotypes: a severe early-onset form, a milder childhood onset and adult onset form.
Patients and Methods: Retrospective analysis of 15 cases (aged: 2 months-7 years) detected in neonatal period in Poland since 2006.
Results: In the study cohort VLCADD was detected post mortem in one child, and then confirmed in his younger brother, one patient was symptomatically diagnosed (apathy and hypoglycemia, liver dysfunction) and one identified as abnormal due to maternal VLCADD. Newborn screening results of the remaining 12 cases revealed C14:1 in range 0.98-7.2 μmol/L. VLCAD activity was 8.7-30.4 % of mean value in healthy individuals. DNA analysis was available in 7 cases; p. Val283Ala (legacy name V243A) mutation was detected on 7 alleles (2 homozygotes and 3 heterozygotes). Early dietary intervention (LCT restriction/MCT supplementation) was introduced in 9 patients. Among them only one experienced a severe decompensation at age of 13 months.
Conclusion: Patients with VLCADD detected by NBS may remain asymptomatic with preventive measures. Enzymatic and molecular analysis should be helpful in adjusting treatment and outcome prediction. Our findings confirm that p. Val283Ala mutation is the most common mutation in patients detected by NBS, resulting in enzyme activity above 10 %.

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Medium-chain acyl-CoA dehydrogenase deficiency in a patient with a complex congenital heart defect

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Background: Medium-chain acyl-CoA dehydrogenase deficiency (MCADD) is an inborn error of mitochondrial fatty acid oxidation (FAO). Here we report a unique clinical course in MCADD affected newborn with a severe complex congenital heart anomaly.
Case report: Unexpected home birth of hypotrophic Romani (gypsy) girl was complicated by umbilical cord rupture and infected amniotic fluid. Medical rescue team transported the newborn to regional hospital. She was immediately referred to Pediatric Cardiac Centre with suspicion of cyanotic heart defect. At the first day of life, a complex congenital heart anomaly (double outlet right ventricle, transposition of the great arteries, ventricular septal defect) was disclosed. Successful baloon atrial septostomy was performed but ventricular arrhythmia occured. At age of 10 days, the girl was positively identified for MCADD by national expanded newborn screening. Our follow up biochemical testing revealed disease typical metabolites in dried blood spot, serum and urine. Molecular analysis confirmed homozygosity for prevalent mutation c.985A>G. Definitive heart surgery correction was planned later.
Conclusion: It was of great interest that this patient with MCADD and complex congenital heart defect survived without metabolic decompensation despite very critical conditions including interventional heart surgery.

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Pilot experience of an external quality assurance for acylcarnitines in serum/plasma

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Background: ERNDIM and CDC offer an external quality assurance (EQA) scheme for qualitative and quantitative acylcarnitines (ACC) in blood spots, respectively. However, ACC are usually determined in plasma/serum samples in some biochemical genetics laboratories. A pilot interlaboratory comparison experience between some European laboratories was initiated using serum/plasma samples from patients with a known diagnosis of organic aciduria or fatty acid oxidation (FAO) defect. Sixteen different samples with a short case report were circulated. Participants were asked to precise the method used to quantify diagnostic ACC, and to give reference values, possible diagnosis and advice for further investigations.
Results: Although the reference and pathological concentrations of ACC varied among laboratories, elevated marker ACC for PA, IVA, MCAD and MADD were correctly identified by all participants allowing the diagnosis of these diseases. Conversely, the increases of dicarboxylic acylcarnitines were not always identified and therefore the diagnosis was not correct as exemplified in cases of malonic aciduria and HMG-CoA lyase defect. Misinterpretation occurred in those labs that did not derivatize, separate isomers, or use MRM acquisition. However, some of these labs suggested further analyses to achieve the diagnosis.

Conclusion: This pilot experience highlights the importance of an EQA scheme for ACC in plasma.

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Patients with medium-chain acyl-CoA dehydrogenase deficiency experience oxidative stress – a metabolomic study

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Background and objectives: The pathophysiological mechanism underlying liver and skeletal muscle involvement in medium-chain acyl-CoA dehydrogenase deficiency (MCADD) patients is poorly understood. We used an untargeted metabolomic approach to identify biochemical changes in MCADD patients.
Materials/Patients and Methods: Dry blood spots of controls (n=25) and patients (n=25) were extracted by methanol/water (1/1, v/v). Supernatants were analysed by LC-MS method with detection by Orbitrap Elite operated in positive full scan mode at 120.000 resolution within range of 70–1200 m/z. Data were processed by R software using packages XCMS, CAMERA, muma (www.bioconductor.org) and pls (http://cran.r-project.org).
Results: Patients were clearly distinguished from controls in principal component analysis and loading plot derived from orthogonal partial least square discriminant analysis pointed at known medium-chain acylcarnitines (C6, C8, C10:1) as well as three phosphatidylcholines. Structures of these compounds were confirmed by multistage fragmentation as PAzPC (PC (16:0,9:0 (COOH))), PC (18:0,5:0 (COOH)) and PC (16:0,8:0 (COOH)). In order to confirm importance of these compounds patient (n=25) and control (n=250) samples were measured by FIA-TMS (API 4000) method in MRM mode. Calculated p-values for PAzPC, PC (18:0,5:0 (COOH)) and PC (16:0,8:0 (COOH)) were 1.927e-14, 2.391e-15 and 3.354e-15 respectively.
Discussion/Conclusion: Elevated oxidized phospholipids show presence of oxidative stress in MCADD patients.

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Favourable long-term evolution of ACAD9 deficient patient treated with riboflavin

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The acyl-CoA dehydrogenase 9 is a recently identified acyl-coA dehydrogenase that demonstrates maximum activity with unsaturated long chain acyl-CoA. Here we report the long-term clinical evolution of one of the patients already reported by Haack et al. (2010). This is the second child of non consanguineous parents, his older sister died at 1 month from multiorgan failure due to severe metabolic acidosis and hyperlactatemia. Deficiency of complex I and V of the mithocondrial respiratory chain was diagnosed on muscle biopsy obtained peri-mortem. At birth our patient had hypoglycemia, severe metabolic acidosis and hyperlactatemia. A vitamin cocktail (riboflavin, thiamine, biotin, coenzyme Q) and carnitine was started at 4 days of life. Despite therapy he had mildly increased lactate (4–6 mM n.v.<1.8). At 3 months left ventricular hypertrophy was recognized. He had mild hypotonia, and a normal psychomotor development. ACAD9 deficiency was diagnosed and confirmed by molecular analysis (Phe44Ile/Arg266Gln) when he was 5 years old. From then on he was treated only with riboflavin. At 9 years he had normal growth (25th percentile for weight and height), he attended primary school with good performance. He had mild stable cardiomegaly with good cardiovascular function, brain MRI, abdomen ultrasound and DEXA were normal.

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Reliable diagnosis of carnitine palmitoyltransferase IA deficiency by plasma acylcarnitine profiling

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Background: Carnitine palmitoyltransferase IA (CPT-I) deficiency is a rare inherited disorder of the carnitine cycle (MIM #255120). Hypoketotic hypoglycemia, hepatic encephalopathy, hepatomegaly, seizures and coma are prominent presenting clinical features. Screening relies on plasma or blood spot free carnitine (C0) and acylcarnitine concentrations, but may be missed easily. The ratio of C0 over C16- plus C18-acylcarnitines seems to have high diagnostic value in blood spots. We investigated the diagnostic value of C0, long-chain acylcarnitines (LC-acylcarnitines: C16-, C16:1-, C18-, C18:1- plus C18:2-acylcarnitines) and the ratios C0/(C16+C18) and C0/(LC-acylcarnitines) in plasma.
Patients/Methods: We compared 9 acylcarnitine profiles of 4 CPT-I deficient patients with those of 4692 samples from patients suspected of or with an inherited disorder of metabolism. Ninety-five percent cut-off values were calculated for the mentioned markers. Sensitivity (Se) and Specificity (Sp) of the markers were calculated based on CPT-I patient samples and the whole patient population.
Results: C0 concentrations were normal in all patient samples. Se and Sp were lowest for C0 and highest for the C18-, C18:1 and sum of LC-acylcarnitines. Diagnostic accuracy significantly increased when combining the sum of LC-acylcarnitines with the ratio C0/(LC-acylcarnitines).
Conclusion: A combination of low LC-acylcarnitines with high C0/(LC-acylcarnitines) in plasma has high diagnostic value for CPT-1 deficiency.

P-259

Mitochondrial dysfunction in isolated long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency

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Isolated long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD) is a mitochondrial fatty acid β-oxidation disorder, which affects the oxidation of long chain fatty acids. LCHADD is clinically characterized by cardiomyopathy, hepatopathy and sometimes myopathy, neuropathy and pigmentary retinopathy. The main pathophysiological determinants are believed to be the resulting energy deficiency and the accumulation of toxic long-chain 3-hydroxy fatty acids. Nevertheless, we can still not explain why patients homozygous for the most common mutation may exhibit very different clinical phenotypes. In order to disclose the main molecular pathways modulated in LCHADD we applied a global proteomic approach to the protein profiling of mitochondria isolated from fibroblasts of two patients homozygous for the most common mutation (c.1528 G>C), with different clinical outcomes. Data reveal a metabolic reprogramming that results in a shift to glycolysis, modulation of apoptotic pathways and of the mitochondrial antioxidant defence system. The patient with the severe phenotype showed increased

ROS levels and MnSOD expression while the other showed borderline ROS increase and MnSOD down-regulation. Our data highlights the main molecular pathways modulated in LCHADD as well as the role of ROS and ROS buffering in phenotype severity.

15. Disorders of pyruvate metabolism and the Krebs cycle

P-260

Fumaric aciduria case

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Background: Fumaric aciduria refers to a mitochondrial disorder, due to lack of fumarase activity, and manifesting as progressive encephalopathy, hypotension, dyspnea, seizures and lactic acidosis.
Case report: − 10 days old, was examined in the ICU. History of the disease: 2 hours after birth dyspnea and diffuse hypotonia appeared. Hypotension increased, depression of reflexes, respiratory failure, cardiomyopathy, arrhythmia, hepatopathy, anemia (hemoglobin 88 g/L, platelets 86*109/L), hypoglycemia.
Examination: malnutrition, pallor, marbled skin, triangular face, flattened chest, valgus feet. Blood tests: lactate ↑2.63 mmol/l, ↑ammonia 122 μmol/l, ↑glutamate, ↑glycine, ↓tyrosine, ↓isoleucine, ↓tryptophan. Gas chromatography test: urine ↑↑fumarate 641.29 mmol/mol creat, ↑↑ lactate 2606.75 mmol/mol creat, ↑↑ oxoglutarate 3491.7 mmol/mol creat; ↑succinate. Periventricular leukomalacia.
The diagnosis: fumaric aciduria (fumarase deficit), secondary hyperammonemia. With a diet and metabolic therapy the child's condition has stabilized; hypotonia persisted, hypodynamy, cardiomyopathy.
Conclusion: Acute deterioration of the child after birth with the development of hypotension, cerebral depression, it is necessary to carry out tests to exclude congenital defect of metabolism, including mitochondrial dysfunction.

P-261

Ketone bodies: a therapeutic option to replace ketogenic diet in PDH deficiency?

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Background: Ketogenic diet is the first line therapy for dystonia and other features of PDH deficiency and intractable seizures in a number of disorders, including GLUT1 deficiency. The effects of ketogenic diet are supposed to partly be mediated by its ultimate metabolites, i.e., ketone bodies. Because of limitations of this high fat diet, we investigated if oral administration of ketone bodies (racemic 3-hydroxybutyrate) could effectively replace the ketogenic diet.
Results: In three patients with GLUT1 deficiency, progressive partial substitution of ketogenic diet with 3-hydroxybutyrate led to clinical deterioration in terms of seizures and myoclonus frequency. By contrast, two patients with PDH deficiency showed dramatic improvement in terms of reduced frequency of dystonic crises and fatigability. In both children, 3-hydroxybutyrate fully replaced the ketogenic diet. Ketone body levels correlated negatively with plasma lactate levels (r-squared=0.59). In fibroblasts from PDH deficient patients, administration of 14C-labeled 3-hydroxybutyrate increased CO2 production consistent with improved Krebs cycle activity.
Conclusion: These results strongly argue for a direct beneficial effect in energy metabolism for ketone bodies in PDH deficiency. In GLUT1 deficiency, the results are consistent with proposals that additional metabolic requirements (possibly Krebs cycle anaplerosis) and mechanisms unrelated to energy metabolism may be involved.

P-262

Ketogenic diet application in PDH deficiency during the course of 6 years

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Objective: To evaluate effects of a strict ketogenic diet (4:1) in a patient with X-linked PDH deficiency during the course of 6 years.
Case report: The disease revealed itself during the first months of age with psychomotor delay, infantile spasms, myopathy, swallowing difficulties and respiratory failure. Hyperintense lesions on T2-weighted head MRI were found. Activity of pyruvate dehydrogenase complex activity was found to be decreased in myocytes and skin fibroblasts. Diagnosis of pyruvate dehydrogenase deficiency was further confirmed by revealing missense mutation in PDHA1 gene. Ketogenic formula, sodium bicarbonate and a trial with thiamine was applied from the age of 9 months resulting in markedly improved general state, dysphagia, seizures, hypotonia and psychomotor development during the first 6 months. In biochemical parameters, the most pronounced improvement was observed on hyperlactacidemia, diminishing from maximal values of 15 mmol/l to normal ranges. Further improvements were considerably less after this initial period. Episodes of intercurrent ilnesses were complicated by marked deteriorations, however, regaining of lost skills was generally reported by parents with convalescence.
Conclusion: Ketogenic diet was successful during the course of 6 years in a patient with PDH deficiency.

P-263

Pyruvate dehydrogenase complex deficiency: characterization of variant proteins in a search for alternative therapies

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Pyruvate dehydrogenase complex (PDC) catalyzes the conversion of pyruvate to acetyl-CoA, a key reaction in aerobic metabolism. Three catalytic, one structural and two regulatory subunits are assembled in this mitochondrial complex. The α-subunit of the E1 component (α2β2) is pivotal for active and cofactor binding sites formation, being a target of tight catalytic regulation. Missense mutations in PDHA1, the gene encoding E1α, are the most frequent cause of PDC deficiency (PDCD), which displays a broad clinical spectrum.
Following recovery of a PDCD patient carrying the E1α p. R224G variant upon arginine aspartate intake, we undertook a biochemical and biophysical characterization of E1 variants. The recombinant proteins p. F205L, p. R224G, p. R349C and p. R349H (α and α+β) were expressed

in E. coli BL21 (DE3) in the absence or presence of arginine, in conditioned medium supplemented with thiamine, at 30 °C. E1α or E1α+β levels were assayed by immunoblotting, their conformational stability by differential scanning fluorimetry (DSF), and E1 activity by spectrophotometric method.
A beneficial effect of arginine was observed upon PDCE1 variants, translated in an increase in protein levels, especially p. R224G, and in E1 activity. DSF assays revealed that the impaired conformational stability of p. R224G was restored to WT levels in the presence of arginine.
PEstOE/SAU/UI4013/11FCT; SFRH/BD9172/12

P-264

The spectrum of pyruvate oxidation defects

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Pyruvate oxidation deficiencies (PODs) are frequent mitochondrial diseases (~20 % of patients). PODs are not only caused by PDHC deficiency but also by various disorders in the pyruvate oxidation route. From our experience and a literature survey, we found 557 patients with POD and a genetic defect. 76 % belong to PDHC (thereof 78.7 % PDHA 1, 9.5 % DLD, 6.4 % PDHX, 4 % PDHB, 1.4 % DLAT), 22 % to cofactors, 1.5 % to regulation and 0.5 % to mitochondrial pyruvate import. PODs are underestimated due to several reasons: Not all diagnostic centers measure PDHC routinely. Even the most frequent PDHA1 deficiency can biochemically be missed due to X-inactivation. Cofactor defects can be missed, since functional investigations of pyruvate oxidation are not performed routinely. More recently an increasing number of patients was diagnosed by genetic screening. PDHC deficiency including regulation and import affect mainly the glucose dependent CNS, PNS and skeletal muscle, other PODs with combined defects affect also other organs like heart, lung and liver. The spectrum of clinical presentation of PODs is still expanding. PODs are therapeutically interesting since PDHC can be bypassed with ketogenic diet and some cofactor deficiencies can be supplemented. Chaperone therapy and PGC1a stimulation is still a matter of further investigations.

16. Mitochondrial disorders: nuclear encoded

P-265

Neonatal liver failure due to deoxyguanosine kinase deficiency; a report of 4 patients

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Background: Hepatic involvement is a common feature in childhood mitochondrial disorders, particularly in neonates. Respiratory chain disorders may present as neonatal acute liver failure, hepatic steatohepatitis, cholestasis, or cirrhosis with chronic liver failure of insidious onset. In recent years, specific molecular defects have been identified, with the promise of genetic and prenatal diagnosis. Mutations in the DGUOK gene are the major causes of mitochondrial DNA depletion syndromes associated with hepatocerebral syndrome.
Case Reports: Our study describes the clinical and laboratory features of 4 infants carrying DGUOK mutations. The patients were 2, 2, 3 and 6 months of age and molecular genetic analysis showed homozygous c.34C>T (p. Arg12X), c.130G>A (p. E44K), c.679G>A (p. E227K), p. K236Afs*4 (c.706_707+2delAAGT) mutations respectively. Two mutations were novel. Common findings were progressive cholestatic liver failure, hypoglycaemia, hyperlactacidaemia, hypotonia, rotatory nystagmus, elevated serum tyrosine, alanine and ferritin levels. Cranial MRS showed elevated lactate peak in one patient. Liver tissue studies revealed: cholestasis, iron deposits, microvesicular steatosis and fibrosis/cirrhosis. All patients died with multiorgan failure.
Conclusion: Mitochondrial etiology should be suspected in infants/neonates with acute liver failure, hypotonia and lactic acidemia. DGUOK gene study should be performed in patients with hepatocerebral presentation and nystagmus. Early diagnosis is important for genetic counselling.

P-266

Dysphagia, malnutrition and gastrointestinal problems in carriers of the m.3243A>G mutation

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Introduction: Previous research has shown that dysphagia and gastrointestinal problems occur frequently in patients carrying the m.3243A>G mutation, however the exact frequency and severity have not been determined. We hypothesize that adult patients have an increased risk for malnutrition.
Methods: In this observational study we evaluated the presence of gastrointestinal problems and dysphagia in a cohort of patients carrying the m.3243A>G mutation. The severity of the general disease involvement was classified using the Newcastle Mitochondrial Disease Adult Scale (NMDAS). Gastrointestinal involvement, dysphagia and the risk for malnutrition were scored using the Gastrointestinal Symptoms Questionnaire and the Malnutrition Universal Screening Tool. The data are compared to healthy controls.
Results: Height, weight and BMI of these patients were lower than the national average ($p<0.05$). Seventy-nine patients (86 %) suffered from at least one gastrointestinal symptom, mainly flatulence, hard stools or bloating. Both frequency and severity of symptoms were significantly increased compared to controls. Forty-five percent of the patients reported (mostly mild) dysphagia. Solid foods cause more problems than liquids.
Conclusion: Gastrointestinal problems and dysphagia are common in patients with the m.3243A>G mutation. The severity of gastrointestinal problems as well as disease severity is associated with a decreased BMI and risk for malnutrition.
Conflict of Interest declared.

P-267 Withdrawn

P-268

Whole exome sequencing confirms Leigh syndrome in a patient showing little biochemical evidence of a mitochondrial disorder

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Background: Leigh syndrome (LS) is caused by mutations in one of more than 30 genes; most of which are associated with the mitochondrial respiratory chain (MRC). Aim: To identify the genetic cause of disease in a patient with an overall clinical picture of Leigh syndrome.
Patient & Methods: A girl with clinically suspected diagnosis of LS was first hospitalized at 2 years because of febrile seizures and partial left ptosis, with subsequent left-sided arm and leg weakness, scoliosis and worsening dystonia at 6 years. Brain MRI showed symmetrical putaminal abnormalities, raised lactate on brain magnetic MRS, but normal in blood and cerebrospinal fluid. Whole exome sequencing (WES) and functional studies were performed to identify the causative gene.
Results: WES uncovered compound heterozygous mutations in the NADH dehydrogenase ubiquinone flavoprotein 1 [NDUFV1](c.1162+4A>C, which causes skipping of exon 8, and c. G640A; p. Glu214Lys), both previously associated with complex I deficiency and LD. Despite normal complex I enzyme activity in patient muscle, liver and fibroblasts, the protein level of NDUFV1 was significantly reduced by 75 % on Western blotting.
Conclusion: WES can be used to provide a definitive diagnosis of a suspected MRC disorder even in cases where MRC enzyme activity is apparently normal.

P-269

Whole exome sequencing identifies novel compound heterozygous mutations in PNPT1 in affected siblings with a mitochondrial phenotype

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Background: We undertook a gene discovery project using whole exome sequencing (WES) in a non-consanguineous family with two affected boys with severe intellectual disability, sensorineural deafness, optic atrophy, an axonal neuropathy and chronic lung disease.
Methods: WES was performed using the Illumina HiSeq2000 platform. Target regions were captured using the Agilent SureSelect Human All Exon 50 Mb Kit.
Results: WES identified compound heterozygous missense variations in exons 9 (p. Gln254Lys) and 19 (p. Ala510Pro) of PNPT1 (Polyribonucleotide nucleotidyltransferase 1) in both boys. PNPT1 encodes for the protein PNPase, which is involved in the transport of small RNAs to mitochondria. Mutations in PNPT1 have previously been reported to affect RNA import into mitochondria, mitochondrial protein translation, combined oxidative phosphorylation defects (OMIM 614932) and autosomal recessive deafness (OMIM 614934). Our variations were predicted to be damaging and in vitro studies revealed a clear reduction in PNPT1 protein and mRNA expression in patient fibroblasts. Furthermore, patient fibroblasts showed reduced mitochondrial respiratory chain complexes I and IV protein levels and enzyme activities, and a reduction in total mitochondrial protein synthesis.
Conclusion: Using WES, we have identified novel, probably pathogenic variations in PNPT1 in this family. Lentiviral rescue studies are being undertaken to provide further evidence of pathogenicity.

P-270

An early manifestation of LBSL (leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation) syndrome, case description

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Background: In leukoencephalopathy, mutation in gene DARS2 is associated with this syndrome. The gene is located on the long arm of chromosome 1 (1q 25.1), the disease is associated with deficiency of mitochondrial aspartyl - tRNA synthetase.
Case report: 1 year and 5 month old boy, with static kinetic and psychospeech development delay, excess body weight (16 kg). The child, from a first pregnancy complicated by a threatened miscarriage, was delivered at 36–37 weeks by caesarean section. Birth weight was 3300 g. Examination revealed decreased muscle tone, muscle strength, and absent tendon and periosteal reflexes, nystagmus, ataxia. ENMG – the neuropathic type of changes, myopathic syndrome. MRI of the brain - using T1, T2 and FLAIR- modes - white matter lesions of the brain and cerebellum. The karyotype - 46,XY. Phosphorylation rate was decreased - 111.8 mmol/min/mg of protein. Amino acid levels, blood lactate were normal. GC-MS analysis showed no evidence of an organic aciduria or a disorder of fatty acid oxidation. Partial analysis of the DARS gene by sequencing: in 5 gene locus - mutation c492+2 T-C in the heterozygous state.
Conclusion: In our case, the disease manifested in a child in whom we were only able to find a single mutation and who developed obesity by one year of life.

P-271

A new case with resistant hypoglycemia, hypertrophic cardiomyopathy, and encephalopathy due to mitochondrial TSFM gene defect

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The TSFM gene encodes the mitochondrial translation elongation factor Ts (EF- Ts). Our patient was diagnosed prenatally with hypertrophic cardiomyopathy and developed severe lactic acidosis and hypoglycemia at age 24 hours. Fatty acid oxidation defect (FAOD) was initially suspected and it was excluded as repeated investigations of acylcarnitine profiles that were found to be normal. He developed generalized muscle hypotonia, seizures, encephalopathy at age six days and one day later died due to respiratory failure. With these findings mitochondrial disease was clinically suspected and autopsy revealed cytochrome c oxidase deficiency in myocardial fibers, smooth muscle fibers and hepatocytes. A homozygous mutation c.997C>T; p. Arg333Trp in TSFM gene was detected by whole exome sequencing. Previously three cases were reported with the same mutation presenting different clinical features, one with hypertrophic cardiomyopathy, one with encephalopathy and one with liver dysfunction. Our case had an additional finding, resistant hypoglycemia. These findings suggest a broad clinical spectrum associated with mitochondrial translational deficiencies and that in the presence of such symptoms, if consanguinity exists, next-generation sequencing will be a

useful guiding method for the detection of mutations in nuclear genes causing mitochondrial diseases.
The study was supported by TÜBITAK (Project No 111S217).

P-272

Identification of a novel mitochondrial-tRNA modifier (MTO1) gene mutation by exome sequencing analysis

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The patient developed drowsiness, disturbance of gait, and seizures at the age of 18 months. She had reached appropriate developmental milestones for her age. At admission, physical examination showed mild cardiac murmur and hepatomegaly 2 cm below the right costal margin, hypotonia and decreased deep tendon reflexes. She had significant head lag.
Lab. studies: Blood pH 6.89 and HCO3 5.2 mmol/L, lactate 62.1 mg/dL, and pyruvate 1.12 mg/dL. Urine organic acids showed lactic ketosis with increased 3-OH butyric acid, 2-OH butyric acid, lactic acid and pyruvic acid, homovanillic acid and 4-OH phenyllactic acid (542 μmol/L). Acylcarnitine profile was normal. Cardiac investigation showed incomplete right bundle block on electrocardiogram and nonobstructive hypertrophic cardiomyopathy, decreased right ventricular function. Brain MRI showed generalised cortical involvement and minimal cerebellar atrophy. Mitochondrial enzyme diagnostics revealed low complex I, III and IV activity. Whole exome sequencing analyses revealed a novel p. R464C mutation in Mitochondrial-tRNA modifier (MTO1) gene. Exome sequencing analyses are very effective way for identification of disease causing genes and mutations in patients with rare genetic diseases. The study was supported by TÜBITAK (Project No 111S217).

P-273

A novel mitochondrial translation defect leading to deafness and cutis laxa is caused by mutations in a mitochondrial ribosomal protein subunit

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Mitochondrial diseases form a heterogenous group of metabolic disorders, caused by defects in nuclear or mitochondrial DNA, that lead to defective OXPHOS function. Mutations in numerous mitochondrial genes, mainly nuclear encoded, have been described to cause disease in humans. A relatively new subclass within this spectrum is formed by genes encoding mitochondrial ribosomes. So far defects in MRPS22, MRPS16 and MRPL3 have been identified. Although these defects are clinically heterogeneous, they all lead to combined complex deficiencies and in two of the three defects skin abnormalities have been shown. In a patient with progressive sensorineural deafness, cutis laxa, failure to thrive and normal intellect we have identified compound heterozygous mutations in a mitochondrial ribosomal protein subunit by using whole exome sequencing. We detected a combined deficiency in complex I, III and IV of the OXPHOS system in muscle, fibroblasts and liver. The phenotype in this patient is relatively mild and distinctive from all other mitochondrial defects. The wrinkled skin was similar to the progeroid skin findings in the mitochondrial PYCR1 defect. We suggest that cutis laxa could be a clue towards mitochondrial translation defects.

P-274

3-Methylglutaconic aciduria as a marker in mitochondrial syndromes

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Introduction: 3-methylglutaconic acid (3-MGA) is a biochemical marker for a heterogeneous group of 3-methylglutaconic aciduria syndromes and includes several IEM characterized by increased urinary excretion of 3-MGA. Several distinct types are described.
Patient 1: 4-year-old girl, IUGR, neonatal hypotonia, lethargy and weak reflexes progressing with severe failure to thrive, moderate psychomotor delay and spastic tetraparesis. Investigations disclosed hyperlactacidaemia and high L/P ratio and the organic acids mainly 3-MGA. MRI suggested Leigh syndrome. Respiratory chain showed decreased activity of several complexes. A leucine load revealed an increase in the levels of leucine and 3-MGA clearly not in favour of a type I defect.
Patient 2: A 20-year-old boy who presented with recurrent infections neutropaenia, dilated cardiomyopathy and weakness. He grew well, has cyclic neutropenia associated only minor infections and no myopathy or heart failure. Lactate levels are usually normal and organic acids show only slightly elevated levels of 3-MGA. Molecular studies of TAZ gene confirmed Barth syndrome.
Comments: Expanding clinical spectrum of the 3-MGA-uria types are described but excretion is unrelated to clinical severity or disease course. Probably OXPHOS dysfunction influences NADP-NADPH-dependent enzymes, such as the 3-methylglutaconyl-CoA hydratase and could explain the presence of 3-MG aciduria in some patients with mitochondrial disorders.

P-275

Ethylmalonic encephalopathy: a novel deletion mutation in the ETHE1 gene

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Background: Ethylmalonic encephalopathy (EE) is an autosomal recessive inherited disorder caused by mutations in the ETHE1 gene, and characterised by progressive encephalopathy, recurrent petechiae, acrocyanosis and chronic diarrhea. We here report a novel homozygous deletion of exons 5, 6, and 7 in the ETHE1 gene in a patient with EE.
Case Report: A 22 months-old-male patient from a consanguineous family presented with psychomotor retardation, hypotonia, chronic diarrhea, relapsing petechiae, and acrocyanosis. His older sister had died with similar clinical findings at the age of four years. Acylcarnitine analysis showed elevated butyrylcarnitine, isovalerylcarnitine, 3-hydroxyisovalerylcarnitine, and hexanoylcarnitine. Urinary organic acid analysis revealed markedly increased excretion of ethylmalonic acid, and moderately increased excretion of isobutyrylglycine, isovalerylglycine, 2-methylbutyrylglycine, and adipic acid. He died from cardiopulmonary arrest at the age of 23 months although oral metronidazole, and N-acetylcysteine treatments were given in addition to supportive therapies.
Conclusions: The ETHE1 gene contains seven exons. Frameshift, stop, splice site, missense mutations, deletion of exon 4, and whole gene deletion have been detected in patients with typical EE. We here describe a novel homozygous deletion of exons 5, 6, and 7 in the ETHE1 gene in a patient with classical findings of EE.

P-276

Normal amount of complex III subunits in three patients with TTC19 deficiency

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Background: Recently, TTC19 was reported as an assembly factor of respiratory chain complex III. So far, 10 patients from 6 families with TTC19-deficiency were reported. Most patients presented with slowly progressive olivo-ponto-cerebellar involvement.
Case report: We describe three boys from three unrelated families with TTC19-deficiency. Patient 1 was characterized by a neonatal onset and rapidly progressive course. Patients 2 and 3 showed a slower progression with cerebellar symptoms and mental retardation. Lactate elevation was found only occasionally, and was normal in CSF in patient 3. On MRI, all patients showed predominant bilateral basal ganglia and brain stem involvement.
Results: Muscle biopsy revealed isolated complex III deficiency in patients 1 and 2 (30 % of control values), but was normal in patient 3. Immunoblot analysis revealed a total loss of TTC19 protein in all patients. However, complex III subunits were found in normal amounts.
Conclusion: The three patients described here expand the clinical spectrum of TTC19-deficiency with regard to the early age of onset and the bilateral involvement of basal ganglia. Interestingly, TTC19 deficient patients can present with normal complex III activity and assembly.

P-277

Milder clinical course of TMEM70 mutation in two siblings

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The TMEM70 gene defect was recently identified as a novel cause of autosomal recessive ATP synthase deficiency. Here we report two siblings with ATP synthase deficiency who have been followed since birth after presenting with neonatal hypertrophic cardiomyopathy and developmental delay. They were born to consanguineous parents of Turkish origin. Both pregnancies were complicated by severe oligohydroamnios and IUGR during the third trimester. Laboratory investigations showed consistently elevated serum lactate fluctuating between 3.7-5.6 mmol/l (normal< 2). Biochemical investigations revealed significant amounts of urinary 3-methylglutaconic (3-MGC) and 3-methylglutaric (3-MGA) acids. In these siblings, we identified a previously reported homozygous splice site mutation, c.317-2A>G in the TMEM70 gene. In two siblings, there was no significant history of metabolic crisis. Although initial reports have illustrated that most of the affected individuals harbor the c.317-2A>G mutation mainly due to a founder effect in the Roma ethnic group, these two siblings who are carrying the same mutation are of Turkish origin.

P-278

A patient with cardiomyopathy and giant mitochondria caused by pathogenic mutations in CHKB

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Megaconial muscular dystrophy is a rare autosomal recessive disorder, leading to a delay of gross motor and speech development, caused by pathogenic mutations in the gene encoding choline kinase beta (CHKB). Half of the patients develop dilated cardiomyopathy. Microscopic examination of skeletal muscle shows a particular pattern of enlarged and peripherally displaced mitochondria. Evaluation of OXPHOS activities in skeletal muscle from previously reported patients has not shown a specific pattern of abnormalities. We report on a 10-year-old boy from consanguineous descent who presented with mild muscle weakness and progressive dilated cardiomyopathy palliated by heart transplantation. Molecular analysis revealed the presence of a homozygous nonsense mutation (c.248_249insT; p. Arg84Profs*209) in CHKB. Light microscopic examination of skeletal muscle showed the typically enlarged mitochondria displaced to the periphery of the cells. Evaluation of OXPHOS activities by BN-PAGE followed by in gel activity staining revealed normal activities in skeletal muscle and a generalized slight decrease of activities in heart muscle. In both tissues, the presence of subcomplexes of complex V was seen, probably resulting from disassembly of complex V. We conclude that this is the first report in heart muscle from a CHKB deficient patient showing disassembly of complex V due to dysfunctional phosphatidylcholine synthesis.

P-279

Nuclear-encoded defects of mitochondrial translation: clinical, biochemical and genetic heterogeneity

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Background: Nuclear-encoded defects causing impaired mitochondrial protein synthesis constitute a relatively new group of recessively inherited mitochondrial disorders.
Patients and Methods: Whole exome sequencing was used to identify nuclear-encoded defects of mitochondrial translation in 10 patients with heterogeneous phenotypes. We sought to understand genotype:phenotype correlations in this cohort using deep clinical and biochemical phenotyping, and expression profiling of genes involved in mitochondrial translation.
Results: We describe novel AARS2, GFM1, and RMND1 mutations and known ELAC2, MTO1 and c12orf65 mutations affecting mitochondrial translation at various steps. Affected patients show great clinical heterogeneity, but we note some clinical homogeneity amongst subgroups of patients sharing mutations in the same genes: cardiomyopathy (AARS2, ELAC2 and MTO1); Leigh syndrome (GFM1); peripheral neuropathy (c12orf65) and renal disease (RMND1).
Discussion and Conclusions: Multiple respiratory chain enzyme (RCE) deficiencies are regarded as the biochemical hallmark of mitochondrial translation defects, but in our cohort we observed some patients with

isolated deficiency of complex I or complex IV. Therefore, absence of multiple RCE deficiencies does not exclude a disorder of mitochondrial translation. We found whole exome sequencing to be a robust tool, which overcomes the ascertainment bias of phenotype to genotype diagnosis, and improves the diagnosis of complex, atypical cases.

P-280

A novel mutation in TTC19 associated with isolated complex III deficiency and bilateral striatal necrosis

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Background: Isolated complex III (cIII) deficiency is a rare cause of mitochondrial disorder. Excluding MTCYB, mutations in other structural cIII genes are rare. Instead several mutations have been described in the assembly factor genes BCS1L, TTC19 and LYRM7. TTC19 mutations have been reported in patients with heterogeneous phenotypes ranging from early onset neurodegenerative disorders to adult forms with psychiatric manifestations and cerebellar ataxia.
Case report: We report a boy born from consanguineous parents, with normal psychomotor development and mild language delay. At 4 years of age he presented gait ataxia. At 9 years, a brain MRI showed bilateral striatal necrosis. The disease worsened to the present condition characterized by tetraparesis, dystonia, severe cognitive impairment. Biochemical analysis revealed isolated cIII deficiency in muscle. The molecular tests showed a novel homozygous rearrangement in TTC19 (c.782_786delinsGAAAAG) resulting in a frameshift with premature termination (p. Glu261Glyfs*8). Western blot analysis demonstrated the absence of TTC19 protein in patient's fibroblasts.
Discussion and conclusion: We describe a novel deleterious mutation in TTC19, a nuclear gene rarely associated with mitochondrial disease to date. We confirm the heterogeneous clinical presentations of TTC19-mutated patients and suggest the TTC19 mutational screening in all patients with cIII deficiency, regardless of the phenotype.

P-281

Mitochondrial phosphate carrier deficiency: new patients, new phenotypes and increased 3-methylglutaconic acid

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Background: Recently, we described for the first time defects of the mitochondrial phosphate carrier in 5 patients with muscular hypotonia, hypertrophic cardiomyopathy and lactic acidosis. This carrier transports inorganic phosphate necessary for ATP synthesis in mitochondria. Here we add patients from 3 further families.
Case reports: Patient 1 is an adult female with reduced exercise capacity since early childhood with muscular hypotonia, hypertrophic cardiomyopathy and 3-methylglutaconic aciduria. Histological investigation of a muscle biopsy showed increased lipid content and high respiratory chain activities. One sibling died at age of 9 months. Patient 2 and 3, two boys both with an age of one year, also manifested with hypertrophic cardiomyopathy and generalized skeletal myopathy already from the neonatal period on.
Results: Exome sequencing revealed pathogenic mutations in SLC25A3, affecting the isoform expressed in skeletal and heart muscle in patient 1 and 2. Patient 3 had compound heterozygous mutations in parts of the protein, that are not spliced in a tissue-specific way.
Conclusion: We describe three further patients with a phosphate carrier deficiency, with novel mutations. The cardiomyopathy was critical in all patients during the neonatal period, but a favorable outcome with normal intellectual development was found in longer surviving individuals.

P-282

Leukoencephalopathy is a common finding in childhood onset mitochondrial disease

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Introduction: Grey matter abnormalities and stroke like lesions on neuroimaging are considered classical for mitochondrial disorders. We report neuroimaging findings in our cohort of children with suspected mitochondrial neurological disease.
Aim: To establish the frequency of white matter changes in our cohort of children with suspected mitochondrial neurological disease.
Methodology: We retrospectively reviewed the data of children with suspected mitochondrial neurological disorder over a 6 year period (April 2007–2013). Information on clinical, neuroimaging, biochemical and molecular genetic diagnosis was recorded.
Results: In our cohort of 108 children with suspected mitochondrial neurological disease, 47 (43.5 %) had white matter changes. 30/47 (63 %) had isolated white matter changes, 12/47 (25 %) had white matter and basal ganglia changes and 5/47 (10 %) had findings of atrophy and white matter involvement. White matter changes were predominant even in the group with confirmed molecular genetic diagnosis or respiratory chain enzyme abnormalities.
Conclusion: In our cohort of suspected mitochondrial disorders, neuroimaging demonstrated predominantly white matter abnormalities. The finding of leukoencephalopathy in a child with neurological and multisystem involvement should therefore prompt a thorough evaluation for mitochondrial disease.

P-283

A novel missense mutation in the AGK gene: Sengers syndrome

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Background and objectives: Sengers syndrome also known as cardiomyopathic mitochondrial DNA depletion syndrome-10 is an autosomal-recessive disorder characterized by cataracts, hypertrophic cardiomyopathy and myopathy. It is caused by lack of the mitochondrial protein acylglycerol kinase due to the mutations in the AGK gene.
Case report: Our patient was born to consanguineous parents after an uneventful pregnancy. Bilateral cataracts was noticed when he was five days old, and operated. His second problem failure to thrive was settled at the age of four months. When he was 7-months-old, routine echocardiography revealed cardiomegaly. Severe hypertrophic cardiomyopathy was firstly documented and was thereafter rapidly progressive. Muscular

hypotonia and motor retardation were detected. Routine hematological, biochemical, and metabolic investigations were within normal limits. Electromyography and cerebral MRI were also normal.
Results: During the etiological investigation, a muscle biopsy revealed fatty infiltration in the muscle fibers and myopathic changes. A novel homozygous missense p. K99N (c.297G>T) mutation detected in the AGK gene is compatible with the diagnosis of Sengers Syndrome.
Conclusion: We would like to report this patient in order to emphasize the severe clinical presentation with hypertrophic cardiomyopathy and to highlight this novel mutation as a cause of a rare disease, Sengers syndrome.

P-284

Mitochondrial disorders associated with mitochondrial DNA polymerase gamma mutations in a neurologic adult population

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Introduction: Human mitochondrial diseases associated with POLG mutations comprise heterogeneous phenotypes, from neonatal to adulthood onset, associated with different heredity patterns, mainly associated with different intergenomic signaling defects.
Case Reports: We present four adult patients with POLG mutations. P1 - 37 years-old female, presenting pan-dysautonomia, myoclonic epilepsy and muscular pain. She has heterozygous POLG mutation, c.1393G>A, and TYMP heterozygous mutation, c.3428A>G, associated with multiple mtDNA deletions and decreased OXPHOS activity of complexes III and II+III in muscle only. P2 - 43 years-old female, presenting progressive external ophthalmoplegia since 15 years-of-age, dilated cardiomyopathy, ovarian insufficiency and generalized mild myopathy. She has a heterozygous POLG mutation c.3708G>T (p. G1236H), multiple mtDNA deletions and combined OXPHOS deficiencies (I, IV and V). P3 - 39 years-old male, with mild abnormal mental development. He has two heterozygous POLG mutations, c.3428A>G and c.3708GG>T, multiple mtDNA deletions and complex II deficiency in muscle. P4 - 24 years-old male, with progressive muscular incapacity. He is tetraplegic, has sensory-motor polyneuropathy and progressive external ophthalmoplegia. He presents a heterozygous POLG mutation c.3708G>T, mtDNA depletion and complexes IV and V deficiencies in muscle.
Conclusion: Recessive POLG mutations are more frequent in childhood. In adult patients, heterozygous, single or combined, mutations are more common.

P-285

Mitochondrial disorder phenotype caused by compound heterozygous digenic mutations

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Introduction: Mitochondrial diseases associated with mutations in POLG gene include both early-childhood-onset and late-adulthood-onset diseases. Compound heterozygous mutations and digenic mutations have been identified in several diseases.
Case Report: We present a 37 years-old female patient with progressive symptoms since 21 years of age. She had pan-dysautonomia (cardiac, gastro-intestinal and urinary), severe muscular pains upon exercise and myoclonic epilepsy. Diagnostic tests revealed: electroencephalography - left frontal epileptic focus; MRI spectrocophy - normal; hyperlactacidemia 2.9; electromyography - abnormal sympathetic skin response. Mitochondrial studies in muscle showed decreased OXPHOS activity of complex III and II+III (37 % and 27 %, respectively, of mean control value corrected for citrate synthase). Genetic analysis revealed no point mutations and presence of multiple deletions of mtDNA, in muscle only. Analysis of nuclear genes involved in bigenomic interactions showed the presence of two heterozygous mutations: one in TYMP gene: c.1393G>A (p. A465T) and another in POLG gene: c.3428A>G (p. E1143G). Other possible disorders were excluded.
Conclusion: This patient has symptoms of pan-dysautonomia like MNGIE syndrome, plus other symptoms suggestives of mitochondrial disorders. According of Wang et al. 2011, the compound heterozygous digenic mutations described in this patient are probably pathogenic, acting synergistically and are causative of this phenotype.

P-286

Fatal outcome in 5 newborns with TMEM70 deficiency and severe persistent pulmonary hypertension (PPHN)

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Objective: In a recent study, a high incidence of persistent pulmonary arterial hypertension (PPHN) in patients with TMEM70 deficiency has been reported. There were five newborns affected by PPHN among nine detected TMEM70 patients (Catteruccia M. et al., Mol. Genet. Metab., 2014).
Methods: We eveluated clinical and laboratory data of five newborns of Romani origin with coincidence of PPHN and TMEM70 deficiency. All children were homozygous for c.317-2A>G mutation in TMEM70 gene.
Results: All five patients were born mildly premature with respiratory insufficiency after the birth. Four of them had hypertrophic cardiomyopathy. The clinical and echocardiographic findings confirmed the PPHN diagnosis. Sildenafil and surfactant was administered, whilst in 3 cases high frequency oscillation and inhaled nitric oxide were necessary. Haemodynamics needed to be stabilised by vasopressors. Severe lactic acidosis (plasma lactate 4.5-44.3 mmol/l) and hyperammonaemia (97–293 μmol/l) persisted. All children died whether in the acute phase of PPHN, or within 27 days of life, with constantly persisting signs of PPHN despite adequate management in the Intesive Care Unit.
Conclusion: We proved severe PPHN as a frequent complication occurring in TMEM70 deficiency. Due to high mortality in the neonatal period TMEM70 deficiency should be considered in any newborn with fatal PPHN.

P-287

Clinical, biochemical and histochemical diagnosis of mitochondrial respiratory chain disorders in Egyptian children

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Background and objective: Mitochondrial Respiratory Chain (RC) disorders are a growing group of disorders with a large variety of clinical presentations ranging from well defined clinical syndromes to non specific manifestations such as failure to thrive and seizures. This study aimed to describe the clinical, biochemical and histochemical spectrum of 9 Egyptian patients with confirmed mitochondrial RC disorders.
Patients and Methods: Fifteen patients clinically and radiological suspected to have mitochondrial RC disorders, were referred to the Inherited Metabolic Disease Unit laboratory, Cairo University Children's hospital. Using muscle biopsy homogenate, histochemical staining of cytochrome oxidase and succinate dehydrogenase and spectrophotometric assay of RC complexes were done.
Results: Nine patients were confirmed to have RC deficiency. Four patients showed marked complex I deficiency, one patient showed marked complex IV deficiency, one patient showed combined complex I and complex IV deficiency and 3 patients showed mild complex I deficiency.
Conclusion: The presence of 9 positive cases out of 15 cases confirmed to have RC deficiency points to the high prevalence of these disorders among Egyptian paediatric patients. With the advent of next generation sequencing technology, mitoexome and whole exome sequencing represents an appealing approach for elucidation of the molecular basis of these disorders among Egyptian patients.

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Mitochondrial neurogastrointestinal encephalomyopathy: case report of a new mutation and treatment with peritoneal dialysis

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Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is a rare autosomal recessive metabolic disorder caused by a deficiency of thymidine phosphorylase due to mutations in the nuclear gene TYMP. Thymidine phosphorylase deficiency leads to plasma and tissue accumulations of thymidine and deoxyuridine which generate imbalances within the mitochondrial nucleotide pools, ultimately leading to mitochondrial dysfunction. MNGIE is characterized clinically by leukoencephalopathy, external ophthalmoplegia, peripheral polyneuropathy, cachexia, and enteric neuromyopathy manifesting as gastrointestinal dysmotility. It lacks an established treatment and the prognosis is traditionally poor.
We present herein the case of a 9 year-old patient with MNGIE, discuss his clinical, radiologic, molecular features. He lost weight progressively, had persistent vomiting and gastrointestinal dysmotility. Genetic investigation identified a novel homozygous TYMP gene mutation (IVS6+ 1G>C). Peritoneal dialysis successfully treated our patient who had normal renal function during the follow-up period of 6 months. Peritoneal dialysis, therefore, should be considered especially in medically compromised patients as a supportive treatment to improve clinical conditions before allogenic bone marrow transplantation.

P-289

Neonatal polyuria and renal failure : new features of MEGDEL syndrome

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Background and Objectives: MEGDEL (3-methylglutaconic aciduria with deafness, encephalopathy, and Leigh-like) syndrome is a mitochondrial disorder recently associated with recessive mutations in SERAC1. Our objective was to describe new clinical features of MEGDEL syndrome.
Case report: This 6 year-old girl was the first child of consanguineous Turkish parents. She exhibited an acute neonatal deterioration with severe lactic acidosis and hepatic cytolysis. Initial evaluation revealed major polyuria and renal failure with 3-methylglutaconic aciduria. Symptoms and biological findings progressively improved with symptomatic treatment but lactic acidosis and high lactate to pyruvate ratio as along with 3-methylglutaconic aciduria persisted. At 8 months of age, a subacute neurological regression occurred with severe hypotonia, dystonia with extrapyramidal movements and failure to thrive. Brain MRI revealed necrotizing lesions in basal ganglia strongly suggestive of a Leigh syndrome. At 2 years of age, sensorineural deafness was documented. MEGDEL syndrome was suspected and sequencing analysis of SERAC1 identified an already reported homozygous mutation.
Discussion and conclusion: Neonatal polyuria and renal failure have not been reported to date in SERAC1 defective patients. Such neonatal kidney findings expand the clinical spectrum of MEGDEL syndrome.

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Pyruvate dehydrogenase E3-binding protein (PDHX) deficiency diagnosed by next generation sequencing

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Background: Mitochondrial disorders are difficult to diagnose due to genetic and phenotypic heterogeneity. Pyruvate dehydrogenase deficiency (PDH-D) causes neurologic dysfunction and lactic acidosis. Over 80 % of PDH-D cases result from mutations in PDHA1, the X-located gene for the E1-alpha subunit. Autosomal recessive deficiency of the E3-binding protein, encoded by PDHX, is rare. Establishing this diagnosis is difficult requiring enzymatic analyses, Western blot and molecular genetic confirmation. We report on a patient with this rare form of PDH-D diagnosed by next generation sequencing (NGS).
Case report of a now 20-months-old female with severe global developmental delay. This eutrophic and normocephalic full-term newborn was admitted on day 10 of life because of vomiting and failure to thrive. Muscular hypotonia, increased lactate in blood and CSF, and a lactate peak on spectroscopy indicated a mitochondrial disorder although MRI was otherwise unremarkable and no cardiomyopathy was found. A panel consisting of 174 nuclear encoded genes associated with mitochondiopathies was used. We identified homozygosity for a known PDHX mutation; both consanguineous parents were heterozygous.
Conclusion: This case indicates that NGS is a powerful diagnostic tool for patients with high suspicion for a mitochondrial disorder, allowing simple detection also of rare subtypes of mitochodriopathies.
Conflict of Interest declared.

P-291

Heterozygous PDHA1 mutations with presumed skewed X-inactivation are associated with clinical disease but normal PDH activity in muscle biopsy

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Objective: Pyruvate dehydrogenase (PDH) defects are often inherited X-chromosomally with mutations in PDHA1. Hemizygous males present usually with severe clinical phenotype and strongly decreased PDH activity in muscle biopsy. Heterozygous females are often asymptomatic but sometimes present with clinical symptoms of PDH defects, like lactic acidosis, neurological symptoms, and typical brain alterations on MRI.
Patients and Results: We diagnosed six female patients with heterozygous pathogenic mutations in PDHA1. Clinical symptoms were variable and comprised developmental delay, seizures, microcephaly, ataxia and lactic acidosis. Despite of the clinical symptoms, PDH activity in muscle biopsy was in the normal range in 5 of 6 patients. Fibroblasts were investigated showing skewed X-inactivation by immunostaining.
Conclusion: If clinical symptoms point to a PDH defect genetic testing is justified despite normal PDH activity in muscle biopsy. Investigation for skewed X-inactivation may be helpful to confirm the pathogenic significance of heterozygous PDHA1 mutations.

P-292

Mitochondrial disorders: the challenge of the diagnosis

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Background: Mitochondrial disorders are a heterogeneous group of defects affecting the energy generating system of body cells. The need for invasive procedures makes the establishment of a diagnosis a major challenge to clinicians.
Objectives: To gather clinical manifestations, diagnostic procedures and determine which factors contribute to definitive diagnosis in a cohort of Portuguese patients.
Materials and Methods: Retrospective study of 28 children (16 males) with mitochondrial diseases followed at a Portuguese Pediatric Department between 1999–2014.
Results: Symptoms began at a median age of 72 days [1–5400]. Neurological (n=26), cardiac symptoms (n=14), and failure to thrive (n=17) were the most frequent clinical manifestations. 13 patients had lactic acidosis and 12 had L/P>25. Brain images were abnormal in 16 patients (5 with spectroscopic lactate peacks). Muscle biopsy (n=27) revealed ragged-red-fibers (n=4) and subsarcolemnic aggregates (n=1). Respiratory chain complex (RCC) activities evaluation identified 11 isolated and 17 multiple RCC deficiencies. In 4 patients genetic confirmation was achieved.
Discussion: No reliable screening biomarker was found to establish definitive diagnosis. Identification of a molecular pathogenic defect can overcome the ambiguities often seen with biochemical and histological evaluations, although genetic analysis remains a challenge. Definitive diagnosis is important for treatment options and genetic counselling for affected families.

17. Mitochondrial disorders: mtDNA

P-293

Kearns Sayre Syndrome: Multiple ligation-dependent probe amplification (MLPA) detection of a large mitochondrial DNA deletion, missed by long-range PCR

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Background: Mitochondrial diseases are genetic disorders which cause respiratory chain dysfunctions. The complexity of the mitochondrial machinery and its dual genetic origin make them a heterogeneous group of illnesses with a challenging diagnostic approach. Genetic diagnosis starts looking for rearrangements in the 16.5 kb mtDNA. To detect deletions, long-range PCR has become the most popular method, replacing the use of Southern blot. MLPA is used to detect copy number variation in nuclear genes, but its use in mtDNA has not yet been applied widely.
Case Report: A Kearns Sayre Syndrome patient with a large mtDNA deletion that was missed by long-range PCR (because the primers used were located within the deletion) but detected by MLPA, with Southern blot and sequencing corroboration. MLPA results were analyzed using 3 internal control probes located in the minor arc for the normalization process, which increased the sensitivity of the method, finding the deletion in muscle and blood. The heteroplasmy level detected by MLPA was comparable to that measured by Southern Blot.
Conclusions: As shown in this report, MLPA with an enhanced normalization process, has advantages over other methods: low cost, easy methodology, primer set does not change outcome, accurate deletion localization, possibility to detect mutations in the same assay.

P-294

MELAS syndrome caused by mutation m.10158 T>C in mt-ND3 gene

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Introduction: MELAS syndrome (mitochondrial myopathy, encephalopathy, lactic acidosis, stroke-like episodes) is a progressive mitochondrial disorder with heterogeneous clinical features. It is caused by mutations in mitochondrial DNA.
Patient: The female now 25 years old, at the age of 20 the disease started with generalized tonic-clonic epileptic seizures, loss of vision and hearing, limb weakness and ataxia. Now the patient is wheel-chair bound and is presenting with head-tremor and episodes of status epilepticus. MRI shows lesions in the frontal, parietal, occipital, temporal lobes and in the

cerebellum. Lactate – 7 mM/L, serum level of fibroblast growth factor 21 was also high (>2000 pg/mL). Patient was negative for frequent MELAS mutations and for POLG gene mutations. Whole-mtDNA sequencing was undertaken recently using IonTorrent. We found de novo heteroplasmic m.10158 T>C mutation in the highly conserved region of the NADH dehydrogenase subunit 3. The level of heteroplasmy in blood cells was 30 %.
Conclusion: m.10158 T>C has been reported in association with Leigh-syndrome, but never linked with MELAS. Probably the low heteroplasmy level delayed the age of the manifestation in our patient and blurred the clinical picture. Our case shows the importance of mtDNA sequencing for the diagnosis of mitochondrial diseases, even when the phenotype is a quite recognisable mitochondrial syndrome.

P-295

Muscle mitochondrial enzyme activities normalized to mtDNA content are age-related and gender-specific

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Mitochondrial DNA content (mtDNA/nDNA ratio) was repeatedly found as a useful biomarker of mitochondrial biogenesis and it was suggested to be used as a normalization factor similarly to citrate synthase activity.
The aim was to characterize activities of cytochrome c oxidase (COX), succinate-coenzyme Q reductase (SQR) and citrate synthase (CS) in skeletal muscle normalized to mtDNA content (NA) with respect to the age and gender of analysed individuals.
Material and methods: 205 samples of muscle biopsies from mitochondrial disease free patients were included. Their age was between 2 weeks and 78 years (98 females, 107 males). mtDNA was quantified by qPCR. COX, SQR and CS activities were measured in isolated mitochondria spectrophotometrically. Data were analyzed by Kruskal-Wallis (KW), Wilcoxon and Spearman (S) correlation tests. Results: Significant positive correlation between COX, CS and SQR activity and mtDNA content was found (S test, p=0.000, p=0,000, p=0.008). In addition, negative age-dependent NA for COX, SQR and CS were found in males (KW test, p=0.010, p=0.005, p=0.013).
Conclusions: The changes in activities or respiratory chain complexes normalized to mtDNA content in muscle may reflect gender-specific changes of mitochondrial function during ageing.
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P-296

Molecular and cellular analysis in an unusual LHON case

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Background and objectives: Leber's Hereditary Optic Neuropathy (LHON) is a mitochondrial cytopathy characterized by optic atrophy due to retinal ganglion cell degeneration; additional neurological abnormalities may be found (LHON-plus). Our group reported (Grazina et al., 2007) a family with the primary mutation m.11778G>A in all members from the maternal lineage, but only the proband disclosed LHON-plus symptoms. This work aims to clarify the pathogenic mechanism underlying this mutation.
Patients and Methods: Analysis of the mitochondrial respiratory chain activity, mtDNA copy number, electron microscopy (EM) morphology and complex I assembly were evaluated, providing insights on the mitochondria function. Alterations in mitophagy and vesicle trafficking were also investigated using immunofluorescence.
Results: Reduction of mtDNA content was detected. Despite being fully assembled, activity of complex I is reduced and complex II is increased. Autophagy seems to be unaffected and mitophagy is under analysis. EM study showed unusual findings, suggesting organelle crosstalk anomalies. Protein intracellular trafficking defects are being characterized.
Conclusion: It was possible to rule-out assembly defects as the cause for complex I deficiency underlying the patient's phenotype.
This study was financed by "Fundação para a Ciência e a Tecnologia" (PTDC/DTP-EPI/0929/2012 and PEst-C/SAU/LA0001/2013-2014).

P-297

Study of mitochondrial DNA in the cerebrospinal fluid of mitochondrial patients

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Background and objectives: CSF mtDNA quantification has been recently described and detected as a biomarker of Alzheimer disease. We aimed to quantify CSF mtDNA in patients with mitochondrial disorders (MD).
Patients & methods: 24 paediatric patients fulfilling definitive MD criteria (12 genetically confirmed) were included. In patients and in a control population, CSF cell free mtDNA was measured using quantitative PCR as described (Podlesny 2013). Previous studies show normal CSF mtDNA values from 30 to 70 copies/μl.
Results: 15 patients had abnormal CSF mtDNA values. In 10 patients they were low (0-29/ul): 2 mtDNA depletions (POLG, SUCLA2), 3 mtDNA mutations (1 MELAS, 2 MERRF), 2 Leigh syndromes (LS), 1 mtDNA deletion, 1 multiple OXPHOS, 1 PDHE1. 5 patients had high values (91-156/μl): 2 LS, 1 NARP (mt DNA mutation), 2 severe encephalopathies. 13 out of 15 patients with abnormal values, had severe clinical courses and early death. Normal levels where found in 9 other diverse MD with better outcome and early death in only 2 cases.
Conclusion: Abnormal CSF mtDNA levels were related to severe clinical courses. The two patients with mtDNA depletion syndromes had also low CSF mtDNA values. Further studies are necessary to understand CSF mtDNA usefulness in MD.

P-298

Complex V investigation in frontotemporal lobar degeneration: molecular and biochemical analysis

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Background and objectives: Frontotemporal Lobar Degeneration (FTLD) is the second most common early-onset dementia. Mitochondrial DNA (mtDNA) mutations have been involved in neurodegeneration. Our study aims to sequence MT-ATP8 and MT-ATP6 genes and evaluate the involvement of ATP content and its correlation with OXPHOS complex V activity in FTLD.
Patients and Methods: A sample of 30 patients (14 females; age range: 38–82 years) with diagnosis of probable FTLD. Total DNA was extracted from blood and MT-ATP genes sequencing was performed. The complex V activity in lymphocytes was performed by spectrophotometry and plasma ATP concentrations were determined by bioluminescence. An age-matched control group was included. Statistical analysis was performed using Graph-Pad Prism 5.0.
Results: In 18 patients, 21 different alterations were identified, 10 missense (3 possibly deleterious). There is a negative correlation between ATP-synthase activity and ATP levels.
Conclusion: Our results report new data regarding mtDNA variations in FTLD patients. The negative biochemical correlation could be due to reversal ATPase activity, previously described. The present work supports the idea that bioenergetics impairment may be both the cause and the consequence of neurodegeneration in FTLD. Supported by "Fundação para a Ciência e a Tecnologia" (PTDC/SAU-EPI/121811/2010 and PEst-C/SAU/LA0001/2013-2014).

P-299

Respiratory complex IV deficiency associated with a homoplasmic novel m.8187G>A mitochondrial COII mutation

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Mutations in mitochondrial complex IV subunits are seldom found in cytochrome-c oxidase deficiency. We describe a case with hypotonia, developmental delay, brain atrophy, growth retardation and congenital lactic acidosis due to isolated complex IV deficiency. An apparent homoplasmy for m.8187G>A (100 % mutant; p. G201E) in muscle was detected in the mitochondrial COII gene by Sanger sequencing which was not previously reported. Glycine at amino acid position 201 of the COII protein is evolutionary conserved from yeast to human and is predicted to be damaging in silico. While mothers of affected offspring are usually heteroplasmic in mitochondrially inherited disorders, our proband's mother did not have the mutation in blood. This implies the possibility of either low level mutant heteroplasmy in the mother at a level beyond the detection limit of Sanger sequencing or a spontaneous de novo mutation in the proband; consistent with pathogenicity of the novel m.8187G>A sequence variant. Quantification by massively parallel NextGen sequencing could evaluate for possible low level heteroplasmy for this variant in the mother or less homoplasmy in her affected son.

18. Other disorders of energy metabolism, creatine disorders

P-300

Anaplerotic therapy using triheptanoin improves brain energy metabolism in patients with Huntington's disease

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Background: Energy deficit has been greatly implicated in the pathophysiology of Huntington's disease (HD). Our previous work has indicated a need to refill the Krebs cycle using anaplerotic therapies. We wished to obtain a proof-of-concept of the therapeutic benefit of triheptanoin using a functional biomarker of brain energy metabolism previously validated in HD.
Methods: 31P brain magnetic resonance spectroscopy (MRS) was coupled with the activation of the occipital cortex to measure the levels of phosphocreatine (PCr) and inorganic phosphate (Pi) before, during, and after a visual stimulus. We performed 31P brain MRS in 10 patients at the early stage of HD and 10 controls. HD patients were then treated for one month with triheptanoin.
Results: We confirmed an increased Pi/PCr ratio ($p=0.022$) during brain activation in controls – reflecting increased ATP synthesis – followed by a return to baseline levels during recovery ($p=0.008$). In HD patients, we confirmed an abnormal brain energy profile before treatment. After one month on triheptanoin, the MRS profile was greatly improved in HD patients with increased Pi/PCr ratio during brain activation ($p=0.004$).
Conclusion: This study suggests that triheptanoin is able to correct the bioenergetic profile in HD patients' brain at an early stage of the disease. Conflict of Interest declared.

P-301

Partial GAMT deficiency leads to axonal hypersprouting and natural cell death disruption via mild GAA accumulation in a 3D model of organotypic brain cell culture

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GAMT deficiency is the most severe of the creatine deficiency syndromes, characterized by creatine deficiency in CNS and guanidinoacetate accumulation. Every patient diagnosed so far was found with negligible GAMT activity (0-4 % residual activity). However, GAMT deficiency may be under-diagnosed if mutations allow sufficient residual activity to avoid creatine deficiency but enough guanidinoacetate accumulation to be toxic.
As neuropathological mechanisms are poorly known, we developed a new RNAi-induced GAMT-deficient model in 3D organotypic rat brain cell cultures by AAV2 transducing a GAMT-specific shRNA. Cultures were infected at DIV0 (AAV2/GAMT MOI: 1000), and followed during one month (harvests: DIV8, 18, 28).
RNAi led to 85 % decrease of GAMT protein, which was insufficient to generate creatine deficiency. However, this partial GAMT deficiency generated a mild guanidinoacetate accumulation intracellularly (45.7 versus 4.0 nmol/mg prot) and extracellularly (9.0 versus 0.9 μM), which led to axonal hypersprouting and decrease in natural apoptosis, followed later by induction of non-apoptotic cell death. All these guanidinoacetate-induced neuropathological effects were prevented by creatine co-treatment.

These findings show that mild guanidinoacetate accumulation without creatine deficiency is sufficient to significantly affect CNS development, and suggest that among GAMT deficiencies, more may be uncovered through guanidinoacetate increase without creatine deficiency.

P-302

Pharmacological inactivation of complex IV (COX) leads to decreased sirtuin levels

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Background: Sirtuins are a group of NAD^+-dependent deacetylases. In humans, seven different members with specific subcellular localisations (nuclear: Sirt1, Sirt6, Sirt7; mitochondrial: Sirt3, Sirt4, Sirt5; cytoplasmic: Sirt2) are known. Target proteins of the sirtuin family belong to signalling pathways, transcription factors, energy metabolism and ROS detoxification. Previously, we found significant reductions of Sirt1, Sirt3 and Sirt4 in fibroblasts from patients with mitochondriopathies. As mitochondriopathies are biologically heterogeneous diseases, we used a standardised pharmacological inactivation of cytochrome c-oxidase (COX).
Methods and results: Fibroblasts from healthy donors were incubated for 5 days with sodium cyanide and analysed at transcript-, protein-, and enzyme-levels, intracellular NAD^+ was measured spectrophotometrically. After COX-inhibition, Sirt1 and Sirt3 were significantly decreased at enzyme and transcript levels, to a lesser extent at protein level in a dose-dependent manner. Results were compared to cellular oxygen consumption.
Conclusion: Our results confirm a link between COX-deficiency and the (mitochondrial) sirtuin levels. This could be of relevance in mitochondriopathies, especially COX-deficiency and in healthy individuals under hypoxic conditions. Compromised sirtuin activities may protect the cell by limiting formation of oxygen radicals. In future studies we will examine if pharmacological manipulation of the sirtuins can restore COX-activity.
Conflict of Interest declared.

P-303

Transient massive D-lactic excretion in a newborn with multiple mitochondrial dysfunction

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A five months-old boy was admitted on the 9th day of life with transient hypoglycaemia, anaemia (Hb 8.6 g/dl) and persistent lactic acidosis (pH −6.97; lactate-15 mM), after progressive feeding difficulties and hypotonia. Treatment included antibiotics, cardiotonics, mechanical ventilation, inhaled nitric oxide due to pulmonary hypertension and blood transfusions. Causal congenital cardiopathy was excluded. Septic workup was negative. Acylcarnitine profile was normal. Huge amounts of D-lactic (>10,000 μmol/mol creat) and L-lactic (531), as well as 2-hydroxybutyric and other organic acids suggestive of mitochondrial dysfunction, were found. Muscle biopsy revealed many COX negative fibers and impairment of OXPHOS complexes IV, II+III and V (2.4 %, 18.8 %, 8.1 %, respectively, of mean control value corrected for citrate synthase) and pyruvate dehydrogenase deficiency (20 % of lower normal range). MtDNA deletions screening was negative. EEG showed burst-suppression pattern, although no seizures were noticed. Brain MRI was normal. Thiamine, riboflavin, biotin and ketogenic diet were tried, without success. On bicarbonate supplement, he has failure to thrive and axial hypotonia. D-lactic acid is no longer excreted. Known causes of D-lactic acidosis, including intestinal bacterial overgrowth, were investigated, but negative. Although a genetic diagnosis was not yet achieved, this patient certainly has a multiple mitochondrial dysfunctions syndrome, which requires further investigation.

P-304

U-guanidinoacacetate/creatinine ratio (U-GAA/crn) exhibits substantial U-pH and gender effects in contrast to U-creatine/creatinine (U-CR/crn)

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Background: The U-CR/crn and U-GAA/crn ratios used in screening for cerebral creatine deficiency syndromes (CCDS) show strong and distinctly different age dependence. By appropriate mathematical modelling these disease markers can be corrected for age and converted to z-scores, which are ideal to identify statistically and biologically significant relationships to other covariables.
Objectives: Examine the influence of U-pH and gender on the marker specific z-scores.
Material and Methods: Age corrected z-scores from 12480 reference individuals without known CCDS were obtained from five diagnostic laboratories. U-pH was available in 4821 samples that were selected for multiple regression analysis.
Results: The mean z-score for U-GAA/crn increased by +0.248 units for every unit increase in U-pH (P=8.8-40) and females showed on average + 0.231 unit higher z-score values than males (P=1.79-14). The corresponding coefficient for change in U-CR/crn was +0.064 with respect U-pH (P=5.00-4). For U-CR/crn the gender effect was only significant in adults.
Conclusion: U-CR/crn and U-GAA/crn have specific age profiles but also differ with respect to gender and U-pH. The gender effect may be related to muscle mass, and the U-pH effect may be due to different creatine and guanidinoacetate metabolism and transport within renal tubular cells.

P-305

Creatine transporter (SLC6A8) deficiency is amenable to treatment

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Background: Creatine transporter deficiency (CTD; SLC6A8 deficiency) is an X-linked inborn error of creatine metabolism characterized by reduced intra-cerebral creatine, intellectual developmental delay disorder, behavioral disturbance, seizures, and hypotonia. Treatment includes supplementation with creatine, L-arginine and/or L-glycine. Unlike other disorders of creatine metabolism, the efficacy remains controversial.

Objectives & Methods: We present our systematic literature review (2001–2013) comprising 7 publications, collectively describing 25 patients and 3 additional cases treated at our institution.
Results: Treatment regimens varied: creatine only (n=2); L-arginine only (n=7); combination (n=19). Median treatment duration 34.6 months (range 3mos-5 yrs5); evidence level IV. Ten patients (36 %) demonstrated response to treatment (increase in cerebral creatine and/or improved clinical parameters). All patients with increased cerebral creatine also showed clinical improvement. The majority of patients with clinical improvement had detectable cerebral creatine prior to treatment. 90 % of the patients who improved were initiated on treatment at age<9 years.
Conclusions: Acknowledging study limitations, we conclude that CTD is amenable to treatment—particularly in milder cases with residual brain creatine, and therefore probable residual protein function. We propose standardization of low threshold screening for CTD to allow early treatment initiation with standardized monitoring. High dose L-creatine is the mainstay; L-arginine/glycine should be considered.

19. Disorders of purines, pyrimidines and nucleic acids

P-306

Biochemical and genetic analysis of Cohen syndrome: an earlier diagnostic strategy

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Background and objectives: Cohen syndrome (CS) is a rare autosomal recessive disorder with several characteristic features such as obesity, mental retardation, distinctive facial appearance and ocular abnormalities. Broad clinical spectrum and the age dependent appearance of some clinical signs contribute to a delay in the diagnosis of most CS. The aim of this study is to find clues for an earlier diagnosis using metabolic analysis profile.
Case report: A 6 month old female patient came to medical attention because of global delay and congenital anomalies. General appearance of the patient was overweight with multiple skeletal defects. Cleft palate, micrognathia, frontal bossing, short extremities especially on the legs, small hands and feet, and short stature was noted.
Results: Chromosome study, array comparative genome hybridization, and tandem mass spectrometry for metabolic disease screening were normal. Metabolic defect workup was performed and plasma amino acid profile demonstrated increased beta-alanine, which was also reported in a CS patient in 1994. Whole exome sequencing revealed two VPS13B mutations and confirmed the diagnosis of CS. Conclusion: CS is related to pyrimidine metabolism and shows hyper-beta-alaninemia. Complete metabolic workup should be performed on all infants with global delay and skeletal abnormalities for an earlier diagnosis of CS.

P-307

Complete dihydropyrimidine dehydrogenase deficiency: Variable presentation in the Irish population

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Introduction: Dihydropyrimidine dehydrogenase deficiency (DPD), a rare inborn error of pyrimidine catabolism presents with a spectrum of clinical signs.
Methods: A retrospective review of 7 patients diagnosed with DPD in TSCUH, Dublin highlighting clinical presentation and outcome.
Results:
(1) 15 month old girl, presented with an episode of ketotic hypoglycaemia. (2) 4 day old girl, presented with focal seizures in the neonatal period. Her parents were consanguineous Irish Travellers. (3) 5 year old boy, presented with 3 episodes of hypoglycaemia during viral illnesses. He had mild dysarthria, otherwise was developmentally normal. Parents distantly related Irish Travellers. (4) 9 month old girl, presented with failure to thrive. Her twin brother unaffected. (5) 4 year old boy, presented with autistic features and seizures. (6) 6 month old boy, presented with a febrile seizure; he was developmentally normal. (7) Sibling of patient 6. Urine organic acids showed abnormal excretion of thymine and uracil.
Mutational analysis of the DPD gene confirmed 5 patients to be homozygous for IVS14+1G>A and two (Patient 6 and 7) were compound heterozygous for IVS14+1G>A and c.2846A>T. These cases demonstrate the heterogeneity of this disorder.

P-308

Intrastriatal administration of hypoxanthine alters bioenergetic in striatum of young and adult rats

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Lesch–Nyhan disease (LND) is an inborn error of purines metabolism caused by deficiency of enzyme hypoxanthine-guanine phosphoribosyltransferase, resulting in accumulation of hypoxanthine. Although, mechanisms of brain dysfunction in LND are poorly understood, it is believed that hypoxanthine may contribute to neurological damage found in patients affected. In the present study we standardized an ex vivo model through of the administration intrastriatal of hypoxanthine to Wistar rats. Using this model, we evaluated bioenergetic parameters (Complex II (CII), Succinate dehydrogenase (SDH), cytochrome c oxidase (COX), pyruvate kinase (PK), creatine kinase (CK) and ATP levels) in striatum of young and adult rats. Rats (21 and 60 days old) received hypoxanthine (10 mM) or saline (control) and were decapitated 30 minutes after.
Results showed that hypoxanthine injection to young rats promoted an increase in CII and SDH activities, while PK and CK's activities were decreased in striatum. In adult rats, hypoxanthine promoted an increase in CII and SDH activity and a decrease in COX and CK activities and ATP levels in striatum. These findings suggest that hypoxanthine promotes bionergetic misbalance that might be related, at least in part, to the pathophysiology of LND.
Supported by CNPq, FAPERGS and CAPES.

20. Lipid and lipoprotein disorders, porphyrias

P-309

Abetalipoproteinemia in two boys aged 10 years and 11 months and effect of therapy

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Two boys with ABL were diagnosed in gastroenterology department of Scientific Center of Children's Health in Moscow. The first one was born to consanguineous marriage in a family from Dagestan; the family's first child died at 2 months of age from severe diarrhea. Our patient received parenteral nutrition in infancy, and then his status improved on a low fat diet. He was late diagnosed at 10 years of age. He looked like 6y/o, suffered from recurrent bronchitis, rhinitis, keratopathy, atrophic gastritis, stage IV cirrhosis. Acanthocytosis 60-85 % in different fields of view was detected. Ophthalmologist confirmed retinitis pigmentosa. The second patient, an 11 month-old boy, weighed 3,2 kg, with severe protein-energy malnutrition like Kwashiorkor. He had severe ataxia, tremors, fatty, foul-smelling stools. and 20 % acanthocytosis. Consistent with severe weakness, ataxia, hyporeflexia, discoordination, diffuse muscular hypotonia, he could not hold his head. Lab data: hypocoagulation due to vitamin K deficiency, cholesterol less than 30 % below the lower limit (VLDL and LDL were ten fold reduced), hypotransferrinemia, hypoalbuminemia, and lymphocytopenia. Vitamin E deficiency was covered by fat soluble tocopherol; they also received vitamin K, B12, A, and D. Within 8 months our first patient had grown 8 cm, stopped hurting and improved generally. Within 5 months, our second patient achieved a weight of 8.5 kg.

P-310

Homozygous familial hypobetalipoproteinaemia due to a novel APOB gene mutation

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Homozygous familial hypobetalipoproteinaemia (Ho-FHBL) is a rare co-dominant disorder characterized by extremely low levels of low density lipoprotein cholesterol (LDL-C) and apolipoprotein B (apoB). Most patients with Ho-FHBL have mutations in APOB gene resulting in truncated apoBs. Some patients are asymptomatic, while others have fatty liver, intestinal fat malabsorption and neurological dysfunctions. An 11 year old boy was referred to our hospital due to elevated liver enzymes. Physical examination was negative. Parents were first degree cousins. Lipid profile was as follows: total cholesterol 71 mg/dl, HDL-C 58 mg/dl, LDL-C 10.2 mg/dl, triglyceride 11 mg/dl, VLDL-C 2.2 mg/dl, apolipoprotein A1 1.36 g/L (1.07 - 1.79), apolipoprotein B<0.229 g/L (0.55 - 1.4). Abdominal ultrasonography revealed grade I hepato-steatosis. The proband's parents and sister had reduced levels of LDL-C and Apo B. The analysis of APOB gene showed that the patient was homozygous for a novel nucleotide insertion in exon 26 (c.6714insT) causing a frameshift leading to the insertion of a premature termination codon in apoB mRNA. The translation product of this mRNA is a truncated protein of 2211 amino acids (vs. 4536 amino acids of the normal apoB-100). This truncated apoB, designated apoB-48.74, is a novel mutation.

P-311

Homozygous familial hypercholesterolaemia (Homo-FH) in Greece: Epidemiological data from 29 patients

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Homo-FH is a rare IEM. The mode of inheritance is mainly autosomal dominant with a frequency of 1:1.000.000 births. The most frequent gene causing FH is LDL–receptor deficiency, with about 1300 mutations known.

3012 FH families (clinical or molecular criteria) were screened for 3–5 continuous generations each, over a period of 24 years. The molecular analysis was performed by PCR and sequencing.

We present 29 patients in Greece (27 families) age 2–30 years old today. Age at diagnosis varied from birth till 6 years. Tendon xanthomas at the extremities were usually the first clinical symptoms (27/29) and corneal arcus appeared in 21 of them. Stenosis of aorta presented in 23/29 at the age of 6–8 years. Cholesterol levels ranged from 620 to 2,000 mg/dl. 56 % of the patients were compound heterozygotes. Eighteen mutations were found in the LDLR gene, the most frequent being Genoa, Afrikaner −2, Greece-2, Greece-1, San Francisco and Sicily. There was no consanguinity in the families and their origin was from different parts of Greece.

Homo FH is more frequent in the Greek population than expected (rate of births 100.000 per year) and probably it could be under-diagnosed as Hetero FH is in all countries.

P-312

Ichthyoses as inherited disorders of lipid metabolism

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Inherited ichthyoses represent large and heterogeneous group of disorders of epidermal cornification. Common forms include ichthyosis vulgaris (IV) and recessive X-linked ichthyosis (RXLI), with a prevalence of 1:250 and 1:4,000. Autosomal recessive congenital ichthyosis (ARCI) is rare with a prevalence of 1:200,000. Eight genes have been identified to be causative of ARCI, including TGM1, ABCA12, NIPAL4, CYP4F22, ALOX12B, ALOXE3, PNPLA1, LIPN, and CERS3. IV is caused by mutations in the FLG gene, which are inherited as an autosomal semidominant trait. In 90 % of RXLI patients, there is a deletion affecting the STS gene. This deletion can extend to adjacent genes, which may lead to more complex disease. Mentioned genes encode a wide spectrum of epidermal proteins, including enzymes of lipid metabolism.

Methods: Clinical, pathological (electron microscopy, immunohistochemistry), and genetic analysis of Czech patients with ichthyosis.

Results: Analysis was performed in 52 unrelated patients and causal mutations were identified in 39 of them. We confirmed ARCI in 28 patients: 12 patients had mutations in ALOX12B, 5 patients in ALOXE3, 5 patients in NIPAL4, 4 patients in CYP4F22, 2 patients in TGM1. We have 7 and 4 patients with mutations in FLG and STS, respectively. Funded by the projects NT14585-3 and CZ.1.05/1.1.00/02.0068

P-313

A novel disorder of phospholipids biosynthesis: expanding a new category of inherited metabolic diseases

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Introduction: Several syndromes with hypo/hypergonadotrophic hypogonadism and ataxia have been described, however there is a remarkable clinical heterogeneity among them.
Objective: To report a new phospholipid disorder in a Brazilian family caused by mutations in the neuropathy target esterase gene (Patatin-like phospholipase domain containing 6, PNPLA6) and examine its role in the metabolism of lysophospholipids. Methodology: Biochemical and molecular investigations were undertook in patients from a kindred affected by a complex neurological disorder, presenting mainly as a spinocerebellar ataxia with hypogonadism and blindness. After molecular analysis for known genetic spinocerebellar ataxias and exclusion of other inborn errors of metabolism (IEMs) associated with cerebellar disease, whole exome sequencing (WES) was performed.
Results: Mutations in the PNPLA6 gene were identified in all affected patients. They showed visual loss (chorioretinal dystrophy) accompanied by progressive cerebellar ataxia and primary hypogonadism. Brain MRI showed cerebellar and pons atrophy. Motor axonal neuropathy was identified in all of them.
Conclusions: Neuropathy target esterase (NTE) is a serine hydrolase located on the ER involved in the hydrolysis of lysophosphatidylcholine. Since lysophospholipids appear in different tissues and have many physiological roles, e.g. in myelination, NTE deficiency has profound biological consequences in the central and peripheral nervous system.

P-314

Outpatient human hemin (Normosang®) therapy for acute intermittent porphyria: Trials and tribulations

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Background and objectives: Acute intermittent porphyria (AIP) remains an orphan disorder. Heme therapy has been beneficial in reducing abdominal pain, peripheral neuropathy and neuropsychiatric symptoms however this therapy is provided only to inpatients in Ontario and other provinces in Canada.
Case report: Our metabolic clinic currently follows 10 patients with AIP. One patient a 52 years old man with AIP with R225X mutation has been started on Normosang® on a once in two weeks as outpatient therapy.
Results: Prior to initiation of Heme therapy our patient was hospitalized about ten to twelve times per year with acute symptoms of abdominal pain and psychiatric etiology. For 18 months he received 250 mg Normosang® monthly for 3–4 days as an inpatient. For the past ten months he has been receiving prophylactic Normosang® outpatient therapy at 250 mg every two weeks. He has not had any hospitalizations for acute porphyria episode since the prophylactic therapy.
Discussion/Conclusion: Prophylactic heme therapy has prevented acute symptoms however the ongoing management of AIP remains complex with the participation of several specialist services including gastroenterology, hematology, psychiatric, and neurology but there are few clinics dedicated to porphyria management.

21. Peroxisomal, sterol and bile acid disorders

P-315

Interleukin 1 blockade with canakinumab for hyperimmunoglobulin D and periodic fever syndrome

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Introduction: Hyperimmunoglobulinemia D and periodic fever syndrome (HIDS; MIM# 260920) is a rare autosomal recessive autoinflammatory condition caused by mutations in the MVK gene, which encodes for mevalonate kinase. There is no standard treatment for HIDS.
Case report: We report on a 2 year-old Austrian boy with recurrent episodes of fever, febrile seizures, arthralgias, and splenomegaly. Rash and abdominal pain were also seen occasionally. During attacks an acute-phase response was detected. Clinical and laboratory improvement was seen between attacks. These findings led to the tentative diagnosis of HIDS. Sequencing of the MVK gene showed a homozygous c.1129G>A (p. Val377Ile, also known as V377I) mutation in the child, while the healthy non-consanguineous parents were heterozygous. The mutation is known to be associated with HIDS. Therapy with nonsteroidal anti-inflammatory drugs during attacks had poor benefit. A further febrile episode resulted in status epilepticus. Treatment with canakinumab was initiated and a final dose of 4 mg/kg every 4 weeks resulted in the disappearance of febrile attacks and a considerable improvement of patient's quality of life during a 6-month follow-up period. The drug has been well tolerated, and no side effects were observed.
Conclusion: Treatment with canakinumab is a therapeutical option for patients with HIDS.

P-316

Infantile Refsum Disease: the influence of dietary management on plasma levels of phytanic acid

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Background: Infantile Refsum disease (IRD), characterized by early-onset neurologic and hepatic dysfunction, is the less severe phenotype of the Zellweger spectrum disorders (ZSDs). We describe the clinical course of a patient with IRD who has a mild increase in plasma very long chain fatty acids (VLCFA) levels but significantly abnormal plasma phytanic acid.
Case report: A girl, born from consanguineous parents, was referred at 3 years-old to the Medical Genetics consultation due to developmental delay, retinitis pigmentosa, sensorineural deafness, pyramidal syndrome, and cranio-facial dysmorphy. Neonatal hypotonia, jaundice and increased hepatic enzymes had been observed. Increased plasma levels of VLCFA, which were initially borderline, in combination with increased phytanic acid and pristanic acid levels and a deficient activity of dihydroxyacetone-phosphase acyltransferase in fibroblasts, confirmed the clinical diagnosis of ZSD. Nutritional advice and follow-up was proposed aiming at decreasing phytanic acid dietary intake. Plasma levels of phytanic acid were within the normal range, at seven and twelve months after starting the proposed dietary treatment, and the developmental evaluation revealed slight progress without regression.
Discussion: Although, after dietary treatment implementation, a biochemical improvement was observed, the long-term benefit of this approach remains to be elucidated.

P-317

Disruption of redox homeostasis provoked in vivo by phytanic acid in cerebellum from young rats

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Phytanic acid (Phyt) is a branched-chain fatty acid that accumulates in some peroxisomal disorders as Refsum's disease (RD) and Zellweger syndrome. Although affected patients usually present neurological dysfunction, including cerebellar symptoms mainly in RD, the pathogenesis of these disorders is still poorly established. Therefore, the aim of this work was to investigate the in vivo effects of an acute intracerebellar injection of Phyt on important parameters of redox homeostasis, namely thiobarbituric acid-reactive substances (TBA-RS) levels (lipid oxidation), sulfhydryl content (protein oxidation), reduced glutathione (GSH) concentrations and the activities of the antioxidant enzymes glutathione peroxidase, glutathione reductase, glucose 6-phosphate dehydrogenase, superoxide dismutase and catalase (antioxidant defenses) in rat cerebellum. Phyt increased TBA-RS levels, reduced GSH concentrations and changed the activities of the antioxidant enzymes. The data strongly indicate that redox homeostasis is disrupted in cerebellum in vivo by Phyt. It is therefore presumed that alterations of redox cellular status may represent a pathomechanism contributing at least in part to the pathophysiology of RD and other peroxisomal disorders in which this fatty acid accumulates.

P-318

Analysis of CYP27A1 gene mutations

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Background and objectives: Xanthomatosis cerebrotendinous (XCT) is a metabolic disorder caused by mutation in CYP27A1; 2q35 gene encoding sterol 27 – hydroxylase enzyme that acts in synthesis of bile acids. Its deficiency results in the accumulation of cholesterol and cholestanol. Treatment with chenodeoxycholic acid shows good results, with reversal of disease progression. The aim of this paper is to describe two siblings affected by the disease with the clinical characterization and genotype analysis.
Case Report: Two brothers, 31 y and 29 y, presented with a history of failure to thrive, chronic diarrhea, cataracts, learning disabilities, thickening of the Achilles tendon and neurological regression with pyramidal syndrome and global cerebellar syndrome. Brain MRI findings were hyperintensity in the lateral periventricular white matter and cerebellar dentate nuclei. Spectroscopy showed lipid peaks.
Results: CYP27A1 gene mutations were sought by direct sequencing using capillary electrophoresis. The two brothers were found to have 2 heterozygous sequence variations (p.T306M and c.1263+1 G>A), considered clinically important.
Conclusions: Being a metabolic disorder whose treatment can stop the progression of symptoms, it is very important to make early diagnosis to minimize the clinical and improve the quality of life of patients.
Conflict of Interest declared.

P-319

Easy and fast HPLC-MS/MS method for the determination of plasma pipecolic acid

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Introduction: Pipecolic acid (PA), is an important biochemical marker for the diagnosis of peroxisomal disorders. PA is also a factor responsible for hepatic encephalopathy and a possible biomarker for pyridoxine-dependent seizures (PDS). We present an easy and fast PA quantification method, by liquid chromatography-tandem mass spectrometry (HPLC-MS/MS), that involves no derivatization.
Method: Plasma sample (100 μL) is extracted with CH3CN (500 μL) containing Phenylalanine-d5 (PHE-D5) 2 mM as internal standard, vortexed and centrifuged. The supernatant is analyzed by HPLC-ESI-MS/MS (AGILENT 1200 and API3200) in the positive-ion mode using MRM scan type. HPLC column is a LUNA 3 m HILIC Phenomenex. Buffer A: Ammonium formate 5 mM; Buffer B: ACN/H20 90:10 containing Ammonium formate 5 mM. PA retention time is 4.77 min.
Result & Conclusion: Linearity, recovery, LLOD, LLOQ, accuracy and precision are R20.995 (range 0.005-30 mM), 93 %, 0.010 μM, 0.050 μM, CV% 1.6, CV% 3.2 intraday and CV% 3.4 interday respectively. Clinical validation is obtained analyzing 5 samples with peroxisomal disorders (range 14.6-37.1 μM; mean 23.7) and 25 controls (range 0.51-5.15 μM; mean 1.8). The method is rapid and easy therefore can be applied to monitoring PA using a small plasma sample.

P-320

Analysis of the profile of saturated and unsaturated VLCFA during LO treatment

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X-linked adrenoleukodystrophy (X-ALD/AMN) is a disorder of peroxisomal β-oxidation characterized by accumulation of very-long-chain fatty acids (VLCFA) in tissues which is associated with neurodegeneration nervous system. Oral administration of Lorenzo's Oil (LO 4:1, glyceroltrioleate/glyceroltrierucate) decrease serum VLCFA levels. The purpose of the study was to evaluate the profile of VLCFA in X-ALD patients on LO administration.
Patients and Methods: Eighteen patients with ALD/AMN received LO combined with low-fat diet. Serum VLCFAs were analyzed by GC methods.
Results and Conclusion: In 16 patients the serum VLCFA levels were normalized after six weeks of administration. Serum C26:0 levels were significantly reduced 46 % to 76 % and C24:0 - 45 % to 72 %.
Simultaneously, C24:1 (lignoceric acid) and C22:1 (erucic acid) were increased (average 360 % and 510 % respectively). The impact of such high levels of these acids is not clear for the clinical outcome of the disease. In 2 patients who did not use regularly LO only a slight lowering of VLCFA levels was observed of about 10 to 30 %.
Regular administration of LO reduces of the VLCFA to normal levels. Systematic monitoring of the profile of saturated and unsaturated VLCFA enables the assessment of the optimal dose of LO.

P-321

Contribution of sterol blood profile in a case of sitosterolemia

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A 8 years old girl, consulted in February 2010, because of joint inflammation symptoms. Childhood was without abnormalities. Her parents have hypercholesterolemia, the father and grandfather have diabetes mellitus, and the mother had gestational diabetes. The first hypothesis was juvenile arthritis, but treatment was ineffective. Quantification of cholestanol showed a high plasma concentration of 123 μmol/L. Treatment was started with chenodeoxycholic acid 500 mg/day, because of suspicion of cerebrotendinous xanthomatosis. The patient continued to have abdominal pain and diarrhea episodes. Moreover there was no abnormality in the CYP27A1 gene. So a plasma sterol profile was performed. Analysis shown a sitosterol concentration of 362 μmol/L. Sequencing of ABCG5 and ABCG8 genes has shown an abnormality in ABCG5 gene: a sequence variation (E452K) was found. Then the patient was treated with ezetimibe 10 mg/day, which led to the improvement of the clinical symptoms. In conclusion, a complete sterol profile is a promising approach, which provides more information to the clinician and so leeds to a better diagnosis.

P-322

Studies of fatty acid metabolism in peroxisomal disorders: some clues emerging using deuterated fatty acids as substrates

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Fatty acid (FA) β-oxidation in cultured fibroblasts has been used for the diagnosis of peroxisomal and mitochondrial defects. Objective: In order to follow up the FA degradation and elongation following the diagnosis of peroxisomal disorders (PD), we used a deuterated substrate, $C20d_{39}$, which can be metabolized by peroxisome and mitochondria.
Methods: Fibroblasts from peroxisomal patients (XALD, Biogenesis disorders (BD), peroxisomal β-oxidation defects (PBO)), CPT2 patient and controls were incubated 72 h in a media supplemented with $C20d_{39}$ and carnitine. Deuterated acylcarnitines and total deuterated FAs were determined in the media by ESI-MS/MS and GLC respectively.
Results: Saturated acylcarnitines $C26d_{39}$-C12days, although minor components respect to the total FAs, reflected β-oxidation as well as elongation from the substrate, showing increased C22days and C14days-C4days in XALD, decreased C16days and increased $C24d_{39}$ in BD and increased C26days and $C18d_{35}$-C14days in PBO. Moreover, the ratio $C22d_{39}$+ C24days/$C18d_{35}$+$C16d_{31}$ was altered in studied PD respect to control and CPT2; specifically the ratio C16days/$C20d_{39}$ was diagnostic for BD, C18days/$C20d_{39}$ for PBO and C14days/$C16d_{31}$ for XALD.
Conclusion: In addition to a specific marker for each PD, the most relevant events were the increased acylcarnitines C14days-$C4d_7$, indicative of secondary mitochondrial alteration in XALD, and the peroxisomal origin of increased acylcarnitines in PBO.

P-323

Zellweger syndrome spectrum: a report of 10 Tunisian patients

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Background: Zellweger Syndrome Spectrum (ZSS) is a group of inherited peroxisomal diseases that share similar symptoms but vary in severity. Aim: to study clinical characteristics and outcome of ZSS.
Methods: We report 10 cases of ZSS diagnosed in a Tunisian pediatric department.
Results: 8 patients had neonatal onset of symptoms and 2 cases presented psychomotor regression at the age of 3 and 10 months. All patients had severe hypotonia and 9 presented with seizures, the most common form was myoclonic seizures (3 cases). Characteristic dysmorphic features were found in 4 cases and hepatomegaly in 3. Nine patients had abnormal neuroimaging: hypoplastic corpus callosum in one case, hydrocephalus in 2 cases, white matter abnormalities in 5 cases and abnormalities of gyration in 1 case. Radiological examination revealed calcific stippling of the patellas in 2 cases. The very long chain fatty acids levels were increased in all cases. Dihydroxyacetone phosphate acyltransferase (DHPAT) activity was very low in 2 cases and molecular studies demonstrated a new mutation in PEX26 gene in 1 case.
Conclusion: The poor prognosis of ZSS incites pediatricians to consider this disorder in etiological investigations of early onset hypotonia even in the absence of typical clinical or imaging features.

22. Lysosomal disorders: mucopolysaccharidoses, oligosaccharidoses

P-324

Natural history and clinical assessment of Taiwanese patients with mucopolysaccharidosis IVA

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Background: Mucopolysaccharidosis IVA (MPS IVA) is a rare lysosomal storage disorder caused by N-acetylgalactosamine-6-sulfatase deficiency, which catalyzes a step in the catabolism of glycosaminoglycans, keratan sulfate and chondroitin-6-sulfate. This disease has a variable age of onset and rate of progression.
Methods: A retrospective analysis of medical records of 24 patients with MPS IVA (11 males, 13 females; current mean age±SD, 12.6±6.6 years; age range, 1.4-29.4 years) seen at 6 medical centers in Taiwan from January 1996 through June 2013 was performed. Results: Mean ages of onset of symptoms and confirmed diagnosis were 2.0±1.6 and 5.7± 4.5 years, respectively. The most prevalent clinical manifestations were kyphosis (100 %), pectus carinatum (96 %), and abnormal gait (93 %). Eight patients (33 %) experienced at least one surgical procedure with the most common being ear grommet insertion (25 %), adenoidectomy (17 %), and tonsillectomy (13 %). At the time of the study, 8 out of 24 patient (33 %) have died at the mean age of 17.2±7.7 years.

Conclusions: An understanding of the natural history involved in MPS IVA may allow early diagnosis of the disease. Adequate evaluations and timely management may improve clinical outcomes and quality of life.

P-325

MPS IV A - patient's profile - objective evaluation of the body stature

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Morquio syndrome A (mucopolysaccharidosis type IVA) is an autosomal recessive, life-limiting lysosomal storage disease characterized by deficient activity of the enzyme galactosamine-6-sulfatase. Body stature abnormalities are common initial presenting symptoms and may facilitate timely diagnosis.
Objective: To describe a typical phenotype of patient with MPS IV A.
Materials and Methods. The analysis of 11 somatometric and 14 craniofacial features was performed for 23 patients with MPS IV A (7 girls and 16 boys). All patients presented with typical clinical features of MPS and had a diagnosis of MPS type IV A confirmed by biochemical and molecular analysis. Two-tailed t-tests were used to compare mean values for body length and weight at birth between patients with MPS IVA with the general population. Individual anthropometric data were standardized in order to show the actual degree and direction of deviations.
Results: Mean values for body height and body weight at birth were greater than in general population. Dwarfism was a result of short trunk and short lower limbs. The head and neck was relatively long, tucked between the narrowed shoulders. Chest had typical features.
Conclusions: Characteristic body stature of MPS IV A patients allows to distinguish them from other mucopolysaccaridoses and bone dysplasias.

P-326

Birth body length and weight at birth in patients with MPS I, MPS II and MPS VI

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Mucopolysaccharidoses (MPS) are a group of lysosomal storage disorders leading to a short stature and severe joint and bone disease.
Objective: To compare mean values for birth body length and weight between patients with MPS I, II, VI and general population.
Material and Methods: A retrospective analysis of birth anthropometric data was performed for patients (n=103) with MPS I (18 boys); MPS II (56 boys) and MPS VI (11 boys; 18 girls). All patients had a diagnosis of mucopolysaccaridosis confirmed by biochemical and molecular analysis. Two-tailed t-tests were used to compare mean values for body length and weight at birth between patients with MPS I, II, VI and the general population.
Results: Mean values for birth body length and weight for all studied groups were greater than in general population. For body length the differences were statistically significant, while for body weight they were greater than in general population, but not statistically significant. The pathomechanism of this phenomenon remains unclear.
Conclusions: At the time of birth, all MPS patients were larger than the healthy population. High birth weight can be suggestive of MPS disease and should raise suspicion aiding in early disease recognition.

P-327

The Morquio A Registry Study (MARS): improving the understanding of Morquio A syndrome

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Morquio A syndrome (mucopolysaccharidosis IVA; MPS IVA) is an autosomal recessive lysosomal storage disorder with multisystem involvement. Enzyme replacement therapy (ERT) with elosulfase alfa has recently become available as a treatment option for this progressive disorder. The Morquio A Registry Study (MARS) was established to further characterize the natural history of Morquio A syndrome and to evaluate the long-term efficacy and safety of ERT. This voluntary multicenter, multinational, observational study will collect and analyze demographic, clinical and laboratory data from patients with a confirmed diagnosis of Morquio A syndrome for up to 10 years. Additionally, the long-term efficacy and safety of elosulfase alfa will be assessed in (i) patients who completed the extension study of the Phase 3 clinical trial (www.clinicaltrials.gov/; NCT01415427), and (ii) patients who completed a clinical trial for pediatric subjects less than 5 years of age (www.clinicaltrials.gov/; NCT01515956). The effects of elosulfase alfa on pregnancy, including maternal, neonatal, and infant outcomes, will also be monitored. Work is currently underway to recruit approximately 50 sites across 20 countries. Information from the Registry is expected to facilitate more uniform management and care of Morquio A patients.
Conflict of Interest declared.

P-328

Molecular testing of 163 patients with Morquio A syndrome (mucopolysaccharidosis IVA; MPS IVA): 39 novel GALNS gene mutations

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Morquio A syndrome is an autosomal-recessive lysosomal storage disorder caused by deficiency of N-acetylgalactosamine-6- sulfatase (GALNS) encoded by the GALNS gene. Mutations occur throughout GALNS and often are identified only in one patient/family, complicating mutation detection and interpretation.
7 laboratories reported mutations in GALNS from Morquio A patients and 2 clinicians directly reported molecular results. Individuals received a diagnosis of Morquio A prior to and independent of molecular results. GALNS mutations were checked against reported SNPs.
Molecular testing of 163 Morquio A patients identified 99 separate changes, of which 39 are previously unpublished, novel GALNS

mutations associated with Morquio A: p. Val16Glu, p. Leu36Arg, p. Asp40Asn, p. Val48Gly, p. Glu51Lys, p. Pro81Leu, p. Ala84Glu, p. Leu91Pro, p. Gly116Val, p. His145Tyr, p. Phe156Leu, p. His166Arg, p. Gly201Glu, p. Leu214Pro, p. Phe216Ser, p. Thr235Lys, p. Ser264Thr, p. Asn289Asp, p. Arg380Gly, p. Gly415Val, p. Ile416Thr, p. Pro420Arg, p. Ala492Thr, p. Gly500Ser, p. Cys507Phe, p. Glu126Ter, p. Trp141Ter, p. Tyr209Ter, p. Arg251Ter, p. Pro357ArgfsTer21, p. Leu372SerfsTer6, p. Tyr385Ter, p. Gln414Ter, p. Val427SerfsTer13, p. Glu477_Gln485del, Complex del-dup (duplication from intron 5-intron 9, deletion in intron 10), c.120+1 g>c, c.405_422+1del, c.758+4a>t. We also identified 26 SNPs.
Reporting Morquio A-associated GALNS mutations improves genetic counseling and diagnosis capability. We recommend also to molecularly test both parents. While molecular testing provides useful diagnostic and genetic counseling information, enzyme activity testing of GALNS, along with other enzymes, remains the standard for diagnosis of Morquio A.
Conflict of Interest declared.

P-329

Morquio A syndrome (mucopolysaccharidosis IVA; MPS IVA): A review of 277 gene mutations curated in a new GALNS locus-specific database

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Morquio A is caused by deficient activity of N-acetylgalactosamine-6-sulfatase (GALNS) resulting from mutations in GALNS . GALNS mutations are numerous and heterogeneous. To aid detection and interpretation of mutations, we summarize published mutations from 541 Morquio A patients, together with 81 published mutations not described as genotypes from individual Morquio A patients, and report a new public-access GALNS locus-specific database.
277 unique GALNS mutations were identified from 1091 alleles. Most alleles (79 %) are missense. Even the most frequent mutations are uncommon. The three most common alleles (R386C, I113F, G301C) together only represent 14 % of alleles. Significant geographical and/or ethnic origin-based allele frequency variability exists. 11 % of alleles are insertions/deletions, 6 % intronic, 4 % nonsense. Most Morquio A-associated mutations have only been reported 1–2 times. 48 % of patients are homozygousfora GALNS mutation, 39 % heterozygous, and 13 % have only one mutation detected.
Mutation detection and genotype-phenotype challenges are in part due to the heterogeneity of GALNS mutations and lack of multiple families with the same mutations. Parental testing is encouraged. Reporting new alleles facilitates distinguishing pathogenic from benign mutations. The standard for Morquio A diagnosis is still deficient GALNS enzyme activity measured in leukocytes or fibroblasts, together with normal control enzyme activities.
Conflict of Interest declared.

P-330

Molecular screening of common mutations in IDUA gene in patients with mucopolysaccharidoses I from Ukraine

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Introduction: Mucopolysaccharidoses I (MPS I) is caused by the deficiency of lysosomal enzyme α-L-Iduronidase. It appears as a result of mutations in the IDUA gene. The most common mutations in the IDUA gene causing MPS I are Q70X and W402X.
Materials and methods: Biochemical methods include Cetylpyridinium Chloride Precipitation Test, thin-layer chromatography (TLC) of GAGs and α-L-Iduronidase activity assay in blood leukocytes. Molecular methods include PCR followed by PDRF analysis of common mutations.
Results and discussion: The diagnosisof MPS I has been confirmed biochemically in 16 patients from different regions of Ukraine: 10 patients have Hurler phenotype, 1 patient – Hurler/Scheie and 5 patients – Scheie. The frequency of Q70X and W402X mutations was 41 % (13/32) and 9 % (3/32) respectively. These mutations have been found only in patients with the severe Hurler type of MPS I. In patients with mild Scheie and Hurler/Scheie types of MPS I the Q70X and W402X mutations have not been found. The comparative assessment of the common mutations frequency has been conducted with the population of Ukraine and other countries.
Conclusion: The Q70X and W402X mutations in the IDUA gene are the most common in Ukraine (50 %) and detected with frequency close to Europe and Russian Federation accordingly.

P-331

The G116V GALNS mutation is common in patients of Pakistani origin with mucopolysaccharidosis type IVa and is associated with a more severe spinal phenotype at presentation

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Objectives and Methods: In our centre, many MPS IVa patients are of Pakistani origin and harbour a common mutation. Patient records of 28 MPS IVa patients treated at our hospital between 2000–2013 were reviewed to assess if this mutation is associated with a more severe skeletal phenotype.
Results: 24/28 patients had Pakistani origin. 17 were homozygous for the G116V GALNS mutation and had family members from the Saleh Khana region of Pakistan. G116V-homozygous patients were diagnosed earlier than other patients: (median 16 m [index cases 23.5 m] vs 24 m [index cases 30 m]). First symptoms were recognised earlier in G116V-homozygous patients: (median 8.5 m vs 12 m). The lumbar spine was abnormal at presentation in all G116V-homozygous patients but only 77 % of others and required/requires surgery in 29 % of the G116V-homozygous patients compared to 9 % of others. Cervical cord compression occurred similarly across both groups but was recognised earlier (median 30 m vs 113 m) with earlier intervention (median 5y vs 10.5y) in the G116V-homozygous group. Four G116V-homozygous patients have died (median 8.6y) compared to one non- G116V-homozygous patient (at 23y).
Conclusions: The G116V mutation is common amongst MPS IVa patients who originate from the Saleh Khana region of Pakistan and is associated with a more severe spinal phenotype.

P-332

Intrafamilial variability in the clinical manifestations of Hunter syndrome: a preliminary analysis of brother pairs in the Hunter Outcome Survey (HOS)

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Background and objectives: Phenotypic variability has been reported among family members with Hunter syndrome. Data from the Shire-

sponsored Hunter Outcome Survey (HOS) were used to compare phenotypic features in brother pairs.
Patients and methods: As of January 2014, data were available from 77 patients aged >5 years at last visit, who had families with ≥2 brothers and were followed prospectively in HOS. Where families had >2 brothers, the two firstborn were included (70 patients, 35 pairs). Most patients (60/70) had received idursulfase.
Results: Median age at last visit for the older and younger brothers was 19.4 and 14.8 years, respectively. Most phenotypic features had the same status in both brothers, although several discordant pairs were identified. Cognitive status at last visit was the same in 27/34 pairs; the majority (19/27 pairs) were cognitively intact. In most pairs, behavioural problems, hyperactivity, hydrocephalus, sleep apnoea and CPAP/BiPAP use were reported as absent in both brothers. Hearing status was the same in 28/34 pairs (hearing loss in 18/28 pairs). However, hernia status differed in 19/34 pairs.
Conclusion: The status of some phenotypic features differed in several brother pairs. Analysis of these discordant pairs will provide further insight into intrafamilial variability in Hunter syndrome.
Conflict of Interest declared.

P-333

The natural history of growth in patients with Hunter syndrome: data from the Hunter Outcome Survey (HOS)

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Background and objectives: Normal/accelerated growth in early childhood and short final height are reported in patients with Hunter syndrome. We analysed the natural history of growth and related parameters, and the impact of disease severity, in a large group of individuals with Hunter syndrome.
Patients and methods: Natural history data from males followed prospectively in the Hunter Outcome Survey (Shire-sponsored, global, observational registry) not receiving growth hormone or enzyme replacement therapy, or before treatment start, (N=676; January 2014) were analysed (reference data: CDC, Nellhaus, Tanner).
Results: Analysis of all first-reported values revealed short stature by 8 years of age, with progressive growth impairment from ~5–6 years. Patients were typically heavier than peers until ~8 years; older patients often weighed less than peers. BMI was above average until ~14–16 years; head circumference was generally above average in patients of all ages. Consecutive measurements analysis found no evidence of a pubertal growth spurt. Logistic regression modelling suggested correlation between cognitive involvement (yes/no in HOS) and increased weight, BMI and head circumference, but not reduced height.
Conclusion: This description of growth and associated parameters in untreated patients with Hunter syndrome will be a useful basis for future analyses of treatment effects.
Conflict of Interest declared.

P-334

Classification of IDS gene mutations in patients in the Hunter Outcome Survey (HOS)

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Background and objectives: Many different mutations in the iduronate-2-sulfatase gene (IDS) have been reported in patients with Hunter syndrome. This analysis classified IDS mutations in a large number of patients enrolled in the Shire-sponsored Hunter Outcome Survey (HOS).
Patients and methods: IDS mutations in 324 unrelated males with Hunter syndrome (52 % with a severe phenotype, 48 % with an attenuated phenotype) were classified according to the latest Human Gene Variation Society nomenclature. In silico analysis was performed on novel mutations to predict whether they would damage iduronate-2-sulfatase structure.
Results: We found 192 different mutations spread throughout the IDS gene. Missense mutations were the most common (49.5 %), followed by small insertions/deletions (24.5 %), nonsense mutations (12.5 %), splice-site mutations (10.9 %) and gross deletions/rearrangements (2.6 %). Almost half of the mutations, found in 33 % of the patients, were novel; most of these were predicted to damage iduronate-2-sulfatase structure. Private mutations were found in 40 % of all patients; only 27 % had recurrent mutations (found in ≥7 patients). Genotype–phenotype correlation was feasible in 73 % of patients.
Conclusion: IDS mutations associated with Hunter syndrome are extremely heterogeneous. This analysis of IDS mutations in a large group of individuals with Hunter syndrome will facilitate investigation of genotype–phenotype correlations.
Conflict of Interest declared.

P-335

Optimisation of keratan sulfate separation by using peltier system following an improved rapid isolation of urinary glycosaminoglycans in small volume urine samples

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The high resolution electrophoresis (HRE) micro method described here enables a clear separation of keratan sulfate (KS) from chondroitin sulfate (CS) based on the effect of cold buffer using low voltage. For optimized precipitation of GAGs in urine 300 μg GAG containing urine sample volume is the most appropriate concentration that gives the best precipitation with 1000 μL cetylpridinium (CPC)/citrate buffer. GAGs HRE banding patterns are more clear, easily identified and provide a guide for the enzyme analysis in the MPS disorders. In order to maintain a cool medium, Peltier system is used. Cooling sides of the Peltier system are in contact with aliminium plate 20 X 30 cm where the electrophoresis tank is placed on. The system is installed with 6 peltiers/30 W supported by the 380 watt power supply. The temperature of buffer in the electrophoresis tank can be cooled down to 8–10°. This procedure allows GAG isolation and high resolution GAG electrophoresis to be easily performed in routine clinical diagnostic laboratories by giving us a distinct pattern of GAGs in MPS I, MPS II, MPS III, MPS IV, MPS VI cases.

P-336

Biochemical properties of acid α-glucosidase in dried blood spots: difference between healthy and Pompe disease individuals

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Acid alpha-glucosidase (GAA) is a lysosomal hydrolase that degrades glycogen molecules. When GAA activity is deficient, glycogen accumulates intralysosomally causing Pompe disease (PD). Early diagnosis of PD is an essential step to the treatment to be more effective. For this, we have developed screening methods which measure the activity of lysosomal enzymes directly on dried blood spots (DBS). The aim of this study was to characterize the biochemical and kinetic properties of GAA in DBS samples from healthy controls and PD patients and determine how precise the technique is through the coefficients of variation (CV). GAA activity was determined according Castilhos et al. (2011). The results showed: optimal pH=4.4, Km=10.2 mM, Vmax=11.2 nmol/h/mg prot and a significant reduction of activity since 20 min at 50 °C from controls and optimal pH=4.2 and a significant reduction of activity since 1 min at 50 °C from PD patients. Significant differences between groups were observed to optimal pH and thermostability. In addition, the CV values below 20 % were established for measuring GAA. The results here presented can certainly contribute to improving Pompe disease screening as well as initiating the investigation process, besides a more precise and reliable diagnosis.

P-337

Survival and causes of death in patients with Hunter syndrome: data from the Hunter Outcome Survey (HOS)

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Background and objectives: Survival and causes of death were compared in treated and untreated patients with Hunter syndrome using data from the Hunter Outcome Survey (Shire-sponsored, global, observational registry).
Patients and methods: This analysis included 647 patients followed prospectively in HOS who had received enzyme replacement therapy with idursulfase (Shire) for a median of 48.7 months, of whom 77 died after enrolment, and 131 untreated historical patients who died before enrolment (January 2014).
Results: The 50 % survival estimate was higher for treated (34.6 years) than untreated patients (13.6 years). Median age at death was 17.0 and 13.6 years, respectively. In the untreated group, age at death increased over time (median, 11.7 and 13.8 years in those who died 1950–1985 [n=29] and 1986–2010 [n=102], respectively). The most commonly reported cause of death in treated patients was respiratory failure (36.4 %), followed by 'other' (31.2 %). In untreated patients, pneumonia/chest infection was the most commonly reported cause of death (29.0 %), followed by 'other' (25.2 %) and respiratory failure (23.7 %).
Conclusions: Estimated survival was higher in treated patients than in untreated historical patients in this analysis. Developments in supportive care over the years may have improved management of Hunter syndrome, even in untreated individuals.
Conflict of Interest declared.

P-338

Identification of early manifestations predicting central nervous system involvement in patients with Hunter syndrome: data from the Hunter Outcome Survey (HOS)

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Background and objectives: The development of neurological manifestations associated with a severe phenotype can be difficult to detect in young children with Hunter syndrome. This analysis investigated early manifestations associated with development of central nervous system (CNS) disease.
Patients and methods: The prevalence of manifestations reported before 2 years of age was compared in 435 patients aged ≥5 years followed prospectively in the Hunter Outcome Survey (a Shire-sponsored, multinational, observational registry) categorized according to last functional classification by clinical impression as attenuated (normal/borderline, n=230) or severe (educable/trainable/profound, n=205; January 2014 data).
Results: Median age at onset of signs and symptoms was 2.4 and 1.3 years in the attenuated and severe groups, respectively. Some manifestations (both somatic and neurological) were reported in a greater proportion of patients before 2 years of age in the severe than the attenuated group. Other than cognitive problems, the greatest differences (≥10 percentage points) were in hyperactivity, behavioural problems, upper airway infections, nasal obstructions, rhinorrhea and coarse facial features.
Conclusions: This analysis identified some early manifestations that were frequently reported in children with Hunter syndrome who later developed CNS disease, and may, with the support of additional investigations, aid prediction of progression to severe CNS involvement.
Conflict of Interest declared.

P-339

Proficiency of mucopolysaccharidosis diagnostics in the ERNDIM urine MPS scheme

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Background. The ERNDIM Urine MPS scheme has started in 2010 and was established a full ERNDIM scheme in 2012. The aims of this External Quality Assurance (EQA) scheme are (1) to evaluate diagnostic proficiency in urine MPS screening and (2) to assist IEM diagnostic laboratories in improving or maintaining their skills in MPS screening.
Methods. In 2010–2013 we distributed 26 authentic urine samples, 21 MPS samples and 5 normal controls. Participants were asked to perform quantitative and qualitative GAG analysis, to interpret results and to give the most likely diagnosis. Results are scored by the scientific advisor.
Results. From 2010 to 2013, participation increased from 88 to 105 labs. The majority of the participants did report their results: 98/105 in 2013. Diagnostic Proficiency varied strongly across different sample types. Normal control samples were well identified with proficiencies of 85-90 %. Proficiency was generally acceptable (>70 %) in MPS I, II, severe III and VI samples, while relatively poor results were obtained for MPS IV (proficiency 56-64 %), mild III (49-69 %) and VII (14 %).
Conclusion. The ERNDIM urine MPS scheme demonstrates the need for improvement in urine MPS screening and has great educational value by providing positive urine samples from these rare disorders.

P-340

Novel splice mutations in IDUA gene in turkish patient with MPS type I

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Mucopolysaccharidosis type I (MPS I) is an autosomal recessively inherited lysosomal storage disorder, resulting from a deficiency of the glycosidase, α-L-iduronidase (IDUA) enzyme. Patients with MPS I are unable to degrade the dermatan sulfate and heparan sulfate, which causes the progressive storage of glycosaminoglycans within lysosomes. The aim of this study was to identify mutational spectrum of IDUA gene in Turkish patients.
Mutations were identified by direct DNA sequencing. Computational programs (ASSEDA and NetGene2) were used to predict putative effects of novel variations. In addition to previously described mutations, two novel missense alterations (c.793-5 C>A and c.793-6 C>G) were detected. Both mutations affect splicing mechanism between intron 6 and exon 7, and have a pathogenic effect. Segregation analysis revealed heterozygosity for family members.
Molecular characterization of the IDUA gene is important for achieving reliable diagnosis of clinical subtypes, improving prognostic prediction, providing accurate carrier detection and developing better therapeutic approaches.
This study was supported by the Scientific Research Unit of Aksaray University (Project No: 2012/05).

P-341

Follow-up study of developmental quotient in the patients with mucopolysaccharidosis type II severe form on treatment with enzyme replacement therapy and hematopoietic stem cell transplantation

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Objective: We analyzed the course of developmental quotient (DQ) in the patients with mucopolysaccharidosis type II (MPS II) trated by enzyme replacement therapy (ERT) or hematopoietic stem cell transplantation (HSCT) to evaluate the efficacy on the brain.
Methods: We collected the DQ records of MPS II severe form patients with HSCT or ERT. The observation periods were 5 years 5 months to 16 years 3 months and 3 years 6 months to 6 years, in HSCT group and ERT group, respectively. The correlation between chronological age and developmental age was analyzed by scatter diagram in each group.
Results: In the patients with ERT, deterioration of developmental age was observed after age five, which was similar to natural history. In HSCT treated patients, the scatter diagram was almost same as ERT patients. However, three out of 30 patients did not show deterioration even after age 5. The values of DQ of these patients before HSCT were 78, 50, 75, and none of them had either cortical atrophy or hydrocephalus.
Conclusion: HSCT is beneficial treatment for the patients with MPS II especially for the patients with brain involvement, when it is performed in early stage.

P-342

Neonatal screening for treatable lysosomal storage diseases on dried blood spots using LC-MS/MS

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Introduction: The interest in neonatal screening for LSDs has increased substantially because the need for early diagnosis improves the therapeutic efficacy of new developed treatments. Due to technological advances (Tandem Mass Spectrometry, MS/MS), the worldwide interest for neonatal LSD screening increased substantially. The results of pilot LSD screening studies show that the current clinical prevalences are underestimated.
Methods: The LC-based method has advantages for expanding the assays to include additional products and internal standards for multiplexing all nine currently available lysosomal enzyme assays in dried blood spots (Gaucher, Niemann-Pick, Fabry, Krabbe, Pompe, MPS-I, MPS-II, MPS-IVA and MPS-VI) as well as allowing other metabolites to be quantified. The method used is modified from Spacil et al., 2012, Clin Chem. Validation of this method to the full set of treatable lysosomal storage disorders has been performed in our laboratory. Case finding of known patients with LSDs is well established. A pilot study of screening in newborns is in progress.
Results: No lysosomal storage disorder could be found at present by screening 9,000 newborns in our population.
In summary, the LC-MS/MS method provides an approach to high-throughput multiplex screening for lysosomal storage diseases that can be implemented in current newborn screening programs.

P-343

Immunological evaluation of patients with mucopolysaccharidosis

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Background and objectives: Mucopolysaccharidosis (MPS) is a group of metabolic diseases caused by deficiency of lysosomal enzymes that degrade glycosaminoglycans. Recurrent respiratory infections are frequently reported in MPS patients. The aim of study is to evaluate immunologically MPS patients to clarify why they are prone to infections.
Methods: Eighteen MPS patients (mean age=13 yr, range 5–32 years) on enzyme replacement therapy (ERT), 88 % male were evaluated (type I=5, type II=9, type VI=4) by measurement of complete blood count and serum immunoglobulins and review of their immunization schedules (BCG, hepatitis B and rubella).
Results: All patients had previous history of pneumonia. Two patients had neutrophils lower than expected and all patients had adequate number of lymphocytes. All patients were vaccinated for BCG, however one patient had lymph node tuberculosis. Only one patient had IgG serum levels lower than 3rd percentile. Three patients had IgM levels on the 3rd percentile. Regarding the qualitative evaluation of immunoglobulins, 1 patient (5.5 %) showed no response to rubella vaccine and 10 (55 %) patients showed no response to hepatitis B vaccine.

Conclusion: The immunological evaluation of MPS patients is mandatory, especially for the high frequency of respiratory infections presented by them. Conflict of Interest declared.

P-344

Maroteaux - Lamy disease: the first case reported from Indonesia

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Background: Maroteaux - Lamy disease (mucopolysaccharidosis type VI) is a rare autosomal recessive lysosomal storage disorder caused by deficiency in arysulfatase B, leading to multiple organ and musculoskeletal abnormality. The objective of the study was to report clinical, radiological and biochemical findings of Marouteax –Lamy disease.
Case Report: A 5 year old boy was referred to our hospital for cholestasis and spondylitis. After complete examination, we also found dysmorphic face, progressive musculoskeletal abnormality, growth and developmental retardation. Growth and development was normal up to one year of age. Thereafter, progressive motor and speech delay started, continued with spasticity of extremities. Later on, the boy became jaundiced. Radiologic findings showed dysostosis multiplex, biochemical testing from Department of Medical Genetics National Taiwan University Hospital revealed an increased level of glycosaminoglycans in the urine and the absence of plasma arysulfatase B activity, confirming the diagnosis of Maroteaux –Lamy disease. Liver biopsy was not performed. Enzyme replacement therapy could not be given due to the cost.
Conclusion: This disease is a very rare case, and this is the first case reported from Indonesia. Prompt investigation to establish the diagnosis should be made, and if possible, start the enzyme replacement therapy.

P-345

Prenatal diagnosis of mucopolysaccharidoses in Egypt: Counseling aspects and diagnostic tools

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Objective: Prenatal Counseling for Mucopolysaccharidoses (MPS) diagnosis.
Methods: 106 pregnancies counseled in last 14 years. All have one or more affected sibling. MPS types were 39 (36,8 %) type I, 15 (14,15 %) type II, 14 (13,2 %) type III, 17 (16 %) type IVa and 21 (19,8 %) type VI. 63 couples counseled in one pregnancy and 20 couples 43 times in successive pregnancies, 18 in 2, one in 3 and one in 4 pregnancies. 91 (85,8 %) consanguineous marriages. 39 (36,8 %) have no normal children. 73 (68,9 %) have no normal boys. Prenatal diagnosis (PD) performed in 83 (78,3 %) pregnancies. Amniocentesis at 14–15 weeks in 39 (47 %) for glycosaminoglycans (GAGS) analysis by 2-dimensional electrophoresis. Chorionic Villus Sampling (CVS) done at 11–12 weeks in 44 (53 %) pregnancies to measure enzyme activity fluorimetrically. In 7 cases, amniocentesis was needed to verify diagnosis of borderline result (5 cases) or because villi not enough (2 cases). 23 (21,7 %) of 106 counseled were not subjected to PD. 14 (60,9 %) did not show up, 4 (17,4 %) had spontaneous abortion, 3 (13 %) came late, one (4,3 %) vesicular mole and one refused. Results: 59 (71,1 %) of these subjected to PD had a normal fetus and 24 (28,9 %) had an affected fetus.
Conclusions: MPS could be diagnosed prenatally. Glycosaminoglycans by 2-DEP is sensitive and accurate. Measuring enzyme activity in chorionic villi is recommended as CVS is done 3–4 weeks earlier than amniocentesis which is more favorable medically, ethically and psychologically.

P-346

Genotype-phenotype correlation in Turkish patients with Mucopolysaccharidosis type VI

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Background and Objectives: Mucopolysaccharidosis (MPS) type VI is a lysosomal storage disorder. Severe, intermediate and mild forms of disease were classified on the basis of the age of onset, the rate of disease progression and the extent of organ involvement. Although the genotype-phenotype correlation has been described for several mutations, it is far from complete. In this study clinical findings and molecular analyses were evaluated to establish genotype-phenotype correlations.
Patients and Methods: In our study, clinical, biochemical and radiological findings in 20 patients with MPS VI were studied. Molecular analyses of the ARSB gene was performed on genomic DNA from patients.
Results: All families have consanguinity. Among them, 16 (75 %) patients had the severe form, while 4 (25 %) patients were mild. Four missense (p.L321P, p.E390K, p.G79E, p.C192R), one frameshift (p.E346fsx13) and two nonsense mutations (p.R160X, p.R191X) were found. Two of them were novel mutations (p.G79E and p.E390K). The most prevelant mutation was p.L321P, accounting for 56 % of all mutant alleles. Patients homozygous for the p.C192R and p.G79E mutations had a mild form of the disease, while patients homozygous for p.R160X, p.R191X, p.L321P, p.E346Sfsx13, p.E390K mutations had a severe form.
Conclusion: In spite of differencies in clinical signs in siblings, disease subtype classification was similar.

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Rare case of mucopolysaccharidosis type II in a female patient

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Background: The mucopolysaccharidoses (MPS) are a group of lysosomal storage disorders caused by deficiency of enzymes catalyzing the degradation of glycosaminoglycans. Manifestation of X-linked MPS II is highly rare in female patients.
Case report: Psychomotor retardation, coarse face and hair with mild hepatosplenomegaly led to a suspicion of mucopolysaccharidosis in a 3-year-old girl.
Results: The electrophoretic profile of glycosaminoglycans in the urine suggested MPS I, II or VI. Subsequent enzyme assay revealed iduronate 2-sulfatase deficiency indicating MPS II. Sanger sequencing of the IDS showed one heterozygous missense mutation (c.1403G>A; p. Arg468Gln). This mutation was not found in patients' parents. We did not find any other mutation (deletion, splicing mutation or recombination IDS-IDS2) which could explain the manifestation of MPS II in a girl. The patient's caryotype was normal. The result of X-inactivation skewing (96/4) examined by HUMARA assay was supported by sequencing of cDNA, where only the mutant allele was represented.
Conclusion: The findings strongly suggest that the manifestation of MPS II in the patient was the result of selective expression of the allele carrying the missense mutation p. Arg468Gln. Supported by MHCZ-

NT14015,MHCZ-DRO-VFN64165, MHCZ-DRO-EU00023761, PRVOUK-P24/LF1/3

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Mucolipidosis Type 2 (I-cell disease) with pulmonary hypertension and difficult airway

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Mucolipidosis II (ML II) is a recessive lysosomal enzyme targeting disorder leading to fatal outcome in childhood mostly due to respiratory insufficiency. Typical cardiac involvement includes thickening and deformation of mitral and aortic valves and dilated or hypertrophic cardiomyopathy. We report the clinical course of a child with confirmed ML II complicated by severe pulmonary hypertension (PH) which is very rare associated with this disorder. This 10 month-old male infant was admitted to PICU with fever, respiratory distress and cyanosis. He was born as the second child of consanguineous Turkish parents. Physical examination revealed the following signs and dysmorphic features which implicated infantile onset lysosomal storage disorder: coarse face, gum hyperplasia, rough voice, joint contractions and hip luxations. Dysostosis multiplex and a globular heart were X-rays findings. Measurements of plasma activity of almost all lysosomal hydrolases were significantly increased. Echocardiographic assessment showed cardiomegaly and severe PH. Patient's endotracheal intubation and airway management were difficult. Patient's endotracheal tube revision was undertaken as follow: guidewire was passed through endotracheal tube. Then the old tube was removed and over the guidewire a new tube was inserted into airway. The patient could not be extubated and he died on the 26th day of his hospital admission due to respiratory failure.

P-349

Mucopolysaccharidosis I – clinical manifestation in 19 patients from the Czech Republic

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Background: Mucopolysaccharidosis I (MPSI) is a lysosomal storage disease caused by alpha-L-iduronidase deficiency. The aim is to describe the natural course and treatment in Czech MPSI patients.
Patients: 19 patients from 18 families (11/8 males/females; Hurler/Hurler-Scheie/Scheie syndromes – 13/4/2).
Results: Hepatosplenomegaly (18/19), craniofacial dysmorphy (16/19), corneal clouding (15/17) and/or dysostosis multiplex (17/19) were observed as the first symptoms. Psychomotor delay (13/19) was present in all patients with Hurler type. Cardiomyopathy with valvular disease (13/17), hearing impairment (8/16), joint stiffness (16/18), carpal tunnel syndrome (5/19) and short stature (8/16) were later symptoms. All children had increased glycoaminoglycans excretion in urine and decreased alpha-L-iduronidase activity in leucocytes. Two mutations in the IDUA were prevalent: p.W402X (n=12); p.Q70X (n=8). Average diagnostic delay was 1.3 year for Hurler patients. Hematopoietic stem cells transplantation (HSCT) with (3)/without (4) short enzyme replacement therapy (ERT) was performed in 8 children, in 6 of them with good clinical results. Four patients with milder type were treated by a long-lasting ERT (3 with good effect, one patient died).
Conclusion: Diagnostic of MPSI in early phases of disease improves prognosis, because correct therapy (HSCT and/or ERT) can be chosen. Prognosis for children with later diagnosis is very unfavorable. The advance diagnosis is very important also for prenatal diagnostic in affected families. Support: RVO-VFN64165.

P-350

Attenuated form of Hunter's syndrome: a challenge to diagnose

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Background: Mucopolysaccharidosis type II (MPSII) is a X-linked recessive multisystem disorder caused by a deficiency of iduronate-2-sulfatase (IDS), which may lead to two clinically distinguishable MPS II forms: severe and mild.
Case report: A 26 year old man of normal intelligence was referred to a geneticist because of mild joint contractures. On examination, slightly coarse face features, contractures of fingers, low height (10‰) were noticed. Increased excretion of urinary GAGs - 21.92 mg/mmol creat (ref 1.6±0.8) and increased dermatan and heparan sulphate on electrophoresis suggested MPSII. Diagnosis was confirmed by low IDS activity and identified novel mutation c.1589 T>A (p. Leu530Ter) in IDS gene (with aminoacid change leading to a stop codon). In detailed review of patient's medical records previously unrecognized hints to diagnosis were found: aortic and mitral valve insufficiency, surgery of inguinal hernia in infancy; pathological humeral fracture and cyst, histopathological investigation of skin biopsy showed vacuoles in epidermal cells.
Conclusions: The multisystem nature of MPS II and the heterogeneity of disease progression may mislead a wide range of specialists and complicate early identification of patients with atenuated form MPSII.

P-351

Thermostability of enzyme N-acetylgalactosamine-6-sulfatase in leucocytes of normals individuals

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Mucopolysaccharidosis (MPS) occur due to the deficiency in the activity of enzymes that catalyze the degradation of glycosaminoglycans. The MPS type IVA (Morquio syndrome) is characterized by accumulation of keratan sulfate and condroitin-6-sulfate, caused by the N-acetylgalactosamine-6-sulfatase (GALNS) deficiency. The aim of this work was to study the effect of temperature (70 °C) on GALNS activity in normal leukocytes using 3 mM substrate concentration. Leukocytes were isolated from 10 ml heparinized blood samples. GALNS activity was measured according to van Diggelen et al. (1990) with 3 mM MU-βGal-6S as substrate. Samples were incubated at 70 °C for 5, 10, 15, 20, 30 and 60 minutes. The results were compared with the samples incubated at 0 °C (100 % activity). We observed that at 70 °C a gradual inactivation of the enzyme occurred, and the residual enzyme activity at 30 minutes is already significantly lower ($p<0.05$). This enzyme seems to be more stable than other lysosomal hydrolases. Its behavior toward heat seems important in the differentiation of normal and homozygous and heterozygous individuals and could be help in the diagnosis of MPS IV A patients.

P-352

Mucopolysaccharidosis type VI in Spain

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Background: Mucopolysaccharidosis VI is a progressive autosomal recessive lysosomal storage disorder. Since 2006 enzyme replacement therapy is available which has improved the prognosis of these patients. In Spain there are 11 patients with MPS VI, all under treatment with ERT. The aim of this review is to present Spanish patients and their evolution before and after therapy with ERT.
Patients: A retrospective, descriptive study of 11 patients diagnosed with MPS VI confirmed by enzymatic and/or genetic studies
Results: 11 patients have been diagnosed with actual ages from 2 to 22 years. Median age at diagnosis was 2 years and at the beginning of therapy 4.5 years. At the time of diagnosis most patients had characteristic phenotype (9/11), signs of skeletal spine involvement (7/11) and hepatomegaly (7/11). All patients started ERT between 18 months and 15 years of age. They have required several surgeries: Porth-a-cath, adenoidectomy, tympanic tubes, carpal tunnel surgery and VP shunt.
Conclusions: All patients have presented a positive clinical course with ERT, with no severe side effects. Despite the benefits of therapy they still need a multidisciplinary follow-up because of the complications that are not reversible with ERT, some of which require various surgical interventions.
Conflict of Interest declared.

P-353

ENT disease and the early diagnosis of mucopolysaccharidoses (MPS) – the HATT project

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Objectives: Several types of MPS, including MPS I, II, and VI, are associated with progressive upper airway obstruction and hearing loss. This project evaluates ENT interventions combined with other disease manifestations as a strategy to accelerate MPS diagnosis.
Methods: The literature was systematically reviewed to determine median age at diagnosis, incidence of ENT interventions, and prevalence and timing of ENT surgery for patients diagnosed with MPS I, II and VI (MPS disorders with specific treatment options).
Results: Median ages at diagnosis for MPS I, II, VI are 2.6, 3.3, and 5 years, respectively. Incidence of ventilation tubes is 40-100 % (MPS II), 23-86 % (MPS I), and 66-100 % (MPS VI). Ranges represent lowest/highest values in literature. Upper airway obstruction incidence is 48-100 % (MPS II), 18-100 % (MPS I), and 45-85 % (MPS VI). Adenotonsillar surgery and ventilation tubes are common interventions in early childhood, that may precede MPS diagnosis.
Conclusions: ENT surgeons could assist in early MPS diagnosis. The HATT project-MPS I, II, and VI Screening in a High-Risk Population With Previous Surgical Repair or Presence of Inguinal and/or Umbilical Hernia in Combination With Pediatric Ear, Nose and Throat Surgery (Adenoidectomy and/or Tonsillectomy and/or Tympanostomy)-will evaluate ENT surgeries, combined with early disease manifestations that may help with early diagnosis of patients with MPS.
Conflict of Interest declared.

P-354

Neurocognitive assessment in 13 patients affected by muchopolysaccharidosis IV A (MPS IV A)

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Background, objectives and methods: The scientific literature reports cognitive involvement in MPS IVA contrary to what was previously thought. To investigate this matter, neurocognitive assessment was performed in 13 MPS IVA patients (pts) with WAIS- R 2pts (37 years (y),28y); WISC III 9pts (6–16y); WPPSI III 1 pt (5y); Griffiths 1 pt (3.5y).
Results: Intelligence quotient (IQ), ranged from 61 to 125 (median 91, mean 91.6, standard deviation (SD) 21). According to ICD10 classification two pts (11y both) had mild mental retardation: 61 and 66 of total (T) IQ and three pts (11y, 12y, 28y) were borderline: TIQ 78, 73, 75. Eight pts had a normal TIQ. For all the pts the verbal IQ (VIQ) (range 65–121; median 96; mean 94.8; SD 21.2) was clearly higher than performance IQ (PIQ) (range 59–124; median 85; mean 88.7; SD 19.6). Four of the 5pts with TIQ lower than 80 had chronic respiratory insufficiency: 1 pt had nocturnal apnea with adenotonsillar hypertrophy and laryngomalacia, the surviving 3 (2 overweight) are on non invasive ventilation.
Conclusions: The VIQ being higher than PIQ is explained by the musculoskeletal and sometimes spinal disease burden. The lowest TIQs might be explained by the hypoxia due to the respiratory insufficiency, an issue which deserves a strict follow up.

P-355

Coenzyme Q_{10} status and other vitamins in mucopolysaccharidosis disease

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Background and objectives: Mucopolysaccharidosis type III (MPS III) is an autosomal recessive lysosomal storage disease. Our aim was to know the nutritional status analyzing vitamins and micronutrients with a special focus on coenzyme Q_{10} (CoQ_{10}) content.
Materials/Patients and Methods: We recruited 9 patients (5 IIIA, 3 IIIB, 1 IIIC; range: 5–17 years; average=11.1 years). All patients were compared with our reference intervals and we selected a control population (n=9) age matched controls to asses data in parallel. Plasma CoQ10 content was analyzed by high pressure liquid chromatography (HPLC) with electrochemical detection. Other antioxidants, amino-acids and other micronutrients were measured as previously reported.

Results: Eight of 9 MPS patients presented a severe deficiency of CoQ_{10} content and low vitamin B6 values whereas vitamin E, A, B1, B12 and folate were normal. Positive correlation was observed between CoQ_{10} and vitamin B6 in MPS patients (r=0.713; p=0.031; Pearson test). When compared MPS patients with matched control population significant differences were observed for CoQ_{10}, vitamin B6, tocopherol and retinol values (U-Mann–Whitney test, p=0.002, p=0.007, p=0.001 and p=0.037 respectively).
Discussion/Conclusion: We conclude that deficiencies of CoQ_{10} and vitamin B6 could be implicated in the physiopathology of the disease and would be restoring for better symptomatic care.

P-356

Acute stroke in a patient with mucopolysaccharidosis type I with increased carotid intima media thickness

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Introduction: We describe a 3-year-old mucopolysaccharidosis type I patient who presented with acute ischemic stroke with ultrasonographic evidence of vasculopathy.
Case Report: 3-year-old MPS I patient was referred due to acute left sided weakness. Laboratory studies including complete blood count, biochemical and coagulation parametres, and autoimmune markers were normal. Transthoracic echocardiography revealed no evidence of thrombi. Carotid artery intima–media thickness (cIMT) was measured as 0.5 mm which was found significantly higher when compared to the reference values for healthy children. This finding suggested vasculopathy. Brain MRI and MR angiography revealed findings consistent with acute stroke in the left temporoparietal area. Genetic analysis revealed heterozygous mutation for Factor V Leiden and MTHFR.
Conclusion: Although GAG accumulation in vessels have been well known in MPS patients, the clinical effects of vascular involvement is still being studied. Vasculopathy, which is a risk factor for stroke, may accompany MPSs. We suggest MPS patients to be routinely evaluated for hypercoagulative factors to initiate prophylactic treatment if needed. Our case is the first MPS patient defined in literature with acute stroke and radiologically proven increased cIMT. Further studies will needed to be performed in order to evaluate the relationship of vasculopathy and stroke in MPSs.

P-357

Cardiac involvement in untreated patients with severe phenotype of Hunter disease

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Introduction: Cardiac involvement is one of the prominent features of Hunter disease (HD). The majority of patients have echocardiographic abnormalities. However, structural changes are not always symptomatic.
Materials and methods: We describe natural course of cardiac disease in six HD patients. Cardiologic evaluation was performed yearly and included clinical, electrocardiographic and echocardiographic assessment.
Results: All included patients have severe phenotype of HD and they haven`t received enzyme replacement therapy yet. Age ranges from 3 do 8 years, and diagnosis was established at median age of 3 years. Majority of patients (5/6) have mitral valve dysplasia with median regurgitation grade of 0.5+. Average left ventricle (LV) end-diastolic diameter z-score is 0.0±2.0. Ejection fraction of LV (70±7 %) and fractional shortening (39±6 %) are normal in all patients. We verified mild thickening of LV posterior wall (z-score 1.8±0.8) while average diameter of interventricular septum is slightly increased (z-score 1.3±0.7). Patient with mutation c.262C>T has severe aortic insufficiency accompanied by dilation of left ventricle. Significant diastolic dysfunction is found in patient with hemizygous deletion encompassing entire IDS gene.
Conclusions: In patients with HD, close echocardiographic assessment is necessary considering the high prevalence of mitral valve dysfunction and left ventricular hypertrophy. Genotype-phenotype correlation regarding cardiac involvement needs further elucidation.

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Fucosidosis case report: rare cause of developmental delay

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Fucosidosis is a rare lysosomal storage disease, resulting from a deficiency of alpha- L-fucosidase. It is an autosomal recessive neurodegenerative disorder caused by mutations in FUCA1 gene. Here we present a 4 year 10 months boy with fucosidosis. He presented with psychomotor retardation. On his physical examination; weight was 3–10 percentile, height was ‹3 percentile (SDS=−3.2), and head circumstance was 50 percentile. He had course face, macroglossia, corneal clouding, hepatosplenomegaly, angiokeratomas, kyphosis, and gibbus deformity. X-ray analysis revealed dysostosis multiplex. He had bilateral hearing loss. Cranial MRI showed bilateral periventricular white matter hyperintensities. Urine GAGs were mildly elevated. Enzyme analysis for muchopolysaccharidoses were normal. Alpha-L-fucosidase enzyme was level 0.02 umol/g/h (N=50-250). According to clinical and laboratory investigation patient had the diagnosis of fucosidosis. There is limited experience in literature for the treatment of fucosidosis. Bone marrow transplantation could be a possibility.

P-359

Outcome after bone marrow transplant with a heterozygous sibling donor in a Hurler patient

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Background: Hematopoietic stem cell transplantation is currently the treatment of choice for Huler syndrome (HS) patients. The risks and benefits of transplantation with a graft from a heterozygous related donor should be considered.
Case Report: A 7-month-old boy was referred with a clinical and biochemical diagnosis of HS, confirmed by genotyping. He had a severe classical phenotype and a subnormal neurological development (DQ: locomotor 85, eye/hand coordination 90, social 92, language not evaluated). Enzyme replacement therapy (ERT) was initiated at 7.5-month-old and a bone marrow transplant (BMT) with a heterozygous sibling donor was performed at 10 months.
Results: The 9-months following BMT were marked by recurrent admissions for a sub-acute digestive graft-versus-host disease grade II successfully treated by corticosteroids, an acute ethmoiditis, a perianal abscess and a glaucoma surgically treated. Donor-recipient chimerism remained always >90 % and

leukocyte enzyme activity was 50 % of control, leading us to discontinue ERT at 9 months post-BMT. Neurocognitive development initially declined but improved again after 12 months post-BMT (DQ at 26-month-old: locomotor 55, eye/hand coordination 69, social 65, langage 65).
Conclusion: Neurologic outcomes could decline in the first months following BMT with a carrier related donor, but have to be studied for a longer period.

P-360

Molecular analysis of Mucopolysaccharidosis type II cases in Mongolia

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Mucopolysaccharidosis type II is an X-linked recessive lysosomal storage disorder resulting from the defective activity of the enzyme iduronate 2-sulfatase (IDS). Hunter disease can vary from mild to severe, depending on the level of enzyme deficiency. Objective: To identify enzyme deficiency of glycosaminoglycans in a case with a familial history.
Method: We diagnosed a patient with clinical symptoms of MPS. The patient was a thirteen-year-old boy. He typically had a coarse face, short stature, big belly with hepato-splenomegaly, dysostosis multiplex, and severe mental, growth retardation and persistent pneumonia. Although he was apparently MPS because of his phenotype, the subtype was not clear. His nephew, and uncle had same symptoms and both of them died at age of thirteen. The grandmother and her two daughters are suspected as a carrier of X linked recessive disorder MPS type II, Hunter syndrome by genealogical study and we measured serum activities of enzymes implicated in mucopolysaccharidoses.
Result: Serum N-acetylglucosaminodase, α-L-iduronidase, iduronate-2-sulfatase, β-galactosidase levels were checked and iduronate-2 sulfatase deficiency was found in this patient. Levels of α-L-iduronidate sulfatase and α-iduronidase were 0.07 μmol/L/hr (normal control: patient control= 136.57: 0.30).
Conclusion: Prenatal biochemical screening test should be undertaken since we have diagnosed familial cases.

23. Lysosomal disorders: sphingolipidoses

P-361

Adult form of Niemann-Pick disease type B: diagnostic pitfalls

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Introduction Niemann-Pick disease type B (NPB) is caused by acid sphingomyelinase (ASM) deficiency. The diagnosis is made by enzyme activity in leukocytes or fibroblasts and by molecular analysis in the ASM gene (SMPD1).
Case Report A 51 years-old male patient presented with splenomegaly, pulmonary fibrosis and portal hypertension. Onset of symptoms was at age nine. High resolution computed tomography showed a pattern of interstitial lung disease producing an image of "crazy paving".
Results ASM residual activity was 40 % of normal in leucocytes. Bone marrow examination revealed sea-blue vacuolated histiocytes. The diagnosis was confirmed by clear deficiency of ASM activity in cultured fibroblasts and positive Filipin staining, as well as by molecular testing.
Discussion Mild "late onset" adult form of NPB due to [p. Arg610del] mutation can be missed based on ASM enzyme activity in leukocytes (activity up to 40 % of control). Bone marrow examination may be a key step in the diagnostic process by revealing the presence of sea-blue histiocytes. Enzyme analysis in cultured skin fibroblasts as well as genotyping are recommended particularly for late onset cases with borderline ASM activity.

P-362

Clinical features, laboratory characters and outcome of three Iranian infantile GM1 Gangliosidosis

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Background: GM1 gangliosidosis is a lysosomal storage disease due to deficiency of β-galactosidase caused by mutations in its gene (GLB1) located on chromosome 3p21.3, leading to a neurodegenerative condition classified into three main clinical forms based often on residual enzyme activity. Infantile form with the lowest (<3 %) enzyme activity leads to death before the age of 4 yr. A recently developed strategy using pharmacological chaperones appears to be more rapidly available for clinical application than gene and substrate reduction therapy.
Results: 2 males and one female. Age at diagnosis: 11,7,8 months. Consanguinity: 2/3. Neonatal jaundice: 3/3. Both males had hydrocele and lower limb edema from birth. Neurodevelopmental delay within the first 3 months of birth, cherry red spots, generalized Mongolian spots, hypotonia and hepatosplenomegaly: 3/3. None had dysmorphic features at birth. Moderately elevated liver enzymes, high alkaline phosphatase (>2000 U/L) and normal acid phosphatase: 3/3. Enzyme levels: 0.02, 0.17, 0.07 nmol/spot 21 hours (N:0.5-3.2). 1&3 expired before genotyping. Case 2 developed seizure at 21 month, expired at 26 months and GLB1 genotyping showed: c.1071del, p. Phe357Leufs*26.
Conclusion: Considering severe progressive neurodegenerative nature of the disease and lack of effective therapy early diagnosis and GLB1 genotyping has an important role for genetic counselling.

P-363

GM2-gangliosidosis, AB variant: clinical, ophthalmological, MRI and molecular findings

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GM2-gangliosidosis, AB variant is a very rare form of GM2-gangliosidosis due to a deficiency of GM2 activator protein, associated with autosomal recessive mutations in GM2A. Less than 10 patients, confirmed by molecular analysis, have been described in the literature.
A 12 month old Hmong girl presented to the Neurometabolic clinic for evaluation of global developmental delay, hypotonia and cherry red spots. The parents are not known to be consanguineous. Her examination was remarkable for hypotonia with hyperreflexia and excessive startling. The head circumference was normal. An extensive neurometabolic evaluation was negative.
Developmental regression began at 14 months of age. Retinal examination at 16 months of age disclosed 4+ cherry red/black spots with "heaped up" whitish infiltrate surrounding both foveae but no evidence of optic atrophy or peripheral retinal abnormalities. Repeat MRI scan at 17 months of age revealed delayed but interval myelination associated with

abnormal signal intensity of the bilateral thalami presenting as T2 hyperintensity of the posterior thalami in the region of the pulvinar nuclei and T2 hypointensity in the anterior thalami. Sequencing of the GM2A gene revealed a homozygous c.160 G>T mutation, predicted to result in a premature protein termination p. Glu54*.

P-364

Are we controlling our patient's pain?

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Background: Fabry disease is a rare X-linked, autosomal recessive lysosomal storage disease, caused by a deficiency in the activity of the enzyme, α-galactosidase A (α-Gal A). One symptom of Fabry disease is episodes of pain which has a profound effect on quality of life.
Objectives: To identify if pain is well controlled in Fabry patients on enzyme replacement therapy. Standard: All patients with Fabry disease should be pain free.
Methods: 24 of 30 patients completed the Brief Pain Inventory (BPI) questionnaire, measuring severity and interference of pain. Results were analyzed using mean pain scores and Mann–Whitney U non-parametric test of mean difference.
Results: 29 % of patients experienced pain on the day of completing the questionnaire. Common sites of pain reported were hands and feet. Patients on pain relief medication had higher pain scores. There was no significant difference between male and female pain scores.
Discussion: Pain is inappropriately managed in patients with Fabry disease. Severe pain scores were obtained in patients on pain relief medication; this may be because pain medication is only initiated when pain is severe.
Conclusion: Pain is not appropriately managed in patients with Fabry disease and so further research should be carried out to address this.

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Early infantile GM1-gangliosidosis: presentation of 3 unrelated Romanian patients

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GM1-Gangliosidosis is an autosomal recessive lysosomal storage disease characterized biochemically by deficient beta-galactosidase activity with accumulation of ganglioside substrates in lysosomes and has 3 distinctly different phenotypic presentations: an infantile variant (type 1), a juvenile form (type 2), and an adult or chronic form (type 3). Clinically, patients show variable degrees of neurodegeneration, ophthalmological and dysmorphic features and skeletal involvement. We present three unrelated cases (two females and a male) diagnosed in our hospital by clinical features, peripheral blood smear and they were confirmed by documentation of deficient enzymatic activity of beta-galactosidase in the peripheral blood. Molecular diagnosis was not done. First case was a boy, suspected to have this diagnosis at age of 8 months, because of facial dysmorphism, hepatosplenomegaly and rapid progression of the symptoms with the development of seizures and general neurologic deterioration. The female patients were recognized with this condition at age of 3 and 2 months, respectively, presenting coarsening of facial features, visceromegaly and generalized oedema. Exact prevalence studies for this disorder are not available for the Romanian population. There is a need to increase awareness about this rare condition to ensure accurate diagnosis and appropriate management, genetic counseling and prenatal diagnosis for the families.

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The spectrum of Krabbe disease in Greece - laboratory experience

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Krabbe disease is a lysosomal disorder associated with deficient activity of β-galactocerebrosidase. We present 19 cases diagnosed in Greece. β-Galactocerebrosidase activity was measured in white blood cells using a radioactive and/or a fluorogenic substrate. Chitotriosidase activity was assayed in plasma. Age of diagnosis was 4 months to 42 years whereas reported age of onset was 3 months to 7 years. Chitotriosidase activity was elevated in 11/17 cases. β-Galactocerebrosidase activity was severely reduced (0.0 – 0.05 nmoles/mg protein/hr) in all cases irrespective of the substrate used. DNA analysis was carried out in 13 patients by analysis of the coding region and exon flanking sequences of the GALC gene and checked by restriction enzyme analysis. The identified mutations included the previously reported mutations: p. I250T (c.749 T>C) (10/26 alleles), c.1113+6555del 32 kb (5/26 alleles), and p. D187V (c.560A>T) (1/26 alleles), and the novel mutations: p. K139del (c.411-413delTAA), found in homozygosity in an infantile case and in heterozygosity in two juvenile patients (4/26 alleles), p. D610A (c.1829 A>C) (2/26 alleles); c.535 583-1G>C (1/26 alleles), and p. W132X (c.396 G>A) (2/26 alleles). In conclusion, a considerable delay in the diagnosis was observed in late-onset cases. Mutation p. I250T accounts for>35 % of the mutations identified. Chitotriosidase can be useful in the diagnosis of Krabbe disease.

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Molecular verification of patients with suspicion of Niemann–Pick disease type C

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Background: Niemann-Pick disease type C (NPC) is an autosomal recessive disorder in the group of lysosomal storage diseases. The disease is caused by mutations in 2 genes: NPC1 and NPC2. This is the first molecular investigation of Russian patients.
Methods: PCR and direct sequencing of all the exons and nearest intronic regions of NPC1 and NPC2 genes were performed by ABI3500 bioanalyzer.
Results: 200 families were included in our investigation. 18 molecular diagnosis were confirmed. Patients had different forms of the disease from early infancy to adult form. Among others we found known common mutations: in NPC1 – p. Pro1007Ala (3 patients), p. Ser954Leu (4 patients), in NPC2 – r. Cys93Phe (1 patient). In 4 patients only 1 mutation was found in the heterozygous state, so more research is needed. In some patients we discovered new mutations leading to disease: c.326insT, c.1625_1626insTG, c.2164_2169insTGGATC, c.2196_2197insT, c.2972_2973delAG, p. Thr477Met, p. Ile837Thr, p. His897Gln, p. Asp1050Asn, p. Gly1073Ser.
Conclusion: The main medicine used in the treatment of NPC is Miglustat (Zavesca, Actelion). Based on confirmed molecular diagnosis many

patients started to receive treatment, which can improve and stabilize their condition. Molecular diagnostic is very important to confirm the clinical diagnosis of NPC and start therapy early.

P-368

Development of a novel substrate reduction therapy with CNS access for treating neuronopathic Gaucher disease

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Gaucher disease (GD) is caused by reduced glucocerebrosidase activity, which results in accumulation of non-metabolized substrates primarily in the viscera only (non-neuropathic) or in the CNS as well (neuronopathic). Enzyme replacement therapy has been used to successfully treat the visceral manifestations of GD for over 20 years; however there is no such treatment for the CNS disease. Substrate reduction therapy (SRT) through inhibition of glucosylceramide synthase (GCS) also improves the visceral aspects of non-neuronopathic GD. Here, we describe the use of novel inhibitors of GCS with CNS access in mouse models of neuronopathic GD. In the 4 L;C* mouse, CNS gliosis and elevated substrate levels (glucosylceramide and glucosylsphingosine) occur prior to death at ~45 days. Oral administration of a CNS-accessible GCS inhibitor delayed CNS histopathologic findings and substrate accumulation with a concomitant ~30 % increase in lifespan. In the conduritol B epoxide (CBE)-induced mouse model of neuronopathic GD, similar gliosis, accumulation of lipids and ataxia were observed. SRT resulted in attenuation of all the neuropathologic manifestations in the continuously CBE treated mouse including astrogliosis, microgliosis, substrate accumulation and ataxia. These results strongly support the development of SRT for the treatment of neuronopathic GD, particularly in patients with greater residual glucocerebrosidase activity.
Conflict of Interest declared.

P-369

Atypical beta galactosidase molecular finding in a juvenile GM1 gangliosidosis patient

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Background: GM1 gangliosidosis, that arises from beta-galactosidase (GLB1) deficiency, is a very rare lysosomal storage disease. The GLB1 gene encodes for two different proteins: GLB1 and elastin binding protein (EBP), a cell surface protein involved in elastic fiber assembly.
Objectives: We aim to characterize a GM1 gangliosidosis patient, affected by the juvenile form of the disease, presenting an atypical molecular analysis.
Methods and Results: The patient was characterized at a clinical and biochemical level and the GLB1 gene coding region and intron-exon boundaries were analyzed. Two mutations were identified: the new c.931G>A (p. Gly311Arg), and the known c.797A>G (p. Asn266Ser). Both mutations were characterized in silico and, surprisingly, the known mutation, although exonic, could reasonably induce an aberrant splicing product. We deepened this hypothesis by Real time, RT PCR and immunofluorescence analyses. The hypothetic aberrant product induced by the c.797A>G (p. Asn266Ser) genetic lesion, missing four bases compared to the wild type transcript, was not identified. However, a reduction of EBP and GLB1 were identified at mRNA and/or protein level.
Conclusions: We stress the importance to consider all the clinical and molecular aspects in analysing GM1 gangliosidosis patients in order to identify and characterize atypical molecular findings.

P-370

A Niemann-Pick disease Type C suspicion index tool to aid diagnosis in paediatric patients

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Background and objectives: Niemann-Pick disease type C (NP-C) is a life limiting lysosomal lipid storage disorder caused by mutations in NPC1 or NPC2 genes. A suspicion index (SI) tool was developed to help clinicians achieve early diagnosis. The tool accurately predicts NP-C in patients >4 years of age, but performs poorly in paediatric patients (≤4 years old). This study aimed to develop a novel NP-C SI tool specifically for paediatric patients.
Methods: Symptomatology data from 200 paediatric patients (categorised as: NP-C cases [confirmed NP-C positive; n=106]; NP-C non-cases [NP-C suspected but confirmed negative; n=31]; and controls [NP-C not suspected; n=63]) were retrospectively collected. Relationships between individual symptoms and likelihood of confirmed NP-C diagnosis were defined by statistical modelling. The final tool was developed using the most predictive symptoms of NP-C in paediatric patients.
Results: The paediatric NP-C SI tool discriminates well between NP-C cases, NP-C non-cases and controls. Receiver operating characteristic curve analysis demonstrates superior sensitivity and specificity of the paediatric NP-C SI tool versus the original tool in paediatric patients.
Conclusion: The paediatric NP-C SI tool will lead to improved early diagnosis and management of NP-C in paediatric patients, allowing therapy initiation at onset of neurological symptoms.
Conflict of Interest declared.

P-371

Improving NP-C screening: identification of seven key discriminatory signs and symptoms

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Background: Niemann-Pick disease Type C (NP-C) is a neurodegenerative disorder, characterised by a wide range of visceral, neurological and psychiatric symptoms. A suspicion index (SI) tool based on 21 signs and

symptoms was developed to aid identification of patients aged >4 years who may warrant diagnostic testing. It was hypothesised that identification of key discriminatory signs and symptoms may allow a simplified prediction of NP-C.
Methods: Retrospective analyses were performed using the original SI tool cohort to identify key discriminatory signs and symptoms that relate to a positive diagnosis of NP-C.
Results: Seven key signs and symptoms were identified: prolonged, unexplained neonatal jaundice or cholestasis; isolated unexplained splenomegaly (±hepatomegaly); vertical supranuclear gaze palsy; gelastic cataplexy; pre-senile cognitive decline and/or dementia; psychotic symptoms (hallucinations, paranoid delusion and/or thought disorder); and parent, sibling or cousin with NP-C. The presence of 2 of these 7 signs was found to be sufficient to correctly identify patients with NP-C.
Conclusion: Combinations of key signs and symptoms have positive discriminatory power, and indicate a high suspicion of NP-C. Identifying these signs and symptoms may help to simplify screening and improve NP-C detection rates in patients aged >4 years.
Conflict of Interest declared.

P-372

The Niemann-Pick disease Type C suspicion index: development of a revised tool with improved predictive ability

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Background: The Niemann-Pick disease Type C (NP-C) suspicion index (SI) tool was developed to help identify patients warranting further investigation for NP-C. It assigns risk prediction scores (RPS) based on the presence of individual symptoms. We aimed to refine the calculation of RPS using combinations of symptoms, to improve the tool's predictive power.
Methods: The NP-C SI tool dataset (N=216: 71 NP-C cases; 64 NP-C non-cases; 81 controls) was retrospectively analysed. Statistical modelling was used to determine RPS of individual and combination symptoms, based on their frequency in each subgroup. The five highest RPS for individual or combination symptoms were weighted to calculate a new total RPS.
Results: The refined tool provides a probability analysis for NP-C; a patient's RPS is compared with those in the NP-C SI database, providing a quantitative assessment of suspicion. The revised tool discriminated well between cases, non-cases and controls. Receiver operating characteristic curve analysis confirmed improved discriminatory performance versus the original tool.
Conclusion: The increased discriminatory power of the revised NP-C SI tool is anticipated to improve screening and increase detection rates. Comparison of RPS with scores within the database will provide more granularity and information about disease severity in individual patients.
Conflict of Interest declared.

P-373

Differences in Niemann-Pick disease type C symptomatology at diagnosis observed in patients of different ages

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Background: Niemann-Pick disease type C (NP-C) is a neurodegenerative disorder characterised by a wide range of visceral, neurological and psychiatric symptoms. The disease can present at any age, and can vary considerably between patients. The aim of the present study was to provide an indication of clinical presentation of NP-C at time of diagnosis, in patients of different ages within a large cohort.
Methods: Retrospective analysis of symptomatology at time of diagnosis in 164 patients with NP-C was performed. Individual NP-C symptoms were descriptively summarised and correlations between symptoms and age were defined. A cluster analysis was used to determine the inferred relationship between each symptom in paediatric or non-paediatric patients and control subjects.
Results: These analyses provide a clinical picture of NP-C at time of diagnosis in patients of different ages; visceral symptoms predominate in paediatric patients and neurological/psychiatric manifestations in older patients. Cluster analysis also reveals differences in the relationships between signs and symptoms between patients with NP-C and control subjects.
Conclusion: The clear differences in symptomatology observed at different ages within a cohort of patients with NP-C, and between these patients and control subjects will help clinicians to identify patients with NP-C more effectively in the future.
Conflict of Interest declared.

P-374

Movement disorders in Niemann Pick type C disease: myoclonus as a constant feature in a series of six patients with different phenotypes

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Background: Niemann Pick type C disease (NPC) is an autosomal recessive sphingolipidose caused by mutation in NPC1 or NPC2 gene. NPC is characterized by visceral, neurological and psychiatric manifestations. Cerebellar ataxia, dysphagia, cognitive impairment, vertical supranuclear gaze palsy and cataplexy are common neurological symptoms. Movement disorders have been rarely reported and are more frequent in adult forms.
Patients and Methods: We described 6 patients: two with late infantile, one juvenile form and three adults with different symptoms. All patients presented mutations in NPC1. In addition to clinical evaluation, the patients were recorded by EEG polygraphy to verify and characterize the presence of movement disorders. At the time of our study, two patients were treated with miglustat, the others started treatment after evaluation.
Results and conclusion: We found in all patients a complex movement disorder always including myoclonus: dystonia in 2/6, tremor in 1/6 and myoclonus in 6/6 patients. Myoclonus was a constant feature in spite of their heterogeneous phenotype. Our report suggests that myoclonus may occur as a common neurological feature of NPC. It should be considered a diagnostic hallmark in this heterogeneous disease and its measure could be a possible instrumental parameter in the follow up of treated patients.

P-375

Coexistence of Niemann Pıck type B and familial mediterranean fever in a Turkish patient

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Niemann-Pick disease type B (NPD-B) is an inborn error of metabolism where reduced acid sphingomyelinase activity leads to multisystem disease with survival into adulthood. The natural history of NPD-B is progressive hepatosplenomegaly and gradual deterioration of pulmonary function. Familial Mediterranean fever (FMF) is a rare inherited autosomal recessive autoinflammatory disorder characterized by recurrent and self-limited episodes of fever and painful serositis. We describe a case who has NPD-B and FMF, concomitantly. An 11 months old boy was referred due to hepatosplenomegaly and prolonged fever. In laboratory analyses thrombocytopenia, hypertriglyceridemia and elevated transaminases were found. Bone marrow biopsy showed foam cells. Sphingomyelinase enzyme level was 0.003 nmol/17 hour/mg protein. We found homozygote c.409 T>C mutation in SMPD1 gene and diagnosis of NPD-B was established. During follow-up period, he developed recurrent prolonged fever attacks. We suspected FMF and detected homozygote M694V mutation on MEFV gene. Our patient benefitted from colchicine treatment. Coexistence of NPD-B and FMF is a rare situation. Although recurrent fever could be thought to be a manifestation of NPD-B due to pulmonary infections; in the case of positive inflammatory markers without proof of infection, FMF should be kept in mind.

P-376

Collagen turnover biomarkers in Fabry disease cardiomyopathy

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Background and objectives: Cardiomyopathy is the most common cardiac abnormality seen in Fabry disease (FD). Since myocardial fibrosis is one of the histological hallmarks of FD cardiomyopathy the assessment of the collagen turnover biomarkers is paramount.
Methods: FD patients with left ventricle hypertrophy and age- and sex-matched controls were studied. All patients and controls underwent echocardiography and markers of collagen type I and III synthesis and degradation were measured. Since type I collagen is a major component of bone, bone-specific alkaline phosphatase (b-AP) and tartarate resistant acid phosphatase 5b (TRAP5b) were also assessed.
Results: Ten patients and ten controls were evaluated. Levels of propeptide of type I procollagen (PICP) were significantly higher in FD patients ($p=0.015$) as also the PICP:b-AP ratio ($p=0.002$). The PICP to C-terminal telopeptide of type I collagen ratio (balance between collagen synthesis and degradation) was non-significantly increased in FD ($p=0.258$) and the matrix metalloproteinase-2 (MMP-2) was significantly lower in FD ($p=0.028$).
Conclusion: Collagen type I synthesis is increased in FD cardiomyopathy and there is a statistical trend for the synthesis to prevail over degradation, related to the inhibition of the MMP. As in hypertrophic cardiomyopathy this profibrotic state is likely to be critical in FD cardiomyopathy.
Conflict of Interest declared.

P-377

Glomerular and tubular damage biomarkers in Fabry disease

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Background and objectives: In Fabry disease (FD) nephropathy storage occurs in all renal cells (endothelial, glomerular, interstitial and tubular cells). These histologic findings can occur with minimal or no alterations on standard renal tests, so alternative markers of glomerular and tubular dysfunction are crucial.
Methods: FD patients with chronic kidney disease and age- and sex-matched controls were studied. All patients and controls underwent kidney function evaluation (glomerular filtration rate [GFR] and albuminuria) and markers of glomerular (transferrinuria and collagen type IV) and tubular (α1-microglobulin, N-acetyl-glucosaminidase and alanine aminopeptidase) damage were measured.
Results: Ten patients and ten controls were evaluated. All the patients had GFR<60 ml/min/1.73 m^2 and 9 had albuminuria>30 mg/g creatinine. Markers of glomerular damage were significantly higher in FD patients (transferrinuria $p=0.001$; collagen type IV $p=0.001$). A similar result was achieved for markers of tubular injury (α1-microglobulin $p=0.006$; N-acetyl-glucosaminidase $p=0.001$; alanine aminopeptidase $p=0.005$).
Conclusion: The kidney damage in FD affects the glomeruli and the tubules. This is the first evaluation of tubular dysfunction markers in FD. These results suggests further study of these markers which have shown promise in the early detection of nephropathy in other diseases such as diabetes, so should also be pursued in FD.
Conflict of Interest declared.

P-378

Clinical profile of females with Anderson-Fabry disease in a Brazilian community

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Background and objectives: Anderson-Fabry Disease (AFD) females were thought to be asymptomatic or to develop only minor manifestations. However several studies reported that heterozygous females do develop substantial symptoms. We aim to describe the clinical findings in female patients carrying a null mutation.
Patients and Methods: We analyzed the clinical profile in 53 AFD female patients with a severe phenotype.
Results: The patients, median age of 30 y (7y-68y), showed early symptoms: 74 % presented acroparesthesia, median age 10y (4y - 51y). Hypohidrosis was present in 72 %, angiokeratomes in 23 %, depression in 45 %. Heart MRI was performed in 43 patients: 51 % normal, 49 % abnormal. Brain MRI was performed in 41 patients: 51 % normal, 49 % abnormal. Proteinuria in 64 %. Mutation analysis showed 35 % of c.1095delT, 35 % c.365del7, 21 % p. W47X, 4 % p. W004X and 4 % unknown.
Discussion/Conclusion: All mutations found are expected to cause a severe phenotype (null mutations). Our female patients showed early symptoms of the disease, most frequently acroparesthesia and proteinuria. They also showed high incidence of cardiac and neurologic

abnormalities. Females with a null mutation have a significant risk for major organ involvement and decreased quality of life and must be monitored and treated accordingly.

P-379

Kidney cysts and Fabry disease: two distinct entities or one single pathology?

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Background and objectives: Fabry Disease (FD) is caused by deficiency of the lysosomal enzyme alpha-galactosidase A which results in the accumulation of globotriaosylceramide (Gb3) in different cell types including different kidney structures. We describe a rare association of cysts in multiple organs and FD.
Patients and Methods: Patient 1, female, 64y, diagnosed at 56y by familial screening. Fatigue was the only symptom at diagnosis. She had proteinuria, and normal creatinine clearance. Brain magnetic resonance imaging (MRI) showed microangiopathy. Patient 2, male, 37y, patient 1's son, diagnosed at 30y, presented acroparesthesia, angiokeratomas, proteinuria, and normal creatinine clearance. The brain MRI showed mild cortical atrophy. Both patients underwent abdominal computerized tomography (CT).
Results: Patient 1, presented on CT, multiple hypodense cysts in hepatic parenchyma, one spleen cyst, and multiple cysts in both kidneys. Patient 2, presented on CT, multiple hypodense cysts in hepatic parenchyma, one spleen cyst, one kidney cyst and one prostatic cyst.
Discussion/Conclusion: Kidney cysts occur as distinctive genetic disorders or in association with inherited or acquired kidney pathologies. There are a few reports of occurence of cysts in FD patients, and the relationship between both remains unclear. Our findings support a possible association.

P-380

Determination of alpha-galactosidase A biochemical properties as auxiliary tool in Fabry disease diagnosis

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Fabry disease (FD) is a lysosomal disorder, caused by a deficiency in the enzyme alpha-galactosidase A (GLA), resulting in progressive accumulation of glycosphingolipids within lysosomes. Considering the importance and the difficulty still present in the biochemical diagnosis of FD, the aim of this study was to establish and compare the biochemical and kinetic properties of GLA in dried blood spots (DBS), plasma and leukocytes samples of FD patients and healthy subjects to evaluate the possible use of these parameters as an auxiliary tool in the diagnosis of this disease. Fluorometric assays using the different sample materials were performed in order to compare and characterize GLA activity in terms of optimal pH, Km and Vmax and heat stability. A difference was observed between the Vmax of FD patients and healthy controls using DBS, plasma and leukocyte samples ($p<0,05$ for DBS, $p<0,001$ for plasma and $p<0,01$ for leukocytes). In leukocytes, pre-incubation at 50 °C for 60 min was effective to differentiate FD patients from healthy controls ($p<0,001$). The results obtained in this study can be used as an auxiliary method to the FD diagnosis, especially in cases of patients whose GLA activity is within normal range.

P-381

Novel mutations and prevalence of Fabry disease (FD) determined via screening studies on dried blood spot samples (DBS) from hemodialysis patients in Turkey

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Patients with FD may present with end-stage renal disease (ESRD). FD is possibly underdiagnosed in patients with ESRD. We present the results of the largest screening study for FD via enzymatic analysis in DBS among ESRD patients undergoing hemodialysis treatment in different regions of Turkey. The study results are compared with the result of he first preliminary study published in 2013 by us. We also report the overall prevalence for FD among hemodialysis patients studied in Turkey.
Methods: A total of 3985 hemodialysis patients including 2849 patients from 32 dialysis centers in 10 different cities of Turkey in the second study, and 1136 patients from 16 dialysis centers in Ankara in the first preliminary study, were screened for FD. In both studies, screening for α-galactosidase A (α-Gal A) deficiency was performed using a DBS on filter paper. GLA gene sequence analysis was performed in patients with low enzyme activity.
Results: Of the 3985 patients (54.4 % male, 45.6 % female), 24 had low α-Gal A activity. A total of four male patients (two patients in each of the two studies) was diagnosed with FD by enzymatic and molecular analysis. Two novel mutations [hemizygous c.638C>T (p. P214S) in exon 5 and hemizygous c.157_158delinsC (p. Asp53Profs*68)] were identified.
Conclusion: The overall prevalence of FD was 0.1 % among hemodialysis patients.

P-382

The results of enzyme studies in the diagnosis of lysosomal storage diseases: 5 year experience at Gazi University, Ankara, Turkey

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Introduction and Aim: Lysosomal storage diseases (LSDs) are a group of approximately 50 rare inherited metabolic disorders that result from defects in lysosomal function. We report the patients diagnosed through enzymatic analysis for LSDs in the Pediatric Metabolism Laboratory of Gazi University, Ankara, Turkey, between October 2005 and April 2014.
Methods: Lysosomal enzyme activities were determined by fluorometry using 4-methylumbelliferryl (4 MU) substrate or spectrophotometry using p-nitrocathecholsulphate in leukocytes.
Results: Seven hundred twenty-five out of 4518 patients referred with suspected LSDs were diagnosed with LSDs. Patient numbers were: 158 Gaucher disease, 138 Niemann-Pick disease A/B, 57 Fabry disease, 195 mucopolisaccaridoses (31 MPS type I; 40 MPS type II; 59 MPS type IIIB; 20 MPS type IIIC; 5 MPS type IIID; 46 MPS type VI and MPS type VII), 41 mucolipidosis, 38 metachromatic leucodystrophy, 27 GM1-gangliosidosis, 30 GM2-gangliosidosis, 10 Wolman disease, 7 alpha-fucosidosis, 6 mannosidosis, 4 Krabbe disease, and 4 Pompe disease.
Conclusion: Because most of LSDs are autosomal recessively inherited they have a high incidence in countries where consanguineous marriages are widespread, like Turkey (21.2 %). In recent years, there has been important progress in the treatment of LSDs which encourages hope for the patients.

P-383

Neurological manifestations in patients with Gaucher disease type 1

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Background and objectives: Gaucher's disease type 1 (GD1), a lysosomal storage disorder, has been recently associated with Parkinson's Disease (PD). We studied GD1 patients with regard to extrapyramidal signs (including DaTscan®), eye movements abnormalities and cognitive impairment, and compare them with patients with PD and heterozygous GBA mutations.
Methods: Cross-sectional study, including all patients in our hospital with the diagnosis of GB1 and PD patients with GBA mutations.
Results:We observed 5 patients with GD1 and 4 patients with PD and heterozygous GBA mutations. In the group with GD1, the mean age was 52.2 and the mean age of first symptoms/signs was 24. None of the patients had subjective complaints of tremor, 4 had mild extrapyramidal signs (mean UPDRS-III 3.8), the mean MoCA score was 24 and in 2 patients abnormalities in horizontal saccades were detected. In the group with PD and heterozygous GBA mutations, the mean age was 69.25 and the mean age of first parkinsonism signs was 60.50. All patients had a bradykinetic-rigid parkinsonism and mean MoCA score was 14.25. All the DATscan results are not yet available.
Conclusion: Besides the usual phenotype of the disease, the clinician must be aware of possible neurological manifestations in non-neuronopathic GD.

P-384

Gaucher disease: Levels of inflammatory factors in patients with and without enzyme replacement therapy

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Gaucher disease (GD) is a lysosomal storage disease caused by deficiency of the enzyme β-glucosidase (GBA). Activity of chitotriosidase is highly increased in GD and its activity decreases with enzyme replacement therapy (ERT). Deficiency of GBA causes accumulation of glycosylceramide which causes a neuroinflammatory response associated with activation of inflammatory markers and cytokines. This study aimed to determine the levels of TNF-a and IL-6 in plasma of patients with and without ERT. Three groups of subjects were analyzed: GD patients without ERT (G), GD patients under treatment (GT), and controls (C). Blood was collected with heparin and plasma was isolated by centrifugation. Plasma levels of IL-6 and TNF-α were determned by enzyme-linked immunosorbent assay (ELISA). Group G showed significantly higher IL-6 (474.6 pg/mL) than the other groups (354.7 and 385.2 for groups C and GT, respectively) and TNF-α (1075.7 pg/mL) was significant lower than in group C (1297.5 pg/mL). The TNF-α level from group GT did not differ significantly from the others (1186.6 pg/mL). In this context, IL-6 could be responsible for stimulating the expression of other anti-inflammatory cytokines, reducing the expression of TNF-α. Thus, IL-6 could be used as a biomarker during treatment by ERT.

P-385

Fabry disease screening study in hypertrophic cardiomyopathy: results of a pilot study

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Introduction: The results of the Belgian Fabry Study I (BeFaS I) suggested that Fabry disease may play a role in up to 1 % of young patients with stroke. In screening studies for this disease in (left) hypertrophic cardiomyopathy, including one Belgian study recently reported, 1-5 % patients were found. We report the results of a screening study performed in our population.
Methods: Analysis of the cardiology database revealed 244 subjects (age: 30–60 years) who matched the inclusion criteria of minimum interventricular and/or posterior left ventricular wall thickness of 13 mm. Male subjects (155) were screened biochemically through measurement of α-Gal A enzyme activity in bloodspots; in the female subjects (89) α-Gal A gene analysis was performed.
Results: In two female subjects the p. D313Y mutation was found. No Fabry patient could be detected.
Conclusions: Our findings demonstrate the importance of developing targeted screening protocols for the identification of Fabry patients. The significance of the p. D313Y mutation remains unclear: the prevalence of this mutation is at least 1 % in patients with (cardio-) vascular disease (BeFaS, Portystroke). The finding of this mutation in patients appears to be a risk factor in developing cardiovascular disease.

P-386

Misdiagnosis as Fabry disease: challenges In the diagnosis

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Fabry disease can present with a broad spectrum of clinical features, and the diagnosis can be challenging. Previous studies explored a number of misdiagnosis such as rheumatic fever, gout, gastro-esophageal reflux, growing pains, petechia, irritable bowel disease and psychogenic disease. The frequency of misdiagnosis is decreasing because of the rising awareness of Fabry disease. On the other hand this can cause factitious diagnosis of Fabry disease especially in patients with a positive family history. Here we represent two cases with misdiagnosis as Fabry disease.
Case 1: A 21 year-old male patient, previously healthy, was admitted to the cardology unit with fatigue, weakness and palpitation. Echocardiography revealed left ventricular hypertrophy. Based on cardiac involvement, Fabry disease wasvconsidered as diagnosis, and mutation analysis revealed a homozygous mutation in the GLA gene (c.-30G>A). the patient was referred to our outpatient clinic.
Case 2: A 20 year-old female patient was admitted with headache, fatigue and pain in arms and legs since childhood. Enzyme activity of α-galactosidase was found to be 148,88 pmol/spot'21 h (N: 450–2000) and the patient was referred to our outpatient clinic. After clinical and laboratory investigations the patients were diagnosed as glycogen storage disorder type 3 and ornitine transcarbamylase deficiency, respectively.

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Evaluation of plasma cholestane-3β,5α,6β-triol and 7-ketocholesterol in patients with Niemann-Pick type C disease and with other cholesterol metabolism related-disorders

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Oxysterols are products of cholesterol oxidation due to increased oxidative stress which has been reported in Niemann-Pick type C (NP-C) mutant fibroblasts, tissues, and patients. Recently, cholestan-3β,5α,6β-triol (C-triol) and 7-ketocholesterol (7-KC) have been proposed as diagnostic biomarkers of NP-C, representing an alternative diagnostic tool to the more invasive and time-consuming fibroblast filipin test. To test this hypothesis, we evaluated by LC-ESI-MS/MS plasma oxysterols, as dimethylaminobuthyrate ester derivatives, in 13 confirmed NP-C patients as well as patients with other lysosomal storage diseases (LSD) and cholesterol metabolism-related disorders (CMRD). All NP-C patients showed increased C-triol levels (median 55.5 ng/ml; controls 1.6-23.1); 7-KC was also elevated (median 120.0 ng/ml; controls 10.2-40.4) with the exception of 2 samples showing values within normal limits. In 2 Smith-Lemli-Opitz patients, C-triol was normal (3.2-7.4 ng/mL) whereas 7-KC was markedly increased (235–141 ng/mL); the patient with acid lipase deficiency showed increase of both C-triol (23.6 ng/mL) and 7-KC (66.2 ng/mL). Five LSD patients (GM1, Gaucher, Krabbe) showed normal levels of both oxysterols. Our study shows that C-triol and 7-KC are reliable biomarkers of NP-C disease, allowing a rapid and easy diagnosis. Careful clinical evaluation is needed to distinguish NP-C from other CMRD which may display elevation of plasma 7-KC.

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10 years therapy with agalsidase A: Prevention of renal failure in male Fabry patients

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Introduction: Aim of this study was to investigate the effect of long-term enzyme replacement therapy (ERT) with agalsidase α on renal function in patients with Fabry disease (AFD) compared to natural history data. These data show that AFD patients develop end stage renal disease (ESRD) with increasing age.
Patients and methods: This retrospective analysis included 24 males, receiving agalsidase α over a period of 10 years. Mean age at onset of ERT was 37 years (range 18–49). Renal function was measured by eGFR at baseline, 1, 2, 5, and 10 years after start of ERT. Patients were grouped by age into group A ≤30 and group B >30 years of age at start of ERT.
Results: Measurement of eGFR revealed a yearly increase of 2.15 ml/min/1.73 m2 in group A and a decrease of 1.59 ml/min/1.73 m2in group B. Kaplan-Meier analysis showed that in the Mainz cohort 25 % of the patients developed ESRD at the age of 52 years, whereas in the natural history study 25 % of the patients developed ESRD already at the age of 44 years.
Conclusion: This study suggests that timely initiation of ERT prevents the loss of renal function in young male patients with AFD.
Conflict of Interest declared.

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Severe cardiac involvement in a homozygous D409H Gaucher patient despite enzyme replacement therapy

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Background: Gaucher's disease (GD) is a clinically heterogeneous sphingolipidosis caused by mutations in the gene encoding lysosomal glucocerebrosidase, resulting in storage of glucosylceramide in RE cells. A homozygous D409H mutation has been associated with a particular phenotype which includes oculomotor apraxia and cardiac valvular calcifications in late childhood.
Case report: We report a seventeen-year-old patient who was diagnosed with GD at the age of 45 days because of massive hepatosplenomegaly and who is homozygous for the mutation D409H. Enzyme replacement therapy (ERT) was started at the age of 2 months and has been well tolerated. Clinically he developed at the age of 6 an oculomotor apraxia. He showed normal cardiac evaluations until the age of 14 when he complained of thoracic pain and was diagnosed with severe aortic stenosis. From then on he has undergone an aortic replacement, three coronary by-passes, several stent implantations and thrombolytic intravascular treatments. He has chronic ischemic cardiopathy requiring frequent admissions to our Coronary Unit but can follow his normal day activities when at home and with assistance at school.
Conclusion: To our knowledge this is the youngest D409H homozygous patient treated with ERT from infancy. Treatment hasn't prevented the development of rapidly progressive cardiac involvement.

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Isovaleric acidemia and Niemann Pick Type C coexistence in a patient and a new mutation for Nieman Pick Type C

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Introduction: Although inborn errors of metabolism (IEM) are rare disorders, there is a significant increse in their rate especially in countries where consagineous marriages are widespread. We report the case of a patient with jaundice and hepatosplenomegaly who was later diagnosed as isovaleric acidemia (IVA) and Niemann Pick Type C (NPC) disease concomitantly.
Case Report: 2-month-old boy with consangineous parents was admitted with jaundice and hepatosplenomegaly. Laboratory investigations revealed elevated liver enzymes, chitotriosidase and elevated alpha feto protein. Tandem mass spectrometry showed elevated isovaleryl carnitine, and urine organic acid profile showed an increased concentration of isovalerylglycine. With the suspicion of IVA, genetic testing for the IVD gene was performed; it showed a previously reported p. A314V (c.941C>T) homozygous mutation. Bone marrow biopsy showed macrophages with abnormal cholesterol storage. Lysosomal enzyme studies of β-glucosidase, acid-sphingomyelinase, and acid-lipase were normal. Genetic analysis for NPC revealed a novel homozygous frameshift mutation in the NPC1 gene: p. P733Sfs*10 (c.2196_2197insT).
Conclusion: Our patient is the first reported case with coexistence of IVA and NPC. A novel mutation for NPC is also reported. Despite of being a rare situation, the fact that a metabolic pathology may accompany another should be kept in mind.

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Molecular testing of 19 Polish patients with Niemann-Pick C disease

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Background: Niemann-Pick type C (NPC) is a neurodegenerative lysosomal storage disease caused by incorrect intracellular lipid trafficking. Mutations in two genes are responsible of NPC: NPC1 (95 % cases) and NPC2 (5 % cases). Filipin staining in cultured fibroblasts and chitotriosidase activity are biochemical markers commonly used in clinical practice in diagnostic algorithm of NPC, apart from DNA analysis.
Objective: Molecular genetic analysis of 19 patients with clinically diagnosed NPC disease.
Results: 19 NPC patients with genetically confirmed diagnosis were studied. Clinically 14 patients presented with early infantile onset, 6 with juvenile onset NPC disease. Direct sequencing of genomic DNA was used to identify NPC1/NPC2 mutations. All identified mutations were in the NPC1 gene. 26 different disease-causing mutations were found: 7 deletions and 19 missense mutations. 12 mutations have not been previously described. The mutation S954L was observed in 4 patients and was associated with a juvenile onset form. The mutation 2316_2317insT was found in 4 patients (3 juvenile and 1 early onset form).
Conclusions: Direct sequencing of the NPC1/NPC2 genes allows accurate diagnosis of NPC. Our study indicates possible genotype-phenotype correlations in NPC. Further study are needed

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Is NPC2 more common than NPC1?

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Background: Niemann Pick type C (NPC) disease is a lysosomal storage disorder affecting the intracellular trafficking of unesterified cholesterol. So far it was shown that 95 % of NPC patients show mutations in the NPC1 and ony 5 % in the NPC2 gene.
Case report: We identified an 18 year old girl with a typical NPC phenotype with first neurological symptoms at the age of 12y. Symptoms included decline of neurocognitive and motoric performance, vertical gaze palsy, weight loss >12 kg in one year, dysarthria and dysphagia.
Results: Biomarkes for NPC (cholestan-3β,5α,6β-triol and chitotriosidase activity) were normal. A genetic analysis revealed a homozygous splice mutation in NPC2, with an allele frequency of 0.9 % (http://evs.gs.washington.edu/EVS/) and a homozygosity frequency of 1:12.000. Three different abnormal protein variants are synthesized. At least one of those proteins is expected to provide residual function. A filipin staining in fibroblasts showed a cholesterol accumulation typical for NPC disease.
Conclusion: The frequency of this mutation indicates that NPC is ten times more frequent than currently estimated and that NPC2 disease is more frequent than NPC1. Although an early therapy allows slowing of disease progression, unsufficient knowledge of the disease and normal biomarkers prevents early recognition of this treatable metabolic disorder.
Conflict of Interest declared.

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Unusual bone presentation in a 4 years old female with Gaucher disease (GD) type 3

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Background: Bone manifestations are common in GD patients but only few cases showed involvement of short bone. We present a new case of vertebral body involvement in a child with GD 3.
Case Report: S.T., female, was diagnosed with sickle cell trait at 9 m because of microcytic anemia. At age 3y splenomegaly was identified which was ascribed to CMV infection. After 1y, because of hepatosplenomegaly, anemia and thrombocytopenia, bone marrow aspiration was performed showing foam cells leading to the diagnosis of GD (high ACE, glucocerebrosidase absent on DBS). Molecular analysis confirmed GD3 (Rec Ncil+N188S). Two days before ERT, fourteen after diagnosis, she developed intensive back pain and deambulation refuse without fever, normal cell count and CRP. Lumbar MRI during acute episode showed signal hyperintensity (T1 and T2) of the entire L1 vertebral body. She needed analgesic drugs iv for 5d. ERT was started. After 3 months the area of hyperintensity was still present on MRI but reduced without collapse.
Conclusions: We present a peculiar case of haemorragic infarct of a short bone in a 4y old child with a recent diagnosis of GD 3. We highlight the importance of early diagnosis because ERT could prevent bone crises but cannot reverse skeletal changes.

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HPLC-MS method development for Fabry disease biomarkers analysis

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Background and objectives: Fabry disease is an X-linked lysosomal storage disease caused by the deficiency of α-galactosidase A, resulting in glycosphingolipids accumulation in body fluids and different organs. Globotriaosylceramide (Gb3), globotriaosylsphingosine (Lyso-Gb3) and their analogues have been identified and quantified as biomarkers for the disease severity and treatment efficacy. The current study aimed to develop HPLC-MS methods in order to identify and quantify FD biomarkers.
Materials and Methods: Human Fabry patients' plasma and urine samples were processed using solid phase extraction. The samples were then analysed for levels of Lyso-Gb3 and its analogues using HPLC-ESI-MS.
Results: Extraction recovery was 90 % for urine and 70 % for plasma. Reverse phase-HPLC methods were optimised with an isocratic elution of (0.1 % formic acid/50 % acetonitrile) and flow rate of 3 μL/min. A multiple reaction monitoring mode MS method was optimised for identification and quantification of metabolites showing limit of detection of 10fmoles Lyso-Gb3. Lyso-Gb3 and 7 analogues were detected showing comparable fragments. These analogues vary from Lyso-Gb3 due to a modification in the sphingosine moiety.
Conclusion: We have established an HPLC-ESI-MS approach for analysis of Lyso-Gb3 and its analogues. Pilot data shows low levels of these biomarkers are quantified in urine and plasma.

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Biological study of pediatric forms of Gaucher disease in Moroccan patients

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Gaucher disease is an inherited glycolipid storage disorder due to lysosomal beta-glucocerobrosidase deficiency. We report here our experience of clinical and biological diagnosis in 27 Moroccan patients (15 days to 14 years) from 22 families and evaluate the correlation genotype/phenotype. The diagnosis was confirmed by beta-glucocerebrosidase measurement in peripheral blood leukocytes in 18 boys and 9 girls: 10 of Type 1, 7 of Type 2, 10 of Type 3. The consanguinity rate is of 70 %.
Type 1 patients present with splenomegaly; hepatomegaly; anemia and thrombopenia, associated to growth retardation in 75 % of cases before age of five years. Although, osseous lesions has not been observed in our patients.
All Type 2 patients died after showing neurological defects.
In Type 3, 7 patients died before the development of neurological complications. Only two patients were submitted to enzyme replacement therapy.
Molecular analysis showed that all patients of type 2 and 3 have the L444P mutation at homozygous state, while in patients of type 1, various heterogeneous mutations were observed. This study is the first one in Morocco.
So, we recommend the screening of the L444P mutation as simple tool of molecular diagnosis of neurological cases of Gaucher disease and also genetic counseling.

24. Lysosomal disorders: others

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Bone complications in Egyptian patients with Gaucher disease type 1 and 3 and role of Enzyme replacement and bisphosphonate therapy in improving their condition

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Forty-three patients, 7 Gaucher disease (GD) type 1 (1 R35+D39Q/R359Q, 3 N370S/N370S, 2 unknown) and 36 GD type 3 (29 L444P/L444P, 4 D409H/D409H, 3 D409H/L444P) were assessed for bone disease and followed for a 3–7 year period. They included 6 adults (5 GD type 3, 1 GD type 1) and 38 children with mean age of 10.1 years. Patients received enzyme replacement therapy (ERT), imiglucerase 40–60 IU/kg/2 weeks with interrupted dosing≤50 % during the 2 year period of worldwide enzyme shortage. Twenty five (71.4 %) patients had no bone symptoms whereas 17 (40.5 %) had bone aches, 4 (11.4 %) children had fractures and 2 (5.7 %) avascular necrosis of femur. Bone mineral density (BMD) ranged from −1.2 to −3.1 SD in 33 children, was normal in five and not done in three . Most patients manifested after enzyme shortage probably due to the cumulative effect of ERT. Eight children with BMD>2.5 SD +/− complications received bisphosphonates with normalization of BMD and resolution of symptoms within 2 years. ERT had no effect on BMD but maintained its stationary course. Ten patients have pectus carinatum deformity and 7 kyphoscoliosis. This is the first report of bone involvement of a large number of children with GD type 3, with normalization of BMD on receiving bisphosphonates.

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Prognostic utility of rapid leucocyte-based assay of alpha-glucosidase cross reactive immunological material (CRIM) patterns in patients with Pompe disease

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Background: CRIM status is a prognostic indicator in Pompe disease. CRIM (−) patients have no detectable alpha-glucosidase (GAA) and a worse outcome. We developed a rapid CRIM assay, facilitating timely immunomodulation preceding enzyme replacement therapy (ERT) in CRIM (−) patients. Further, we hypothesised that GAA bandform patterns in CRIM (+) patients carried additional prognostic information.
Methods: A Western-blot CRIM protocol was optimised for blood samples. Correlations were investigated between CRIM status, GAA bandform pattern and clinical and echocardiographic parameters, urinary tetrasaccharide and bloodspot alpha-glucosidase activity.
Results: CRIM was determined in 29 infants (0.3-12months) and 6 adults. All adults were CRIM (+) with detectable processed GAA (76 kDa), but 3/6 had no precursor GAA (110 kDa). 7/29 infants were CRIM (−). There was no correlation between CRIM and age, baseline tetrasaccharide or alpha-glucosidase activity. In 5/22 CRIM (+) infants only the precursor GAA 110 kDa band was detected. One patient had a poor outcome with no echocardiographic response and died at 10 months. 3/22 CRIM (+) infants had all bandforms detectable. A patient had significant echocardiographic and motor improvement after 3 months ERT.
Conclusion: Rapid CRIM testing has facilitated timely detection of CRIM- patients and instigation of immunomodulatory therapy. Differential bandform patterns in CRIM positive patients may yield further prognostic information.
Conflict of Interest declared.

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^{1}H-MRS as a tool to monitor glycogen accumulation in the central nervous system: A case of infantile Pompe disease

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Background: Pompe disease leads to the accumulation of glycogen in muscle and nerve cells throughout the body. Proton magnetic resonance spectroscopy (^{1}H-MRS) is a non-invasive method of determining brain metabolites in vivo. Here we report an infantile Pompe disease, whom we used ^{1}H-MRS to assess the degree of the accumulation of glycogen in the central nervous system (CNS).
Case report: The patient is an 11-year-old Japanese who was diagnosed as Pompe disease at age 8 months. He has received enzyme-replacement therapy (ERT) since age 2 years. MRI of the brain was performed at 6 and 11 years of age, revealing enlargement of T2WI hyperintensity area during 5 years. A ^{1}H-MRS study demonstrated a decreased peak of N-acetyl aspartate, and abnormal peaks at 3.6-3.9 ppm. and 5.3 ppm, which suggested the accumulation of glycogen in the deep white mater. At age 11 years, a decreased NAA peak and similar abnormal peaks were also observed in the basal ganglia and the cerebellum, as well as in the deep white matter.
Discussion: This is the first study to demonstrate glycogen peaks in the brain ^{1}H-MRS in a case of Pompe disease. ^{1}H-MRS would be one of the diagnostic tools to evaluate the efficacy of current ERT and novel CNS-targeted therapies.

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Analysis of plasma oxysterols by LC-MS/MS as mono-dimethylglycine derivatives in Niemann-Pick Type C patients

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Background. Increased levels of plasma cholestane-3β,5α,6β-triol (CT) and 7-ketocholesterol (7-KC) have been found in NPC patients, suggesting CT and 7-KC as biomarkers of NPC disease.
Objective. Setting up the methodology by LC-MS/MS to corroborate the previous findings in NPC patients.
Material and methods.CT and 7-KC were analyzed by LC-MS/MS after derivatization with dimethylglycine. We identified the mono-dimethylglycine derivatives of CT and 7-KC as the most abundant peaks in the oxysterol profile. The method for both compounds was linear up to 800 ng/mL with a low limit of detection (1 ng/mL) and quantification (3 ng/mL). Within-day and between-day CV were <8 % and recovery was ≥92 %. CT and 7-KC were significantly higher in NPC patients (n=13) than in controls (n=107). The highest levels of oxysterols in patients with more severe clinical phenotypes. One asymptomatic NPC individual also showed an increase of CT and 7-KC. CT was specific for NPC disease while 7-KC was high in other diseases (n=16).
Conclusions: Our method has a good analytical performance. CT and 7-KC are good biomarkers for the diagnosis of NPC and correlate with the severity of the disease. In our hands, CT is a specific biomarker of NPC disease.

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Diagnosis of Fabry disease: Selection criteria for case-finding studies

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Background and Objectives: Because specific enzyme replacement therapy is available for Fabry disease (FD) patients, identification of affected patients is important. Therefore there is strong reason for establishing screening programs and/or case-finding studies among the high-risk patient groups.
Materials and Methods:This prelimenary study aimed to explore the prevalence of signs and symptoms of FD in patients who attended four outpatient clinics (Internal Medicine, dermatology, neurology, urology) of Kirikkale University Hospital.
Results: The study group was consisted of 1012 patients (601 female, 59,4 %; 411 male, 40,6 %) with a mean age of 40.5±19.24 years (range 17–95 years). A short questionnaire based on the symptom checklist of the Fabry Community was used to collect the data. Heat/cold intolerance (42,3 %), exercise intolerance (34,5 %), gastrointestinal problems (27,2 %) and acroparethesia (28 %) were the most prevalent symptoms. This study confirms that some previously described symptoms are significantly more frequent in patients with a family member that has had heart or renal disease.
Conclusion: This was a baseline study to identify symptoms that could be used for case-finding studies. We suggest that presence of one high-frequency symptom plus a family history of heart or renal disease may be used as suitable selection criteria.

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Niemann-Pick Type C disease with NPC2 gene mutation

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Background: Niemann-Pick diseases are hereditary neurovisceral lysosomal lipid storage disorders, of which the rare type C2 almost uniformly presents with respiratory distress in early infancy.
Case report: We report a 5-months-old infant with Niemann-Pick type C2 (NPC2) disease with early and fatal respiratory distress. He admitted to our emergency service with respiratory failure. He was the second child of unrelated parents; the mother had three siblings who died of unknown cause. On physical examination there was failure to thrive, hepatosplenomegaly, respiratory distress and hypotonia. Metabolic work-up demonstrated normal levels of sphingomyelinase, acid lipase and beta-glucosidase. Chitotriosidase activity was elaveted. Lung involvement was observed on both X-ray and CT images of the lung. Foamy hystiocytes were seen in bone marrow aspiration. Molecular analyses showed a homozygous c.352G>T (p.E118X) mutation in the NPC2 gene.
Conclusion: Severe and early respiratory distress is more likely to be associated with the rare NPC2. While NPC2 gene mutations have been reported in 5 % of patients worldwide, they have been more frequently observed in our patients compared to other countries. NPC should be suspected in patients with lung involvement which is resistent to treatment and with systemic involvement such as hypotonia and hepatosplenomegaly.

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Lysosomal acid lipase (LAL) activity and stability in dried blood spots: Brazilian reference values

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Background and objectives: Lysosomal acid lipase (LAL) deficiency leads to two different lysosomal storage disorders: Wolman disease and Cholesterol ester storage disease. The aim of this study was to determine LAL activity in dried blood spots (DBS), as well as its stability, in a Brazilian population sample to enable the use of this assay as a diagnostic tool.
Methods: LAL activity was determined by a fluorimetric assay, according to Hamilton et al., (2012), with slight modifications.DBS were collected from 37 healthy subjects and 4 LAL deficient patients. LAL stability was evaluated by measuring enzyme activity from 3 control samples, stored at 4 °C, during 5 months.
Results: LAL activity in healthy volunteers ranged between 23.56 and 199.67 pmol/punch/h and was not detectable in all patients. After 5 months from sample collection, LAL activity in DBS decreased up to 65 %.
Discussion/Conclusion: Our assay clearly differentiated healthy subjects from patients with LAL deficiency, showing that DBS samples can be used as a diagnostic tool. Care should be taken when LAL activity is detectable but below 23 pmol/punch/h and when stored DBS are used for the assay. In these cases, another enzyme must be analyzed and a new DBS collection is recommended.
Financial Support: CNPq, AFIP and IGEIM.

P-403

A review of our patients with Pompe disease: four identified novel mutations

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Pompe disease is an autosomal-recessive lysosomal storage disorder caused by a deficiency of acid alpha-glucosidase. Accumulation of glycogen in various tissues leads to a progressive skeletal muscle weakness which results in a decline of locomotive and respiratory functions. Infantile patients show first symptoms within the first months of life with heart failure and a profound muscle weakness. Without enzyme replacement therapy, most of infantile patients die within the first year of life.
We reviewed a total of 9 patients. The mean diagnostic age was 4.3 months. Hypotonicity was a common feature in all, and hypertrophic cardiomyopathy was detected in 8 of 9 patients. In one patient who is free of cardiac disease, relapsing respiratory failure was present. The activity of the enzyme alpha-glucosidase was markedly low in all patients. GAA sequencing detected four homozygous mutations (p.R854X, c.2019C>A, c.1396-1397insT and p.Q682R), one heterozygous mutation (c.670C>T) and two heterozygous mutations coexisting in one patient (c.1061del+c.2015G>A). 4 patients were deceased on follow-up; the mean ERT duration of the remaining 5 patients was 13 months. The mean increase in percentage EF was 17 %. Mutations p.Q682R, c.2019C>A, c.1061del, c.2015G>A, and c.1396-1397insT, are novel mutations that have not been previously reported in Pompe disease.

P-404

Epidemiology of lysosomal storage diseases in Sweden

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Background and objectives: Lysosomal storage disorders (LSDs) are a group of more than 50 inherited diseases. Effective treatments are emerging making early diagnosis increasingly urgent. The aim was to determine the incidence of clinically diagnosed lysosomal storage diseases in Sweden.
Materials and Methods: Compile the number of patients with LSDs diagnosed during 1980–2009.
Results: A total of 433 patients were diagnosed during the 30 year period. The compiled incidence calculated from the last 20 years was 1:6 100 births. Most common was Krabbe disease (1:39 000) followed by Gaucher disease (1:47 000), metachromatic leukodystrophy (1:58 000) and Salla disease (1:63 000). Gaucher disease is more frequent in Sweden than other European countries, which is caused by a founder effect of the mutation (p.L444P) present in the northern part of Sweden.
Metachromatic leukodystrophy follows the pattern of other countries being one of the most common LSDs. Salla disease, which is very rare elsewhere, was the fourth most common, which stems from a founder mutation in the Salla region in northern Finland brought to Sweden by immigration.
Conclusion: The collective incidence of LSDs is essentially equal to other European countries but the disease pattern is somewhat different.

P-405

Diagnosis of variant late-infantile neuronal ceroid lipofuscinosis (CLN6) using a NGS inherited disease panel

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Background: A 5-year old Pakistani boy of consanguineous parents presented with neuroregression, myoclonic epilepsy, global developmental delay and ophthalmologic changes, and was referred for testing for neuronal ceroid lipofuscinosis (NCL). Infantile NCL (CLN1) and classical late-infantile NCL (LINCL; CLN2) were excluded by biochemical studies.
Method: Molecular analysis was performed using the Illumina TruSight™ 552-gene Inherited Disease Panel, which contains the NCL genes CLN3, CLN5, CLN6, MFSD8 (CLN7), CLN8 and CTSD (CLN10). DNA was sequenced on an Illumina Miseq system. Variants in these genes were assessed using Integrative Genomics Viewer (IGV) and Illumina VariantStudio.
Results: A single nucleotide duplication (c.316dupC) in exon 4 of the CLN6 gene was detected and homozygosity confirmed by Sanger sequencing. This change is predicted to cause a frame shift and premature stop codon (p.Arg106Profs*26), and is consistent with the diagnosis of LINCL. No pathogenic changes were observed in any of the other NCL genes.
Conclusion: The use of a NGS panel provided an efficient and successful means of screening multiple NCL genes. The implementation of this technology will enable rapid molecular testing in a range of patients with a known or suspected inherited metabolic disorder.

P-406

Infantile nephropathic cystinosis with severe gastrointestinal manifestations: a case report

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Nephropathic cystinosis, an inherited lysosomal storage disorder, is caused by defective lysosomal cystine transport. Intracellular storage of free cystine results in widespread tissue damage and organ dysfunction. We present an 8 months old boy who was referred for hypotonia, motor developmental delay and failure to thrive. Family history revealed that his sister died from severe malnutrition, dehydration and electrolyte imbalance with severe metabolic acidosis at 2 months old. Our patient appeared normal at birth. He had frequent vomiting, diarrhea and abdominal distension afterwards. Hepatomegaly was present.
Generalized aminoaciduria was detected. Abdominal ultrasonography revealed bilateral nephrolithiasis and hydronephrosis. Leukocyte cystine level was increased at 2.3 nmol half-cystine/mg protein (<0.3 nmol). A p.thr260Ile homozygous mutation was detected in the CTNS gene. After the diagnostic onset of infantile nephropathic cystinosis, oral cysteamine therapy was initiated (50 mg/kg/day). Continuous total parenteral nutrition was required for electrolyte imbalance, insufficient oral nutrition and abdominal distension. The patient died from central catheter infection at 9 months old.
Gastrointestinal dysfunction, an extra renal manifestation of infantile nephropathic cystinosis, is rarely considered besides renal and other involvement, but it can cause morbidity and mortality and must be taken into account more often in these patients.

P-407

Case presentation: a girl with cholesterol ester storage disease

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Cholesterol Ester Storage Disease (CESD) is an autosomal recessive disease characterized by deficiency of lysosomal acid lipase (LAL). Sometimes hepatomegaly, detected usually during early childhood or the neonatal period, is the most prominent and sometimes only symptom. Here we report a 3 year old girl who was followed up with hepatomegaly and abnormal liver function tests (LFTs) in various hospitals who was diagnosed with CESD in our center. She was born at term by vaginal delivery, birth weight was 2800 g. The parents are non-consanguineous. At age 6 months, abnormal LFTs were found and thought to be due to an upper respiratory tract infection (URTI). One year later, abnormal LFT's were again observed when she presented with abdominal pain. Liver was palpable 2 cm below the right costal margin. Viral markers, sweat test, α-1 antitrypsin, creatinine kinase, metabolic screening, autoimmune parameters and ceruloplasmin levels were all in normal range; however, liver biopsy revealed minimal portal fibrosis. Diagnosis was made by showing decreased acid lipase activity 0,01 (0,37-2,3 nmol/spot/hr) in dried blood spot. CESD is rarely seen; many cases may not be diagnosed because of paucity of symptoms. CESD should be considered in the differential diagnosis of patients with isolated hepatomegaly or increased LFTs.

P-408

Molecular analysis of patients with Pompe disease

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Pompe disease is an autosomal recessive disorder which refers to lysosomal storage diseases. It is caused by mutations in GAA gene that encodes the lysosomal alpha-1,4 glucosidase. Its deficiency leads to the accumulation of non-hydrolyzed glycogen in lysosomes of the muscle tissue which is followed by the symptoms of progressive muscular dystrophy.
In the course of the selective screening for six lysosomal enzymes by tandem mass spectrometry (1521 patients were researched) the low alpha-1,4 glucosidase enzyme activity in dried blood spot specimens 0.52±0.19 (0.13-0.81) was found in 10 patients (normal range 1–25 μM/L/h). In the process of molecular genetic testing of the GAA gene in 10 patients the diagnosis of Pompe disease was confirmed. The homozygous c.525delT and p.Gly334Ser mutations were found in two of the ten patients. Another 15 heterozygous mutations (mutation c.-32-13 T>G were found in 2 patients), eleven of which were described previously were found in 8 patients, four substitutions never described before: c.2077_2078dupA, c.1379_1380insCGA, c.1951_1952delGGinsT and c.2799+4A>G, which was found in proband and sibling with the late-onset Pompe disease. The measurement of alpha-1.4 glucosidase activity using tandem mass spectrometry can be successfully introduced into a routine screening laboratory.

P-409

Increased level of sphingomyelinase activity in dried blood spot in patients with mucolipidosis type II/III

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Background: Lysosomal storage disorders (LSD) comprise a group of rare genetic diseases in which a deficit of specific hydrolases leads to the storage of undegraded substrates in lysosomes. Enzyme activities can be measured in dried blood spots by MS/MS quantification of the reaction products obtained after incubation with specific substrates.
Methods: Since 2012 we perform high-risk patient screening for 6 lysosomal enzymes in our laboratory. A quadruplex solution is used for screening for Mucopolysaccharidosis type 1, Pompe, Krabbe and Fabry diseases, while a separate solution is used for Niemann-Pick A/B and Gaucher diseases.
Results: Among 1521 DBS samples analysed in the laboratory, 3 had high level of sphingomyelinase activity 235±108 μmol/L/h (normal values 7.7±4.4 μmol/L/h). Whole blood samples were used to measure other lysosomal enzymes. In all cases mucolipidosis type II/III was confirmed biochemically by high levels of several lysosomal acid hydrolases in plasma.
Conclusion: This results indicate that the mass spectrometry methodology is useful for the diagnosis of mucolipidosis II/III.

P-410

Oxysteroles in Niemann-Pick Type C: limitations of sensitivity and specificity

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Introduction: Porter et al. reported 2010 that the oxysteroles 7-keto-cholesterol (7KC) and cholestan-3β, 5α, 6β-Triol (Triol) are specific biomarkers for Niemann-Pick Type C.
Methods: We investigated oxysteroles in plasma/serum of patients with NPC (n=18) and for the first time in patients with Niemann-Pick Type B (NPB) (n=2) and Cholesteryl Ester Storage Disorder (CESD) (n=1).
Results: 15 NPC patients with confirmed biochemical and genetic diagnosis showed distinct elevated oxysteroles (7 KC: Median: 440 μmol/l, Triol: Median: 148,5 μmol/l, normal range for 7KC: <250; Triol<100)). 3 patients with clinically and biochemically confirmed diagnosis of NPC, who had none or only one mutation in the NPC1-/NPC2-gene, revealed normal concentrations of oxysteroles. Patients with other lysosomal storage disorders (NPB and CESD) also showed distinct elevated concentrations of oxysteroles (NPB: 7KC 820/Triol 407; CESD 7 KC 600/Triol 200).
Discussion: Our investigations refute the assumption of Porter et al. that oxysteroles are specific and sensitive biomarkers for the diagnosis of Niemann-Pick Type C. Our data revealed that oxysteroles may also be elevated in other lysosomal storage disorders. Furthermore normal oxysteroles may not exclude the diagnosis of NPC. However oxysteroles are useful screening tools in the differential diagnosis of NPC.

P-411

Negative urinary excretion of free sialic acid in Salla disease-a case report of a 5 year old girl

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Background: Salla disease is an autosomal recessive free sialic acid storage disease that is extremely rare outside of Finland. The SLC17A5 gene encodes sialin, an anion/cation symporter, which may also function as a glutamic acid/aspartate transporter in the brain. Transport of free sialic acid through lysosomal membranes is impaired. CNS manifestations may be due to disturbances in glutamatergic/aspartergic neurotransmission.
Case report: We report on a 5 year old Swiss girl, who presented with slowly progressive spastic paraparesis, mild ataxia and global

developmental delay from the age of 2 years. First cMRI at age 4 years showed marked supratentorial hypomyelination with thin corpus callosum. ^{1}H-MRS revealed increased lactate within the white matter and basal ganglia. Urinary excretion of free sialic acid was significantly elevated on one occasion, but normal in a subsequent sample. Genetic analysis confirmed compound heterozygosity for the finish founder mutation c.115C>T and a known splice site mutation c.291G>A in the SLC17A5 gene.
Discussion: Leukodystrophies with hypomyelination are a group of genetic defects, distinguishable from classical demyelinating disorders by imaging techniques. Salla disease belongs to the differential diagnosis of hypomyelination. Our observation stresses the fact, that sialic acid excretion is an unreliable biomarker for selective screening of Salla disease.

P-412

Clinical utility of oxysterol cholestane-3β,5α,6β-triol measurement by LC-MS/MS as a biomarker for Niemann Pick C disease: The UK experience

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Background: Biochemical detection of Niemann Pick type C is dependent on filipin staining on fibroblasts and plasma chitotriosidase neither of which is sensitive nor specific. Cholestane-3β,5α,6β-triol, an abundant oxidative product of cholesterol, has been validated as a biomarker for NPC in other countries. We evaluated its clinical utility in two NPC Specialist Centres (paediatric & adult services) in the UK.
Method: Plasma cholestane-3β,5α,6β-triol was detected by LC-MS/MS (Jiang X et al., 2011) in mutation-confirmed NPC type 1 subjects (n=15; aged 2 months to 44 years) and control subjects (n=70; 1 week to 69 years).
Results: Interassay precisions were 12 % at 24.4 ng/ml; 7 % at 228.1 ng/ml. 95th Confidence intervals in the control and NPC groups were 10.9–34.0 ng/ml and 43.2–453.9 ng/ml respectively. A cutoff of 34.0 ng/ml showed 100 % sensitivity and 94 % specificity. Cholestane-3β,5α,6β-triol was highest at 1170 ng/ml on a 7-day old infant who had visceral involvement (neonatal hepatitis and jaundice) and did not survive infancy. A positive correlation with clinical severity was observed in two adult siblings with juvenile onset.
Conclusion: Cholestane-3β,5α,6β-triol by LC-MS/MS is a rapid, highly sensitive and specific screening test for NPC. Dependency on filipin staining for diagnosis would be reduced. Early detection of this previously under-diagnosed disease becomes possible.
Conflict of Interest declared.

P-413

Improved diagnostics of Niemann Pick type C by oxysterol analysis

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Background: The diagnosis of Niemann Pick type C (NPC) disease usually takes several months or years. Available screening methods – including filipin staining in fibroblasts and analysis of the chitotriosidase activity in plasma- are unspecific and insensitive, leading to several false positive and false negative results.
Methods: In an ongoing study with more than 1000 plasma samples examined during the last two years, cholestan-3β,5α,6β-triol was evaluated as biomarker for a rapid diagnosis of NPC. Plasma was collected from confirmed NPC patients, patients with the suspicion of NPC disease and heterozygote parents and siblings. Plasma levels of cholestan-3β,5α,6β-triol were detected by GC/MS analysis.
Results: Samples of confirmed NPC patients served as internal quality control and showed increased levels of plasma cholestan-3β,5α,6β-triol (<0.05 ng/μl, range 0.07-0.22 ng/μl). In 37 samples of patients with NPC suspicion, elevated cholestan-3β,5α,6β-triol was evident. A genetic analysis was initiated after positive cholestan-3β,5α,6β-triol results, leading to a rapid diagnosis of NPC1 (n=36) or NPC2 (n=1). Elevated cholestan-3β,5α,6β-triol levels were also present in additional 24 samples, where the genetic result is still awaited.
Conclusion: With correct sample handling, plasma cholestan-3β,5α,6β-triol may serve as sensitive and specific biomarker leading to a rapid diagnosis of Niemann Pick type C disease in 37 patients.
Conflict of Interest declared.

P-414

Early clinical presentation of Danon disease in a boy with a novel mutation of the LAMP2 gene

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Danon disease is an X-linked dominant disorder caused by deficiency of lysosomal-associated membrane protein 2 due to LAMP2 gene mutations. Main clinical characteristics are myopathy, cardiomyopathy and intellectual disability. Our patient was followed due to permanent aminotransferases elevation since 2 months of age. He presented to us at the age of 2.5 years with concentric hypertrophic cardiomyopathy, elevated CK and delayed psychomotor development. Acid alpha-glucosidase was normal. Pathohistological analysis of muscle tissue showed subsarcolemmal a+D24nd intermyofibrillar glycogen depositions. During the next years he became hypotonic with muscle wasting and mental difficulties leading to the diagnosis of ADHD. His heart function was stable, CK permanently elevated, and aminotransferases higher than expected for degree of muscle affection, but without other signs of hepatopathy. PAS staining of liver tissue also showed increased glycogen content. Selective family screening revealed elevated CK and hypertrophic cardiomyopathy in his 7-years old brother who afterwards also developed axial muscle weakness and learning difficulties. Western blot analysis showed the absence of LAMP2 protein in proband's muscle tissue. The Danon disease was further confirmed by sequence analysis of the LAMP2 gene, detecting a novel c.987 T>A mutation that leads to premature stop codon and truncated protein. Their mother also has cardiomyopathy.

P-415

Successful prenatal diagnosis in infantile free sialic acid storage disease (ISSD) caused by a deletion in SLC17A5 gene classified as a variant of unknown significance (VUS)

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Background: ISSD is caused by autosomal recessive mutations in the SLC17A5 gene. We here report a case with a deletion mutation in the SLC17A5 gene which was classified as a variant of unknown significance in a mutation database, and successful prenatal diagnosis of the subsequent sibling.
Case Report: A couple whose second child had died from ISSD presented at 8 weeks of gestation. The index patient had been diagnosed ISSD at the age of 8 months after presenting with failure to thrive, microcephaly, coarse facial appearance, fair hair, hepatosplenomegaly, and increased urine free sialic acid content (108 μmol/mmol creatinine). He was homozygous for a deletion of at least 1325 bp and at most 18668 bp spanning exon 1 and intron 1 in the SLC17A5 gene; both parents are heterozygous for the deletion. No mutation was detected at 10 weeks of gestation in the subsequent fetus, and a healthy boy was born. Urine sialic analysis revealed normal results at 3 months of age. He is now 9 months old with normal growth and development.
Conclusions: Described deletion mutation was previously classified as VUS in a mutation database. We here report that the deletion causes ISSD and report successful prenatal diagnosis of the subsequent sibling.

P-416

Microalbuminuria in children and adolescents with Fabry disease in the Fabry Outcome Survey: occurrence and association with other Fabry features

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Background and objectives: The clinical course of renal involvement in children with Fabry disease (FD) is not well described. Renal parameters in children in the Fabry Outcome Survey (FOS; an international registry sponsored by Shire) were evaluated together with other FD manifestations.
Patients and Methods: This retrospective study analysed data (extracted August 2013) from children in FOS treated or not with agalsidase alfa. Left ventricular mass index (LVMI), estimated glomerular filtration rate (eGFR), microalbuminuria (MAB), and FOS Mainz Severity Score Index (FOS-MSSI) were assessed.
Results: MAB was present in 24/52 (46.2 %) girls and 22/50 (44.0 %) boys, mainly ≥12 years of age. Girls with MAB had significantly higher maximum LVMI ($p=0.013$) and lower minimum eGFR ($p=0.002$) versus those without MAB. Baseline median FOS-MSSI was higher in children with MAB (girls 13.5; boys 13.5) compared with those without MAB (girls 11.0; boys 10.5), although these differences were not significant in this small sample.
Discussion/Conclusion: Data suggest MAB occurs frequently in children with FD and it appears to be associated with greater cardiac and renal involvement in girls and with a trend to overall higher FOS-MSSI score. Detection of MAB may be a simple test to identify children at risk for FD progression.
Conflict of Interest declared.

25. Lysosomal disorders: treatment, enzyme replacement therapy

P-417

The Australian ATHOME™ infusion service experience

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Background and objectives: Evaluate the Shire funded ATHOME™ infusion service for eligible Australians prescribed intravenous agalsidase alfa ghu (REPLAGAL®) (AAG) or velaglucerase alfa ghu (VPRIV®) (VAG) for Fabry and Gaucher Disease respectively. ATHOME enrolment is organized by treating physicians for patients after a minimum 12 AAG or 3 VAG in hospital infusions.
Patients and Methods: ATHOME Coordinator arranges an IV administration trained registered nurse to deliver, prepare, administer and monitor infusion safety in the home or workplace. Physicians receive written reports after each infusion. Records of infusion timings, retention rates and patient numbers are collated by the nurses and managed by the ATHOME Coordinator.
Results: ATHOME commenced in Australia July 2010 for AAG patients. In May 2013 it was extended to VAG patients. Total enrolments to 28 February 2014 are 30 AAG and 12 VAG patients. Patient retention to ATHOME over the length of the program has been 86.7 % and 75.0 % with an adherence of 97.9 % and 98.1 % of planned infusions administered, 89.7 % and 86.9 % delivered within 2 days of due date for AAG and VAG respectively.
Conclusion: ATHOME infusion service successfully offered enrolled patients the convenience and flexibility to receive treatment in home or workplace environment with high adherence.
Conflict of Interest declared.

P-418

Next-generation enzyme replacement therapy for Fabry disease: co-formulation of migalastat HCl with a proprietary recombinant human α-galactosidase A leads to enhanced enzyme uptake and GL-3 reduction in Fabry mice

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Fabry disease is an X-linked lysosomal storage disorder caused by a deficiency in α-galactosidase A (α-Gal A) activity, which leads to progressive accumulation of lysosomal globotriaosylceramide (GL-3) in multiple tissues. Previously we demonstrated that oral pre-administration of the pharmacological chaperone migalastat HCl (1-deoxygalactonojirimycin HCl; AT1001) improves the pharmacological properties of marketed recombinant human α-Gal A (rhα-Gal A) via binding and stabilization, and leads to improved enzyme uptake and GL-3 reduction in Gla knock-out (KO) mice.

Here we show that migalastat HCl has the same stabilizing effect on a proprietary rhα-Gal A (JR-051) ex vivo. Importantly, in Gla KO mice, a single intravenous (IV) administration of JR-051 co-formulated with migalastat HCl led to increased plasma exposure and tissue uptake of rha-Gal A, as well as a greater GL-3 reduction in disease-relevant organs compared to the administration of JR-051 alone. Furthermore, repeat IV administration studies demonstrated increased potency of JR-051 upon co-formulation with AT1001. In addition, immunohistological examination confirmed increased uptake of co-formulated JR-051 into disease-relevant cell types/structures. Taken together, these data demonstrate the potentially beneficial effects of co-formulating JR-051 with migalastat HCl into a single IV product, thus warranting clinical investigation of this next-generation enzyme replacement therapy in Fabry patients.
Conflict of Interest declared.

P-419

Effect of galsulfase enzyme replacement therapy on growth of patients with mucopolysaccharidosis VI (Maroteaux-Lamy syndrome)

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Background and objective: Growth failure and short stature are consistent features of mucopolysaccharidosis VI (MPSVI). Standard growth charts for the rapidly and slowly progressing MPSVI population show a gradual increase in height up to age 10-12y in the rapidly progressing, and throughout the teenage years in the slowly progressing patients (Harmatz et al. 2014, submitted). We studied the effect of galsulfase enzyme replacement therapy (ERT), the recommended standard of care for MPSVI, on growth in MPSVI patients.
Methods: Cross-sectional and longitudinal height for age data from 168 ERT patients who participated in various clinical trials and cross-sectional studies was compared to MPSVI standard growth charts. The rapidly and slowly progressing patients were defined by pre-ERT urinary glycosaminglycan levels of>and ≤200 μg/mg creatinine as assayed by Whitley method and defined in Swiedler et al. (2005).
Results: Rapidly progressive MPSVI patients who started ERT before age 6y showed significant increase in growth (~10 cm increase in height by age 9y). Rapidly progressing patients who started ERT between ages 6-9y and slowly progressing patients who started ERT before age 3y, also showed a benefit of galsulfase on growth.
Conclusion: Early initiation of galsulfase ERT improves growth of MPSVI patients.
Conflict of Interest declared.

P-420

Galsulfase for mucopolysaccharidosis type VI (MPS VI): analysis of clinical data since 2000

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Objective: Evaluate efficacy/safety of galsulfase enzyme replacement therapy (ERT) in MPSVI.
Methods: Summarized data from clinical trials, 10y-followup (Resurvey Study; mean 6.8±2.2y ERT) and clinical surveillance program.
Results: Endurance parameter 12-minute walk test (12MWT) showed significant improvement in mean distance of 92 m at week 24 over placebo (clinical trials); in Resurvey, patients (n=46) walked 65.7 m more (in 6MWT) versus baseline. Forced vital capacity (FVC) increased by 17 %(n=33); forced expiratory volume (FEV1) increased by 11 %(n=33) (clinical trials; 96 weeks). In Resurvey, patients 50 % of patients but were manageable with premedications and/or altering infusion rates.
Conclusions: Data support galsulfase safety; demonstrate improvements in endurance, cardiopulmonary function, growth; and long-term survival.
Conflict of Interest declared.

P-421

Desensitisation to galsulfase for the treatment of recurrent infusion associated reactions in a child with mucopolysaccharidosis type VI (MPSVI)

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Objectives: Enzyme replacement therapy (ERT) with galsulfase is an effective disease-altering treatment for MPSVI. Antibody development against infused enzymes is well-described and may adversely affect clinical response to ERT. We report a 10-year-old MPSVI patient who developed recurrent, severe infusion-associated reactions (IARs) after 5 years of ERT which only became tolerable with corticosteroid premedication leading to associated side-effects. High titres of non-neutralising anti-galsulfase IgG antibodies were present. We report our experience of using a desensitisation protocol in this patient.
Methods: The standard dose of galsulfase (1 mg/kg/dose) was reduced 100-fold (0.01 mg/kg/dose) and infused at standard rates over at least four hours in hospital. The dose was doubled each week, reaching the standard dose after 8 weeks. Premedication with loratadine and paracetamol was continued but no corticosteroids were given.
Results: No IARs occurred during desensitisation. Occasional, less frequent and severe IARs have occurred during the following year when the patient returned to home infusions. Corticosteroid premedication has not been restarted.
Conclusions: We conclude this desensitisation protocol appears to have been helpful in this patient. Previous studies report success with slowing infusions to 20 hours. This is not practical when ERT is infused at home and desensitisation may be a more practical solution.

P-422

Long-term velaglucerase alfa enzyme replacement therapy in children with type 1 Gaucher disease

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Objective: To evaluate velaglucerase alfa's long-term safety and efficacy in children with type 1 Gaucher disease.
Methods: Patients completing the trials TKT032, TKT034 or HGT-GCB-039 were offered enrolment into HGT-GCB-044 (extension study) and received every-other-week velaglucerase alfa infusions (open-label).
Results: 24/95 who enrolled in HGT-GCB-044 were ‹18 years old (range 4–17 years; n=7 TKT032, n=9 TKT034, n=8 HGT-GCB-039). During HGT-GCB-044, treatment duration varied (10.1-57.1 months); 7/24 experienced ≥1 study drug-related adverse event (AE); three serious AEs were reported in one patient (all unrelated to treatment, but one [convulsion] fatal); no patient withdrew due to an AE. The safety profile in children was consistent with that seen in the adult patients and AEs were generally similar in pattern. Nine children switched from long-term imiglucerase to velaglucerase alfa therapy 12 months before entry into HGT-GCB-044; primary efficacy variables (haemoglobin concentration, platelet count, spleen volume, liver volume) were stable in the 12-month core trial (TKT034) and HGT-GCB-044. Fifteen children started enzyme replacement (velaglucerase alfa or imiglucerase) 9 or 12 months before entry into HGT-GCB-044, in TKT032 or HGT-GCB-039; their efficacy variables continued to improve in HGT-GCB-044.
Conclusion: Efficacy variables were stable or continued to improve in children in this extension study. No new safety concerns were identified.
Conflict of Interest declared.

P-423

A multicentre, open-label study of velaglucerase alfa enzyme replacement therapy in patients with Gaucher disease in Japan

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Objective: To evaluate the safety and efficacy of velaglucerase alfa treatment in Japanese patients with Gaucher disease (GD).
Methods: Patients received every-other-week, intravenous infusions (open-label) in a 12-month study, HGT-GCB-087, which was open to enrolment to treatment-naïve patients and patients switching from imiglucerase, and designed to enrol neuronopathic and non-neuronopathic GD patients. Switch patients received the same dose (units/kg) as previously received for imiglucerase.
Results: N=6 switching from imiglucerase (48.9-60.0 U/kg, 8.9-14.2 years' imiglucerase) enrolled (no treatment-naïve patients): four male children (aged 11–15 years), one 26-year-old male, one 39-year-old female. 3/6 experienced adverse events (AEs) considered possibly or probably study drug-related: eczema (n=1), nausea and vomiting (n=1), retinal detachment (serious AE) and proliferative retinopathy (n=1). No discontinuations due to AEs occurred. One type I and two type III GD patients had abnormalities in neurological function at Baseline, and the same abnormalities at 12 months. Median (range) changes in haemoglobin and platelet count and percent changes in normalized liver and spleen volumes were −0.05 (−0.7, 1.0) g/dL, -6.2×10^9/L (−12, 64), +0.75 % (−6.9, 16.1) and +2.65 % (−12.1, 27.0), respectively. Bone mineral density Z-scores in the adults were stable.
Conclusion: Japanese patients switching from imiglucerase were clinically stable over 12 months. Velaglucerase alfa was generally well tolerated.
Conflict of Interest declared.

P-424

Evaluation of therapeutic efficacy in Fabry disease using Lyso-Gb3 as an indicator

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Patients with Fabry disease (N=43) were treated with agalsidase alfa underwent plasma Gb3 and lyso-Gb3 measurement in the following 4 groups: those with the classic type of Fabry disease receiving monotherapy with agalsidase alfa (Group Ia) or patients switched from agalsidase beta to agalsidase alfa (Group Ib); those with heterozygous Fabry disease receiving monotherapy with agalsidase alfa (Group IIa) or patients switched from agalsidase beta to agalsidase alfa (Group IIb). In Group Ia, plasma Gb3 and lyso-Gb3 levels decreased after treatment initiation and in particular, lyso-Gb3 remarkably decreased over time during the treatment period. Group IIa also showed the similar reduction in lyso-Gb3. These findings confirm the efficacy of ERT with agalsidase alfa. In Groups Ib and IIb, plasma Gb3 and lyso-Gb3 levels pre and post switch were similar and agalsidase beta did not differ from agalsidase alfa in therapeutic effects. Although evaluated with limited number of patients, our results have indicated that lyso-Gb3 may be an indicator for therapeutic efficacy in Fabry disease and in addition, that ERT with agalsidase alfa is effective. We will continue the evaluation and increase the number of patients.

P-425

Evaluation of the efficacy of enzyme replacement therapy for cardiac hypertrophy in Fabry disease

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Patients with Fabry disease (N=31) were treated with agalsidase alfa underwent echocardiography to measure IVSd, LVpwd, EF, LV mass, and BNP in the following 4 groups: those with the classic type of Fabry disease receiving monotherapy with agalsidase alfa (Group Ia) or patients switched from agalsidase beta to agalsidase alfa (Group Ib); and those with heterozygous Fabry disease receiving monotherapy with agalsidase alfa (Group IIa) or patients switched from agalsidase beta to agalsidase alfa (Group IIb). In addition, patients were divided into 2 groups, i.e. those with (Group H) or without (Group C) cardiac hypertrophy. In the switch groups (Groups Ib and IIb) and the group with cardiac hypertrophy (Group H), LV mass was significantly reduced. In the group without cardiac hypertrophy (Group C), cardiac hypertrophy did not develop after treatment initiation and their disease conditions did not worsen. EF was maintained and IVSd, LVpwd, and LV mass reduced, indicating that agalsidase alfa reduced the metabolic products accumulated in cardiac muscle. BNP was also reduced. Although evaluated with limited number of patients, our results indicated that regular echocardiography and BNP measurement are useful for monitoring treatment progress and that ERT with agalsidase alfa is effective.

P-426

Evaluation of enzyme replacement therapy for impaired renal function in Fabry disease

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Patients with Fabry disease (N=35) were treated with agalsidase alfa underwent regular measurement of urine protein/Cr ratio, urinary Alb/Cr ratio, and eGFR. The efficacy of agalsidase alfa was evaluated according to the severity classification stated in the Guidelines for Chronic Kidney Disease (CKD). The urine protein/Cr ratio was kept within the normal range in Group A1 (<0.15 g/gCr), showed tendency towards improvement in Group A2 (0.15–0.49 g/gCr), and showed tendency towards worsening in Group A3 (≥0.5 g/gCr). The urinary Alb/Cr ratio provided the similar results. The eGFR was kept within the normal range in Group G1 (≥90 mL/min/1.73 m²) and showed tendency towards improvement in Group G2 (60–89 mL/min/1.73 m²). These results indicate that the start of ERT in the stage of mild renal function impairment may inhibit subsequent progress of renal disorder and thus lead to improvement. Although evaluated with limited number of patients, our results indicate that regular measurement of urine protein/Cr ratio, urinary Alb/Cr ratio, and eGFR are useful for monitoring treatment progress and that ERT with agalsidase alfa is effective. We will continue the evaluation and increase the number of patients.

P-427

Is premedication necessary before desensitization in an infant with Pompe disease having alglucosidase alpha anaphylaxis?

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Pompe disease (PD) is an autosomal recessive disorder resulting from lysosomal acid α-glucosidase deficiency. Enzyme replacement therapy (ERT) has known to improve cardiomyopathy,motor skills, and functional independence, and to prolong survival in patients with pompe diasease.Other than recombinant human acid alpha-glucosidase (rhGAA),there are currently no alternative treatments for PD.Desensitization is indicated in patients who had life threatening hypersensitivity reactions.We present a one year old boy with PD who had previously experienced anaphylaxis during rhGAA medication. Epidermal tests with rhGAA concentration of 1: 1000 and 1: 100 were negative four weeks after anaphylaxis.Intradermal test with rhGAA at concentration of 1: 1000 was positive with 7*8 mm induration with surrounding hyperemia. Premedication used for radiocontrast media allergies was applied prior to desensitization protocol.We performed desensitization protocol with a dosage of 10 mg/kg weekly with serial dilutions that individually prepared and delivered based on patient's clinical manifestations and tolerance.During the first week in the 10th and 12th steps of desensitization, our patient developed urticaria on his ears,eyes,face and rarely on his trunk.After repeating premedication no interruption of drug infusion was necessary.No reaction was observed in the second week of desensitization.We emphasize that premedication prior to desensitization enables us to give the ERT to the patient successfully who had rhGAA anaphylaxis.

P-428

Taliglucerase alfa 36-month clinical safety and efficacy in treatment-naïve patients

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Background: Taliglucerase alfa is an enzyme replacement therapy approved for treatment of adults with Type 1 Gaucher disease (GD) and is the first approved plant cell–expressed recombinant therapeutic protein. Safety and efficacy were evaluated in treatment-naïve adult patients in study PB-06-001 and the extension study PB-06-003. Here, we report long-term safety and efficacy results of 26 treatment-naïve adult patients with GD.
Methods: Patients were randomized to receive taliglucerase alfa 30 or 60 U/kg every other week. Spleen volume, liver volume, hemoglobin concentration levels, platelet counts, and chitotriosidase activity were assessed through 36 months.
Results: At 36 months of treatment, taliglucerase alfa (30; 60 U/kg, respectively) resulted in decreases in mean multiples of normal (MN) of spleen volume (50.0 %; 64.6 %) and liver volume (25.1 %; 24.4 %) and mean chitotriosidase activity (73.5 %; 83.0 %) with mean increases in hemoglobin concentrations (1.8 g/dL; 3.0 g/dL) and platelet counts (29,783/mm^3; 71,700/mm^3). All treatment-related adverse events (AEs) were mild/moderate and transient. The most common AEs were nasopharyngitis, arthralgia, upper respiratory tract infection, headache, and pain in extremity.
Conclusions: These 36-month results of taliglucerase alfa in treatment-naïve adult patients with GD extend the taliglucerase alfa clinical safety and efficacy dataset.
Conflict of Interest declared.

P-429

Taliglucerase alfa in adult patients with Gaucher disease who were previously treated with imiglucerase: 36-month safety and efficacy results

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Background: Taliglucerase alfa is the first approved plant cell–expressed recombinant human protein and is an enzyme replacement therapy indicated for treatment of adults with Type 1 Gaucher disease (GD). Here, we report taliglucerase alfa long-term safety and efficacy results in adult GD patients previously treated with imiglucerase.
Methods: Patients with stable disease were switched from a stable dose of imiglucerase to the same dose of taliglucerase alfa given every other week. Spleen/liver volumes, hemoglobin concentration, platelet counts, and chitotriosidase activity were assessed through 36 months.
Results: Mean (SE) values at baseline and study end were as follows, respectively: spleen volume (n=11), 4.6 (1.2) and 3.7 (0.9) multiples of normal (MN); liver volume (n=12), 1.0 (0.1) and 1.0 (0.1) MN; hemoglobin concentration (n=14),13.4 (0.4) and 13.3 (0.3) mg/dL; platelet counts (n=15), 171,222 (24,032) and 172,467 (23,067)/mm^3; and chitotriosidase activity (n=10), 12,206 (4,934) and 6,551 (3,018) nmol/ml*hr. All treatment-related adverse events (AEs) were mild/moderate and transient.

Most common AEs were nasopharyngitis, arthralgia, upper respiratory tract infection, headache, and pain in extremity.
Conclusion: Mean disease parameters were similar at baseline and following long-term treatment with taliglucerase alfa, suggesting ongoing disease stability in adult GD patients previously treated with imiglucerase.
Conflict of Interest declared.

P-430

Clinical response to eliglustat in treatment-naïve patients with Gaucher disease type 1: Post-hoc comparison to imiglucerase in a real-world setting

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Background/Objective: To compare long-term treatment response to eliglustat and imiglucerase in treatment-naïve patients with Gaucher disease type 1.
Patients and Methods: Four-year data from eliglustat-treated patients in an open-label study (NCT00358150, N=26) and 9-month data from a randomized, double-blind, placebo-controlled study (ENGAGE: NCT00891202, n=20 in eliglustat arm) were compared to 75 matched imiglucerase-treated patients enrolled in the ICGG Gaucher Registry who had received at least 15 U/kg/2 weeks.
Results: Baseline hematologic parameters were similar in the two groups but eliglustat patients had slightly larger spleens and livers. Time course and degree of improvement were similar for eliglustat- and imiglucerase-treated patients for most parameters. After 4 years, mean spleen volume decreased by 63 % and 48 %, mean liver volume decreased by 27 % and 30 %, mean platelet count increased by 95 % and 99 %, and mean hemoglobin level (g/dL) increased by 2.27 and 0.71 in eliglustat and imiglucerase patients, respectively. Mean improvements in lumbar spine and femur z-scores were consistently higher in eliglustat-treated patients; however, bone data were limited from imiglucerase-treated patients.
Conclusion: This post hoc analysis suggests that eliglustat, in treatment-naïve patients, results in improvements in organ volumes and hematologic parameters that are comparable to those observed with imiglucerase in a real-world setting.
Conflict of Interest declared.

P-431

Effects of pre-symptomatic initiation of enzyme replacement therapy for infantile-onset Pompe disease :comparison in two siblings

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Pompe disease is an autosomal recessive lysosomal glycogen storage disease caused by a deficiency of acid α-glucosidase. Infantile-onset Pompe (IPD) disease is characterized by progressive cardiomegaly, skeletal muscle weakness, delay of motor function and respiratory insufficiency, and die before the first 1 year of life. Enzyme replacement therapy (ERT) has been shown to improve symptoms and life expectancy of IPD patients. We report on two siblings with IPD in this study. The siblings initiated ERT at 4 months (older sister) and 12 days (younger sister) of age, and we compared their outcomes after 6 and 3 years of treatment respectively. At the start of treatment, the older sister showed typical symptoms of IPD. Now she is 7 years old, bedridden and needs the invasive ventilation due to progressive muscular weakness.Younger sister was initiated ERT at 12 days after birth without any symptoms of IPD. She acquired natural motor development and has no IPD symptoms at 3 years old. These results show that early ERT initiated at pre-symptomatic IPD is effective in preventing disease progression of IPD. Early diagnosis and ERT introduction can result in better clinical outcomes. Therefore, newborn screening is necessary to allow IPD patients to be treated as early as possible.

P-432

Chemical chaperone treatment for galactosialidosis: chaperone effect of NOEV on β-galactosidase activities in galactosialidosis fibroblasts

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Background: Galactosialidosis is a rare lysosomal storage disease caused by a combined deficiency of β-galactosidase (β-gal) and neuraminidase secondary to a defect of another lysosomal enzyme protective protein/cathepsin A (PPCA). There is no available treatment for human galactosialidosis so far. We have previously reported the chaperone effect of N-octyl-4-epi-β-valienamine (NOEV) on mutant β-gal proteins and G_{M1}-gangliosidosis model mice. Here, we found that NOEV caused stabilization and normalization of β-gal in PPCA deficit patients.
Materials and methods: Four patients were diagnosed by low β-gal and neuraminidase activities and low expression of protective protein and confirmed by direct sequencing of CTSA gene. Patients' skin fibroblasts were treated with NOEV and subjected to enzyme assay.
Results: In vitro NOEV treatment caused 70-80 % heat stabilization for patients' skin fibroblasts at 48 °C. In cultured SF with NOEV treatment showed high β-gal activity up to 300 nmol/h/mg protein. Even after washing out of NOEV treated skin fibroblasts with normal medium for 72 hours the activity did not change remarkably.
Conclusions: Low concentration of NOEV is effective to normalize β-gal activity in skin fibroblasts of galactosialidosis. These results suggested that chaperone compound might have a beneficial effect in part on the pathophysiology of this disease.

P-433

Comorbidities and pharmacotherapies in patients with Gaucher disease type 1 (GD1)

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Background: There are currently two treatment options for patients with GD1: enzyme replacement therapy (ERT) and substrate reduction therapy (SRT). Efficacy of these therapies can differ between patients, due to comorbid conditions and/or pharmacotherapy for comorbidities. This study examined common comorbidities and pharmacotherapies in patients with GD1.
Methods: A PubMed literature search of articles on GD1 published in the last 10 years was conducted to determine comorbidities. Prescriptions issued to patients receiving ERT in Germany and the USA during 2011

and/or 2012 were examined and concomitant pharmacotherapies descriptively summarised.
Results: The literature search revealed a wide range of comorbidities, which included disorders with known links to GD1 (e.g. Parkinson's disease and haematological malignancies) and other conditions, such as neurological, pulmonary, metabolic, cardiovascular and immunological disorders. Commonly-prescribed pharmacotherapies included analgesics and antibiotics, although a wide range of drug classes were represented. Notably, a large proportion of the prescribed drugs in Germany and the USA are known to interact with the hepatic enzymes, CYP2D6 (19 % and 15 %, respectively) and CYP3A4 (both 40 %).
Conclusion: This study highlights the diversity of comorbidities and pharmacotherapies in GD1, and reinforces the need to consider potential drug–drug interactions when determining appropriate treatment for individuals with GD1.
Conflict of Interest declared.

P-434

Prompt diagnosis and early treatment of patients with Fabry disease is still a clinical challenge

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Objective Increasing evidence suggests starting enzyme replacement therapy (ERT) early in the course of Fabry disease (FD) for optimal results. We evaluated whether time from first symptoms to diagnosis/treatment onset has decreased.
Methods Patients in the Fabry Outcome Survey (FOS, Shire) identified as the first FD patients diagnosed in families with several or no FOS members, who had available diagnosis dates and received ERT, were included. Evaluable data on time of first symptoms, diagnosis and treatment were analysed for 2 periods since ERT commercialisation (2001–2006; 2007–2013).
Results Overall demographics: N=467; median age at diagnosis, 43.0 (interquartile range [IQR]=29.0-54.0) years; 12.6 % aged ‹18 years; 58 % male. There was no significant change in delay between symptom onset and diagnosis (median [IQR] 10.5 [2.0-26.0] versus 9.0 [1.0-22.0] years). Median age [IQR] at symptom onset significantly increased after 2006 (14.5 [7.0-39.0] versus 26.0 [11.0-46.0] years; P=0.001). Median delay [IQR] between diagnosis and treatment onset after 2006 significantly reduced from 2.4 [0.8-5.6] to 1 [0.6-1.5] year (P<0.001).
Conclusion The delay in FD diagnosis has not decreased in recent years while ERT appears to be started sooner after diagnosis. Age at FD symptom onset increased over time, likely due to inclusion of late-onset variants. The data show room for improvement remains.
Conflict of Interest declared.

P-435

Improved respiratory function in a mouse model of Pompe disease treated with BMN 701

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Objectives: Pompe disease is a rare neuromuscular disorder caused by deficiency of acid α-glucosidase, resulting in accumulation of glycogen in muscle tissues leading to myopathy and respiratory muscle weakness. BMN 701, IGF2 tagged rhGAA, enhances rhGAA uptake. This study evaluates the effects of BMN 701 administration on respiratory parameters in a Pompe mouse model.
Methods: A Pompe mouse model ($Gaa^{tm1Rabn}$) contains a disrupted acid α-glucosidase gene and recapitulates clinical features of the human disease. Effects of weekly intravenous administration of 12 or 20 mg/kg BMN 701 on respiratory parameters were monitored in $Gaa^{tm1Rabn}$ mice and wild-type mice using whole body plethysmography under normoxic and hypercapnic conditions.
Results: Following four weeks of BMN 701 treatment (20 mg/kg), $Gaa^{tm1Rabn}$ mice demonstrated increased tidal volume, minute volume and peak inspiratory flow with no compensation in respiratory rate and inspiratory time during hypercapnic conditions compared to vehicle treated $Gaa^{tm1Rabn}$ mice. Dose related decreases in glycogen levels in diaphragmatic muscle were observed in treated animals.
Discussion: Administration of BMN 701 in adult Pompe mice normalized respiratory function and decreased glycogen levels in the diaphragm. These results are consistent with improvements in respiratory muscle strength observed in late onset Pompe patients treated with BMN 701.
Conflict of Interest declared.

P-436

ENCORE: a randomized, controlled, open label non-inferiority study comparing eliglustat to imiglucerase in Gaucher disease type 1 patients stabilized on enzyme replacement therapy: 24-month results

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Background/Objectives: Eliglustat is a novel oral substrate-reduction therapy in development for adults with Gaucher disease type 1. This Phase-3 trial (ENCORE, NCT00943111, Genzyme, a Sanofi company) compared eliglustat with imiglucerase. We report on the 12-month primary analysis period (PAP) and the first 12 months of the extension period (EP).
Patients and Methods: Randomized (2:1 eliglustat:imiglucerase), controlled, open-label non-inferiority trial of 159 patients previously receiving ERT for ≥3 years who had met therapeutic goals. The primary efficacy endpoint was percent of patients remaining stable after 12 months (using a pre-specified composite of spleen, hemoglobin, platelet, and liver parameters). All patients received eliglustat in the EP.
Results: Eliglustat was non-inferior to imiglucerase: 85 % of eliglustat and 94 % of imiglucerase patients maintained all four goals (lower bound of 95 % CI of difference [−17.6 %] within the pre-specified [−25 %] non-inferiority margin). Overall, 91 % of patients completed 12 months of the EP (99/106 eliglustat-eliglustat patients and 46/53 imiglucerase-eliglustat patients) and the majority have maintained stability in all parameters. Most adverse events were mild or moderate in severity. No new safety concerns have arisen after 24 months.
Conclusions: Most ENCORE patients maintained clinical stability while on eliglustat for 12 or 24 months.
Conflict of Interest declared.

P-437

Long term clinical outcomes in patients with Fabry disease receiving enzyme replacement therapy

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BACKGROUND: Fabry disease (FD) is a disorder of glycosphingolipid metabolism resulting from α-galactosidase A deficiency and accumulation of globotriaosylceramide. Although enzyme replacement therapy (ERT) has been available for over a decade, little data has been presented regarding its long-term effects. This study aims to analyse clinical outcomes in a group of FD patients on long-term ERT.
METHOD: A review of clinical and pathological data of 15 male patients who participated in a Phase II Clinical Trial of agalsidase alfa and have subsequently received ERT for up to 15 years. Renal, cardiac and neurological adverse events and overall mortality were analysed and the ability of the Fabry International Prognostic Index (FIPI) to predict these events was examined. Individual cardiac and renal function measurements were also collected.
RESULTS: A cardiac event occurred in 8/15 patients, of which 2 had a myocardial infarction and 2 required a pacemaker. 5/15 patients reached end-stage renal failure, and 4/15 patients suffered a stroke. 3/15 patients died since the original trial. More events occurred in the group with the highest baseline FIPI.
CONCLUSION: 12/15 patients experienced an adverse clinical event during 15 years ERT. Further understanding of which patients will benefit most from ERT is required.
Conflict of Interest declared.

P-438

Late onset Tay Sachs disease: Different response to the pyrimethamine treatment of two siblings' fibroblasts

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Background and objectives: Pyrimethamine (PYR) has been described as a possible pharmacological chaperone (PC) for Late Onset Tay Sachs disease (LOTS). LOTS results from mutations in the gene HEXA encoding the alpha subunit of beta-hexosaminidase A (HexA). Recently two clinical trials with PYR showed an enhancement in plasma HexA activity in some of their patients depending on their genotype. The aim of this study was to analyse PYR treatment response in fibroblast of two siblings affected by LOTS disease.
Patients and Methods: Patients were two siblings with biochemically and genetically confirmed LOTS. Fibroblasts derived from both patients were treated with different concentrations of PYR, and enzymatic activity was determined by fluorimetry.
Results: Surprisingly, treatment with PYR increased up to 1.13 fold the residual enzymatic activity in sibling 1 and up to 2.1 fold increase in sibling 2. In both cases the increase of the residual enzymatic activity was dose dependent although sibling 2 responded more efficiently to treatment.
Discussion/Conclusion: Our results point out that other factors besides genotype might be influencing PYR response. We strongly recommend pre-clinical studies in vitro in fibroblasts derived from each future patient before attempting any treatment.

P-439

Uptake and intracellular handling of DLHex-DGJ, a potential chaperone for activity enhancement of GLB1 (ß-galactosidase) gene products

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Objective: Morquio B disease (MBD) and GM1-gangliosidosis (GM1) are caused by defects in the gene coding for lysosomal ß-galactosidase (ß-gal). In certain GLB1-variants, fibroblast activity against 4-methyl-umbelliferyl substrates increases >10-fold upon culture in the presence of a competitive ß-gal inhibitor and pharmacological chaperone, DLHex-DGJ[1]. This may be due to normalized subcellular distribution and processing of ß-gal precursors, but the metabolic fate and subcellular localization of DL-HexDGJ is so far unknown.
Methods: Uptake of DLHex-DGJ into GM1 fibroblasts was measured by tandem mass spectrometry and compared with the formation of mature enzyme by Western blotting and enzyme activity.
Results: An optimum of uptake into cells was reached within 12 hours. No evidence for intracellular retention was obvious upon chase experiments. In contrast, precursor maturation and increase of catalytic activity of ß-gal appeared with several hours of delay and required up to 48 hours of chaperone exposure.
Conclusion: The diverse time courses of chaperone uptake, enzyme maturation and activity are hardly explainable by improved folding and trafficking of pre-existing enzyme precursors alone. Further data, e.g. on subcellular distribution of DLHex-DGJ are necessary to understand its effects on sensitive ß-gal mutants.
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P-440

Development of a next-generation ERT, ATB100C, for Fabry disease

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Objectives: To develop a next-generation enzyme replacement therapy (ERT) for Fabry disease using the CHART™ (Chaperone Advanced Replacement Therapy) platform technology.
Methods: A next-generation Fabry ERT based on CHART™, in which the migalastat chaperone binds and stabilizes alpha-Gal A in its properly folded and active form (denoted ATB100C), may result in increased uptake of active enzyme into target tissues and improved tolerability. A Phase 1 pharmacokinetics (PK) study of IV migalastat (Study 018), will be followed by a Phase 1/2 study to evaluate the activity, safety, and PK of ATB100C (Study 019).
Results: The Phase 1, randomized, DBPC, single IV dose study is evaluating the safety, tolerability, PK, and, in a 2-way crossover arm, the absolute bioavailability of migalastat in healthy volunteers (Study 018). This study will identify the optimal dose of migalastat for use in ATB100C, and will inform the design of Study 019 in Fabry subjects. Key measurements in Study 019 will include plasma lyso-GB_3, antibodies, plasma α-Gal A activity, and PK of migalastat and ATB100C.
Conclusions: Study 018 will characterize the PK of IV migalastat, and inform dosing for further study with ATB100C. Study 019 is designed to provide proof-of-concept for this next-generation ERT in Fabry disease.
Conflict of Interest declared.

P-441

ENGAGE: A phase 3, randomized, double-blind, placebo-controlled, multi-center study to investigate the efficacy and safety of eliglustat in adults with Gaucher disease type 1: 18-month results

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Background/Objectives: ENGAGE (NCT00891202, Genzyme a Sanofi company) is a randomized, double-blind, placebo-controlled, Phase-3 trial investigating the efficacy and safety of eliglustat, an investigational novel oral substrate-reduction therapy.
Patients/Methods: Forty untreated adults with Gaucher disease type 1 and splenomegaly and thrombocytopenia and/or anemia were randomized 1:1 to receive eliglustat or placebo during a 9-month primary analysis period (PAP). All patients received eliglustat during a 9-month open-label extension (EP). The primary efficacy endpoint was percent change in spleen volume (multiples of normal). Other efficacy measures included hemoglobin, liver volume, and platelets.
Results: In the PAP, eliglustat was superior to placebo in all primary and secondary endpoints; no patients discontinued due to an adverse event. For 18/20 patients who received 18 months of eliglustat, mean improvements from baseline continue (spleen: −45 %, hemoglobin: +1.02 g/dL; liver: −11 %; platelets: +58 %). For 20/20 former placebo patients, mean improvements after 9 months of eliglustat were consistent with improvements in eliglustat patients in the PAP: spleen: − 31 %; hemoglobin: +0.79 g/dL; liver: −7.3 %; platelets: +40 %. No new safety concerns were identified.
Conclusion: ENGAGE met its primary and secondary efficacy endpoints and patients from both treatment arms have showed continued improvements in the first 9 months of the EP.
Conflict of Interest declared.

P-442

Does enzyme replacement therapy induce an acute improvement in exercise-tolerance in late-onset Pompe patients?

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Background: Late-onset Pompe disease (LOPD) is a glycogen lysosomal storage disorder, due to acid alpha-glucosidase (GAA) deficiency, characterized by progressive muscle damage. The aim of this study was to investigate the acute effect of enzyme replacement therapy (ERT) on exercise tolerance.
Patients and Methods: Nine patients with LOPD, chronically treated, performed a constant work rate submaximal exercise (22.8±15.6 watt) on a cycle-ergometer, the day before (B) and the day after (A) the ERT infusion. GAA activity and Creatine Kinase (CK) were measured in blood.
Results: No differences in variables related to exercise tolerance were observed in B (heart rate [HR] 120±24 b/min, gas exchange ratio [R] 0.98±0.08, rates of perceived exertion [RPE] 2.6±3.2) vs. A (HR 123±17, R 0.98±0.07, RPE 3.0±3.7). The same occurred for the O2 cost of exercise (pulmonary O2 uptake 0.88±0.25 L/min in B vs. 0.90±0.25 in A). GAA activity was higher in A (31.6±11.2 nmol/mg/h) vs. B (5.0±2.7). CK slightly decreased in A (448±71 U/L) vs. B (488±65).
Conclusion: In LOPD patients ERT infusion is not associated with an acute improvement in exercise tolerance. Moreover, exercise tolerance does not decrease during the 2-week interval between infusions, despite a significant decrease of GAA activity in blood.

P-443

Antibody response to investigational intrathecal enzyme replacement therapy with idursulfase-IT in pediatric mucopolysaccharidosis II (MPSII) patients with cognitive impairment

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Background: Fifteen cognitively impaired MPSII patients received monthly doses of 1, 10 or 30 mg of investigational intrathecal idursulfase-IT in an ongoing phase 1/2 study and extension (exposure: 18–52 months). Patients continued receiving weekly intravenous idursulfase.
Results: 7/15 patients had no baseline CSF or serum anti-idursulfase antibodies and did not develop antibodies after idursulfase-IT exposure. Six patients had CSF and/or serum antibodies prior to idursulfase-IT exposure; their antibody status/titer remained essentially stable on treatment. One patient, with high-titer serum and CSF antibodies prior to idursulfase-IT exposure, experienced a moderate increase in CSF titer during the trial. Antibodies did not appear to impact CSF glycosaminoglycan levels in these patients. Another patient, with moderate baseline titers of CSF and serum antibodies, experienced a large increase in both titers after the first idursulfase-IT dose. His CSF antibodies displayed neutralizing activity. The initial decrease in his CSF glycosaminoglycan level on treatment was followed by a gradual increase and stabilization at ~40 % of pre-treatment values.
Conclusion: Antibody status/titers generally remained stable in most patients who received investigational idursulfase-IT. An increase in pre-existing CSF and serum antibodies in one patient was accompanied by a decreased pharmacodynamic response. The clinical relevance of this needs further investigation.
Conflict of Interest declared.

P-444

Cardiac manifestations of Fabry disease in classical and cardiac variant (Chinese hotspot IVS4+919G>A) patients: data from the Fabry Outcome Survey

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Background and objectives The clinical course of cardiac variant Fabry disease (FD) is not well known, although it is reported to manifest later than classical FD. We compared cardiac manifestations, focusing on left ventricular hypertrophy (LVH), in classical and cardiac variant FD.

Patients and Methods This retrospective study analysed Fabry Outcome Survey data (extracted August 2013; sponsored by Shire) from patients with classical Fabry (from Taiwan and Germany) and Chinese hotspot IVS4+919G>A (IVS4; from Taiwan) mutations. Age at onset of cardiac symptoms was evaluated.
Results IVS4 patients: n=45, 32 male, mean (SD) age at database entry 58.7 (14.5) years; classical FD patients: n=150, 51 male, 35.2 (18.8) years. Mean [SD] onset age (years) of LVH occurred later in IVS4 males (55.9 [11.3]) versus classical FD males (33.8 [12.5]; P<0.001), and in classical females (50.3 [13.1]) versus classical males (33.8 [12.5]; P<0.001). Mean [SD] onset age (years) of LVH was comparable between IVS4 males (55.9 [11.3]) and females (57.4 [13.7]; P=0.745), and between IVS4 females (57.4 [13.7]) and classical females (50.3 [13.1]; P=0.141).
Discussion/Conclusion Although classical males had an earlier age-at-onset of LVH than IVS4 males, interestingly, no significant difference was found among IVS4 males, IVS4 females and classical females.
Conflict of Interest declared.

P-445

Effect of sebelipase alfa after 2 years in adults with lysosomal acid lipase deficiency

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Background: Lysosomal acid lipase (LAL) deficiency in children and adults presents with dyslipidemia, elevated transaminases, and hepatomegaly due to abnormal accumulation of cholesteryl esters and triglycerides.
Methods: Eight LAL-deficient patients who completed a phase 1/2 sebelipase alfa study received 4 once-weekly infusions in an extension study before transitioning to every-other-week infusions.
Results: Sebelipase alfa produced rapid, sustained reductions in 6 patients with 2-year data. Compared with baseline values in the phase 1/2 study, mean decreases in alanine aminotransferase and aspartate aminotransferase were 58 % (p=0.031) and 28 % (p=0.219), respectively. Mean decreases in low-density lipoprotein cholesterol and triglyceries were 54 % (p=0.031) and 31 % (p=0.063), respectively. Mean increase in high-density lipoprotein cholesterol was 18.4 % (p=0.125). Most adverse events (AEs) to date across all patients were mild/moderate and unrelated to sebelipase alfa. Infusion-related reactions were uncommon, with the majority being gastrointestinally related and mild. To date, no sebelipase alfa-related serious AEs have been reported and no anti-drug antibodies have been detected.
Conclusion: These results suggest that long-term, every-other-week sebelipase alfa dosing improves abnormal serum lipid profiles and normalizes serum transaminases in LAL-deficient patients. A phase 3 clinical trial is underway to further study the safety and efficacy of sebelipase alfa in children and adults.
Conflict of Interest declared.

P-446

Plasma oligomeric alpha-synuclein levels in patients with Gaucher disease is attenuated by duration of enzyme-replacement therapy

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Gaucher disease (GD) is caused by mutations in the GBA gene, encoding glucocerebrosidase. Today enzyme-replacement therapy (ERT) is the standard treatment for GD. GD patients and carriers of GBA mutations are at increased risk of Parkinson's disease. However, it remains unclear whether this link is due to oligomerization of neuronal protein alpha-synuclein. 41 GD patients (median age 15, range 1–71, 17 males) and 40 sex and age matched controls were involved. Patients were divided into three groups: patients without ERT (N=9), who receive ERT less than 5 years (N=17) and who received ERT 5 years and more (N=15). Oligomeric alpha-synuclein plasma level was measured using ELISA (Human Synuclein OLIGO kit Roboscreen). The level of alpha-synuclein oligomers was significantly elevated as in untreated GD patients (median 37,6 pg/ml, range 6,5-440,4 pg/ml, p=0.001) so in patients with ERT less than 5 years (median 29,2 pg/ml, range 4,6-357,3 pg/ml, p=0.001) compared with controls (median 6,0 pg/ml, range 2,3-103,1 pg/ml). There was no difference between patients, receiving ERT more than 5 years (median 12,4 pg/ml, range 1,6-444,6 pg/ml) and controls. Plasma oligomeric alpha-synuclein is increased in GD patients and might be considered as biomarker assessing the effect of an ERT.

P-447

Dyslipidemia and sustained transaminase elevations from early childhood are common in lysosomal acid lipase deficiency

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Background: LAL deficiency (LAL D) is an underappreciated cause of cirrhosis, dyslipidemia, and premature atherosclerotic disease.
Methods: Data were compiled by performing a retrospective chart review of children and adults (N=48) with documented LAL D.
Results: The median age (range) of first symptoms was 5.8 (0.0-42.0) years and the median age at diagnosis was 9.5 (1.2-46.1) years. Dyslipidemia or elevated transaminases was reported at age 8.4 and 8.2 years, respectively. Alanine aminotransferase above the upper limit of normal (median 80.5 U/L) was seen in 44/48 patients at the first recorded assessment (on study), and

remained abnormal in almost all (follow-up interval 3–24 months). The highest reported median total cholesterol, low-density lipoprotein cholesterol, and triglycerides were 316 mg/dL, 239 mg/dL, and 219 mg/dL, respectively, and the lowest median high-density lipoprotein cholesterol was 26.5 mg/dL. Although serum lipid improvements were observed in some patients received lipid-lowering therapy, some patients still developed end-stage liver disease and underwent liver transplant. Disease appears to be progressive in some patients despite restriction of dietary fat or use of lipid-lowering therapy.
Conclusion: This study confirms that dyslipidemia and hepatic dysfunction are common in children with LAL D, although the diagnosis is often delayed because of non-specific presentation.
Conflict of Interest declared.

P-448

Migalastat reduces plasma globotriaosylsphingosine (lyso-Gb_3) in Fabry patients: Results from the FACETS phase 3 study

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Objectives: To demonstrate the effect of migalastat on plasma lyso-Gb3 in the FACETS study (NCT00925301).
Methods: A liquid-chromatography-mass-spectrometry plasma lyso-Gb3 method using a novel stable isotope-labeled internal standard, 13C6-lyso-Gb3 was developed (lower-limit-of-quantification: 0.200 ng/mL, 0.254 nmol/L). Plasma lyso-Gb3 levels were retrospectively determined at baseline, month 6, and month 12 in a subset of the subjects. In Stage 1 (baseline to month 6), subjects were randomized to placebo or migalastat. In Stage 2 (months 6–12), all subjects were treated with migalastat.
Results: Baseline plasma lyso-Gb3 levels in subjects with amenable GLA mutations ranged from 1.19-218 nmol/L (mean=45.0 nmol/L; n=31; normal range: 0.374-1.19 nmol/L; n=47). Treatment with migalastat resulted in a statistically significant reduction in plasma lyso-Gb3 ($p=0.0033$ by ANCOVA comparing migalastat to placebo in Stage 1; $p<0.0001$ by ANCOVA comparing month 6 to month 12 in subjects switched from placebo to migalastat in Stage 2). The treatment effect was maintained through month 12. The mean percent change after 6 months of treatment with migalastat was −33 % in males and −10 % in females.
Conclusions: Migalastat reduces plasma lyso-Gb3 levels in Fabry patients with amenable mutations, demonstrating the activity of oral chaperone therapy in this disease.
Conflict of Interest declared.

P-449

Safety and pharmacodynamic activity of elosulfase alfa in pediatric patients less than 5 years of age with Morquio A Syndrome (Mucopolysaccharidosis IVA)

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Objective: The study's primary objective was safety evaluation of elosulfase alfa in MPSIVA patients
Methods: Safety was monitored in 15 MPSIVA subjects receiving 52 weeks of infusions (2 mg/kg/week). Urinary keratan sulfate (uKS) and growth were also assessed.
Results: The mean (range) age was 3.1 (0.8-4.9) years at study entry. Majority (96.4 %) of adverse events (AEs) were mild-moderate. Most common drug-related AEs were pyrexia (n=6, 40.0 %) and vomiting (n=5, 33.3 %). Of 8 serious AEs, 1 was drug-related (hypersensitivity). 6/758 infusions (0.8 %) administered led to AEs requiring interruption and intervention with antihistamines and/or steroids; all patients received subsequent infusions. No discontinuations or deaths occurred. Mean (±SD) baseline uKS was 35.9 (±12.32) ug/mg creatinine, and decreased by 43.1 %(±22.15) at Week 52. Standing heights were measured in >2 year-olds, and one 18 month-old, (n=13); lengths measured for all (n=15). Standing heights (centimeters) increased by mean (±SD) 6.7 (±3.76) from baseline. Mean (±SD) baseline Z-scores for height/length (n=15) were −1.6 (±1.61) and at week 52 were −1.9 (±1.62).
Conclusion: In children<5yo, elosulfase alfa exhibited a safety and pharmacodynamic profile consistent with that observed in prior studies. Continued assessments will evaluate the long-term benefit of elosulfase alfa in this population.
Conflict of Interest declared.

P-450

Infusion management of elosulfase alfa for patients with Morquio A syndrome (MPSIVA)

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Objective: Elosulfase Alfa (EA) is an enzyme replacement therapy for MPSIVA. We describe outcomes of infusion associated reaction (IAR) management in MPSIVA patients.
Methods: 7 patients participated in an early access program and received EA infusions of 2 mg/kg/week for 18–31 weeks (median=28 weeks). Patient ages were 2–25 years (median=6 yrs).
Results: Infusion compliance was high; 8 % missed due to illness, surgery, or weather preventing travel. Patients were asked to consume a light meal, and ~30 minutes pre-infusion administered non-sedating antihistamine (s). For patients with history of IARs or other risk factors (e.g., allergies) sedating antihistamine was administered with additional agents. Antipyretics were optional. 5/7 patients experienced IARs as defined by any adverse events (AE) occurring between Infusion onset and 1 day post-infusion, regardless of relationship to treatment. All IARs were classified "mild", occurring in 10 % of infusions. Most were managed with symptomatic treatment and/or infusion rate modification. No discontinuations were required due to an AE. Most common IARs: headache (3.3 %), pallor (3.3 %), emesis (3.3 %), chest tightness (2.7 %), hives (2.7 %), altered heart rate (2.7 %), nausea (2.1 %), light headedness (1.1 %), and anxiety (1.1 %). Those experiencing IARs received/tolerated subsequent infusions. None discontinued treatment.
Conclusion: IARs were mild and manageable in 5/7 patients experiencing them.
Conflict of Interest declared.

P-451

Cardiac outcomes in Fabry disease patients after ten years agalsidase alfa enzyme replacement therapy

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Background and objectives: To evaluate long-term effectiveness of agalsidase alfa enzyme replacement therapy (ERT) in controlling progression of Fabry disease cardiomyopathy.
Patients and Methods: A retrospective chart-based analysis of morphological and functional cardiac changes in patients aged ≥16 years after 10 years' agalsidase alfa ERT.
Results: Mean age at ERT start was 34.7 years (SD 12.8; n=45), median treatment duration was 10.8 years (range 9.6?12.5). New York Heart Association heart failure classifications improved by ≥1 in 22 patients (n=42) and worsened by 1 in 1. Canadian Cardiovascular Society angina scores improved in 15 patients and worsened in 1. Left ventricular mass indexed to height (LVMi) was unchanged after 10 years in those with baseline LVMi <50 g/m$^{2.7}$ (males, mean [SD]: 44.4 [4.6] g/m$^{2.7}$; n=6; females, 39.6 [5.0]; n=8) and in females with LVMi ≥50 g/m$^{2.7}$ (69.2 [14.6]; n=16). In men with baseline LVMi ≥50 g/m$^{2.7}$ (73.7 [17.6]; n=15), improvement, apparent after 1 year, was sustained (10-year least-squares mean change −13.55 g/m$^{2.7}$ [95 % CI −23.05, −4.06]; p=0.0061). Cardiac functional parameters (eg, left ventricular ejection fraction, heart rate) were unchanged after 10 years.
Discussion/Conclusion: In this analysis, 10 years' agalsidase alfa ERT seem to control progression of Fabry disease cardiomyopathy.
Conflict of Interest declared.

P-452

The long-term effectiveness of agalsidase alfa in Fabry disease: a Fabry Outcome Survey analysis

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Background and objectives: Five-year outcomes from Fabry Outcomes Survey (FOS; Shire) for Fabry disease patients receiving agalsidase alfa were compared with published outcomes for untreated patients.[1,2,3]
Patients and Methods: Rates of change in estimated glomerular filtration rate (eGFR) and left ventricular mass indexed to height (LVMI) were evaluated, as were morbidity (time to events) and death.
Results: Data were available for 740 treated patients. After approximately 5 years of follow-up, treated patients with baseline eGFR <60 mL/min/1.73 m^2 had lower mean annualized decline in eGFR (−2.86 [SEM 0.53] mL/min/1.73 m^2/y) versus untreated (−6.8 [1.5]). Mean annualized rates of LVMI change were lower with treatment (males 0.33 [SEM 0.10] g/m$^{2.7}$/y; females 0.48 [0.09]) than without (males 4.07 [1.03]; females 2.31 [0.81]). Events indicating end-organ disease progression occurred later with agalsidase alfa (~15 % risk after 24 months versus ~45 % untreated), as did death (in males, estimated median survival 77.5 y versus untreated 60 y).
Discussion/Conclusion: Our findings suggest long-term benefits with agalsidase alfa in slowing progression of renal/cardiac disease and delaying significant morbidity/mortality in Fabry disease. Confirmatory studies are required.
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Conflict of Interest declared.

P-453

Safety of home treatment during a multicenter, open-label, extension study to evaluate the long-term efficacy and safety of elosulfase alfa in patients with Mucopolysaccharidosis IVA (MPSIVA, Morquio A Syndrome)

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Objective:Enzyme replacement therapy (ERT) with elosulfase alfa (EA) has been approved for the treatment of MPS IVA, a lysosomal storage disorder (LSD) caused by deficiency of N-acetylgalactosamine-6-sulfatase. We investigated the feasibility and safety of home therapy in patients with MPS IVA, as this option is commonly used with ERTs for other LSDs.
Methods: Seventeen MPS IVA patients, 5–18 years, enrolled in a Phase 1/2 extension study of EA at 2.0 mg/kg/wk, evaluating long-term safety and efficacy outcomes.
Results: Thirteen of 17 patients transferred to home treatment with nursing assistance with median transfer time at study week 245 (range week 235–253). Four patients continued ERT in the hospital due to personal preference or logistic considerations. Interim results (as of April, 2014) indicate home infusion of EA was well tolerated. Eighty-four home infusions were performed (infusions per subject ranged from 3–13, mean 6.5). No treatment related serious adverse events (SAE) were reported, with no infusions interrupted or discontinued due to an AE requiring medical intervention. All 13 patients are continuing to receive home infusion ERT.
Conclusion:ERT for MPS IVA administered at home appears to be safe and might lessen the burden of life-long intravenous treatment in these patients.
Conflict of Interest declared.

P-454

The multinational experience on long-term enzyme replacement therapy in children with Fabry Disease

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Fabry disease is an X-linked lysosomal disorder caused by the deficiency of α-galactosidase-A (α-GalA) affecting renal, cardiac and nervous systems. Studies in adults indicate limited efficacy of ERT in treating major complications and reports on the efficacy of ERT in preventing FD related complications in children are not available. This report describes the outcomes from two treatment centers with 13 years of experience.
Methods: A retrospective chart review was performed. Continuous variables were compared with student t-tests while categorical variables were compared with chi square analysis or fisher exact test. McNemar's test was used for paired categorical analysis at different time points.
Results: The mean age of disease onset for 57 children was 6.3 +/− 3.8 years. The mean age of diagnosis was 9.7 +/− 4.4. 38 children had pain at the onset of ERT. Following ERT, 9 patients continued to have pain while 29 no longer had pain (p <0.0001). At baseline, 14 children had abdominal related pain. Following therapy, 3 patients continued to

have abdominal related pain while 11 patients were pain free (p-value= 0.012)
Conclusion: Regardless of gender, the common morbidities in children with FD are related to pain and gastrointestinal problems, and both respond well to therapy.
Conflict of Interest declared.

26. Glycosylation disorders/CDG, protein modification disorders

P-455

COG6-CDG: fourth family and a novel mutation

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Congenital disorders of glycosylation (CDG) are a group of genetic diseases caused by defects in the synthesis of glycans and in the attachment of glycans to proteins and lipids.Two types of protein N-glycosylation disorders can be distinguished:CDG-I (defects in the assembly of N-glycans in the cytosol and the endoplasmic reticulum (ER)) and CDG-II (defects in the processing and maturation of N-glycans in the ER and subsequently in the Golgi apparatus).Mutations in the Conserved Oligomeric Golgi (COG) complex give rise to CDG-II.We describe a 6-month-old girl with COG6-CDG. She is the third child of healthy consanguineous Turkish parents.She presented at 6 months of age with psychomotor disability, growth retardation and dysmorphic features including post-axial polydactyly, broad palpebral fissures,strabismus, retrognathia, metopic synostosis, inverted nipples, hyperkeratosis of the skin and anal anteposition.She also showed chronic diarrhea, hepatosplenomegaly and life-threatening and recurrent infections. The type 2 isoelectric focusing (IEF) pattern of serum transferrin and the abnormal IEF of serum apolipoprotein C-III pointed to a CDG-II, affecting the biosynthesis of both N- and O-linked glycans.MALDI-TOF analysis of serum transferrin glycans showed mainly hyposialylation and hypogalactosylation. Sequencing of the COG subunit genes revealed a homozygous mutation c.1237dupA (p.Ile413Asnfs*5) in COG6, and carrier status in both parents.

P-456

Unraveling growth hormone defect in a CDG-IIx

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Background and objectives: Defective glycosylation leads to decresed levels of insulin-like growth factor 1 (IGF-1), IGF-2, acid-labile subunit (ALS) and insulin-like growth factor binding protein-3 (IGFBP-3), which can contribute to failure to thrive. We present the first CDG IIx patient to be treated with recombinant human IGF-1.
Case report: 16 year-old caucasian male, referred at 6 years of age for development delay, failure to thrive and dysmorphic facial features.Serum transferrin isoelectric focusing (IEF) revealed an abnormal type 2 pattern, with normal Apolipoprotein C-III IEF. Serum transferrin glycan analysis: unspecific pattern with partial hyposialylation and hypogalactosyalylation.At age 12 he presented low stature (SDS −3,07 ‹P 1) and growth velocity 3,89 cm/year, IGF-1 90 ng/ml (N - 202–957 ng/ml) and normal IGFBP3. Clonidine and glucagon test revealed normal growth hormone release and IGF-1 generation test growth hormone resistance. He started treatment with recombinant IGF-1 therapy with good linear growth.
Conclusion: An increase of IGF-1 of ‹15 ng/ml during an IGF-1 generation test is consistent with growth hormone resistance due to GH receptor or postreceptor signaling pathway abnormalities, or even to diminished IGF-1 half-life. This suggests IGF-1 deficiency may be responsible for growth failure in some CDG patients.

P-457

A novel genetic defect connecting cutis laxa to congenital disorders of glycosylation

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Background and objectives: Cutis laxa syndromes form a heterogenous group of inborn errors of metabolism eventually affecting the formation or function of extracellular matrix proteins leading to inelastic and wrinkled skin. Genetic defects in different metabolic pathways have been described, including two disorders of glycosylation: COG7-CDG (wrinkly skin) and ATP6V0A2-CDG.
Case: We present a patient with a unique combination of features in combination with cutis laxa and subtle glycosylation abnormalities. Our patient presented at birth with severely wrinkled skin and progeroid features, an abnormal fat distribution and bilateral cataract. Dysmorphic features at birth, including a triangular face, large ears and downslanting palpebral fissures, resembled those seen in ATP6V0A2-CDG. Furthermore, he suffered from severe cardiomyopathy, which improved spontaneously over the years as did his skin phenotype. Psychomotor development was severely delayed. Growth parameters were normal after birth, however, started to deviate from the age of 2 years. Brain MRI revealed white matter abnormalities and widened ventricles. Biochemical analysis showed hypercholesterolemia and a CDG type II pattern on transferrin isofocusing and mass spectrometry.
Results/Conclusion:Exome sequencing revealed a variation in a subunit of the V-ATPase proton pump, from which another subunit (a2) is deficient in ATP6V0A2-CDG, thereby further bridging cutis laxa and CDG.

P-458

Screening of congenital disorders of glycosylation in Russia

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Congenital disorders of glycosylation (CDGs) are genetic multisystem disorders caused by a large group of defects in glycosylation of biomolecules. CDG should be looked for in any unexplained syndrome. Primary diagnosis of CDG involves glycoprofiling of glycoproteins, mainly serum transferrin. We developed an analytic method for punch card testing based on the method for plasma.

Transferrin fractions were analyzed in plasma and blood spots of patients with unclear metabolic syndromes by isoelectric focusing (IEF) in agarose gel. Visualisation entailed immunofixation and Coomassie staining. IEF was combined with genetic analysis. So far 300 patients have been studied. Three of them showed a type 1 IEF profile of transferrin. In all these patients PMM2 mutations were detected. Two of these patients were compound heterozygote for missense mutations F157S/F183S and G57R/R141H, respectively; the third was homozygote for N101K. We also found 1 patient with a type 2 IEF profile. The genetic defect is not found yet.
Screening for CDG is now vailable for the first time in Russia. Genetic and clinic heterogeneity makes detection of glycosylation defects difficult. Because of the use of punch cards, our method allows very simple transport of samples and selective screening of large numbers of unclear cases throughout the country.

P-459

Expanding the phenotype of congenital disorders of glycosylation: Late-onset hereditary spastic paraplegia caused by GM2 synthase deficiency

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Introduction: Hereditary spastic paraplegias (HSPs) comprise a complex and heterogeneous group of neurological disorders. Although most HSP cases are due to genes involved in axonal growth or vesicular trafficking, there is a somtimes overlooked group of HSPs caused by inborn errors of metabolism (IEMs). Adrenomyeloneuropathy and cerebrotendineous xanthomatosis are among the known metabolic causes of HSPs.
Objective: To report a new hereditary metabolic cause of HSP in a Brazilian family caused by deficiency of the enzyme beta-1,4-N-acetylgalactosaminyl transferase 1 (B4GALNT1). Methodology: After exclusion of the more common IEMs associated with HSPs, and molecular analysis of SPG11/15 genes, whole exome sequencing (WES) was performed.
Results: Mutations in the B4GALNT1 gene were identified in all affected individuals. Patients have a relatively late onset spastic paresis, mild intellectual disability, strabismus and psychiatric disturbance. Male hypogonadism was also noticed. Brain MRI showed nonspecific white matter changes in older patients.
Conclusions: Although there are many IEMs involved in ganglioside catabolism that present as neurodegenerative disorders, this enzyme deficiency is the second human disorder identified in the pathway of ganglioside biosynthesis and the first glycosylation defect with mainly spastic paraplegia presentation, expanding the neurological involvement in this growing group of IEMs.

P-460

Hits identification of pharmacological chaperons that increase the thermal stability of missense folding mutations in PMM2-CDG

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Congenital Disorders of Glycosylation (CDG) are a group of multisystemic metabolic diseases resulting from defects in the protein glycosylation pathway. Phosphomannomutase 2 (PMM2) is the affected protein in CDG-PMM2 disorder, the most common CDG, for which currently there is no effective treatment. The screening of target mutations for specific therapies through the functional analysis of PMM2 mutations suggested that protein misfolding is the most common disease-causing mechanism in PMM2-CDG. By this technique there were identified a number of destabilizing mutations such as p.V44A, p.D65Y, p.R162W, p.C241S, p.F207S and p.T237M which are promising candidates for testing pharmacological chaperones and proteolysis modifiers. In order to identify pharmacological chaperone we have done a high-throughput screening from 100000 library's compounds validated with the wild-type protein by differential scanning fluorimetry (DSF). We have selected 15 hits which specifically stabilize the wild-type protein. Then, we have evaluated the effect on its activity which suggested that the selected hits are not inhibitors of the PMM2 activity. We have also evaluated the effect on stability using DSF and a transcription and translation system revealing that most of them stabilize the normal and mutant proteins significantly. Our preliminary results point out several promising compounds which increase wild type and some mutant's protein half-lives.

P-461

Epilepsy in congenital disorders of glycosylation: a neurophysiological study

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Background and objectives: Congenital disorders of glycosylation (CDG) represent a heterogeneous group of diseases usually characterized by mild to severe encephalopathy. Epilepsy may be present in CDG but extensive descriptions are lacking. We aimed to analyze seizure characteristics and EEG pattern in a cohort of CDG patients with different genetic backgrounds.
Patients and methods: We reviewed epilepsy history in 15 CDG children (8 PMM2-CDG, 2 ALG9-CDG, 3 RFT1-CDG, 1 ALG6-CDG, 1 COG5-CDG) aged 1–18 years. All patients underwent ictal and interictal polygraphic video-EEG recording.
Results: Epilepsy was the presenting symptom in 14 out of 15 patients. Age at onset was between 3 weeks and 4 years. Two patients showed drug-resistant epileptic spasms, 3 a phenotype resembling malignant migrating partial seizures of infancy (MMPI), while 9 had drug-responsive febrile and later afebrile partial or generalized tonic-clonic crises. Intercritical EEG showed in three patients a picture of hypsarrhythmia and multifocal epileptiform abnormalities in the other ones.
Discussion/Conclusion: Epilepsy seems to be a frequent symptom in CDG regardless of the genetic background. Severity of epilepsy relates to the age of onset. MMPI may be a presentation of epilepsy in CDG, requiring diagnostic differentiation from other well defined genetic causes of epileptic encephalopathy.

P-462

Measuring catalytic activity for wild type and mutant PGM3 by monitoring consumption or production of GlcNAc-6-P using mass spectrometry

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Background: Human phosphoglucomutase 3 (PGM3) catalyzes the reversible conversion of N-acetyl-D-glucosamine-6-Phosphate (GlcNAc-6-P) to N-acetyl-D-glucosamine-1-Phosphate (GlcNAc-1-P) during the synthesis of uridine diphosphate N-acetylglucosamine (UDP-GlcNAc),

a common substrate for assembly of N-glycans. PGM3 mutations in humans cause a congenital disorder of glycosylation.
Objectives: To measure catalytic activity for wild type and mutant PGM3 by monitoring consumption or production of GlcNAc-6-P by mass spectrometry. Material and Methods: PGM3 cDNA constructs (wild type and mutants) were transformed into E. coli BL21 (DE3) RIL Codon Plus cells for overexpression and subsequently purified. PGM3 activities were assayed in a standard reaction mixture. The effect of the mutations on PGM3 was then tested by mass spectrometry.
Results: A Multiple Reaction Monitoring (MRM) mode identified a fragment (m/z 138) specific for the substrate (GlcNAc-6-P) from the molecular ion (m/z 300) common to both the substrate and the product (GlcNAc-1-P). PGM3 mutants were differently affected with respect to forward and backward activity.
Conclusion: The method differentiates directional enzyme activity and can be applied to analogues enzymes, e.g. PGM1 and PMM2 that also catalyze transfer of phosphate between the 1 and 6 positions of the hexose ring.

P-463

Movement disorders: an under-recognized sign in Congenital Disorders of Glycosylation

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Background and objectives: Congenital disorders of glycosylation (CDG) represent a heterogeneous group of neurometabolic diseases characterized by mild to severe encephalopathy. Movement disorders (MD) are not considered as a common symptom in CDG. We looked for the presence of MD in a cohort of genetically-confirmed CDG patients, correlating them with disease progression, quality of life (QoL) and neuroradiological findings.
Patients and methods: We studied 15 genetically-confirmed CDG children, aged 1-18y. All patients underwent: standardized neurofunctional scales (Gross Motor Fuction Motor scale-GMFM; Movement Disorder Childhood Rating Scale-MDCRS); the Nijmegen paediatric CDG rating scale (NPCRS); Pediatric QoL scale (PedsQL); and a 1,5 T Brain MRI, focused on 4 regions of interest (cortex, white matter, basal ganglia, cerebellum).
Results: 10/15 patients showed significant impairment at MDCRS and GMFM. Dystonia was the most frequent MD, followed by ataxia. Worse scores correlated to higher NPCRS scores and lower PedsQl scores. Functional scores correlated with the rate of cerebral/cerebellar atrophy and white matter involvement, but not with basal ganglia damage at MRI.
Conclusion: MD seem to be more frequent then expected in CDG patients and affect general function and QoL. We suggest a role for brain cortico-subcortical circuits alterations in the genesis of MD in CDG.

27. Neurotransmitter disorders

P-464

The iNTD registry: A new clinical database of patients with inborn neurotransmitter, pterin and folate disorders

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Background:Inherited defects of biogenic amines, tetrahydrobiopterin (BH4) and folate metabolism lead to progressive neurological dysfunction in early infancy. Immediate diagnosis and treatment may result in improved outcome. To date there is no standardized systemic evaluation of diagnostic processes, therapeutic approaches and long term outcome of affected patients.
Methods: In 2013 the initiative "International Working Group on Neurotransmitter Related Disorders (iNTD)" including eight metabolic centers from seven European countries was founded. Its major goal is to set-up a web-based patient registry for inherited defects of biogenic amines, pterin and folate metabolism providing a basis for improving our understanding of the epidemiology, genotype/phenotype correlation and outcome of these diseases, their impact on the quality of life of patients, and for evaluating diagnostic and therapeutic strategies. The modular registry is based upon the IT technology of the EU-funded projects E-IMD and E-HOD. Based on the evaluation of current diagnostic and therapeutic strategies, consensus care guidelines will be developed.
Conclusion: The establishment of the iNTD registry is a major precondition to improve research and our knowledge on these rare diseases, to understand their impact on the quality of life of patients and their families, and to systematically evaluate current diagnostic and treatment strategies.

P-465

Homovanillic acid in cerebrospinal fluid of 1388 children with neurological disorders

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Background and objectives: To determine the prevalence of dopaminergic abnormalities in 1,388 children with neurological disorders, also analysing their clinical, neuroradiological and electrophysiological characteristics. Patients and
Methods: We studied biogenic amines in 1,388 cerebrospinal fluid (CSF) samples from paediatric neurological patients from various Hospitals. Correlations among CSF homovanillic acid (HVA) values and other biochemical, clinical, neuroradiological, and electrophysiological parameters were investigate.
Results: Twenty one patients with primary dopaminergic deficiencies were identified. Of the whole cohort, 20 % of patients showed altered HVA. Abnormal CSF HVA values were found in various neurological disorders such as pontocerebellar hypoplasia, perinatal asphyxia, central nervous system infections, mitochondrial disorders, and other inborn errors of metabolism and genetic diseases. HVA levels were overlapping between primary and secondary dopamine deficiencies. Prevalence of low CSF HVA levels was statistically higher in neonatal patients. Concerning neuroradiological traits, abnormalities in white matter were associated with low CSF HVA.
Discussion/Conclusion: No clear limits for CSF HVA values pointing towards primary diseases can be stated. We found twenty five neurologic diseases with consistent HVA alterations. No neuroimaging traits were associated with low HVA values, except for white matter abnormalities.

P-466

The spectrum of movement disorders under 3 years of age in inherited disorders of monoamine metabolism

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Background: Movement disorders represent one of the clinical hallmarks of congenital defects of monoamine metabolism, in association with neonatal encephalopathy, developmental delay, autonomic dysfunctions and neuropsychiatric symptoms.
Patients and Methods: We retrospectively evaluated the spectrum of movement disorders in children under the age of 3 years in a cohort of 27 patients with inherited disorders of monoamine metabolism referrred to our institution between 1996 and 2014. Selection criteria included: a) a definitive biochemical and genetic diagnosis of a disorder of biogenic amine synthesis or transport; b) an adequate amount of data concerning clinical history and serial video recordings.
Results: Rigid-hypokinetic syndrome (17/26 patients) and dystonia (13/27 patients) were the prominent early movement disorders in our sample. These two clinical presentations were more constant in AADC deficiency (4/4 patients showed a rigid hypokinetic syndrome) and in PTPS deficiency (6/8 patients showed dystonic posture). Oscillatory movements including myoclonus (3/27 patients with PTPS deficiency) and tremor (1 patient with SR deficiency) were rarely observed. Mean age of onset of movement disorder symptoms was 2 years and 2 months (age range: 1 months-16 years).
Conclusions: The early spectrum of movement disorders in congenital deficiencies of biogenic amine metabolism is characterized by dystonia and rigid-hypokinetic syndrome without a disease-specific pattern.

P-467

Response to L-Dopa in a group of paediatric patients with secondary dopaminergic deficiencies

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Background and objectives: Low concentration of dopaminergic metabolites in the cerebrospinal fluid (CSF) is a secondary phenomenon in different neurological disorders. We investigated the clinical response to L-Dopa in a group of patients with secondary dopaminergic deficiencies.
Patients and Methods: We analyzed the medical records of 16 paediatric patients with severe encephalopathies and low CSF homovanillic acid (HVA) treated with L-Dopa. Primary deficiencies were excluded.
Results: Neurological disorders included hypoxic ischemic encephalopathy (HIE) (7), encephalopathy of unknown aetiology with extrapyramidal signs (4), early-onset encephalopathy of unknown aetiology with spastic tetraparesis (2), mitochondrial encephalopathy (1), Pelizaeus-Merzbacher-like syndrome (1) and neonatal seizures (1). Mean age at start of treatment: 27, 2 months. Mean dose used: 4, 8 mg/kg/day. Mean treatment duration: 26, 7 months. Disorders with good L-Dopa response were: HIE (n=4), encephalopathy associated with extrapyramidal signs (n=3) and mitochondrial encephalopathy (n=1). The improvement was mostly in motor function (tone, tremor, dystonia). Two HIE patients got worse on therapy.
Conclusion: Half of our patients with low HVA levels have shown some benefit from L-Dopa. The response was variable in HIE. Improvement was more marked during the first year of treatment. In extrapyramidal encephalopathies the improvement seems to be more sustainable.

P-468

Brain studies in a human fetus with tyrosine hydroxylase deficiency

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Background and objectives: The tyrosine hydroxylase deficiency (THD) "B" phenotype is characterized by a severe encephalopathy with suboptimal L-dopa response. We studied the expression of synaptic proteins in the brain of a fetus with mutations that produced a "B" phenotype in her sister.
Materials: Fetal brain tissue from a 16-week miscarriage was dissected and immediately frozen at −80 °C. Genetic analyses revealed the TH mutations p.R328W and p.T399M previously found in the sister affected by a severe "B" phenotype. TH, VMAT1, VMAT2, AADC, BDNF and PSD95 were quantified by Western blot in different cortical areas, mesencephalon, protuberance, cerebellum and suprarenal gland (SG). Results were compared with an age-matched control fetus.
Results: TH, AADC and VMAT2 were under-expressed in all sections. VMAT1 was under-expressed in cortical areas, protuberance and SG. PSD95 and BDNF showed no differences compared to control.
Discussion: Normal post-synaptic density and BDNF suggest preserved volume of synaptic contacts and neuronal growth at this early neurodevelopmental stage. However, dendritic markers are needed to assess other branching and synaptic aspects. AADC, VMAT 1/2 underexpression suggest compensatory problems in these proteins, which could worsen the already deleterious dopaminergic defect. We are currently studying other biomarkers to better understand neurodevelopmental abnormalities in this disease.

P-469

Disruption of tyrosine hydroxylase in PC12 and its effect on dopamine synthesis: a model for the study of tyrosine hydroxylase deficiencies

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Background: Tyrosine hydroxylase is an enzyme that catabolizes the conversion of tyrosine into L-DOPA. This is the first and limiting step in the synthesis of catecholamines, a group of neurotransmitters involved in important neurological processes such motor control, sympathetic nervous system response, motivation or attention. Mutations in the TH gene cause tyrosine hydroxylase deficiency. Patients with this rare metabolic disorder cannot produce the levels of L-DOPA and derived catecholamines required for normal brain function.
Objective: Development of a cell culture model for the study of tyrosine hydroxylase deficiencies.
Materials and methods: Tyrosine hydroxylase was downregulated in the catecholaminergic cell line PC12 using commercial ShRNAs. Neurotransmitters were measured by HPLC. Proteins were analyzed by Western blot.
Results: The effect of disruption of tyrosine hydroxylase expression on dopamine synthesis was studied in relation to the basal levels present in rat PC12 cells. Tyrosine hydroxylase ablation did not alter other enzymes of the pathway. However, dopamine synthesis was reduced all cases.

Human tyrosine hydroxylase can be expressed in PC12 cells by transfection.
Conclusions: PC12 cells are a suitable model for the study of tyrosine hydroxylase deficiency and preclinical assays of treatments for this syndrome.

P-470

A rare cause of severe hypotonia in childhood: Aromatic L-aminoacid decarboxylase deficiency

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Aromatic L-amino acid decarboxylase (AADC) deficiency is a rare inborn error of biogenic amine metabolism which combines deficiencies of serotonin, dopamine and catecholamines. Main clinical features are developmental delay, muscular hypotonia, dystonia, oculogyric crises and additional extraneurological symptoms. Patients are often diagnosed late or misdiagnosed, especially those without dystonia and oculogyric crises. Here we represent a 25 months old female patient with severe hypotonia and seizures diagnosed as AADC deficiency. She was referred to the Pediatric Metabolic Diseases unit for evaluation of severe hypotonia, neuromotor delay and convulsions. She had been followed at the Pediatric Neurology clinic since age 4 months. On admission she was severely hypotonic with normal deep tendon reflexes, showing deterioration during day, and had developmental delay. Laboratory investigations including transferrin isoelectric focusing and plasma very long chain fatty acid profile were unremarkable. There was extraaxial space increase frontotemporaparietal region in brain MRI, and normal EMG. CSF studies revealed low neurotransmitter metabolites and elevated 3-O-methyldopa but normal L-dopa. AADC was the most probable cause but as L-dopa level was normal another sample was taken which revealed mild increase in l-Dopa. Serum enzyme activity was found to be lost completely. L-dopa, pyridoxal-5-phosphate and serotonin uptake inhibitors were commenced with mild response to treatment.

P-471

Analysis of cerebrospinal biogenic amine metabolites as a good tool for diagnosis of neurotransmitter disorders in patients with extrapyramidal syndrome

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Background: Biogenic amines (BA) are related to central and autonomous nervous system functions with a diverse range of action. They provide control of psychomotor function, thermoregulation, and vascular tone, and play an essential role in emotional stability, memory, appetite, mood and sleep.
Material: In the period of 2008–2013, BA metabolites were analyzed in CSF of 117 patients. Clinical inclusion criteria were: extrapyramidal syndrome of unknown etiology, progressive infantile encephalopathy, and/or drug-resistant epilepsy.
Results: Out of 51 patients with progressive extrapyramidal syndrome, 19 had decreased BA metabolites (9 among them also had epilepsy), 13 patients were finally diagnosed as: aromatic amino acid decarboxylase deficiency (2 patients), GTP cyclohydrolase deficiency (7 patients), 6-pyruvoyltetrahydropterin synthase deficiency (2 patients), sepiapterin reductase deficiency (1 patient), dihydropteridine reductase deficiency (1 patient). In addition, low BA metabolites were found in one case later diagnosed as Niemann-Pick type C disease. Five out of 14 patients with extrapyramidal-pyramidal syndromes had a decrease of one metabolite (mainly serotonin). Fifty two patients with isolated drug–resistant epilepsy had normal BA metabolites.
Conclusion: Extrapyramidal syndrome (sometimes with epilepsy) should be an indication for CSF analysis for BA metabolites, which were decreased in 37 % of cases and allowed a specific diagnosis in 25 % of patients.

P-472

Monoamine profile in the cerebrospinal fluid of neuropaediatric patients with cerebellar ataxia

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Background and objectives: The cerebellum contains neurons utilizing monoamines and other neurotransmitters. We investigated CSF monoamine metabolites in a series of patients with cerebellar ataxia.
Patients and methods: CSF 5-HIAA and HVA concentration were measured by HPLC in 40 patients with cerebellar ataxia. Mean age: 5.5 years (1–16 years). Etiology was diverse: 1 histiocytosis, 1 cortical displasia, 2 opsoclonus- myoclonus, 2 mitochondrial disorders, and other genetic disorders (mutations of GA1, SLC2A1, NTNG1, UB3A, TITF1, NARP). Etiology was unknown in 70 % of patients. Diverse clinical findings were related to neurotransmitter profiles.
Results: Serotoninergic and dopaminergic alterations were present in 52.5 % (21) of our patients. 16 patients (10 younger than 6 years of age) had abnormal 5-HIAA concentration (14 low and 2 high). 5/21 had isolated low HVA concentration and 5/21 had alterations of both, HVA and 5HIAA. MRI findings in patients with low 5HIAA showed cerebellar atrophy (7/14) and white matter alterations (4/14). 4/7 patients with low 5HIAA and cerebellar atrophy had a progressive disease.
Conclusions: Neurotransmitter alterations (especially low 5-HIAA levels) were present in more than 50 % of our patients with cerebellar ataxia. Abnormalities were more frequent in patients with young age, abnormal brain MRI, and progressive ataxia.

P-473

Dopamine, serotonin, gamma aminobutyric acid and its correlation with vitamin B6, in the CSF from neuropaediatric patients

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Background/Objectives: Pyridoxal 5'-phosphate is the active form of vitamin B6 and acts as the cofactor of multiple enzymatic reactions. Among the most important functions is the particpation in the non-limiting step in the synthesis pathway of the neurotransmitters dopamine, serotonin and GABA. We investigated the B6 status and its possible relation with the neurotransmitter profile in the CSF of 20 patients with different neuropaediatric disorders.
Patients and Methods: 20 samples from 20 patients with concomitant quantitation of dopamine, serotonin, B6 and GABA were analysed. 10 of those patients had early onset epilepsy. Other diagnosis were tyrosine hydroxylase deficiency, Aicardi-Goutières syndrome, 5 patients with epilepsy, secondary hyperphenylalaninemia and 2 with developmental delay.
Results: No correlations were observed with PLP and HVA, 5-HIAA and GABA. A statistical positive correlation between HVA and 5-HIAA was found, as expected. Four patients had low CSF PLP values but they did not present alterations in neurotransmitters and GABA concentrations. No positive correlation between GABA and HVA was found.
Discussion/Conclusion: No correlation of B6 and neurotransmitters was found, possibly due to its role in general brain homeostasis. Alterations in NT and B6 profile were more frequently detected in early stages and neonatal period.

28. Disorders of vitamins, cofactors and trace elements

P-474

5-methyltetrahydrofolate degradation is accelerated by exposure of neuronal cells to selenium and prevented by ascorbate. Implications for understanding the pathogenesis and treatment of cerebral folate deficiency

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Background: A number of disorders are associated with cerebral folate deficiency (CFD) including disorders directly affecting 5-methyltetrahydrofolate (5-MTHF) transport and mitochondrial disorders, particularly Kearns-Sayre syndrome (KSS). In KSS, failure of active transport has been proposed as the mechanism. However, other factors, such as oxidative stress, may contribute to loss of CSF 5-MTHF. An increased concentration of CSF selenium has been reported in patients with KSS. In view of this, we evaluated the effects of selenite on 5-MTHF availability.
Methods: Stability of 5-MTHF was assessed in pooled CSF exposed to sodium selenite. Neuronal (SH-SY5Y) cells were incubated with selenite and the rate of disappearance of 5-MTHF from extracellular medium documented. Effects of co-incubation with ascorbate were also studied.
Results: Selenite (2–20 μg/L) had no direct effect on 5-MTHF stability in CSF. In contrast, a significant increase (+20 %) was observed in rate of 5-MTHF disappearance from extracellular medium of selenite treated (10–20 μg/L) SH-SY5Y cells. This was prevented by the antioxidant ascorbate (150 μM).
Conclusion: Intracellular metabolism of selenite, by neuronal cells, may lead to increased oxidative stress and hence may be a contributing factor to the CFD associated with those mitochondrial disorders where an elevated CSF selenium has been documented.

P-475

Human pyrroline-5-carboxylate reductase (PYCR1) forms l-pipecolic acid out of cyclic α-aminoadipic semialdehyde (i.e. Δ1-piperideine-6-carboxylate)

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Previously, we have performed studies on fibroblasts using α-15 N-l-lysine and ε-15 N-l-lysine and the results showed that l-pipecolic acid is solely formed via a novel metabolic route i.e. from Δ1-piperideine-6-carboxylate (P6C). Based upon this finding, we now have performed studies with commercially available human recombinant pyrroline-5-carboxylate (P5C) reductase (PYCR1) to investigate whether this enzyme is responsible for the formation of l-pipecolic acid out of P6C. P6C was prepared out of allysine ethylene acetal and co-incubations of P6C in the presence of PYCR1 revealed that indeed PYCR1 acts on P6C. The chirality of the formed pipecolic acid was verified by GC-MS using a chiral GC column, and this showed that solely l-pipecolic was formed. Additionally, incubations with PYCR1 were carried out using urine samples from individuals with proven Antiquitin/ALDH7A1 deficiency containing vast amounts of accumulated P6C. The combined measurement of P6C and l-pipecolic by LC-MS/MS following incubations with PYCR1, showed a decrease of the P6C signal accompanied with a clear increase of the l-pipecolic acid signal. In summary: PYCR1 acts on P6C generating l-pipecolic acid implying that the currently accepted catabolic l-lysine pathway needs revision.

P-476

Disorders of methylation CblE and G type: Clinical signs and symptoms at presentation and during the course in 24 patients and review of the literature

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Background: The cobalamin E (CblE) (MTRR, methionine synthase reductase) and cobalamin G (CblG) (MTR, methionine synthase) defects are rare inborn errors of cobalamin metabolism leading to impairment of homocysteine to methionine remethylation.
Methods: Physicians caring for patients with a CblE or CblG defect proven in the Basel/Zürich diagnostic laboratory were asked to complete a survey on clinical symptoms and lab data at onset and during the course of the disease.
Results: Data from 11 CblE and 13 CblG patients were included. Failure to thrive, feeding problems, delayed milestones, muscular hypotonia, cognitive impairment and macrocytic anaemia were most frequent symptoms. The average combined score for CNS and eye symptoms per patient increased significantly during the course ($p=.003$). Delayed diagnosis correlated with older age at onset and impaired ability to communicate at follow-up. Feeding difficulties, impaired consciousness and macrocytic anaemia at onset correlated with shorter time to diagnosis. Treatment with variable schemes of betaine, cobalamin, folate/folinate

and methionine resulted in lower homocysteine and higher methionine concentrations.
Conclusions: The combined score for CNS and eye involvement increases over time despite treatment. Treatment strategies are highly variable. Time to diagnosis depends on age and clinical pattern and correlates with outcome parameters.

P-477

CSF MTHF levels in Indian children with pyridoxine dependent seizures, non ketotic hyperglycinemia, mitochondriopathy, and Aicardi Gautier syndrome

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Background: 5-MTHF in cerebral spinal fluid (CSF) is considered an important metabolite. Many diverse metabolic pathways can lead to depletion of CSF 5-MTHF, whose supplementation may help. Objective: CSF 5-MTHF levels were determined in children with neurometabolic disorders and compared with clinical and biochemical features.
Materials and method: Four groups of neurological disorders were studied: pyridoxine dependent seizures (PDS), glycine encephalopathy (NKH), Aicardi Gautiérsyndrome (AGS), and mitochondrial encephalopathy (MitoE). CSF 5-MTHF, CSF pterins, CSF pipecolic acid, plasma and CSF glycine, and urinary organic acids (GC-MS), were analysed.
Result: Out of 7 patients with PDS (elevated CSF pipecolic acid and good response to pyridoxine), 5 had low 5-MTHF levels (44.79±37.62 nmol/l, NR=64–182). 2 of 3 patients with NKH (glycine encephalopathy with elevated CSF & plasma glycine and normal urinary GC-MS) had very low 5-MTHF (42.71±29.58). One patient with AGS (elevated CSF-INFα, elevated neopterin and classical CT-scan), had low 5-MTHF (42.77). 5 patients with MitoE also had low 5-MTHF (32.39±24.93).
Discussion: CSF 5-MTHF levels seem to be affected in many children with various neuro-metabolic disorders. Supplementation with folinic acid may be offered, which is very economical, safe and effective. One child with Aicardi Gautiér syndrome and one child with NKH showed good clinical response.

P-478

Outcome in Polish patients with biotinidase deficiency treated with biotin - twenty years follow-up

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Since 1991, in Poland 18 patients from 15 families were diagnosed with biotinidase deficiency. In fourteen patients diagnosis was suspected on the basis of clinical signs: skin lesions, hyperventilation, seizures, and spasticity. The diagnosis was confirmed by measurement of biotinidase activity in serum. Four patients were diagnosed through family screening. All patients were treated with biotin. Symptomatic patients (range age 4–48 months) received an initial dose of 20 mg biotin/day, gradually reduced to 5 mg/day. Asymptomatic patients began the treatment with a dose of 5 mg/day. All patients were monitored with regard to excretion of 3-hydroxyisovaleric acid by GC/MS. All patients were examined once every year by a neurologist, an audiologist and an ophthalmologist. In 5 patients who had been symptomatic at the time of treatment initiation, progressive optic nerve atrophy treatment was noted despite biotin supplementation. In these patients sensorineural hearing loss was also present. In asymptomatic patients who started biotin administration there were no signs or symptoms of biotinidase deficiency. Biotin treatment slowed the progression of the disease in patients who started treatment after the onset of neurological symptoms. However, nerve atrophy progressed despite the treatment. In contrast, biotin administration in the presymptomatic period prevents the onset of symptoms including optic nerve.

P-479

A rare cause of recurrent infections: Biotinidase deficiency

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Biotinidase deficiency is an inborn metabolic disorder inherited in an autosomal recessive manner. The high consanguinity rates in Turkey contribute to a high frequency of the disease in our country. Based on residual enzyme activity the disease is classified as partial (activity between 10–30 %) or profound (<10 %) biotinidase deficiency. Without biotin replacement therapy, neurologic abnormalities including seizures, hypotonia, sensorineural deafness, alopecia, eczematous skin rash, and candidiasis may occur. We report a 3 year-old girl admitted with recurrent infections, candidiasis and eczematous skin rash. Immunological evaluation was normal. Associated deafness and parental consanguinity were compatible with biotinidase deficiency which was subsequntly proven by enzyme activity measurement. With this report we wish to emphasize that biotinidase deficiency can lead to recurrent infections, candidiasis and cutaneous rash.

P-480

Metabolomic signatures of pyridoxine-dependent epilepsy (PDE)

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Background and Objectives: Most cases of PDE are caused by α-aminoadipic semialdehyde dehydrogenase (i.e. antiquitin) deficiency. Antiquitin deficiency results in the accumulation of AASA, P6C and PA. In vivo, AASA equilibrates with it's cyclic form P6C, and is thought to sequester PLP via Claisen condensation. Cerebral PLP deficiency has a range of deleterious effects, including perturbed metabolism of cerebral amino acids and biogenic amines. We aimed to study the global effects of PDE on metabolic profiles and to gain further insights toward new therapeutic and diagnostic tools.
Materials and Methods: CSF from a genetically confirmed PDE patient was analyzed by accurate-mass LC-MS. The resultant datasets were preprocessed, normalized, scaled and compared against a corresponding non-PDE sample. Features with significant variability ($p<0.05$) were mined against the Human Metabolome Database for metabolite annotation.
Results: Global analysis revealed 7341 total features, with 694 differential data frames identified. So these, 390 corresponded to endogenous human metabolites. We observe significant variation in features associated with lysine metabolism e.g. P6C, AASA, PA, saccharopine, glutamic acid, N,N-dimethyllysine and 3,5-diaminohexanoate. These first data of this type reveal interesting changes in amino acid, lipid and neurotransmitter metabolic profiles related to PDE.

P-481

Deep brain stimulation in the treatment of dystonia secondary to pantothenate kinase-associated neurodegeneration (PKAN)

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Introduction: Pantothenate kinase-associated neurodegeneration (PKAN) is a neurodegenerative disease with iron accumulation in the brain. Deep brain stimulation (DBS) has recently shown that it can be a benefit in the treatment of secondary dystonia refractory to medical treatment.
Case Report: We present a case of an 18 year old boy, with clinical and MRI signs of PKAN. When he was 16 years old he developed dysarthria, swallowing difficulties, progressive and severe dystonia of his right hand and arm that compromised his writing, eating and all functions of the hand. MRI revealeds the "eye of the tiger" sign. The study of the PKAN2 gene showed a heterozygote mutation c.1070G>C (p.Arg357Pro) – Exon 3. A DBS surgery was performed with implantation of electrodes, bilaterally, in Globus pallidus internus. The surgery was safe. The dystonia substantially improved on a short evaluation and the hand became completely functional.
Conclusion: In this patient with PKAN late-onset form, stereotactic procedure with DBS has been found effective in resolving the disabling dystonia and improving the quality of life. There are few cases in the world literature highlighting the usefulness of this technique. The beneficial effect is protracted over months but is sustained, usually, up to 8 years post-operatively.

P-482

Dyskinesias associated with cobolamin therapy

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Involuntary movements may be an initial symptom of neurological damage by both acquired and inherited forms of cobalamin (Cbl or vitamin B_{12}) deficiency. Rarely, they may appear during vitamin B_{12} replacement. Methylmalonic aciduria and homocystinuria, CblC type, is the most frequent inborn error of cobalamin metabolism. Developmental, haematological, neurological, metabolic, ophthalmologic and dermatologic abnormalities are characteristic. Here, we report an 8-month-old female presented with irritability, food aversion, skin hyperpigmentation, resistant diaper dermatitis, psychomotor regression, and seizure episodes. Cranial magnetic resonance imaging showed global cerebral atrophy and retarded myelination. Homozygous c.394C>T (p.R132X) pathogenic variant was detected in the MMACHC gene. A striking dyskinetic orofacial and upper limb twitching developed 5 days following intramuscular cobalamin without electrographic ictal changes. Relentless enough, they needed symptomatic treatment and resolved completely within 2 weeks of clonazepam. The movements are thought to be consistent with a known self-limited 'infantile tremor syndrome' arising during Cbl therapy. The exact pathogenesis of this syndrome remains unclear; however, the sudden availability of cobalamin resulting in intense stimulation of cobalamin and folate pathways and a temporary imbalance of metabolic pathways has been suggested. Early diagnosis and treatment may prevent irreversible neurological damage.

P-483

Diverse dynamics of clinical signs and subtle biochemical findings in thiamine responsive megaloblastic anemia syndrome

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Background: Thiamine responsive megaloblastic anemia syndrome (TRMAS) is a rare autosomal-recessive disorder caused by mutations in the SLC19A2 gene coding thiamine transporter. Only ~80 cases have been described mainly in consanguineous families. TRMAS is characterized by megaloblastic anemia, early onset deafness and non-type1 diabetes.
Case report: 3y boy from non-consanguineous family had normal psychomotor development in infancy but was easy irritable and suffered with common affecto-respiratory spasms. Regress of speech development was noticed on 7thmonth of life. Non-type1 diabetes and profound bilateral hearing loss was diagnosed till 1,5y. During 3rd year of life severe megaloblastic anemia without folic acid or vitamin B_{12} deficiency and bilateral maculopathy developed. The patient had slightly elevated branched chain amino acids (Leu/Ile/Val) in fasting plasma. The diagnosis of TRMA syndrome was suspected, and supplementation with thiamine 100 mg/day was started. The condition of the patient markedly improved several days after the initiation of treatment. Therapy had a positive effect on anemia and control of glycemia; also the psychological status of the child clearly improved. A homozygous SLC19A2 gene mutation c.205G>T (p.Val69Phe) was revealed in the proband, and carrier status was confirmed in the parents.
Conclusion: Clinical heterogeneity, diverse dynamics and a wide spectrum of symptoms are aggravating factors in disease diagnostics.

P-484

Development of a sensitive mass spectrometry-based enzyme assay for the diagnosis of pyridox (am) ine 5'-phosphate oxidase deficiency

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Vitamin B_6 exists in the body as six vitamers: pyridoxine (PN), pyridoxamine, pyridoxal, their 5'-phosphate esters; and the excretion product 4-pyridoxic acid. Pyridoxal 5'-phosphate (PLP) is the active vitamer and an essential cofactor for >140 enzymes, many of which are involved in neurotransmitter metabolism. Pyridox (am) ine phosphate oxidase (PNPO) deficiency causes a deficiency of PLP resulting in severe antiepileptic drug resistant seizures. This disorder is eminently treatable with high doses of PN or PLP. Currently the only way to diagnose this disorder with certainty is to sequence PNPO.
We have developed a highly sensitive UPLC-MS/MS assay enabling quantification of PNPO enzyme activity in 10 μL of fibroblast lysate. Using this method we have confirmed a diagnosis of PNPO deficiency in a patient where only one heterozygous frameshift mutation had been identified despite sequencing cDNA. In the presence of PN, fibroblasts accumulated PN and PNP whilst the concentration of PLP decreased. PNPO activity was dramatically reduced in these cells.
This assay will not only enable investigation of pathogenicity of PNPO sequence variants but could also be used to identify patients with

pyridoxal kinase deficiency. Novel treatments that boost enzyme activity may be trialled in vitro and thus provide better patient-specific treatments.

P-485

Cobalamin E defect - treatment modalities (case report)

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Objective: Cobalamin E (CblE) defect – methionine synthase reductase deficiency – is a very rare disease. The enzyme deficiency causes a defect of homocysteine remethylation. We describe the first patient with CblE defect in Slovakia.
Case report: 16 year-old boy with ADHD, mild mental retardation, and speech problem from the age of 2 years. Macrocytic anaemia was diagnosed at the age of 11 years and was treated with Folic acid without a positive effect. At the age of 13 years, more detailed haematological tests showed hyperhomocysteinemia without methylmalonic aciduria. The next diagnostic steps - biochemical investigations, intracellular cobalamin metabolism analysis and genetic test - confirmed a CblE defect. Treatment included cobalamin, folic acid, betaine, and carnitine, which led to an improvement of anaemia. Neurological and mental status was stable, without deterioration. Plasma homocysteine level decreased from 176 to 120 umol/l only.
Conclusion: Despite changing the type and dose of cobalamin and betaine and a period with low protein high methionine diet, we were unable to decrease the concentration of plasma homocysteine to less than 120 umol/l for a long period of time. The boy has had no thromboembolic event so far. Antihypertension treatment was started because of mild hypertension.

P-486

Molybdenum cofactor deficiency - diagnostic pitfalls on day two of life

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Introduction: Molybdenum Cofactor Deficiency (MoCD) affects the enzymes Sulphite Oxidase, Xanthine Oxidase and Aldehyde Oxidase and results in severe neurological damage mainly due to the accumulation of toxic sulphite.
Case Report: A female infant presented with seizures on day two of life. Her neurological examinations were all abnormal. Metabolic biochemical investigations included a normal urinary urate/creatinine ratio 0.55 mmol/mmol (0.2 – 3.0); plasma amino acids showed a low but unremarkable cystine ‹5 μmol/L (0 – 92) and other amino acids normal, with a comment to repeat samples as infant was less than 3 days old. At two weeks of age plasma urate was markedly low at 3 μmol/L (135–360). A urinary sulphite dipstick tested positive at 60 mg/L. A repeat urinary urate/creatinine ratio was 0.07 mmol/mmol (0.2 -3.0); amino acid analysis showed marked excretion of sulphocysteine and taurine, with plasma total homocysteine and cystine undetectable. These findings were consistent with MoCD.
Discussion: This case highlights the importance of repeating apparently normal plasma and urine metabolic investigations in the first few days of life, if clinical symptoms persist, to allow prompt diagnosis and timely intervention.

29. Miscellaneous

P-487

Fish odour syndrome (trimethylaminuria): Case report

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Primary trimethylaminuria or fish odour syndrome is an inherited metabolic disorder characterized by excessive amount of free trimethylamine, a malodorous volatile aliphatic amine, in urine, breath, sweat and vaginal secretions. It is caused by a decreased capacity of the enzyme flavin-containing monooxygenase 3 which is required for the formation of non-odorous trimethylamine N-oxide. Here we describe a 6 year-old girl with primary trimethylaminuria that who was admitted to the pediatric metabolic unit because of a strong odour of rotting fish noticed by her friends. She had no physical abnormality except a withdrawn personality. Family medical history revealed parental consanguinity and an unaffected sister. Laboratory tests showed normal liver and kidney functions. Urinary organic acid analysis by mass spectrometry and tandem mass analysis were nonspecific. Subsequent molecular analysis of the coding region of the FMO3 gene revealed a homozygous mutation p.S374Vfs*10 (c.1118_1119insA). The parents of affected patients are heterozygotes. We advised dietary modifications including avoidance of choline-rich foods (egg yolk, liver, kidney, peas, soybean, sea fish), a short course of metranidazole to suppress production of trimethylamine from choline in the gut, and mildly acidic soaps to reduce the odour.

P-488

The place of billiscintigraphy in the diagnosis of progressive familial intrahepatic cholestasis (PFIC)

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Progressive familial intrahepatic cholestasis (PFIC) is an autosomal recessive disease with hepatocellular cholestasis and low gamma-glutamyl transpeptidase (GGT) levels compared to other cholestatic diseases with biliary hypertension. PFIC2 caused by mutations in the ABCB11 gene on chromosome 2q24 was confirmed in 7 patients, PFIC1 caused by a mutation in the ATP8B1 gene on 18q21-22, coding for the protein FIC1 known as ATP8B1, was detected in 1 consanguineous boy. Average GGT: 18,3±1,8 U/; ratio conjugated/unconjugated bilirubine: 1/1 (M± m 122±12, and 250±27 μM/l); AFP: 2,32…2500 U/l. Biliary atresia is the most common condition witch must be excluded in all cases of pale stool in infants, which may also be suggestive of PFIC. Ultrasound investigation is useful but not sufficient. The most informative test is radioisotope investigation of the biliary tree, i.e. hepatobiliscintigraphy (HBSG), including cumulative function of hepatocytes at the time of

maximum activity, half-time elimination of isotope, and the advent of the Tc99m-labeled bile in the intestine. We use not only standard time points, but also an additional investigations at 24 hours with half-life correction, test decay of radioactivity above the hepatic region, and increase of radioactivity above the intestine. It is more specific even screen visualization, due to blood and tissue vons. In results, HBSG allowed us to confirm patency of the biliary tract and intrahepatic cholestasis.

P-489

Anxiety and depression in parents of children with inborn errors of metabolism on a restricted diet

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Aim: Parents of children with inborn errors of metabolism (IEM) are faced with a frightening diagnosis and numerous demands associated with caring for these children. The objective of this study was to explore the existence of depression and anxiety in parents of these children.
Methods: The study group consisted of 55 parents (34 mothers, 21 fathers) of children who had IEM with restricted diet [disorders of aminoacid and carbohydrate metabolism], and 26 healthy children. Beck Depression Inventory (BDI) and State-Trait Anxiety Inventory I-II (STAI I-II) were used to assess the parental depression and anxiety.
Results: Depression and anxiety scores were significantly higher in the study group than controls (BDI: 13.7±9.0 vs 5.9±4.2; STAI-I: 39.4±10.1 vs 31.6±8.1; STAI-II 43.5±7.8 vs 36.0±6.7 respectively; $p<0.01$). Parental depression score was positively correlated with anxiety score ($r=0.670$, $p=0.001$). Mothers of patients had significantly higher depression and anxiety scores than fathers (BDI: 15.9±8.9 vs 10.0±8.4; STAI-I: 41.7±9.2 vs 35.8±10.6; STAI-II 44.7±8.3 vs 41.2±6.2 respectively; $p<0.05$).
Conclusion: Our results suggest that a subset of parents may have an anxiety or depressive disorder. Future studies need to determine whether supportive services can reduce the level of anxiety and stress in parents of children with these disorders.

P-490

Usefulness of Vitamin D monitoring in patients with inborn errors of metabolism on protein restricted diet

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Background: Vitamin D is responsible for bone development and other important functions. Inborn errors in urea cycle, organic acidemias and aminoacidopathies such as tyrosinemia and PKU are usually treated with low protein diet along with special formulas containing aminoacids.
Objective: To examine the Vitamin D status in patients on protein restricted diet with or without special formulas as in urea cycle defects, organic acidemias, and aminoacidopathies. Subjects: The study involved 36 cases of IEM with tyrosinemia type1 (8), PKU (4), MSUD (2), citrullinemia type 1 (6), ASA (2), MMA (10), PA (1), IVA (1), and homocystinuria (2). Method: 36 serum samples were analysed for 25 (OH) Vit (D2+D3) using IVD-approved ELISA kits along with serum calcium, phosphorus, alkaline phosphatase, and proteins. In 3 subjects, Vit D levels were analyzed before and after special diet.
Result: We found that patients on special diets had a higher level of Vit D concentration (n=15, 62.862±50.456 ng/ml), with 6 being in the toxic range and 4 deficient. Persons not on any special dietary supplement (n=24, 16.320±9.26 ng/ml) had lower levels and 18 were deficient.
Conclusion: It is necessary to monitor vitamin D concentrations in patients with protein restricted diet with or without special AA supplements, as some could be deficient or have toxic levels.

P-491

Urine organic acids, analysis by GC/MS and autoannotation by Masshunter software

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Background: A large number of metabolic disorders can be detected either directly or indirectly by a GCMS urine organic acid (UOA) profile. The results produced are highly complex and require a skilled and experienced analyst to annotate and interpret. This presents the issue of ensuring that result analysis is consistent between operators and free of human error.
Method: Urine from a healthy volunteer was stripped of existing endogenous organic acids. 45 UOAs and glycine conjugates useful in the diagnosis of inborn errors of metabolism were then used to spike this urine and produce two QC levels. A six point calibration curve was constructed by serial dilution of a stock standard and using seven deuterated internal standards. Mass Spectra from these QC's and patients with known disorders was used to construct a custom library database in Masshunter (Agilent) software.
Results: Our Masshunter Quant method allows semi/full-quantitation of over 45 analytes with average intrabatch precision of 2.95 % and interbatch precision of 8.14 %. The chromatogram is annotated by spectral library matching.
Conclusions: Masshunter has not been used for before for the routine analysis of UOAs, it presents the laboratory with an accurate diagnostic tool for the diagnosis of inherited inborn errors of metabolism.

P-492

Pregnancy outcomes and complications in spanish women with acute intermittent porphyria

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Background and objectives: Acute intermittent porphyria (AIP) results from a partial deficiency of hydroxyl-methyl-bilane-synthase (HMBS). Hormonal changes during pregnancy may cause symptomatic crisis. We assessed complications and outcomes of AIP pregnancies in Spain.
Patients and methods: retrospective charts review of AIP pregnancies followed in our hospital.
Results: 40 pregnancies (from 15 women aged 20–40 years) were identified, resulting in 33 live births and 7 miscarriages including 6 spontaneous miscarriages (17,5 % of pregnancies). AIP crises occurred in 4 pregnancies (12,12 %), one pregnancy was interrupted because of a very severe crisis. No significant differences in newborn weights and lengths between symptomatic and asymptomatic women. Complications in 33 pregnancies in progress included anemia in 21,21 % (7); hyperemesis gravidarum in 15,15 % (5);

gestational diabetes in 9,1 % (3); isolated hypertension in 9,1 % (3); HELLP in 5 % (2), both with confirmed fetus carrying AIP mutation.
Conclusion: Hyperemesis gravidarum (20 % of AIP women) and HELLP (5 % of AIP pregnancies) had a higher incidence (10 times and 5 times, respectively) than expected in the general population (0.3-2 % and 0.9 % respectively) and may be associated with AIP. AIP in the fetus may be another factor predisposing to the development of maternal HELLP. The frequency of the other complications was similar to that reported in the general population.

P-493

Acute intermittent porphyria in a Spanish pediatric population: prevalence and phenotype

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Background and Objectives: The prevalence of acute intermittent porphyria (AIP; MIM# 176000) in Europe is estimated 1-2/10,000. There is evidence for a founder effect in Murcia (southeast Spain). AIP is usually asymptomatic during childhood, in absence of systematic studies.
Objective: analysis of prevalence and phenotype of pediatric AIP in Murcia. Patients and Methods: Study of individuals≤18 years with a genetic diagnosis of AIP in Murcia (total population≤18 years: 320,698 people) by retrospective charts review.
Results: 9 cases (6 male and 3 female). Eight (88 %): 669-698del mutation and one (12 %) with R26C. Average age at diagnosis: 7 years (range: 7 months-16 years). Mean time of follow-up: 23 months (range: 3–40). The children were asymptomatic. Associated anomalies: delayed language acquisition (2 patients), severe intrauterine growth retardation (IUGR) following complicated pregnancy by documented porphyria episodes (1 patient), sideroblastic anemia associated with homozygous p.Gly228Val in the SLC25A38 gene (1 patient) and VACTERL association (1 patient).
Conclusion: The prevalence of AIP in our region (28.06 cases/10,000) is 14 times higher than estimated. No episodes of porphyria have been reported during childhood. Episodes of porphyria in the mother during pregnancy may be a risk factor for IUGR.

P-494

Tryptophan slows MRC-5 and A549 cell growth

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Background: This study examines whether high levels of single amino acids can decrease or prevent cellular growth in cells, supposing that faster growing cell populations would be greater affected.
Materials and Methods: Comparing MRC-5 cells, a lung fibroblast cell line, with A549, a cancer cell line, growth was determined according to visual inspection and protein content following the one-time addition of various amino acids at 2 mM concentration.
Results: In MRC-5 cells, protein levels were not significantly affected by the addition of any amino acid tested over 72 hours except tryptophan. On the other hand, A549 cells (the cancer cell line) when treated with aspartic acid, tryptophan and citrulline had an initial decrease in protein amount as measured at 1 hour following single treatment, but only those exposed to tryptophan continued to be affected 4 to 24 hours following single treatment.
Discussion: This study provides initial data that the A549 cancer cell line is more affected by tryptophan for a greater period of time than a cell line derived from a non-cancer cell, potentially providing a basis for a new therapeutic approach.

P-495

Measurement of fatty aldehyde dehydrogenase activity as rapid diagnostic test for Sjörgren-Larsson syndrome in lymphocytes

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Background and objective: Sjörgren-Larsson syndrome (SLS) is an autosomal recessive disorder characterized by a clinical triad consisting of ichthyosis, spastic di- or triplegia and mental retardation. SLS is caused by a deficiency of microsomal fatty aldehyde dehydrogenase (FALDH), which converts long-chain fatty aldehydes to the corresponding fatty acids. SLS can be diagnosed by demonstrating an FALDH deficiency in cultured skin fibroblasts or by molecular analysis of the ALDH3A2 gene encoding FALDH. However, no rapid diagnostic tool is available for SLS at the moment. For this reason, we developed an enzyme diagnostic screening test for SLS in lymphocytes.
Methods:A previously reported HPLC-method to monitor FALDH activity by formation of pyrenedecanoic acid from pyrenedecanal was adapted and validated for enzyme diagnostics in lymphocytes.
Results:Several parameters were tested including time dependency (linear up to 30 minutes) and protein dependency (linear up to 0.4 g/L), and stability of the enzyme in whole blood and in lymphocyte homogenates. Clinical validation was performed with lymphocytes from three established SLS patients: FALDH activity was below the limit of detection of the enzyme assay.
Conclusion:We have developed a reliable rapid assay to screen for SLS in lymphocytes.

P-496

Two possible new mutations of the POLR3B gene in Russian patients with hypomyelinating leukodystrophies

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Background and objectives: Mutations in POLR3B genes have been reported to cause hypomyelinating leukodystrophies, a rare inherited neurodegenerative disorder characterised by abnormal central nervous system white matter.
Patients and Methods:We investigated the entire coding region and flanking exon/intron boundaries of the POLR3B gene by PCR amplification and sequencing in 3 unrelated Russian female patients with hypomyelinating leukodystrophies.
Results: All patients turned out to be compound heterozygous carriers of two mutations. Two patients (P2 and P3) were found to be heterozygous for the previously reported mutation c.1568 T>A (p.523Val>Glu, CM119256). The nucleotide substitution c.731G>T (p.244Gly>Val, rs199782156) was detected in all three patients in a heterozygous state. According to the NCBI database this nucleotide substitution is a rare single nucleotide variant (G/T- 0.002) with unknown clinical

presentation. One patient (P1) was found to be heterozygous for a previously unreported nucleotide substitution c.2315A>G (p.772Tyr>Cys).
Conclusion: Our results indicate that nucleotide substitutions c.731G>T (p.244Gly>Val) and c.2315A>G (p.772Tyr>Cys) are mutations causing hypomyelinating leukodystrophies.

P-497

Vaccination in patients with inborn errors of metabolism

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Background and objectives: Vaccination is a procedure that has a great impact on reducing illnesses and death. However, the induction of a detectable immune response increases the metabolic needs of individuals. Some diseases are associated with metabolic decompensation during periods of stress, like infection or after vaccination. The aim of this study was an evaluation of the literature on the safety of vaccination in patients with inborn errors of metabolism.
Methods: A non-systematic review was conducted in the electronic databases of MEDLINE through March 2014 using the terms: inborn errors of metabolism and vaccines.
Results: 21 were selected from 251 articles, these being the most representative of the theme.
Discussion/conclusion: Articles show there is a group of diseases with more sick patients, such as leucinosis, organic acidemia, urea cycle disorders, and mitochondrial diseases. In these patients, the vaccines should be performed when the patient is stable, afebrile, accepting the diet. Intervals of at least 15 days must be considered for making each vaccine. Emergency diets can be imposed for up to 72 hours after vaccination, especially in those with the use of live virus. More studies on vaccine response and safe practice are needed.
Conflict of Interest declared.

P-498

BCKD-kinase deficiency: Expanding the disease phenotype with sensorineural hearing loss and transient acrodermatitis enteropathica-like dermatitis

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Background and objectives: Mutations in the BCKDK (branched-chain keto-acid dehydrogenase kinase) gene have recently been described as a cause of comorbid intellectual disability, autism, and epilepsy in three consanguineous families. Two additional unrelated children with persistently reduced body fluid levels of branched chain amino aicds (BCAAs), developmental delay, microcephaly, and neurobehavioral abnormalities, were also reported to carry homozygous BCKDK mutations. As this is a new entity, the disease phenotype and underlying pathophysiological mechanisms are not yet fully understood.
Methods: Whole exome sequencing was performed in a consanguineous family with two siblings (8.5 year-old boy and 6 year-old girl) with intellectual disability, autism, epilepsy, microcephaly, and senserineural hearing loss (SNHL).
Results: Previously described homozygous p.R156X mutation was found on BCKDK. No pathogenic mutations were found in known deafness genes. Both children have persistently low plasma BCAAs and a history of transient acrodermatitis enteropathica-like dermatitis unresponsive to oral zinc supplementation, which was striking in infancy.
Discussion: This is the first description of SNHL and acrodermatitis enteropathica-like dermatitis in BCKDK deficiency.

P-499

Impairment of bioenergetics parameters induced by acute carnosine administration in skeletal muscle of young rats

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Serum carnosinase deficiency is an inherited disorder that leads to an accumulation of carnosine in central and peripheral tissues of affected patients. Considering that patients present severe episodes of dystonia and lethargy and that the pathophysiological mechanisms involved in serum carnosinase deficiency remain poorly understood, we investigated the in vivo effects of carnosine on bioenergetics parameters, namely respiratory chain complexes (I–III, II, and II–III), malate dehydrogenase, succinate dehydrogenase, and creatine kinase activities and the expression of mitochondrial-specific transcription factors (NRF-1, PGC-1α, and TFAM) in skeletal muscle of young Wistar rats. We observed a significant decrease of complexes I–III and II activities in animals receiving carnosine acutely, as compared to control group. However, no significant alteration in respiratory chain complexes, citric acid cycle enzymes, and creatine kinase activities were found between rats receiving carnosine chronically and control group animals. As compared to control group, mRNA levels of NRF-1, PGC-1α, and TFAM were unchanged. The present findings indicate that energy dysfunction occurs in skeletal muscle of rats receiving carnosine acutely, suggesting that it might be a putative mechanism responsible for the muscle damage observed in serum carnosinase-deficient patients.

P-500

Sustained efficacy and tolerability in infants and young children with life-threatening hypophosphatasia treated with asfotase alfa

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Background/objectives: Hypophosphatasia (HPP) results from inactivating mutation (s) in the gene for tissue non-specific alkaline phosphatase (TNSALP). Long-term efficacy and tolerability of asfotase alfa, a recombinant human TNSALP, was evaluated in patients with life-threatening perinatal and infantile HPP.
Materials/methods: Radiographic Global Impression of Change scale (RGI-C, −3 to +3), Rickets Severity Scale (RSS; 0 to 10) and respiratory status were assessed during asfotase alfa treatment (Rx; SC injection, 3x/wk, 1–3 mg/kg) for ≥3 yrs.

Results: Eleven patients (median age 6.8 months) were enrolled; one subsequently withdrew and one died (unrelated to treatment). Median treatment duration was 35 months. Asfotase alfa was well tolerated. Three SAEs were reported as possibly treatment-related; craniosynostosis (a known complication of HPP), conductive deafness and chronic hepatitis. Median RGI-C (+2.50, p=0.008) and median change in RSS (−6.25, p=0.016) showed sustained improvement from baseline at 3 years (n=8). Ten patients required respiratory support at some time during the first 48 weeks, after which three remained on support. Two subsequently discontinued support and one improved to supplemental oxygen. Probability of survival at 3 years was 90 %.
Conclusion: Infants and young children with life-threatening HPP treated with asfotase alfa show sustained improvement in skeletal mineralization and respiratory status for ≥3 years.
Conflict of Interest declared.

P-501

Severe perinatal and infantile forms of hypophosphatasia: A retrospective natural history study

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Background and objectives: Hypophosphatasia (HPP) is a rare metabolic bone disease caused by inactivating mutation (s) in the gene for tissue non-specific alkaline phosphatase. There are only limited data on the natural history of perinatal and infantile HPP.
Materials and Methods: This was a multinational, retrospective chart review study in HPP patients (onset<6 months of age) with respiratory complications, rachitic chest deformity and/or seizures. Primary and secondary outcome evaluations were survival and invasive ventilator-free survival, respectively.
Results: Of 48 patients, 14 had signs of HPP in utero. At the time of diagnosis (median age: 8.6 weeks, range: 0–178 weeks), 32 (67 %) showed rachitic chest deformity. At the time of analysis, 35 (73 %) patients had died. The median time to death was 8.9 months (95 % CI: 5.1-14.1), with a 31 % and 58 % probability of death by 3 and 12 months, respectively. All 10 patients with seizures died. 29 patients required respiratory support, 14 in the first 6 days of life. 19 required invasive ventilation (median time to invasive ventilation: 7.8 months [95 % CI: 2.6-9.9]).
Conclusion: Perinatal or infantile HPP complicated by respiratory compromise, seizures or chest deformity is associated with high mortality.
Conflict of Interest declared.

P-502

New method for the molecular genetic diagnosis of heritable disorders of connective tissue using next-generation sequencing (NGS)

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Background and objectives: Heritable disorders of connective tissue comprise a heterogeneous group of inherited disorders with impaired synthesis or breakdown of proteins of the extracellular matrix of connective tissue, leading to disruption of its structure. The aim of our work was to develop a new method for the diagnosis of twelve most common heritable disorders of connective tissue using NGS. Materials/Patients and Methods: In 24 patients with clinical manifestation of heritable disorders of connective tissue the target regions of genes COL1A1, FBN1, COL1A2, COL3A1, COL5A1, COL5A2, COL2A1, TNXB, FGFR3 and FLNB were examined by NGS. Mutations in these genes cause the 12 most common diseases of connective tissue, i.e. osteogenesis imperfecta type 1,2,3,4, Marfan syndrome, a Larsen syndrome, Stickler syndrome, Ehlers-Danlos syndrome, type 1,2,3,4, achondroplasia, gipochondroplasia.
Results: In 18 patients (75 %) we identified mutations that can lead to the development of inherited connective tissue diseases. 70 % of the mutations were identified in genes encoding collagen chains (COL1A1, COL1A2, COL3A1, COL5A2). 30 % of the identified mutations were not recorded in the database HGMD. All mutations were confirmed by Sanger sequencing.
Discussion/Conclusion: Our method allows the molecular genetic diagnosis of 12 common heritable disorders of connective tissue with a high accuracy and high performance.

P-503

Urinary pyridinoline cross-links in osteogenesis imperfecta

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Background: Osteogenesis Imperfecta (OI) is a connective tissue disorder (CTD) characterized by increased bone fragility and susceptibility to fractures, caused by mutations in 16 genes. The diagnosis relies on expensive and time consuming molecular and biochemical analyses. The ratio of urinary lysyl-pyridinoline (LP) to hydroxylysyl-pyridinoline (HP), two biomarkers of bone resorption, is a well established diagnostic tool for other CTD.
Methods: We evaluated the urinary LP/HP ratio as a screening tool in 35 OI patients from 26 families with proven mutations: 15 COL1A1; 3 COL1A2; 7 LEPRE1; 3 CRTAP; 2 SERPINF1; 1 SP7/OSX; 1 BMP1; and 3 FKBP10.
Results: Mean age at biochemical/molecular analysis was 15.3 years (range: 0.1 – 57 years). 54 % of families showed AD inheritance (COL1A1/COL1A2), while 46 % showed AR inheritance. Compared to control LP/HP ratios we found markedly decreased ratios in patients with mutations in LEPRE1, CRTAP, SP7/OSX, FKBP10; normal LP/HP ratios in OI caused by mutations in SERPINF1 and BMP1. In individuals with COL1A1/COL1A2 mutations LP/HP was normal in 11/18, decreased in 6/18 and increased in 1/18.
Conclusion: LP/HP ratios have the potential to detect some of the recessive forms of OI as a non-invasive, fast, reliable and cost effective screening test, prior to biochemical and/or molecular genetic analyses.

P-504

The extension of molecular genetic diagnostics of congenital adrenal hyperplasia in the Czech Republic

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Background: Congenital adrenal hyperplasia (CAH) is a group of inherited AR disorders caused by enzymatic deficiency which impairs steroid hormone biosynthesis. About 90 % of all CAH cases are due to 21-hydroxylase deficiency (21-OHD) caused by CYP21A2 gene aberrations. Most of the CYP21A2 gene mutations result from recombination events with its pseudogene CYP21A1P. Mutations in the CYP11B1, HSD3B2 and CYP17A1 genes are genetic causes of 11-β-hydroxylase deficiency (11-βOHD), 3-β-hydroxysteroid dehydrogenase deficiency (3-βHSD) and 17-α-hydroxylase deficiency (17-αOHD), respectively.
Methods: For differential amplification of the genes and their homologs, long-range PCR is used (21-OHD, 11-βOHD). Diagnostics of 21-OHD is followed by restriction analysis, secondary PCR, sequencing and MLPA. Further CYP11B1 analysis comprises secondary PCR and sequencing. Molecular diagnostics of 3-βHSD and 17-αOHD include PCR and sequencing.
Results: Molecular genetic testing confirmed the diagnosis of 21-OHD in 342 probands (45,1 %). Currently, we have confirmed the diagnoses of 11-βOHD in one patient and 17-αOHD in two patients.
Conclusion: Molecular genetic testing in our laboratory allows for the identification of the CYP21A2 gene mutations in patients suspected of 21-OHD. Recently, we have broadened the scale of molecular genetic testing of CAH in the Czech Republic by introduction of CYP11B1, HSD3B2 and CYP17A1 genes analyses.

P-505

Two cases of pxoprolinuria: 5-oxoprolinase deficiency?

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The gamma-glutamyl cycle is a six-enzyme cycle that represents the primary pathway for glutathione synthesis and degradation. 5-Oxoprolinase (OPLAH) deficiency is a rare disorder of the gamma-glutamyl cycle. We report two patients who manifested massive excretion of 5-oxoproline in urine. Patient 1: She is 19 months of age. She was born on term by C/S as the third child of her family (first degree cousin marriage). Her siblings (9 year-old sister and 7 year-old brother) were healthy. She had two cousins with methylmalonic acidemia. At the age of two months she had an acute gastrointestinal infection with compensatory metabolic acidosis. High urinary 5-oxoproline was detected. Her weight is 25-50th percentile, height is 75-90th percentile, and systems examinations were normal. Patient 2: She is a 5 year-old girl. She was born on term by C/S. She had congenital nystagmus and strabismus (detected at 6 months of age). Her weight is 75-90th percentile, height is 90-97th percentile, mild ataxia, and mild mental retardation; other systems were normal. Laboratory investigation revealed 5 oxoprolinuria. There was no sign of hemolysis. Two cases with suspicion of OPLAH deficiency; the first case presented with metabolic acidosis and the second had neurological findings.

P-506

Peripheral blood smear and/or bone marrow analysis are simple tools to conduct the diagnosis in inborn errors of metabolism

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Background and objectives: Hematological abnormalities are frequently seen in inborn errors of metabolism (IEMs). However, these are often nonspecific such as macrocytic anemia, pancytopenia or vacuolated lymphocytes. Our objectives were to emphasize the clinical value of peripheral blood smear and/or bone marrow analysis in IEMs.
Methods: Seven patients for whom blood smear and/or bone marrow had helped diagnosis orientation were retrospectively analyzed.
Results: 5 patients had a lysosomal storage disorder (LSD). In 3 of them (Sanfilippo, Morquio A and Wolman diseases), the diagnosis was suspected clinically and blood smear showed storage material thus confirming clinical suspicion. In the other 2, there was no specific clinical suspicion and storage material in blood smear was helpful for precise diagnosis orientation (Morquio B and Pompe diseases). One patient exhibited neonatal pancytopenia with dysmegakaryopoiesis which had been initially overlooked. Later on, this patient was diagnosed with isovaleric acidemia (IVA). One patient had failure to thrive and thrombocytopenia. The bone marrow showed particular hemophagocytosis which helped to confirm the diagnosis of lysinuric protein intolerance.
Discussion/Conclusion: These examples illustrate the value of blood smear and/or bone marrow analysis as simple diagnostic tools to appropriately conduct etiologic investigation in various IEMs.

P-507

LPIN1 mutations cause severe rhabdomyolysis in childhood - the Austrian experience

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Background: LPIN1 mutations are probably causative for 50 % of rhabdomyolysis episodes in pediatric patients. The Lipin-1 protein, expressed in muscle and adipose tissue, is involved in lipid metabolism. Loss of function leads to intracellular accumulation of lipid droplets and free fatty acids, enhanced under inflammatory conditions and eventually leading to cellular death. Treatment options are limited to symptomatic therapy including maintainance of an anabolic state.
Patients: Three girls and one boy, all younger than 5 years at first rhabdomyolysis episode, were diagnosed with LPIN1 mutations in two Austrian hospitals. All were normally developed and had 1 to 4 episodes of rhabdomyolysis. Episodes were triggered by viral infections, excessive physical activity or fasting before anesthesia. Maximal creatine kinase concentration was up to >500.000 IU (0–200 IU). Episodes were treated with glucose to establish anabolism. Unfortunately, one patient died of hyperkalemia-triggered cardiac arrest at 5 years during the third rhabdomyolisis episode. The other three are stable, painfree and have normal muscle strength and function.
Conclusion: Lipin-1 deficiency is the most common known cause of infection-associated rhabdomyolysis in children. Apart from hyperhydration and forced diuresis during rhabdomyolysis, these patients highly benefit from establishment of an anabolic state to prevent cell death.

P-508

Successful bisphosphonate therapy in a family with Torg-Winchester syndrome

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Background: Torg-Winchester syndrome (TWS) is a rare inherited progressive multicentric osteolysis syndrome caused by MMP2 mutation coding for matrix metallopeptidase 2. We present bisphosphonate therapy in three affected siblings.
Patients: They are children of healthy non-consanguineous Turkish parents. Patient 1 (13 years), developed painful swelling and contractures of the small joints of hand and feet with multiple low-impact fractures at age 4 years. Examination showed short stature, a coarse face and hypertrichosis. Radiographs demonstrated general osteopenia, multiple osteolysis, widening of the phalanges and irregular epiphyses. Patient 2 (7 years), and patient 3 (5 years) had an earlier onset and more severe evolution. All three have a homozygous stop mutation in the MMP2 gene.
Bisphosphonate therapy: In patient 1 pamidronate therapy was started at first symptoms and was continued until age 12 years. For compliance reasons therapy was changed to zoledronate. Patient 2 and 3 were only treated with zoledronate. Therapy led to increased bone mineral density and reduced pain in the patients. No more fractures occured. Patient 1 with the most favourable outcome is still able to walk and use her hand for writing.
Conclusion: Bisphosphonate therapy is a suitable option in TWS. Pamidronate might be favourable when compared to zoledronate.

P-509

Metabolic Diet App Suite: Digital medicine to support families with inborn errors of metabolism

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Background: Diets for inborn errors of metabolism (IEMs) are known to be burdensome, with lack of nutrient information often leading to compliancy issues, one-sided nutrition and frustration for children and families. Digital technologies provide the opportunity to develop Metabolic Diet Applications for handheld devices which can help families overcome these problems.
Objective: We aimed to develop Applications comprising nutrient information with options for tracking and exporting food records, to facilitate therapeutic compliance for IEM patients on diets.
Methods & Results: Using Metabolic Pro Database we designed the 1st ever Metabolic Diet Apps Suite tailored to more than 15 different IEMs. Login to the App is personal. Features of each App include: relevant nutrient listing per food item, calculation tracking of food/nutrient intake, summary of food records with graphs, own recipes, and the possibility to export the food records as PDFs. We have performed a study on user friendliness; data was used to improve the Apps, to be launched summer 2014.
Discussion: The Metabolic Diet App suite enhances personalized treatment of IEMs via digital medicine. Especially for adolescents the Apps will be attractive tools, allowing them to better understand and control their disease. Change in adherence will be evaluated.

P-510

Atypical clinical presentation of familial hypomagnesemia with hypercalciuria and nephrocalcinosis: report of two Russian siblings

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Background and objectives: Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC), an autosomal recessive renal tubular disorder, is characterized by the impaired tubular reabsorption of magnesium and calcium and an eventual progression to end-stage renal disease. Typical features are polyuria and polydipsia, hypomagnesemia, hypercalciuria and nephrocalcinosis. Additional symptoms include convulsions, ocular abnormalities and hearing impairment. Recent studies have reported that this disease is caused by mutations in the CLDN16 gene.
Patients and Methods: Russian siblings clinically diagnosed with Familial hypomagnesemia. Laboratory workup for the differential diagnosis of nephrocalcinosis was done: complete urinalysis, including urinary calcium excretion, serum electrolytes, and renal function tests. DNA analysis of each exon of the CLDN16 gene.
Results: We present two sibs from a nonconsanguineous couple with atypical features such as seizures, hearing impairment, autistic features, hypomagnesemia with hypercalciuria and without nephrocalcinosis. Compound heterozygosity for the common CLDN16 mutations c.165_166delGGinsC and c.324+10 T>C was found. Their parents were asymptomatic heterozygous carriers of single mutations.
Conclusion: This is the first report of FHHNC in Russia, and the second mutation reported is novel. To our knowledge FHHNC without nephrocalcinosis has not been described previously. FHHNC should be considered in any patient with a tubular disorder with additional neurological signs such as ataxia, epilepsy, or sensorineural deafness.

P-511

Neopterin antioxidant effects In glial cells

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Neopterin (Neo), a biochemical product of the guanosine triphosphate pathway, is found at increased levels in body fluids of immune system activatived patients. According to the literature, Neo production is restricted to monocyte/macrophage and related cells after IFN-γ stimulation. However the exact cell type, stimulus to production, and role of this endogenous pteridin, are unknown. We investigated the effect of Neo in glial cells exposed to Neo alone or in combination with H_2O_2 or azide for 1 or 3 hours, in order to assess free radical production, Neo and lactate release, and hemeoxygenase-1 (HO-1) immunecontent. Neo pre-treatment prevented free radical generation, when C6 cells were exposed to a pulse of H_2O_2. However when Neo was co-incubated with H_2O_2 the antioxidant property displayed a U curve effect. Additionally, we observed that inhibition of mitochondrial activity led to increased Neo production, lactate release, and HO-1 content in primary striatal astrocytes. Furthermore, the co-incubation of Neo plus azide reduced free radical production and did not avoid the activation of the anaerobic glycolysis. These data indicate that astrocytes may produce and release neopterin when the mitochondrial function is impaired and that this compound might possess antioxidant properties possibly by Nrf-2-mediated inhibition of free radical production.

01. Inborn errors of metabolism: general, adult

A-001

Case of combination of cystic fibrosis with metabolic disorders of fatty acids and sulfur containing amino acids

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Background: Cystic fibrosis has a clinically and genetically heterogeneous polymorphic pathology, which is accompanied by various changes in metabolism. Case report: A boy, 4 years old, from a closely related marriage, with diagnosis of malabsorption, hypoproteinemic edema, pneumonia, endogenous intoxication syndrome, sick since 2 months of age with poor weight gain, stool disorder, lethargy. At 3 months the condition worsened: malnutrition, hypodynamy, hypotonia, episodes of arrhythmia, dyspnea, anxiety, malabsorption, pallor, swelling of the skin. On examination : ↑↑sweat chloride 156, 110 mmol/l sweat. Steatorrhea, reduced trypsin in feces. Mild hepatosplenomegaly, metabolic nephropathy. Lagged behind in development, hypotonia, pneumonia, shortness of breath, coughing, tachyarrhythmia, bloating, stool disorders. At the age of 1 year: hepatomegaly, fibrosis. In blood: ↓methionine 0.015 mmol\l, ↓valine 0.068 mmol\l, ↓glutamine 0.280 mmol\l, ↑homocysteine 7.8 mmol\l; ↑AST 110U/L, ↑ALT 226U/L, ↑LDH 959.84U/L; ↓cholesterol 1.32 mmol/l, ↓iron 5.1 μmol/l, ↓ Ca 1.38 mmol/l, ↓albumin 30.93 g/l. Gas chromatography of urine: ↑↑methylmalonate 54.44 mmol| molCREA, ↑↑suberic acid 461.48U|molCREA, ↑↑oxoglutaric acid 755.73U|molCREA, ↑↑p-hydroxyphenylacetic acid 3466.41 U|molCREA, ↑↑hydroxyphenyllactic acid 1475.6 U|molCREA, ↑↑3-hydroxysebacic acid 157.25 U|molCREA, ↑ethylmalonic acid, ↑phenoxyacetic acid 179.39 U|molCREA, ↑azelaic acid, ↑5-hydroxyindolacetic acid. Conclusion: We need to find comorbidity in patients with multiple organ disorders, in families with incestuous marriages to choose adequate therapy and rehabilitation.

06. Phenylketonuria: general

A-002

Does utilizing the PKU clinical coordinator for a 13 year old PKU patient and her family improve outcome

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Phenylketonuria (PKU) is an autosomal recessive disorder treated for life with a phenylalanine restricted diet and an adjunct therapy called sapropterin dihydrochloride in responsive patients. The traditional PKU diet may be difficult for families to maintain due to cost, convenience and support. The success of phenylalanine levels within treatment range is associated with support at home and school. BioMarin introduced a PKU clinical coordinator (PCC) program where a registered dietitian is available to assist our clinic and patients to ensure individual clinic protocols for treatment are followed. Sapropterin dihydrochloride therapy was initiated in a patient struggling despite close clinic contact and weekly phone calls. The PCC makes home visits, ensures Kuvan is taken daily and provides the food service staff with thorough education regarding the appropriate PKU diet modifications to the breakfast and lunch school program. Frequent blood Phe monitoring is completed with reminders from the PCC to assess improvement in phenylalanine levels. The knowledge of a patient's history and guidance from the clinic creates a team approach that enhances the role of the PCC. This combination of clinic and PCC may lead to better outcomes and greater understanding of the diet for the patient and their support system.
Conflict of Interest declared.

A-003

A systematic review (SR) and meta-analysis (MA) to assess blood phenylalanine (Phe) levels in adults with phenylketonuria (PKU)

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Background and Objectives: Treatment guidelines recommend lifetime maintenance of a low-Phe diet to lower blood Phe levels, reducing the risk of neuropsychiatric symptoms. This SR and MA was performed to assess blood Phe levels in individuals ≥16 years old with PKU.
Methods: The SR of published literature was conducted based on searches in MEDLINE, Embase, and Cochrane Collection databases from January 1980 through June 2013 supplemented by manual searches of reference lists in accepted studies and recent reviews. Random-effects MA were performed to calculate a pooled estimate of blood Phe levels with variance estimates and potential sources of heterogeneity were explored.
Results: The SR identified 61 study arms comprising 1366 PKU adults that reported mean blood Phe levels with variance estimates. The random-effects combined estimate for blood Phe was 1179 μmol/L (95 % CI: 1064–1293 μmol/L). Univariate meta-regression analysis suggested that neuropsychiatric symptoms, PKU treatment (early vs late/untreated), and publication year may account for some of the observed heterogeneity.
Discussion/Conclusion: Blood Phe levels in PKU adults substantially exceed treatment guidelines for lifetime maintenance, confirming previous reports of the difficulty of maintaining a low-Phe diet after adolescence, and supporting the need for medications that lower blood Phe levels in adults with PKU.
Conflict of Interest declared.

A-004

A systematic review (SR) and meta-analysis (MA) to assess the prevalence of neuropsychiatric symptoms in adults with phenylketonuria (PKU)

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Background and Objectives: Numerous reviews have documented the prevalence of neuropsychiatric symptoms in children with PKU with elevated blood phenylalanine (Phe) levels, but a comprehensive review of prevalence in adults has not been reported. This SR and MA was conducted to assess the prevalence of neuropsychiatric symptoms in individuals ≥16 years old with PKU.
Methods: The SR of published literature was conducted based on searches in MEDLINE, Embase, and Cochrane Collection databases from January 1980 through June 2013, supplemented by manual searches of reference lists in accepted studies and recent reviews. Random-effects MA were performed to calculate pooled estimates of the prevalence of reported psychiatric symptoms such as anxiety, hyperactivity, inattention, depression, executive function deficits (attention, cognitive flexibility, inhibitory control), and neurologic symptoms (epilepsy/seizures, tremors).

Results: MA were performed based on 8 study arms (889–945 subjects) reporting psychiatric symptoms, 33 study arms (793 subjects) reporting executive function assessments, and 14 study arms (1028 subjects) reporting neurologic symptoms. The random-effects combined estimates found a higher prevalence in PKU adults compared with unaffected adults for anxiety, depression, hyperactivity, inattention, executive function attention deficit, epilepsy/seizures, and tremors.
Discussion/Conclusion: The high prevalence of neuropsychiatric symptoms in adults with PKU represents a significant disease burden in this population.
Conflict of Interest declared.

08. Sulphur amino acid disorders

A-005

Profiling of genetic variants in the folate-mediated one-carbon metabolism (FOCM) pathway as risk factors for hyperhomocysteinemia in a North Indian cohort

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Objective: Homocysteine (hcy) is a product of folate one carbon metabolism pathway (FOCM). Genetic defects in the metabolic pathway result in elevated hcy concentrations. Hence, we studied the genetic polymorphisms in FOCM pathway in patients with hyperhomocysteinemia and evaluated patients with susceptible diagnosis of hyperhomocysteinemia.
Materials and Methods: Fasting plasma total hcy concentrations were measured using RP-HPLC & genotypic frequencies of MTHFR, MTR, CBS & ENOS genes were ascertained using PCR-RFLP & ARMS-PCR method. p value ‹0.05 was considered significant. Results: Of 176 subjects, 40 had documented hyperhomocysteinemia (22.72 %). Twenty patients are children, mean age at presentation was 6.22 yrs (6.22± 2.83). Commonest presentation was stroke (60 %) and neuroimaging finding was an infarction (30 %). Six cases presented in adolescent age with mean age of 13.6 yrs (13.6±1.57). Commonest reason was stroke (75 %) and seizures (33 %). In adult age, 14 cases with mean age at presentation of 25.43 yrs (25.43±5.97), presented with DVT (29 %), stroke (14 %) and recurrent abortions (14 %). The frequencies of mutant genotypes in MTHFR (C677T & A1298C), MTR (A2756G & C2758G), CBS (T833C) and eNOS (C786T) genes are (10 %&2.5 %), (10 %&27.5 %), 0.0 % and 20 %, respectively.
Conclusions: Elevated hcy concentrations may be a significant contributor for pediatrics and adolescent stroke. Mutations in MTR and eNOS genes confer significant susceptibility to the development of hyperhomocysteinemia in an Indian cohort.

09. Other amino acid disorders

A-006

Early diagnosis of Grawitz tumor due to alkaptonuria follow up

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Background: Alkaptonuria is a rare autosomal- recessive metabolic disorder with highest incidence in Slovakia. The deficit of the enzyme dioxygenase homogentisate results in an accumulation of homogentisate and benzoquinone acetic acid in various tissues. Grawitz tumor is a relatively frequent malignant kidney disease with variable, often poor clinical symptomatology.
Case report: We present a 56 year old obese female patient. From childhood her dark urine and sweat caused the staining of the underclothes. From the second decade she suffered from progressive arthrosis, later from repeated urolithiasis and arterial hypertension. At the age of 44 she was send to metabolic outpatient department. Her clinical picture shows ochronosis of sclera, ears, skin, and severe generalised arthropathy. The diagnosis was confirmed by an extremely high excretion of homogentisate in urine and mutation analysis. Nephrological examination revealed ultrasonographic changes in left kidney. Despite possible traumatological changes due to lithotripsy the diagnosis of tumour was considered and she was operated. First stage of Grawitz tumor was confirmed histologically. Radical nephrectomy was performed with no further therapeutic intervention till now.
Conclusion: Grawitz tumor does not belong to clinical symptoms of alkaptonuria, but thanks to the routine nephrological examination it was revealed at an early stage.

10. Urea cycle disorders

A-007

Two patients with ornitine transcarbamylase (OTC) deficiency - gender differences

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X-linked OTC deficiency was confirmed in 7 y/o boy and 2 y/o girl by DNA sequencing, the girl had skewed inactivated X-chromosome. Both kids had complaints with vomiting, failure to thrive. At two years of age, the boy showed aversion to meat and dairy foods. The girl had tetraplegia, metabolic encephalopathy, lethargy and prolonged (7 days) insomnia, mother was satisfied that daughter slept till 1 year old and seemed very patient. The clinical manifestations began after weaning at 6 months and starting a higher protein diet. The boy's intellect is lower to age, physical status less 3 percentile, cryptogenic hepatitis was the aim of hospitalization. Ammonia concentration in both cases was similar (284 and 249 μg/dl), orotic acid in urine (in boy 1009 mmol/mol creat., in girl 206 mmol/mol creat.). Liver function tests were elevated (ALT 15, AST 5 times above normal in boy; 1,5 and 2 in the girl, respectively). Urea concentration in blood was 3.7 (boy) and 0.8 mmol/L (girl), arginine concentration 15 and 17 mmol/L (ref.range 38–122 mmol/L), glutamine 874 and 802 mmol/L (ref.range 60–800 mmol/l). The investigation of the residual OTC activity in liver would be useful to decide on liver transplantation.

A-008

Use of low dosage of carglumic acid in the treatment of hyperammonemia due to N-acetylglutamate synthase deficiency

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N-acetylglutamate synthase (NAGS) deficiency is an autosomal recessively inherited disorder of the urea cycle. The disorder leads to deficiency of N-acetylglutamate, which is an allosteric activator of carbamylphosphate synthetase. N-carbamylglutamate is a structural analogue of human N-acetylglutamate. The patient is the first child of a consanguineous Turkish couple. At 2 days old he developed irritability and lethargy. He had an elevated ammonia level of 328 mmol/L. Treatment with intravenous glucose, oral sodium benzoate and arginine was started and enteral feeding was stopped. Carglumic acid treatment was initiated with a dose of 100 mg/kg/day. After carglumic acid treatment, the ammonia level was normalized. Mutation analysis revealed classical mutation of the NAGS gene. At 2 years, 3 months old, he attended to emergency unit with an elevated ammonia level of 228 mmol/L due to upper respiratory tract infection. The carglumic acid dose was raised to 50 mg/kg/day, and ammonia level returned to normal within 6 hours. After hyperammonemia resolved, carglumic acid dosage was arranged to 30 mg/kg/day and protein restricted diet was discontinued and blood ammonia levels were closely monitored. The patient's ammonia levels remained within normal limits. The patient is now 3 years old and has no neurodevelopmental abnormalities.

A-009

Citrullinaemia type 1 patient presenting as Sandifer syndrome

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Background: Citrullinaemia type 1 is a rare autosomal recessive urea cycle disorder caused by deficiency of argininosuccinate synthetase.
Case report: Eleven months old infant girl admitted to our hospital with complaint of vomiting and abnormal head posture. On physical examination head was tilted to the right, hand stereotypies and hypotonia were seen. Laboratory examinations were normal except hyperammonemia. Metabolic screening showed elevated level of plasma citrulline. Compound heterozygous c.688G>A/c.793C>T mutations were detected in ASS1 gene and citrullinaemia type 1 diagnosis was made. Low-protein diet, sodium benzoate and arginine therapy were given. After treatment ammonia levels decreased and clinical symtoms disappared.
Conclusion: In spite of large clinical symptoms in citrullinaemia type 1 patients, Sandifer syndrome was the first presentation in this case report.

11. Organic acidurias: branched-chain

A-010

An asymptomatic mother diagnosed with 3-methylcrotonyl-CoA carboxylase deficiency after newborn screening

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Background and objectives: 3-Methylcrotonyl-CoA-Carboxylase (MCC) deficiency is an autosomal recessively inherited disease of leucine catabolism. It is the most commonly observed organic acidemia where tandem mass spectrometry can be performed in newborn screening. The clinic may differ from neurological involvement in newborns to asymptomatic adults. Diagnosis is made by increased 3-hydroxyisovaleric acid in blood and 3-methylcrotonylglycine (3-MCG) in urine.
Case Report: We would like to present an interesting case of a 32-year-old asymptomatic mother, investigated metabolically and diagnosed with 3-MCC deficiency, after a 7-day-old healthy baby referred to our unit with the preliminary diagnosis of organic academia during her extended newborn screening.
Results: All of the metabolic findings of the baby were normal except very low carnitine levels. Her mother's total and free carnitine levels were also extremely low. Urine organic acid analysis revealed excessively increased 3-MCG and 3-hydroxyisovaleric acid. Acylcarnitine profile showed elevated C5 (hydroxy-3-hydroxyisovalerylcarnitine) and decreased C2 (acetylcarnitine). In order to confirm diagnosis of 3-MCC deficiency, molecular analysis was done and IVS3-1G>C/p.T556I compound heterozygote mutation was detected. p.T556I is a novel mutation.
Conclusion: We would like to emphasize performing extended metabolic investigations in case of suspicion of metabolic disease in order to diagnose metabolic diseases both in babies and asymptomatic mothers.

13. Carbohydrate disorders

A-011

A Patient with Glycogen Storage Disease Type Ia: A novel homozygous mutation in G6PC gene

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Glycogen storage disease type Ia (GSD-Ia) is an autosomal recessive disorder resulting in hypoglycemia, hepatomegaly and growth retardation. It is caused by mutations in the G6PC gene encoding Glucose-6-phosphatase. Here we report a case with GSD-Ia with a novel mutation. He is a twelve years old boy and was born on term by C/S delivery. At 3 days of age lower airway tract infection with metabolic acidosis and hepatomegaly were detected. At 3 months of age hypoglycemia, transaminase elevation and lactic acidosis (110.6 mg/dl) were determined. Liver biopsy revealed glycogen storage disease. Glucose-6-phosphatase activity in liver was near lower limit (6.5 nmol/Pi/mg protein, N: 6–25). Recurrent hypoglycemia and metabolic acidosis attacks were detected during his follow up. Patient visited our department at the age of 11 years. On his physical examination growth retardation and hepatosplenomegaly were detected. Laboratory investigation revealed elevated transaminase levels, hypertriglyceridemia, uric acidemia, and high lactic acid levels. Biotinidase activity was 14 nmol/ml/min (200 %). G6PC gene analysis performed and homozygote p.198IlefsX5 (c.592_593delAT) mutation is detected. To the best of our knowledge we are first reporting this mutation in a GSD-Ia patient.

14. Disorders of fatty acid oxidation and ketone body metabolism

A-012

When a common symptom of a neonate becomes an unusual diagnosis: A case report of HMG-CoA lyase deficiency

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3-hydroxy-3 methylglutaryl-coenzyme A (HMG-CoA) lyase deficiency is a rare autosomal recessive inborn error of metabolism characterized by impairment of ketogenesis and leucine catabolism resulting in attacks of metabolic acidosis,nonketotic hypoglycemia,and a characteristic pattern of elevated urinary organic acids: 3-hydroxy-3-methylglutaric, 3-methylglutaconic, 3-methylglutaric, and 3-hydroxyisovaleric acids. Here we report the case of an infant with HMG-CoA lyase deficiency presenting at 7 days of life with a sudden hypoglycemic seizure, metabolic acidosis and hyperammonemia. A female newborn, born at term from consanguineous parents by caeserian section. The baby was discharged the following day, breast feeding well. However, by day 3 of life, the infant was admitted to a primary care hospital for poor suckling and vomiting. Initial laboratory investigations revealed hypoglycemia 23 mg/dl, blood urea nitrogen 69 mg/dl, creatinine 1.49 and blood gas analysis revealed metabolic acidosis (blood pH 7.06, PCO2 17.8 mmHg, bicarbonate 5 mEq/l, base excess −23.5), hyperammonemia 460 μg/dl, hyperlactatemia 3.25 mmol/l (0.9-1.6) and moderate hypertransaminasemia (aspartate aminotransferases 57 U/l, alanine aminotransferases 84 U/l; reference range 10–37). No ketone bodies were detected in urines. Urinary organic acid analysis by mass spectrometry detected massive amounts of 3-hydroxy-3 methylglutaric, 3-methylcrotonylglycine and methyglutaconic acids. Serum total free carnitine was low and 3-hydroxyisovalerylcarnitine (C5-OH) and 3-methylglutarylcarnitine (C6-DC) levels were significantly increased. This pattern led to the final diagnosis of HMG-CoA lyase deficiency.

A-013

Neonatal onset VLCADD with a novel mutation

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Very long chain acyl-CoA dehydrogenase deficiency was first described in 1993.It is an autosomal recessive genetic disorder in which the first step in the mitochondrial β-oxidation spiral of fatty acids for 14–20 carbons is defective. VLCAD deficiency (VLCADD) results in accumulation of tetradecanoyl (C14) to octadecanoyl (C18) acylcarnitines with prominence of C14:1.The phenotype is heterogeneous.There are three clinical forms of VLCAD deficiency based on the age of onset and clinical presentations:(i) neonatal form presents with hypo-ketotic hypoglycemia, liver dysfunction and cardiomyopathy,infantile form presents with episodes of hypo-ketotic hypoglycemia and liver dysfunction, and (iii) adult form presents with exercise intolerance, episodic rhabdomyolysis.The defective gene of VLCAD has been located to the short arm of chromosome 17. To date more than 80 different mutations have been identified. VLCADD may be rapidly fatal due to cardiac and hepatic involvement. Here we report a 4 month old patient with VLCADD with a severe neonatal onset type who presented with hypoglycemia, cardiomyopathy and hepatomegaly. DNA sequencing of ACADVL revealed a yet unreported homozygous non-synonymous mutation (c.1391C>A) in exon 14. Family studies confirmed the disease-causing mutation. However the patient died unexpectedly one month after the diagnosis and appropriate treatment, supposedly from cardiac dysrhythmia secondary to hypertrophy in left ventricle.

18. Other disorders of energy metabolism, creatine disorders

A-014

Red ragged fibres - a rare finding in muscle biopsies in children

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Introduction: Red ragged fibres (RRF) an important marker for mitochondrial disease are an infrequent finding at an early age. We report two children in whom RRF were found in muscle biopsy despite no encephalomyopathy.
Patient 1. 22-month-old girl with previous diagnosis of unclear endocrinological disorder presenting severe hydroelectrolytic decompensation. Investigations showed multiple endocrinopathy with low IGF1, mixed tubulopathy, high lactate and abnormal organic acids leading to a muscle biopsy depicting numerous RRF and low II+III activities of the respiratory chain.
Patient 2. 15-year-old girl with hypoglycaemia, hepatomegaly and high transaminases at an early age followed at six years by acute vascular events secondary to bilateral occlusion of carotid arteries and severe bilateral hearing impairment. Investigations show intermittent proteinuria and glycosuria, excretion of fructose, glucose or galactose and lactic, malic or fumaric acids despite normal lactate. Muscle biopsy showed RRF and low activity of the II+III complexes of respiratory chain.
Comments: We illustrate two children with RRF, an unusual finding in the literature. Although RRF are generally characteristic of mitochondrial encephalomyopathies or primary muscle disorder, none of the patients had these but curiously both had lactic, fumaric and malic acids in the urine, renal leakage and II+III decreased activities in respiratory chain.

21. Peroxisomal, sterol and bile acid disorders

A-015

Treatment of X-linked childhood cerebral adrenoleukodystrophy by the use of bone marrow transplantation

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X-linked adrenoleukodystrophy (X-ALD) is a peroxisomal disorder biochemically characterized by the accumulation of very long chain fatty acids (VLCFA), particularly hexacosanoic acid (C26:0) and tetracosanoic acid (C24:0), in tissues and biological fluids. X-ALD is clinically heterogeneous, with the occurrence of different phenotypes. The most frequent phenotypes are classical (or childhood) X-ALD (cALD), adrenomyeloneuropathy (AMN) and isolated Addison's disease (AD). The gene deficient in X-ALD, ABCD1, codes for a peroxisomal membrane protein that is a member of the ATP binding cassette transporter

superfamily. MRI severity score, designed specifically for X-ALD, correlates with the severity of neuropsychological and neurologic involvement and, when combined with age, provides information of prognostic significance in X-ALD. The proband was an eight year old boy whose brain MRI severity score (Loes score) was 9 showing pattern 1 with a serious prognosis. He developed symptoms from age 7, including visual and auditory disturbances, decreased school performance, adrenal insufficiency, walking difficulties, demyelinization and leukodystrophy. Genomic sequencing revealed homozygous mutation p.R464* (c.1390C> T) in ABCD1 gene. Lorenzo's oil (LO), combined with a VLCFA-poor diet was administered immediately. This patient was successfully transplanted from his father and neurological disease has not progressed further after conventional BMT with full donor-derived engraftment accomplishment. A family pedigree was obtained, and other family members at risk were identified and underwent genetic counselling.

22. Lysosomal disorders: mucopolysaccharidoses, oligosaccharidoses

A-016

One-year enzyme replacement therapy results in Bulgarian patients with a severe form of Hunter disease

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Hunter syndrome (MPS II) is a rare X-linked disease caused by a deficiency of the enzyme iduronate-2-sulphatase (IDS), which results in the lysosomal accumulation of undegraded glycosaminoglycans (GAG) in various tissues and organs. The disorder is divided into two types (IIA and IIB) based on the severity of symptoms. The early-onset severe form of the disease with progression to profound mental retardation, begins in late infancy. A late-onset mild form causes less severe symptoms. Enzyme replacement therapy (ERT) with recombinant iduronate-2-sulphatase (idursulfase) is the first disease-specific treatment for Hunter syndrome. Four boys with severe form of MPS II (aged from 8 to 17 years) were treated with idursulfase (0.5 mg/kg body weight weekly) for a one-year period. By 6 months urinary GAG levels were significantly reduced from baseline in all patients and continued to be slowly reduced and approaching the normal range after a one year. No significant improvements for joint range of mobility and growth were found. During follow-up the cardiac status remained stable in all cases. Only one patient experienced a normalization in size of the liver. Spleen volume was slightly increased in all cases. One patient had thrombocytopenia, the platelet counts normalized within 2 weeks of initiating idursulfase.

A-017

Hurler syndrome in a two years old Indonesian girl

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Background: Mucopolysaccharidosis I is a rare, inherited, progressive disorder caused by deficiency of lysosomal enzyme, Alpha-L-iduronidase. This disease has been classified into severe (Hurler syndorme) and attenuated type (Hurler-Scheie and Scheie syndromes).The objective of the study was to describe a case of Hurler syndrome in a 2 years old girl which was difficult to diagnose early in Indonesia.
Case Report: A 6 month old baby was brought to hospital with recurrent upper respiratory infection and noisy breathing. She was diagnosed as having patent ductus arteriosus, small atrial septal defect and pneumonia. Physical examination showed forehead enlargement, cracked skin, and lips thickening. At 2 years of age she was brought back to nutrition clinic caused by growth failure, developmental delayed and progressive asymmetrical growth of the torso. Radiologic examination revealed disostosis multiplex. Biochemical testing from Department of Medical Genetics National Taiwan University Hospital showed increased excretion of urinary glycosaminoglycans and a low plasma alpha-L-iduronidase enzyme activity, confirming the diagnosis of mucopolysacharidosis type I, and classified as severe type. Enzyme replacement therapy could not be given due to the cost.
Conclusion: Early recognition is important to give earlier intervention, however without treatment, the life expectancy is short, primarily as a consequence of heart and lung problems.

A-018

Early laboratory diagnosis and bone marrow transplantation in MPS type I: A case report

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Background: Heparan and dermatan sulphates accumulate in tissues due to a defect of alpha-L iduronidase in MPS Type I.
Case report: 16-months-old girl was admitted with complaints of waist curvature and nasal obstruction. Coarse facial features, a depressed nasal bridge, large tongue, coarse hair, thick eyebrows, hypertrichosis, corneal clouding, kyphosis, minimal limitation in knee extension, dysostosis multiplex were observed. Urinary GAG electrophoresis showed dermatan and heparan sulphate bands. A marked deficiency of alpha-L-iduronidase was found (0.008 μmol/hour/gram protein). Molecular analysis revealed IVS11+5G>C and exon 14 c.1893delC mutations of IDUA gene. ERT was started at 20 months of age. At 28-months of age bone marrow transplantation was performed from a non-relative, tissue group 4/6 compatible donor. Chimerism in first month was found 85 % and second, third and sixth months were found 100 %. At fifth month normal alpha-L-iduronidase specific activity was observed. Meanwhile, she is 6 years of age with only little hearing loss and going to preschool.
Conclusion: Early laboratory diagnosis is important for effective treatment in MPS. Due to consanguineous marriages (21 %) in Turkey, the incidence is expected to be more. Establishment of laboratory diagnostic tests in the populations that have higher incidence is important.

23. Lysosomal disorders: sphingolipidoses

A-019

Stability of miglustat in InOrpha® flavoured suspending excipient for compounding of oral solutions and suspensions

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Background and objectives: Miglustat (Zavesca®) hard gelatine capsules are approved for the oral treatment of certain patients with type 1 Gaucher disease or with Niemann Pick type disease. We evaluated the chemical and microbiological stability of miglustat in InOrpha® suspending agent – a liquid flavoured excipient – over 4 weeks when stored under refrigerated conditions.
Methods: The constituents of Zavesca® capsules (100 mg miglustat and excipients) were transferred to InOrpha® to produce miglustat 5, 10 and 20 mg/mL suspensions, with and without pH adjustments. Physicochemical and microbiological testing was then performed at 0 hours (baseline), and after 14 and 28 days.
Results: Miglustat 20 mg/mL suspension changed from yellow to brown by Day 14 at pH 7.4–7.6. At pH 7.5, pH-adjusted pure InOrpha® turned brown by Day 9, but remained yellow to Day 28 at pH 4.6 (no pH adjustment). Miglustat 5 mg/mL (pH 6.5) and 20 mg/mL (adjusted to pH 4.4) suspensions remained yellow at days 14 and 28; miglustat 10 mg/mL suspension (pH 7.3) turned brown by Day 9. High-performance liquid chromatography detected no miglustat degradates in the suspensions. Microbial testing showed no proliferation of microorganisms; contamination decreased in most cases.
Conclusion: Miglustat-InOrpha® suspensions 5 mg/mL and 20 mg/mL (pH 4.4) had stable physicochemical and microbiological properties.
Conflict of Interest declared.

A-020

Novel mutation defined in a patient with attenuated form of Niemann-Pick disease

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Niemann–Pick disease (NPD) is a lysosomal storage disorder occuring as a result of lysosomal acid sphingomyelinase deficiency (ASM). Enzymatic dysfunction of this lysosomal enzyme results in the accumulation of sphingomyelin within the lysosomes of affected individuals. Niemann–Pick disease type A and B is caused by mutations in the sphingomyelin phosphodiesterase gene (SMPD1) coding for ASM. A 5 year-old boy presented to our clinic with enlargement of the liver and spleen noticed in a routine medical examination. He did not have any neurological symptoms and he was a successful student in school. Physical examination of the patient revealed a moderate increase in liver size and a significant increase in spleen size. His respiratory system examination was normal. Ophthalmoscopic examination of the patient revealed retinal involvement with cherry red maculae and his bone marrow aspiration revealed foamy vacuolated histiocytes. Niemann Pick Disease was confirmed by the decrement of sphingomyelinase level in leukocytes in a dried blood sample (103 pmol/spot*20 h, reference range: 200–3500 pmol/spot*20 h). Mutation analysis of the SMPD1 gene revealed homozygous mutation in p.L163P (C.488 T>C).

A-021

Adult case of metachromatic leukodystrophy

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Metachromatic leukodystrophy (MLD) is a rare autosomal recessively inherited lysosomal sphingolipid storage disorder, caused by a deficiency of arylsulfatase A (ASA). In general three different clinical forms of MLD can be distinguished: a late infantile form, a juvenile form and an adult form. In the adult form, the initial symptoms are often evoking the diagnosis of a psychiatric disease, especially schizophrenia, as psychotic symptoms and behavioral abnormalities often precede or accompany a decline of intellectual capacities. Here, we present a 21 years old patient with MLD who was admitted with worsening school performance and irritability for more than two years. Although there were behavioral changes, patient's neurological examination was in normal limits. Electromyography findings revealed sensory axonal neuropathy. Echocardiography was normal. MRI findings showed significant atrophy of anterior white matter, anterior corpus callosum and frontal region, consistent with neurodegenerative disease. The urinary organic acid profile and tandem mass spectrometry analysis were normal. Arylsulfatase A enzyme activity was decreased to 6 nmol/s/mg (N: 50–990). A molecular analysis is pending. The rare condition, adult form of MLD, should be considered in the presence of behavioral abnormalities and decline of intellectual capacities in adolescence and young adults.

A-022

Important diagnostic clues for Sandhoff disease: Hyperacusis and cherry red macular spots

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Background: Sandhoff disease is a rare autosomal recessive sphingolipidosis caused by deficiency of hexosaminidase A+B.
Case report: A thirteen-month-old infant girl was admitted with blindness and developmental problems. Examination revealed severe developmental delay, macrocephaly, hypotonia and macular cherry red spots. Hyperacusis was the most prominent clinical finding. The parents were unrelated and there were no siblings. Chitothroisidase activity was normal while hexosaminidase A+B was very low.
Conclusion: Cherry red macular spots and hyperacusis were the most important diagnostic clues for diagnosis of Sandhoff disease.

A-023

A Gaucher patient with portal hypertension: homozygosity for the double D409H+H255Q allele

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Gaucher disease (GD), an autosomal recessive lysosomal storage disease, results from deficient activity of β- glucocerebrosidase, leading to glucocerebroside accumulation in various tissues such as bone marrow, lymphoid tissue, liver and spleen. Portal hypertension and hepatocellular dysfunction have been rarely reported in infantile Gaucher patients. A female infant, born from consanguineous parents and with a previous infant brother who died from congenital hepatitis and pneumonia, was diagnosed as congenital CMV infection at 3 months old. The patient was referred to our clinic because of non-regressed pancytopenia, hypofibrinogenemia, and hepatosplenomegaly despite ganciclovir treatment. Vacuolated lymphocytes

were detected in bone marrow aspiration material. Liver fibrosis was detected on workup. The diagnosis was established as GD, using enzyme studies. Molecular investigation of the patient showed homozygosity for a double mutation [H255Q; D409H]. Enzyme replacement therapy was initiated and was given twice in total. The patient died as a result of portal hypertension and gastrointestinal bleeding at age 8 months. We report the first Turkish patient to have a homozygous double mutation [H255Q; D409H]. Apart from other cases with the same mutation, our patient's neurological involvement was minimal to none, while gastrointestinal pathology (liver fibrosis) was the main factor for deterioration and death.

24. Lysosomal disorders: others

A-024

A novel mutation described in a turkish patient with infantile neuronal ceroid lipofuscinoses

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Neuronal ceroid lipofuscinoses are a heterogenous group of neurodegenerative disorders which are autosomal recessively inherited and characterized by the intracellular accumulation of autofluorescent lipopigments in neurons and other tissues such as retina of the eye. Neuronal ceroid lipofuscinoses are characterized by progressive loss of acquired functions, intellectual disability, myoclonic epilepsy and decline in motor skills. A two year old girl was the first living child of consanguineous parents presented to our clinic with restlessness. Her development had been appropriate until the end of the first year of her life. At first she had an ataxic gait and then she completely lost her ability to walk at the age of 18 months. On physical examination she was conscious; she had significant truncal hypotonia and severe hypertonia in her extremities. Deep tendon reflexes were increased. She did not have a social smile or follow with her eyes. There was no organomegaly. Magnetic resonance imaging of the brain revealed diffuse cerebral atrophy and corpus callosum agenesis. Neuronal ceroid lipofuscinoses was considered as diagnosis, and mutation analysis revealed a novel mutation in the PPT1 gene (p.P238Cfs*56, c.712_713delCC).

29. Miscellaneous

A-025

Functional independence of Taiwanese children with Down syndrome

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Background: Information regarding the functional strengths and weaknesses of children with Down syndrome is important for early intervention programs, agencies providing family support, and educational services.
Methods: We used the Functional Independence Measure for Children (WeeFIM) questionnaire on parents or caregivers of 166 Taiwanese children with Down syndrome to assess their functional skills (101 males and 65 females; median age, 12.7 years; age range, 3.2 to 19.1 years).
Results: The mean total WeeFIM score was 101.2 out of a potential score of 126. One hundred and fifty-four children (93 %) were identified with full trisomy 21, 7 with mosaicism (4 %), and 5 with the translocation type (3 %). The mean total WeeFIM score of each type was 100.6, 111.9, and 102.4, respectively ($p>0.05$). The mean scores for three domains (self-care, mobility, and cognition) were 45 (maximum 56), 33 (maximum 35), and 23 (maximum 35), respectively. Performance was strongest in the mobility domain, but weakest in the cognition domain. The total WeeFIM scores and 18 sub-scores for these three domains all positively correlated with age ($p<0.05$).
Conclusions: The WeeFIM questionnaire may be useful for the monitoring of long-term response to interventions in these children, as well as in subjects with developmental disabilities.

A-026

A child with combined chromosomal abnormality, mitochondrial dysfunction and disordered cobalamin metabolism

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Background: Mitochondrial dysfunction manifests with multiorganic disorders mainly affecting CNS, heart, liver, and muscles.
Case report: 4 month-old child. Development delay: weakly holds her head, lethargy, mild paratrofia. History: first days of life - lethargy, dyspnea, diffuse hypotonia and hyporeflexia. No sucking reflexes. Ultrasound: increased echodensity or the brain, subependymal cyst on the left side 2 mm; operating oval window, open ductus arteriosus. Elevated echodensity of the liver, metabolic nephropathy. Intensive therapy was provided. Hypotension, developmental delay; hydrocephalic syndrome. Tendon reflexes were average on hands, on knees were torpid. Electromyography at ag 3 months: upper limb muscle function reduced due to nerve damage along trunks of the brachial plexus, decreased muscle contraction of the hip; central type hypertonicity. Neurosonography - expanded external cerebrospinal fluid spaces. Karyotype: 47,XX, +mar. In blood: lactate ↑2.69 mmol\l, ↑LDH 545.68U/L, ↓creatinine 22.14mkm/L. Gas chromatography of urine: modified Krebs cycle metabolites; ketosis, ↓ Vit B2, B5, B12. Metabolic therapy - ubiquinone, carnitine, vitamins. General condition improved.
Diagnosis: chromosomal abnormality (marker chromosome), mitochondrial dysfunction, metabolic cobalamin disorder.
Conclusion: combination of chromosome pathology with metabolic disorders. We need to investigate chromosomal aberrations of metabolic status to choose adequate therapy.

A-027

Diagnosis of familial mediterranean fever masked by symptoms of chronic pancreatitis

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Familial mediterranean fever (FMF) is the most common hereditary periodic fever syndrome in populations of Mediterranean origin (Arabs, Turks, Jews, Lebanese, Greeks, etc.). The frequency of heterozygous carriers of MEFV, responsible for the development of FMF, is more than 1/5 of the total population. Case report: 22-year-old Lebanese student of Kharkiv National Medical University complained of acute pain in mesogastrium, nausea and fatigue. Similar episodes had occurred two years earlier, and the diagnosis of chronic pancreatitis was made. Given the cyclical nature of pain and the Mediterranean origin of the patient, molecular genetic testing was performed. by a combination of restriction fragment length analysis and allele-specific hybridization methods (Tibnin Govermental Hospital). The patient is compound heterozygous

for the mutations M694I and M694V in MEFV exon 10. The mutations V726A, E1480Q, M680I, P369S, R408Q, A744S, M680Ib, R761H, R65, F479L, E167D, K695R, and I692del were excluded. FMF was diagnosed in this patient with symptoms of chronic pancreatitis. It is known that the severity of clinical signs may be decreased in heterozygous carriers of mutant alleles. However, given the risk of FMF-associated amyloidosis, rheumatologist clinical supervision and control of serum amyloid were recommended.

A-028

A case of a possible ciliopathy

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Introduction: Ciliopathies comprise a group of disorders associated with genetic mutations encoding defective proteins, which result in either abnormal formation or function of cilia. They can manifest as a constellation of features that include characteristically, retinal degeneration, renal disease and cerebral anomalies. Nephronophthisis the most frequent genetic cause of ESRD in the first three decades of life results from mutations in different recessive genes (NPHP1 to *NPHP11*).

Case Report: 13-year-old girl with a previous history of unclear endocrinopathy admitted for renal failure. She has mild psychomotor development delay, is small for age and later on has mild ataxia. Investigations showed growth hormone deficiency, mildly high lactate, severe pigmentary retinitis, muscle biopsy mild atrophy of type II fibres, slight predominance of type I fibres and very mild respiratory chain complex I deficiency (36.1 %). A renal biopsy revealed glomerular sclerosis and cystic degeneration of tubules suggesting a possible nephronopthisis.

Comment systic kidney diseases get increasingly complex and there is evidence for a genetic and proteomic network with mutations in multiple cilia-related genes The association of the ocular changes, endocrinopathy, a hypothetical nephronopthisis and ataxia led us to think that the primary disorder may be an unknown syndrome or a possible ciliopathy.