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ABSTRACTS

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01. Inborn errors of metabolism in adult

O-001

Alteration of ornithine metabolism leads to dominant and recessive hereditary spastic paraplegia

Mochel F[1], Coutelier M[3], Habarou F[2], Goizet C[5], Durr A[4], Charles P[4], Janin M[2], Saudubray J M[1], Brice A[3], Stevanin G[3], Ottolenghi C[2]

[1]Neurometabolic unit, La Salpêtrière Hosp, Paris, France, [2]Metab Biochem Lab, Necker Hosp, Paris, France, [3]Inserm U 1127, ICM, Paris, France, [4]Dpt Genetics, La Salpêtrière Hosp, Paris, France, [5]Lab Rare Dis, University of Bordeaux, Bordeaux, France

Background: *ALDH18A1* encodes delta-1-pyrroline-5-carboxylate synthase (P5CS), an enzyme that catalyzes the first and common step of proline and ornithine biosynthesis from glutamate. *ALDH18A1* mutations have been described 15 years ago in an autosomal recessive neurocutaneous syndrome comprising mental and growth retardation, cutis laxa, peripheral neuropathy and cataract.
Methods and results: Through exome sequencing, we report two families with autosomal recessive transmission of *ALDH18A1* mutations and predominant complex hereditary spastic paraplegia (HSP), marked cognitive impairment, but no cutaneous abnormality. More interestingly, we also identified monoallelic *ALDH18A1* mutations segregating in three independent families with autosomal dominant pure or complex HSP, as well as in two sporadic patients. Low levels of plasma ornithine, citrulline, arginine and proline in four individuals from two families suggested P5CS deficiency. In loading tests with ^{13}C labelled glutamine, P5CS flux was reduced in cultured fibroblasts from two affected subjects.
Discussion: Besides expanding the clinical spectrum of *ALDH18A1*-related pathology, we describe mutations segregating in an autosomal dominant pattern. We propose that the mechanism of pathology involves mitochondrial ornithine depletion. We suggest including plasma amino acid profiling as trait biomarkers in the work-up of HSP, particularly in dominant cases, as the associated phenotype is not distinct from other causative genes.

O-002

Does nitisinone in alkaptonuria alter non-metabolic outcomes? Experience from the United Kingdom National Alkaptonuria Centre

Hughes A T[1], Milan A M[1], Ranganath L[1]

[1]Dept Clin Biochem, LCL, RLBUHT, Liverpool, United Kingdom

Background: Nitisinone is used in treatment of alkaptonuria (AKU) at the National AKU Centre (NAC), Liverpool, UK. The resulting inhibition of p-hydroxyphenylpyruvate dioxygenase in the tyrosine degradation pathway results in decreased homogentisic acid (HGA) production. Nitisinone, so far, has not been shown to modify the evolution of AKU.
Patients and Methods: 38 AKU patients have attended the NAC, since 2012 (2 mg nitisinone daily). Eleven out of the 38 AKU patients also attended a non-interventional clinical study (2008-2011; mean follow-up of 36.8 months). AKU patients have completed one (n=26) and two (n=17) years of monitoring post-nitisinone with severity of AKU assessed by calculating the All AKUSSI scores allowing comparison pre- and post-nitisinone.
Results: **The** rate of change in AKUSSI was calculated and compared: these were 8±1.8 (pre-nitisinone), 1.2+1.4 (at one year), 1.3+1.0 (two years), with p values of < 0.001 only for the pre-nitisinone group. Plasma HGA showed a positive correlation with age (R 0.29; $p< 0.5$) and with All AKUSSI (R 0.44; $p< 0.002$) (Paired student's T test and Pearson's correlation coefficient).
Conclusion: The results are consistent with a decrease in evolution of AKU post-nitisinone therapy and this is the first data demonstrating a relationship between circulating HGA and the severity of AKU.

02. Novel diagnostic/laboratory methods

O-003

Combined analysis of plasma oxysterol and lyso-sphingomyelin, for Niemann-Pick types A, B and C diagnosis

Raymond K M[1], Turgeon C[1], Ory D[2], Lourenco C[3], Giugliani R[3], Rinaldo P[1], Gavrilov D[1], Oglesbee D[1], Tortorelli S[1], Matern D[1]

[1]Mayo Clinic College of Medicine, rochester, United States, [2]Diab. Cardiovasc. Dis. Ctr, WASHU, St. Louis, MO, United States, [3]Univerisity of São Paulo, Riberao Preto, SP, Brazil

Background: The diagnostic workup of Niemann-Pick type A (NPA), type B (NPB) or type C (NPC) diseases involves specialized biochemical testing often requiring fibroblast cultures or DNA testing that may uncover variants of uncertain significance. We present an effective and efficient method that simultaneously measures cholestane-3β,5α,6β-triol (COT), 7-ketocholesterol (7-KC) and lyso-sphingomyelin (LSM) in plasma as a simpler and less invasive alternative to current diagnostic approaches.
Methods: Acetonitrile containing d7-COT and d7-7-KC as internal standards is added to plasma. Following centrifugation the supernatant is diluted with water and analyzed by LC-MS/MS (10 min/sample).
Results: Pre-analytical and analytical factors were studied and met predetermined acceptance criteria for clinical testing. The assay was applied to plasma from 119 controls, 14 NPA/B, and 14 NPC patients and enabled clear differentiation of controls from NPC and NPA/B but not between NPA and NPB.
Conclusions: Combined analysis of COT, 7-KC and LSM in plasma represents a robust and useful approach to the simultaneous evaluation of NPA, NPB and NPC diseases. It is a less invasive, more efficient, and cost effective alternative to determination of acid sphingomyelinase in leukocytes or fibroblasts for NPA/B, or filipin staining and cholesterol esterification studies in fibroblasts for NPC.

O-004

Ultra rapid biochemical follow-up of therapy for metabolic disorders

De Sain-van der Velden M G M[1], Gerrits J[1], Jans J J[1], Van der Ham M[1], Prinsen H C M[1], Verhoeven-Duif N M[1]

[1]Dept Med Genet, Univ Med Center (UMC), Utrecht, Netherlands

Background: Multiple analytical platforms are used to biochemically monitor therapy of metabolic disorders. These methods are often time-consuming and relatively expensive.
Patients and Methods: For practical reasons we chose dried blood spot samples (DBS) and combined stable isotope dilution high resolution mass spectrometry (HR-MS) with chip-based nanoelectrospray ionization. Metabolites were extracted with methanol. Extracts were measured in positive and negative mode (mass range 70-600 m/z, resolution of 140.000). Using this method we investigated untreated and treated PKU (n=204), tyrosinemia type I (n=72), MCAD (n=60), VLCAD (n=20), PA (n=8), CMAMMA (n=10), MAT1A deficiency (n=10), and controls (n=37).
Results: Good correlations for all markers of therapy control: phenylalanine, tyrosine, methionine, C0, C2-, C3-, C6-, C8-, C10-, C10:1- and C14:1-carnitine, succinylacetone, malonic acid and methylcitrate were observed, when data are compared with the diverse LC-MS/MS methods.
Conclusion: Our data illustrate that HR-MS can simultaneously quantify a great diversity of metabolites extracted from DBS in one run. Our method has many advantages: it is patient-friendly, extremely fast (3 minutes run time and no manual peak integration), and efficient (many methods in one run), without loss of accuracy. The method has been validated and is used for therapy-control in our metabolic laboratory.

O-005

A novel defect in phophatidylinositol 4-kinase type IIα (*PI4K2A*) leads to a metabolic cutis laxa syndrome with choreathetosis

Mohamed M[1], Ngu L H[3], Balasubramaniam S[4], Waugh M[2], Morava E[2 5], Wevers R A[1]

[1]Radboud University Medical Center, Nijmegen, Netherlands, [2]University College London, London, United Kingdom, [3]Div of Med Genet,, Kuala Lumpur, Malaysia, [4]Metab Unit,Princess Margaret Childr Hosp, Subiaco, Australia, [5]Haywards Genet Cent, Tulane University, New Orleans, United States

Background: Cutis laxa, or old looking, sagging skin is a symptom of normal aging in healthy individuals. Interestingly, cutis laxa also occurs as a genetic condition of premature and generalized connective tissue aging, affecting various elastic components of the extracellular matrix. Several types of cutis laxa are inborn errors of metabolism and caused by cutis laxa genes related to metabolic pathways.
Patient and methods: In a patient with cutis laxa, a choreoathetoid movement disorder, dysmorphic features and

intellectual disability we performed whole exome sequencing to elucidate the underlying genetic defect causing this unique clinical phenotype.
Results: We identified homozygeous missense mutations in the phosphatidylinositol 4-kinase type IIα, encoded by *PI4K2A*. Enzyme activity of PI4KIIα in patient fibroblast were severely reduced and lipid mass spectrometry showed that particular molecular species of PI4P and PI(4,5)P2 were decreased.
Conclusions: Phosphoinositides (PI) lipids play a major role in intracellular signaling and trafficking and regulate the balance between proliferation and apoptosis through Akt activation. *PI4K2A* mediates the first step in the pathway synthesizing these PI lipids. Here we describe the first patient with mutations in *PI4K2A* illustrating the importance of this enzyme in the synthesis of PI lipid pools and neurodevelopment.

O-006

A multiplex assay for the diagnosis of mucopolysaccharidoses and mucolipidoses

Langereis E J[1], Wagemans T[1 2], Kulik W[2], Lefeber D J[3], Van Lenthe H[2], Oussoren E[4], Van der Ploeg A T[4], Ruijter G J[5], Wevers R A[3], Wijburg F A[1], Van Vlies N[1 2]

[1]Dept Pediatr Metab Dis, Acad Med Cent, Amsterdam, Netherlands, [2]Lab Gen Metab Dis, Academic Medical Cent, Amsterdam, Netherlands, [3]Transl Metab Lab, Radboud UMC, Nijmegen, Netherlands, [4]Dept of Peds, Erasmus MC, Rotterdam, Netherlands, [5]Dept Clin Genetics, Erasmus MC, Rotterdam, Netherlands

Background: Diagnosis of the MPSs generally relies on quantification of urinary GAGs by dimethylmethylene blue (DMB) assay, although false-negative results have been reported. We present a multiplexed screening test with a high sensitivity for all MPSs, with the potential to identify patients with MLII/MLIII.
Methods: Urine samples of 103 treatment naive MPS/ML patients were analyzed by DMB and a multiplex assay based on enzymatic digestion of heparan sulfate, dermatan sulfate and keratan sulfate and subsequent quantification by LC-MS/MS. Specificity was calculated by analyzing urine samples from a cohort of 39 patients in which screening for inborn errors of metabolism was performed.
Results: All 100 MPS patients were identified by pattern of elevated GAG-levels in the LC-MS/MS assay (sensitivity 100%). Specificity was 92%. DMB screening of the urine was in the normal range in 10 of the 100 patients (sensitivity 90%). Specificity was 97%. All three patients with MLII/MLIII had elevated GAGs in the LC-MS/MS assay while the DMB test was normal in 2.
Conclusion: This multiplex assay provides a robust and sensitive assay for the diagnosis of the complete spectrum of MPSs and has the potential to identify MPS related disorders such as MLII/MLIII. Its performance is superior to that of the conventional DMB assay.

O-007

A rapid and simple method for the diagnosis of Niemann Pick C and other disorders affecting cholanoid metabolism from blood spots

Mazzacuva F[4], Mills P B[4], Mills K[4], Nicoli R[1], Taylor-Te Vruchte D[1], Maekawa M[2], Porter F D[3], Platt F[1], Clayton P T[4]

[1]Dept of Pharmacology, Univ of Oxford, Oxford, United Kingdom, [2]Dept of Pharm Sciences, Tohoku Univ Hosp, Tohoku, Japan, [3]National Institutes of Health, Bethesda, Bethesda, United States, [4]UCL Institute of Child Health, London, United Kingdom

Background: Niemann-Pick C (NPC) is a progressive neurodegenerative disorder often associated with liver disease in early life. However diagnosis can be challenging due to a heterogeneous clinical presentation and there is a need for a simple, rapid, robust test. Previously it has been shown that a patient with NPC had an abnormal bile acid profile.
Objectives: Can bile acid analysis diagnose NPC patients?
Methods: Analysis of plasma/blood spot cholanoids was performed using liquid chromatography tandem mass spectrometry. Bile acids were semiquantitated using MRM based fragmentation patterns: neutral loss of 221 for N-acetylglucosamine conjugates, product ion scan of m/z 74, 80 and 97 for glycine, taurine and sulphated conjugates, respectively.
Results: An unusual bile acid, shown to be diagnostic for NPC, was identified as 3β-hydroxy-7β-N-acetylglucosaminyl-5-cholenoic acid (NPC-BA1) (by comparison with reference compound). Blood spot concentrations of NPC-BA1 were approximately 10-fold higher in NPC1 patients than in controls whilst patients with Smith-Lemli-Opitz syndrome had mildly elevated concentrations. Other abnormal cholanoids in plasma/blood spots were readily detected in patients with inborn errors of bile acid synthesis.
Conclusion: Cholanoid analysis is rapid, simple and capable of high sample throughput with the potential for diagnosis of NPC, SLO, 3β-HSDH and other disorders of cholanoid metabolism.

04. Dietetics and nutrition

O-008

Triheptanoin dramatically improves paroxysmal movement disorders in GLUT1 deficient patients

Mochel F[1], Hainque E[2], Gras D[3], Adanyeguh I M[1], Caillet S[4], Heron B[5], Roubertie A[6], Kaphan E[7], Rinaldi D[1], Ottolenghi C[8], Servais L[9], Roze E[2]

[1]Neurometabolic unit, La Salpêtrière Hosp, Paris, France, [2]Dpt Neurology, La Salpêtrière Hosp, Paris, France, [3]Dpt Neuropediatrics, Robert-Debré Hosp, Paris, France, [4]Dpt Dietetics, La Salpêtrière Hosp, Paris, France, [5]Dpt Neuropediatrics, Trousseau Hosp, Paris, France, [6]Dpt Neuropediatrics, Gui de Chauliac Hospt, Montpellier, France, [7]Dpt Neurology, La Timone Hosp, Paris, France, [8]Metab Biochem Lab, Necker Hosp, Paris, France, [9]Institute Myology, La Salpêtrière Hosp, Paris, France

Objectives: Based on our work with triheptanoin, a compound providing key substrates to the Krebs cycle, we wished to assess its therapeutic effect in patients with glucose transporter type 1 deficiency syndrome (GLUT1-DS).
Methods: We performed an open-label pilot study with three phases of 2 months each (baseline, treatment and withdrawal) in eight GLUT1-DS patients (7-47 years old) with non-epileptic paroxysmal manifestations. We used a patient diary to record motor and non-motor paroxysmal events. Functional ^{31}P-NMR spectroscopy (f-MRS) was performed to measure the levels of phosphocreatine (PCr) and inorganic phosphate (Pi) within the occipital cortex before (rest), during (activation) and after a visual stimulus (recovery).
Results: GLUT1-DS patients experienced a mean of 30.8 (± 27.7) paroxysmal manifestations (52% dystonic events) during baseline that dropped to a mean of 2.8 (± 2.9) paroxysmal manifestations (76% dystonic events) when treated ($p= 0.028$). During withdrawal, patients experienced a mean of 24.2 (± 21.9) paroxysmal manifestations (52% dystonic events) ($p= 0.043$). Consistently, triheptanoin led to the normalization of patients' brain energy profile, i.e. increased Pi/PCr ratio during brain activation compared to recovery ($p= 0.021$), reflecting increased ATP synthesis.
Conclusions: Treatment with triheptanoin resulted in a significant clinical improvement and normalized brain bioenergetics profile in GLUT1-DS patients.
Conflict of Interest declared.

O-009

Intravenous administration of a specific amino-acid mixture in children and adults with acute decompensation of maple syrup urine disease: a prospective observational study

Alili J M[1], Martin C[1], Amchin L[1], Langlet C[1], Cousseau R[4], Ramdani F[4], Henry H[5], Berleur M P[1], Grisel C[3], Guemann A S[3], Servais A[2], Abi-Wardé M T[3], Arnoux J B[3], De Lonlay P[3], Husson M C[1]

[1]Pôle EPHP, AGEPS AP-HP, Paris, France, [2]Dept of Nephrology, CHU Necker AP-HP, Paris, France, [3]Metab Dis Ref Center, CHU Necker, AP-HP, Paris, France, [4]Dept of Pharmacy, CHU Necker, AP-HP, Paris, France, [5]Dept of Pharmacy, CHRU Lille, Lille, France

Background: Patients with acute decompensation of maple syrup urine disease (MSUD-AD) are usually treated with amino-acid mixtures without leucine, valine and isoleucine by oral or nasogastric route. Because enteral intake is not always possible (coma, vomiting or patient refusal), we developed an amino-acid mixture for parenteral use and we evaluated its efficacy and safety.
Methods: In this prospective observational study from March 2010 to April 2015, 21 patients (13 males; 8 females) with 100 MSUD-AD (30 in children;70 in adults) were treated with the parenteral amino-acid mixture in 5 French hospital centers.
Results: At presentation, the mean leucine plasma concentration was 847.3 [38.2 - 3488.3] μmol/L in children and 855.0 [206.1 - 2060.9] μmol/L in adults. Patients were treated with parenteral amino-acid mixture at 1.0 to 2.0 g/kg/day, children during 4.4 [1 - 7] days and adults during 3.8 [1 - 8] days. At discharge, 86% (n=19/22) of children and 98% (n=65/66) of adults had a normalized leucine concentration. Extracorporal filtration was used in 4 children. No side effects were observed. Mean duration of hospitalization was significantly different in children and adults: 6.7 [1 - 13] days and 5.0 [2 - 27] days, respectively (p=0.02).
Conclusion: As an early emergency treatment of MSUD-AD, the amino-acid parenteral mixture appeared effective and safe, providing an alternative to nasogastric route.

O-010

Oral galactose supplementation in PGM1-CDG: as sweet as it sounds?

Gadomski T E[1], Wong S[1], Honzik T[2], Misfeld A[1], Koch D[3], Brocke K[5], Sykut-Cegielska J[4], Bowling F[6], Kozicz T[7], Lefeber D J[3], Morava E[1]

[1]Tulane University Medical School, New Orleans, United States, [2]Charles University in Prague, Dep Peds, Prague, Czech Republic, [3]Radboudumc, Dep. of Neurology, Nijmegen, Netherlands, [4]The Children's Memorial Health Institute, Warsaw, Poland, [5]Helse Stavanger, Overlege, Norway, [6]Royal Children's Hospital, Brisbane, Australia, [7]Radboudumc Dep. of Anatomy, Nijmegen, Netherlands

Background: Phosphoglucomutase 1 deficiency is a new CDG with a multi-system phenotype. The PGM1 enzyme is essential for glycosylation, glucose and galactose metabolism. We report on the first prospective multicenter observational study in 10 patients with PGM1 deficiency receiving dietary galactose supplementation.
Methods: Participants in our pilot study received oral galactose supplementation over 18 weeks. D-galactose intake started at 0.5 g/kg per day, increasing to 1.0 g/kg per day after 6 weeks and to 1.5 g/kg per day after 12 weeks. Maximal daily dose of galactose was 50.0 g, an amount that is within the recommended daily intake.
Results: Observed clinical changes included improved transaminases values, coagulation, thrombolytic and endocrine status. Liver function and coagulation abnormalities restored within weeks on galactose supplementation whereas endocrine changes required longer treatment-periods. No rhabdomyolytic episodes and no progression in cardiac dysfunction occurred on therapy. Creatine kinase and glucose levels remained variable.
Conclusions: Our study confirmed safety and significant beneficial effects of galactose supplementation in patients with PGM1-CDG. A mean daily oral intake of 0.5 g/kg was sufficient to maintain 80-100% of normal laboratory values within the first 18 weeks on supplementation. Intra- and inter-individual compliance remained variable. Controlling hypoglycemia and muscle dysfunction remained challenging.

O-011

Uncooked cornstarch (UCCS) doses in glycogen storage disease type 1a and 1b (GSD1a and GSD1b) from a single centre

Wells R[1], Skeath R[1], Davidson J[1], Abulhoul L[1], Cleary M A[1], Gissen P[1], Grunewald S[1], Dixon M[1]

[1]Great Ormond St Hosp for Child NHS FT, London, United Kingdom

Background: UCCS is an effective treatment that prolongs fasting time in GSD1. Recent publications focus on length of action of UCCS. GSD1 guidelines recommend 1.5-2g/kg/dose of UCCS, given 4-6 hourly. It is important to evaluate methods used to calculate UCCS dosing to avoid over or under treatment.
Objectives: To review UCCS doses (household cornflour) and controlled fasting studies in children with GSD1a/b and determine best practice to calculate UCCS dose.
Methods: A retrospective review of patient case records: GSD1a n=10 and GSD1b n=3. Data was collected on anthropometry, UCCS dose and controlled fasting tolerance studies at single time points within age-bands: 2 < 4y, 4 < 8y, 8 < 12y, 12 < 16y. UCCS dose was calculated as g/kg for actual and ideal (weight corrected to height centile) bodyweight.
Results: Most recent BMI centiles; normal n=3, overweight n=2, obese n=8. Median UCCS dose/kg reduced with age: 1.6 g/kg at 2< 4y, to 0.7 g/kg at 12 < 16y; corrected for ideal weight 2.2 g/kg at 2 < 4y to 1.1 g/kg at 12 < 16y. Fasting tolerance (blood glucose ≥ 3.5mmol/L) increased with age (median 2.5 hours at 2 < 4y, 3.5 at 12 < 16y).
Conclusion: As children with GSD1 are commonly overweight or obese, we recommend UCCS dose/kg be calculated on ideal rather than actual body weight, and monitoring by controlled fasting studies.

O-012

Glycomacropeptide: can we safely advocate its use in children with PKU?

Daly A[1], Evans S[1], Chahal S[1], MacDonald A[1]

[1]Birmingham Children's Hospital, Birmingham, United Kingdom

Introduction: In PKU, glycomacropeptide (GMP), a whey-based peptide may be an effective replacement for phe-free L-amino acid supplements (L-AA) but it contains phenylalanine potentially affecting blood phenylalanine control.
Objectives: A longitudinal, 6 month evaluation of GMP [60% GMP; containing 30 mg phenylalanine/20 g protein equivalent (PE)] vs L-AA (Cg) in 15 children with PKU [median age 11y range (6 -16 y), 8 boys].
Methods: 9 subjects took GMP (GMPg), and 6 took L-AA only. The dose of GMP was adjusted according to blood phenylalanine control; if blood phenylalanine control consistently exceeded target levels, some L-AA was reintroduced. Weekly fasting phenylalanine/tyrosine blood spots were collected. Median annual phenylalanine/tyrosine concentrations were calculated from the previous year.
Results: Both GMPg and L-AAg had a median total PE intake of 60 g; but GMPg took 40g PE from GMP (with 20g PE added from L-AA); L-AAg had 60 g PE from L-AA. In the GMPg, all subjects had higher median phenylalanine concentrations, [pre GMP 270 μmol/L post 390 μmol/L (p=0.004)],

and tyrosine decreased [pre GMP 50 μmol/L post 40 μmol/L (p=0.02)]. L-AAg phenylalanine/tyrosine concentrations remained unchanged.
Conclusions: Phenylalanine/tyrosine control deteriorated when GMP replaced L-AA in PKU children. GMP could only partly replace L-AA supplements due to its adverse impact on phe control.
Conflict of Interest declared.

O-013

Nutritional management of infants with Lysosomal Acid Lipase (LAL) deficiency (Wolman Disease)

White F J[1 6], Gallagher J[1 6], Boyle F[3], Breen C[1], Broomfield A[1], Cawtherley S[1 6], Della Casa R[5], Fecarotta S[5], Ghosh A[1], Holmes V[1 6], Hughes J[3], Jameson E[1], Morris A[1], Parenti G[5], Robertson L[1 6], Schwahn B[1], Stonestreet K[2], Strisciuglio P[5], Swancott A[7], Vara R[2], Walker R[2], Zuppaldi C[5], Jones S A[1], Rojas-Caro S[4], Friedman M[4]

[1]Centre for Genomic Med, St Mary's Hosp, Manchester, United Kingdom, [2]Evelina Children's Hospital, London, United Kingdom, [3]National Centre for IMD, Child Uni Hosp, Dublin, Ireland, [4]Synageva BioPharma Corp, Lexington MA, United States, [5]Paediatrics, Federico II University, Milan, Italy, [6]Dept Dietetics, Royal Man Child Hospital, Manchester, United Kingdom, [7]Dept Dietetics, St George's Hospital, London, United Kingdom

LAL deficiency results in lysosomal accumulation of cholesterol esters and triglycerides. In infants it is a medical emergency with rapid disease progression, usually fatal within the first 6 months of life.
We report our centre's experience with the nutritional management of six infants with rapidly progressive LAL deficiency. All were participants in clinical trials of enzyme replacement therapy (ERT) with Sebelipase alpha (Synageva BioPharma). Presenting symptoms included marked abdominal distension, gross hepatosplenomegaly, diarrhoea, vomiting and marked failure to thrive. At diagnosis nutrition was provided by breast milk (3), normal infant formula (1), amino acid formula (2).
Dietary management is an important component of the overall management of these infants. Our approach is restriction and modification of fat intake using whole protein low fat formula with medium chain triglycerides (MCT) (Monogen®), low fat amino acid based modular feed with MCT or fat modified total parenteral nutrition depending upon individual needs.
Of our cases one was managed with Monogen® alone. Five required modified TPN due to severe malabsorption and poor weight gain. 3 of the 5 were weaned off TPN onto a modular feed with 2 children subsequently tolerating Monogen®.
Five children survive (age 1 – 3years) and have normal anthropometry for age.
Conflict of Interest declared.

05. New metabolic disease groups

O-014

Leigh disease and the valine pathway

Pitt J J[1], Peters H[11], Ferdinandusse S[2], Ruiter J[2], Wanders R J A[2], Yaplito-Lee J[11], Kok F[3], Boy R[12], Korman S H[4], Fitzsimons P E[5], Crushell E[5], Hughes J[5], Yamaguchi S[6], Goto Y[7], Wakamatsu N[9], Yokochi K[8], Yamada K[9], Chen B C[10], Ngu L H[10]

[1]Murdoch Childrens Research Institute, Melbourne, Australia, [2]Academic Medical Centre, Amsterdam, Netherlands, [3]Mendelics Genomic Analysis, Sao Paulo, Brazil, [4]Shaare Zedek Medical Center, Jerusalem, Israel, [5]Temple Street Children's Univ Hosp, Dublin, Ireland, [6]Dep of Ped, Shimane Univ, Shimane, Japan, [7]National Center for Neur and Physch, Tokyo, Japan, [8]Seirei-Mikatahara General Hospital, Shizuoka, Japan, [9]Aichi Human Service Center, Aichi, Japan, [10]Kuala Lumpur Hospital, Kuala Lumpur, Malaysia, [11]Dept of Metabolic Medicine, RCH, Melbourne, Australia, [12]Rio de Janeiro State University, Rio de Janeiro, Brazil

Background: Two disorders affecting valine metabolism have recently been associated with Leigh syndrome (LS): 3-hydroxyisobutyryl-CoA hydrolase deficiency (*HIBCH* gene, OMIM 250620) and short-chain enoyl-CoA hydratase deficiency (*ECHS1* gene), described in 2014. At least 20 *ECHS1* affected families have now been identified, mainly by WES. A wide phenotypic spectrum is emerging in these disorders, ranging from lethality in the first months of life to adult patients who may not fulfil all criteria for LS. Both disorders have biochemical and clinical features in common including secondary deficiencies of respiratory chain enzymes and PDHc, which may confound the diagnosis. Relevant metabolites are not targeted during routine metabolic screening.
Results: We have identified abnormal metabolites derived from methacrylyl-CoA and acrylyl-CoA, highly reactive intermediates in the valine pathway, which appear to be involved in the development of LS. Urine 2,3-dihydroxy-2-methylbutyric, N-acetyl-S-(2-carboxypropyl)cysteine and the

carnitine ester of S-(2-carboxypropyl)cysteine are specific and sensitive biomarkers for both disorders, even in mild cases.
Conclusion: Measurement of 3-hydroxyisobutyryl-carnitine can distinguish between *ECHS1* and *HIBCH* defects and the metabolite levels correlate with clinical severity. These findings highlight the importance of glutathione detoxification, suggesting potential treatments.

06. Phenylketonuria: general

O-015

Effect of *PAH* variants on interallelic complementation in PKU patients

Shen N[1], Schmidt K[1], Thiel C[1], Hoffmann G F[2], Blau N[1]

[1]D. Hoppe Matab Ctr, Univ Child Hosp, Heidelberg, Germany, [2]Dept Pediatr, Univ Child Hosp, Heidelberg, Germany

Background: In phenylketonuria (PKU) patients, the combination of two phenylalanine hydroxylase (*PAH*) alleles determines residual enzyme activity *in vivo*. Inconsistencies in genotype-phenotype correlations have been observed in compound heterozygous patients and a particular combination of two *PAH* alleles may produce phenotype different from expected, due to interallelic complementation.
Methods: A dual eukaryotic vector system was used with two distinct PAH proteins N-terminally fused to different epitope tags. Transient co-expression of two distinct *PAH* variants was processed in COS-7 cells. PAH activity was measured by LC-MSMS, and expression levels of PAH were monitored by western blot. Genotypes were compared with phenotypes from the BIOPKU database (www.biopku.org).
Results: We demonstrate by expression and co-expression of particular variant alleles that interallelic interaction can be both positive and negative. For example, out of >400 missense variants the co-expression p.[I65T;R261Q] (19.21% activity; predicted 44.30%) or p.[I65T;R408W] (14.65% v. 33.48%) are the examples for genotypes with the negative interallelic interaction. The co-expression of p.[E178G;Q232E] (50.54% v.32.36%) and p.[P284S;R408W] (51.65% v. 42.99%) are the examples of positive subunits interaction.
Conclusion: The co-expression of two distinct alleles revealed possible dominance effects (positive or negative) by one of the mutations on residual activity as result of interallelic complementation.

07. Phenylketonuria: treatment, BH4

O-016

Evaluation of long-term safety and efficacy with rAvPAL-PEG for control of blood phenylalanine levels in adults with phenylketonuria (PKU).

Longo N[3], Thomas J A[2], Wasserstein M[6], Burton B K[5], Vockley J[8], Grange D K[7], Hillman R[9], Harding C[10], Dimmock D[15], Shur N[11], Adams D[1], Rizzo W B[12], Whitley C[16], Goodin K M[13], Decker C[14], Bolt K[14], Chen Y[14], Schweighardt B[14], Zori R[4]

[1]Goryeb Childrens Hospital, Morristown, United States, [2]University of Colorado, Aurora, United States, [3]University of Utah, Salt Lake City, United States, [4]University of Florida, Gainesville, United States, [5]Lurie Childrens Hospital, Chicago, United States, [6]Mount Sinai Hospital, New York, United States, [7]St Louis Childrens Hospital, St Louis, United States, [8]Childrens Hospital Pittsburgh, Pittsburgh, United States, [9]University of Missouri, Columbia, United States, [10]Oregon Health Sciences, Portland, United States, [11]Albany Medical College, Albany, United States, [12]Nebraska Medical Center, Omaha, United States, [13]Weisskopf Child Evaluation Center, Louisville, United States, [14]BioMarin Pharmaceutical Inc, Novato, United States, [15]Medical College of Wisconsin, Milwaukee, United States, [16]University of Minnesota, Minneapolis, United States

Background: Phenylketonuria (PKU) is caused by a deficiency of phenylalanine hydroxylase. PEGylated recombinant *Anabaena variabilis* phenylalanine ammonia lyase (rAvPAL-PEG) is a potential enzyme substitution therapy to treat PKU.
Methods: Sixty-seven subjects entered the PAL-003 Phase 2 extension study with 11-24 (mean: 17) weeks of previous rAvPAL-PEG exposure. Dosing was adjusted to maintain blood phenylalanine between 60-600 μmol/L; average weekly dose was 186 ± 157 mg/week (ranging from 1-7 doses/week).
Results: Pre-exposure baseline mean phenylalanine was 1337 ± 327 μmol/L. PAL-003 baseline mean Phe was 1052 ± 545 μmol/L. After 1 year on PAL-003 mean Phe decreased 65% from pre-exposure baseline to 437 ± 452 μmol/L (N=56); this decrease was generally sustained up to 4 years. Mean time to achieving 2 consecutive Phe levels below 600 μmol/L was 26 ± 22 weeks. Long-term treatment was well tolerated. The most common adverse events were headache, injection site reaction, and arthralgia in 58%, 54% and 46% of subjects, respectively. All subjects developed a sustained antibody response against PAL and most developed a transient antibody response against PEG.

Conclusions: Subjects treated with rAvPAL-PEG experienced a substantial and persistent decrease in mean blood phenylalanine. Long-term treatment was well tolerated, with most subjects experiencing mild-to-moderate adverse events.
Conflict of Interest declared.

08. Sulphur amino acid disorders

O-017

Adenosine kinase deficiency: expanding the clinical spectrum and evaluating therapeutic options

Staufner C[1], Lindner M[2], Dionisi-Vici C[3], Freisinger P[4], Dobbelaere D[5], Douillard C[5], Makhseed N[6], Harting I[7], Straub B K[8], Kahrizi K[9], Ballhausen D[10], La Marca G[11], Kölker S[1], Haas D[1], Hoffmann G F[1], Grünert S C[12], Blom H J[12]

[1]Div Neuroped Metab Med, Univ Child Hosp, Heidelberg, Germany, [2]Dep of Neurology, Univ Child Hosp, Frankfurt, Germany, [3]Div of Metab, Bambino Gesù Child Hosp, Rome, Italy, [4]Child Hosp Reutlingen, Reutlingen, Germany, [5]Univ Child Hosp Jeanne de Flandre, Lille Cedex, France, [6]Dep of Ped, Jahra Hospital, Jahra, Kuwait, [7]Dep of Neuroradiology, Univ Hosp, Heidelberg, Germany, [8]Inst of Pathology, Univ Hosp, Heidelberg, Germany, [9]Genetics Res Center, Univ Soc W and R Sc, Tehran, Iran, Islamic Republic of, [10]Cent for molecular diseases, CHUV, Lausanne, Switzerland, [11]NBS,NeuroFarba Dep Meyer Child Univ Hosp, Florence, Italy, [12]Cent for Ped and Adol Med, Univ Hosp, Freiburg, Germany

Background: Adenosine kinase deficiency (ADK deficiency) is a recently described defect at the cross-road of methionine and adenosine metabolism comprising mainly hepatic and neurological impairment and dysmorphism.
Patients and Methods: Clinical and biochemical data of 11 additional patients from 8 families with ADK deficiency were gathered through a retrospective questionnaire. 18 MRI scans of 8 patients and 2 liver biopsies of 1 patient were systematically evaluated.
Results: The main clinical symptoms are mild to severe liver dysfunction with neonatal onset, muscular hypotonia, global developmental retardation and dysmorphism (especially frontal bossing). Most patients have epilepsy and recurrent hypoglycemia due to hyperinsulinism. Major biochemical findings are intermittent hypermethioninemia, increased S-adenosylmethionine and S-adenosylhomocysteine in plasma and increased adenosine in urine. MRIs revealed mostly patterns of initially delayed but ultimately completed brain myelination but brain atrophy. Therapeutic trials with a methionine restricted diet indicate a beneficial effect on biochemical and clinical parameters in 4 patients and hyperinsulinism was responsive to diazoxide in 2 patients.
Conclusion: ADK deficiency generally causes CNS and liver symptoms, but also recurrent hypoglycemia. The clinical phenotype varies from an exclusively neurological to a multi-organ manifestation. Methionine-restricted diet should be considered as therapeutic option.

09. Other amino acid disorders

O-018

Correction of maple syrup urine disease (MSUD) and phenylketonuria (PKU) by transplantation of human amnion epithelial cells

Gramignoli R[1], Skvorak K J[2], Kannisto K[1], Srinivasan R C[1], Vosough M[1], Strom S[1]

[1]Karolinska Institute, stockholm, Sweden, [2]University of Pittsburgh, Pittsburgh, United States

Background: Our group moved hepatocyte transplants to the clinic to treat liver disease, but severe shortage in human hepatocyte limits this technology. We previously reported that human amnion epithelial cells (hAEC) express characteristics of pluripotent stem cells, without expressing telomerase, being immortal, and not tumorigenic. Once transplanted in murine livers, hAEC express human liver genes at levels observed in adult liver.
Objectives: We describe the efficacy of hAEC in correcting 2 mouse models of metabolic liver disease (MSUD and PKU).
Methods: Newborn mice with PKU or MSUD received direct hepatic/splenic injections of hAEC in the early neonatal period. Blood and tissues were analyzed.
Results: MSUD: Over 80% of hAEC-treated animals survived with normal weight gain and behavior, with significantly improvement or normalization of neurotransmitters and of branched chain amino acids levels in serum and brain; while controls died prior day 35th. PKU: hAEC transplants corrected serum (>60%) and brain (>80%) Phe levels in PKU mice.
Conclusions: Many of the amino acid and neurotransmitter abnormalities observed in metabolic liver disease are corrected by hAEC transplants. We are beginning cGMP isolation and banking of hAEC at our Institute for cellular therapy of liver disease in the clinic.

O-019

Mutations in *SLC25A22* as a cause of hyperprolinaemia, epilepsy and developmental delay in children

Reid E S[1], GOSgene C[1], Anderson G[2], Benatti M[2], Footitt E J[2], Cleary M A[2], Mills P B[1], Gissen P[1 2], Clayton P T[1]

[1]UCL Institute of Child Health, London, United Kingdom, [2]Great Ormond Street Hospital, London, United Kingdom

Background: Mutations in *SLC25A22* are known to cause neonatal epileptic encephalopathy and migrating partial seizures in infancy. No biochemical abnormalities have been documented in any of the nine patients described previously.
Case reports: Patient 1 presented in the first month of life with tonic seizures, hypotonia and severe global developmental delay. Patient 2 presented with developmental delay and eye abnormalities at three years of age. Patient 3 presented in the first month of life with myoclonic and tonic-clonic seizures, hypotonia, developmental delay and eye abnormalities. EEG and MRI findings were concordant with previously reported cases. Plasma amino acid analysis showed elevated proline in two patients (P1: 368; P3: 437 – 1195; ref: 85 – 290 μmol/L). Electron microscopy demonstrated widespread fibroblast vacuolation in all patients.
Methods and Results: Whole exome sequencing revealed novel homozygous mutations in *SLC25A22* in all patients. Histochemical staining of patient fibroblasts demonstrated an accumulation of lipids.
Conclusion: Mutations in *SLC25A22* should be considered in children with early-onset epilepsy, developmental delay, hyperprolinaemia and vacuolated fibroblasts. Increased cytosolic lipid synthesis could indicate build-up of cytosolic NADPH and this, alongside hyperprolinaemia suggests impairment of the proline/ pyrroline-5-carboxylate (P5C) shuttle; perhaps *SLC25A22* is a mitochondrial transporter of P5C/ glutamate-γ-semialdehyde as well as glutamate.

O-020

A case of tyrosinemia type I without succinylacetone elevation in a family with early onset liver cirrhosis and hepatocellular carcinoma

Gavrilov D[2], Blackburn P[2], Hickey R[2], Oglesbee D[2], Al-Qabandi W[1], Grompe M[3], El-Youssef M[2], Clark K[2], Roberts L[2], Klee E[2], Ekker S[2]

[1]University of Kuwait, Kuwait City, Kuwait, [2]Mayo Clinic, Rochester, MN, United States, [3]Oregon Science & Health University, Portland, OR, United States

Background: Tyrosinemia type I (TYR I) is autosomal recessive disorder caused by deficiency of fumarylacetoacetase (FAH) and presenting with progressive liver and renal disease. The diagnosis is based on detection of succinylacetone (SUAC). We describe two siblings diagnosed with hepatocellular carcinoma (HCC) secondary to liver cirrhosis. Laboratory testing did not reveal obvious causes for cirrhosis, including negative SUAC.
Methods: Whole exome sequencing performed on the surviving affected sibling and unaffected mother and older brother identified novel mutation in *FAH*. A Sleeping Beauty transposon expression system was used to functionally test if this mutation could rescue the liver phenotype in *Fah* knockout mice.
Results: A homozygous c.424A>G (p.Arg142Gly) mutation in *FAH* was identified in the proband and a younger brother, who also has cirrhosis. The mutant form of FAH could not rescue the *Fah* defect in knockout mice. This novel mutation involves the catalytic pocket of the enzyme, but does not result in increased blood or urine SUAC/tyrosine.
Conclusions: We describe a novel *FAH* mutation that produces clinical phenotype without the usual diagnostic biochemical abnormalities, particularly SUAC. This atypical phenotype suggests that not all cases of TYR I can be identified by traditional biochemical testing, including newborn screening.

O-021

Asparagine synthetase deficiency due to an unexpected molecular mechanism

Faoucher M[1], Poulat A L[2], DesPortes V[2], Pons L[1], Labalme A[1], Lamy R[1], Beche C[1], Till M[1], Vianey-Saban C[3], Lesca G[1], Acquaviva C[3], Sanlaville D[1]

[1]Service de Génétique, GHE,HFME,CHU Lyon, Bron, France, [2]Neuropédiatrie,GHE,HFME,CHU Lyon, Bron, France, [3]Maladies métaboliques,CBPE,CHU Lyon, Bron, France

Background: Asparagine synthetase, encoded by *ASNS* gene, catalyzes the synthesis of asparagine from glutamine and aspartate. Asparagine synthetase deficiency, secondary to mutations in *ASNS* gene, was recently described as a rare autosomal recessive disorder characterized by a severe congenital microcephaly and progressive epileptic encephalopathy.
Case report & Results: A young girl born to a non-consanguineous family presented with epileptic encephalopathy and severe microcephaly (-6DS). Array CGH identified a deletion of 11 Kb at position 7q21.3 in the *ASNS* gene. According to the phenotype and to the slight decrease of asparagine in CSF and plasma, *ASNS* sequencing was performed and found a hemizygous mutation in exon 3

(c.144C>A). It was predicted to change a highly conserved histidine residue, located in a functional domain of the protein, to a glutamine. This mutation was absent from databases of control individuals and in silico predictions were in favor of a deleterious effect.
Conclusions: We conclude that the compound status of the patient (deletion/mutation) explains the patient neurological phenotype. We report a new pathogenic mutation in *ASNS* and a new molecular mechanism with a compound heterozygous genotype responsible for asparagine synthetase deficiency. This case confirms the severe neurological outcome of patients with recessive mutations in *ASNS* gene.

10. Urea cycle disorders

O-022

Non-invasive "biochemical biopsy" of the liver: determination of OTC activity in plasma

Kozich V[1], Krijt J[1], Sokolova J[1], Jesina P[1], Chroma L[1]

[1]Inst IEM, Charles Univ-1st Faculty Med, Prague, Czech Republic

Background: We have shown previously that the predominantly hepatic enzyme cystathionine beta-synthase (CBS) is present in plasma and that LC-MS/MS based assay may be used to diagnose CBS deficiency. In this report we explored whether this approach may be utilized also for the non-invasive enzymatic diagnosis of ornithine transcarbamylase (OTC) deficiency.
Methods: Catalytic activity of OTC in plasma was determined using custom purified deuterium labelled substrate citrulline. Labelled ornithine produced in the OTC reverse reaction was quantified by LC-MS/MS using chloroformate derivatization kit.
Results: In control plasma samples (n=10) the OTC activity was detectable (median 888 nmol/L/hour, range 501-2760). Compared to the median of controls the plasma OTC activity was clearly decreased in 4 hemizygote males (median 2% with a range 1%-29%) and in 7 symptomatic heterozygote females (median 35% with a range 26%-49%), respectively. The OTC activity was slightly decreased in 3 asymptomatic heterozygote females (median 60% with a range 43%-70%).
Conclusion: Activity determination of enzymes released from the liver to plasma using sensitive LC-MS/MS assay appears to be a feasible, non-invasive and fast method to confirm the diagnosis of some inborn errors of metabolism including severe forms of OTC deficiency.
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O-023

Carbonic anhydrase VA deficiency: a not so rare cause of neonatal hyperammonemia

Diez-Fernandez C[1], Rüfenacht V, Santra S, Lund A M[3], Santer R[4], Lindner M[5], De Lonlay P[6], Burlina A[7], Häberle J[1]

[1]Div Metab, Univ Child Hosp Zurich, Zurich, Switzerland, [2]Birmingham Child Hosp, Birmingham, United Kingdom, [3]Center Inherit Metab Dis, Dep Clin Genet, Copenhagen, Denmark, [4]Dep Pediatr, Uni Med Cent Eppendorf, Hamburg, Germany, [5]Uni Kinderklinik Frankfurt, Frankfurt, Germany, [6]Cent Inherit Metab Dis, Hosp Necker, Paris, France, [7]Uni Hosp, Padova, Italy

Background: Carbonic anhydrase VA (CAVA) deficiency, recently described in four patients, was suggested as a novel differential diagnosis for patients with neonatal hyperammonemia. The disease presents an almost unique combination of biochemical findings including hyperammonemia, elevated lactate and ketone bodies, metabolic acidosis, hypoglycemia, and excretion of carboxylase substrates and related metabolites.
Methods: The *CA5A* gene was sequenced in a cohort of 96 patients with neonatal or early infantile hyperammonemia, who had no mutations in the *NAGS* and *CPS1* gene. We developed an expression system using insect cells to study the recombinant wild-type CAVA, all known mutations (published and novel), and three polymorphisms.
Results: In 9/96 patients, mutations (missense/splicing/deletions) in the *CA5A* gene were identified on both alleles. Demonstrating decreased enzyme activity or thermal stability, all mutations were proven to be disease-causing. In patients treated with N-carbamylglutamate, plasma ammonia concentrations rapidly went down. Overall, the follow-up course was mild, necessitating no treatment or N-carbamylglutamate only.
Conclusions: CAVA deficiency is a novel differential diagnosis of neonatal hyperammonemia that seems to be more common than rare urea cycle disorders. The disease exhibits an almost unique combination of biochemical findings. Early identification may allow specific treatment of hyperammonemia in this condition.

O-024

L-aspartate as potential treatment for citrullinemia type 1 patients

Diez-Fernandez C[1], Wellauer O[1], Rüfenacht V[1], Fingerhut R[1], Häberle J[1]

[1]Div Metab, Univ Child Hosp Zurich, Zurich, Switzerland

Background: Citrullinemia type 1 is an autosomal recessive urea cycle disorder caused by mutations in the *ASS1* gene. Of the 64 missense mutations reported, nine affect substrate (aspartate or citrulline)-binding residues, and thus, are potential kinetic mutations whose decreased activities could be rescued by L-aspartate.
Methods: We used an *E. coli* expression system to study all known potentially kinetic *ASS1* mutations plus the most common mutation p.G390R, proven to be inactive. All mutations plus the wild-type enzyme were nickel-affinity purified, their activity and kinetic parameters were measured using tandem mass spectrometry and their thermal stability using Differential Scanning Fluorimetry.
Results: All ten mutants could be expressed and purified. Eight mutants were totally inactive while two exhibited decreased affinity for aspartate and citrulline. In mutant p.A118T the activity could be rescued to ~80% of the wild-type with doses up to 5 mM of L-aspartate.
Conclusions: The activity of *ASS1* kinetic mutations with some residual activity seems to be rescued by L-aspartate administration. Since L-aspartate is already available as a drug, such treatment could be directly implemented for treating certain citrullinemia type 1 patients. However, to better prove this hypothesis, further studies with not so devastating kinetic mutations should be done.

O-025

Towards non-viral minicircle-DNA vector-directed liver gene therapy for ornithine transcarbamylase deficiency (OTCD)

Viecelli H M[1 4], Deplazes S[1 4], Schlegel A[2], Cunningham S[3], Alexander I[3], Häberle J[1 4], Thöny B[1 4]

[1]Div Metab, Univ Child Hosp Zurich, Zurich, Switzerland, [2]Dept Sugery, Univ Hosp Zurich, Zurich, Switzerland, [3]Child Med Res Institute, Westmead, Australia, [4]Child Res Center, Univ Child Hosp Zurich, Zurich, Switzerland

Background: Naked-DNA minicircle (MC) vectors for gene therapy have a better safety profile than viral vectors as they lack any viral or bacterial sequences.
Objectives & Methods: Here we aim at therapy of ornithine transcarbamylase deficiency (OTCD) in the *spf-ash* murine model. Our MC vectors, which have no size limitation, contain an expression cassette with a short (0.3 kb) synthetic liver-specific promoter or the natural/endogenous *Otc* promoter with a length of up to 2 kb, the murine *Otc*-cDNA or the codon-optimized murine *Otc* with or without truncated 5'-intron, and a polyA signal. Naked DNA-MC vectors were delivered to mouse liver by hydrodynamic tail vein or portal vein injection.
Results: Liver extracts of OTCD mice after tail vein injection showed an up to 100 times increased OTC enzyme activity. However, gene expression was mainly found at the perivenous area with only little positive cells in the periportal area where the urea cycle is located. Hydrodynamic injection of MC-vectors via portal vein resulted in elevated transgene expression but at a much lower level than through tail vein injection.
Conclusions: In summary, MCs have the potential to treat OTCD if delivered via the portal vein but further improvements towards higher transgene-expression and delivery efficacy are required.

11. Organic acidurias: branched-chain

O-026

miRNAs as potential biomarkers and key players in the pathophysiology of propionic acidemia

Rivera-Barahona A[1], Fulgencio A[1], Pérez-Cerdá C[2], Perez B[1], Richard E[1], Desviat L R[1]

[1]Centro Biología Molecular Severo Ochoa, Madrid, Spain, [2]CEDEM-UAM, Madrid, Spain

Background: Propionic acidemia (PA) results from a deficiency of mitochondrial propionyl-CoA carboxylase enzyme, leading to a toxic intracellular accumulation of propionyl-CoA and derived metabolites.
Methods & Results: Using a qRT-PCR panel analyzing 752 mouse miRNAs we have found 14 significantly deregulated miRNAs in the liver of two months old *Pcca*$^{-/-}$ (p.A138T) mice, a hypomorphic model of PA. Among them, miR-34a-5p, miR-338-3p and miR-350 were initially selected as candidates to play a role in the disease due to the involvement of their targets in apoptosis, mitochondrial function, oxidative stress response and in cardiac and neurological dysfunction, hallmarks of the disease. The levels of these miRNAs were also found increased in the brain and heart of *Pcca*$^{-/-}$ (A138T) mice at different ages, with a concomitant decrease in BCL2 and p38 proteins, specific targets of miR-34a-5p and miR-350, respectively. Parallel studies of miRNA expression profiling are being conducted in PA patients' plasma samples.
Conclusions: The data generated will allow us to establish correlations and to validate the results obtained in the murine model. Taken together, our results highlight

the possible involvement of miRNAs and their targets in the pathophysiology of PA, paving the way for their future use as biomarkers or development of targeted therapies.

O-027

Long-term N-carbamylglutamate may stabilize metabolic control in patients with propionic and methylmalonic aciduria

Del Rizzo M[1], Furlan F[1], Celato A[1], Giordano L[1], Fasan I[1], Polo G[1], Burlina A B[1]

[1]Div Metab Dis, Dep Ped, Padua Univ Hosp, Padua, Italy

Background & Objectives:We aimed to evaluate the effect of long-term N-carbamylglutamate in patients with propionic aciduria (PA) or methylmalonic aciduria (MMA) who had frequent episodes of metabolic decompensation.
Patients & Methods: Patients with PA or MMA (mut 0 form) who had >5 episodes of metabolic decompensation/year requiring hospitalization in the last 2 years, failure to thrive and poor appetite received N-carbamylglutamate 40 mg/kg/day. The number of metabolic decompensation episodes, weight gain, ammonia levels and propionylcarnitine levels were evaluated pre- and post-treatment.
Results: Six patients with PA (n=4) or MMA (n=2) aged 3-20 years were enrolled in the study. Treatment duration was 2-10 months. The number of episodes of metabolic decompensation decreased after treatment initiation. Ammonia levels were 115-193 μmol/L pre-treatment, decreasing to 17-77 μmol/L post-treatment. Propionylcarnitine levels were similar before and after treatment (24.7-120.2 vs. 31.7-57.2 μmol/L). Patients gained 0.5-4.5 kg in weight over the treatment period. Parents reported improvements in attention, school activity, and appetite, and reduced need for gastrostomy in the patients enrolled.
Discussion/Conclusion: We report for the first time use of long-term N-carbamylglutamate as an adjunct to conventional management, and demonstrate its efficacy in stabilizing metabolic control in patients with severe PA or MMA.

O-028

Pathophysiology of kidney disease in methylmalonic aciduria (MMA)

Schumann A[1 2 3 4], Luciani A[1 2], Hall A[5], Baumgartner M R[2 3 4], Devuyst O[1 2]

[1]Inst Physiol Univ Zurich, Zurich, Switzerland, [2]Zur Cen Integr Hum Physiol, Univ Zurich, Zurich, Switzerland, [3]Rare Dis Init Zurich, Univ Zurich, Zurich, Switzerland, [4]Div Metab Child Res Cen, Univ Child Hosp, Zurich, Switzerland, [5]Inst Anat, Univ Zurich, Zurich, Switzerland

Background: MMA, caused by mutations in the mitochondrial enzyme methylmalonyl-CoA mutase (MCM) or the synthesis of its cofactor adenosylcobalamin, leads to chronic kidney disease (CKD) and end-stage renal failure as a classical long-term complication. The pathophysiology of CKD in MMA is unknown.
Methods: We investigated renal cells obtained from the urine of MMA patients and controls by RT-qPCR, immunoblot analysis, confocal and electron microscopy to study subcellular effects caused by MMA.
Results: Control and patient cells expressed kidney specific tubular markers and MCM activity was not detectable in patient cells. Analysis revealed fragmented, swollen mitochondria with reduced mitochondrial membrane potential in patient cells accompanied by evidence for increased oxidative stress. A marked reduction of TOM20 indicated a loss of mitochondrial mass. These changes were paralleled by elevated levels of p62 and enlarged numbers of LC3 positive vesicles, indicating activation of the autophagy machinery, while PINK1 and parkin, the key players of mitophagy, were reduced. These findings could be reversed by treatment with different compounds.
Conclusion: Patient cells show altered mitochondrial morphology and function. An imbalance of the mito-/autophagy machinery leads to a loss of mitochondrial mass in patient cells and hints at the importance of mitochondrial homeostasis in MMA.

12. Organic acidurias: others

O-029

Combined malonic and methylmalonic aciduria due to *ACSF3* deficiency (CMAMMA) is probably a benign condition

Levtova A[1], Waters P J[2], Buhas D[4 6], Levesque S[2], Auray-Blais C[2], Clarke J T R[2], Laframboise R[3], Maranda B[2 3], Mitchell G A[1], Brunel-Guitton C[1], Braverman N E[4 5 6]

[1]Div Med Gen, Dept Peds, CHU Ste-Justine, Montreal, Canada, [2]Dept Peds, Sherbrooke Univ Hosp Centre, Sherbrooke, Canada, [3]Dept Peds, Laval Univ Hosp Centre, Quebec, Canada, [4]Dept Human Genet, McGill Univ, Montreal, Canada, [5]Mtl Childr Hosp Res Inst, McGill Univ,

Montreal, Canada, [6]Dept Med Gen, Montreal Children's Hospit, Montreal, Canada

Background: Combined malonic and methylmalonic aciduria due to deficiency of *ACSF3* (CMAMMA) is a recently characterized condition whose clinical significance remains controversial. While a variety of clinical manifestations have been described in a group of patients most of whom had been diagnosed following investigation for a clinically suspected inborn error of metabolism, we have previously characterized individuals who had come to clinical attention through the Quebec Provincial Urine Newborn Screening Program and who were asymptomatic.
Methods & Results: In a multicenter natural history study, we describe twenty-three patients with CMAM MA (age range 3 months to 29 years) who had come to clinical attention following the finding of elevated methylmalonic acid levels by the Quebec Provincial Urine Newborn Screening Program. Our cohort of patients, while predominantly French-Canadian, includes four patients of diverse ethnicity, and is characterized by greater methylmalonic than malonic acid elevations and by a benign clinical course.
Conclusion: Because our cohort of patients was identified through a population urine newborn screening program, it is uniquely free of selection bias. The favourable clinical course in our patients suggests that CMAMMA is probably a benign condition, although we cannot exclude the possibility that it may predispose to later-onset disease.

O-030

Development of neuropsychological functions in patients with glutaric aciduria type I

Boy N[1], Heringer J[1], Haege G[1], Glahn E[1], Hoffmann G F[1], Garbade S F[2], Kölker S[1], Burgard P[1]

[1]Div Neuroped Metab Med, Univ Child Hosp, Heidelberg, Germany, [2]Faculty Appl Psych,SRH Univ Appl Science, Heidelberg, Germany

Background: Glutaric aciduria type I (GA-I) is an inherited metabolic disease due to deficiency of glutaryl-CoA dehydrogenase. Cognitive functions have not been studied systematically.
Methods: Thirty patients (ages 5-28 years) were compared with 196 healthy controls (5-28 years) for simple reaction time (SRT), continuous performance (CP), visual working memory (VWM), visual-motor coordination (Tracking) and visual search (VS). Dystonia (n=13 patients) was categorized using the Barry-Albright-Dystonia Scale (BADS). Development of cognitive performance was analysed using a negative exponential function model.
Results: BADS scores correlated with speed tests but not with tests measuring stability or higher cognitive functions. Developmental functions of GA-I patients significantly differed from controls for SRT and VS but not for VWM, and showed trends for CP and Tracking. Asymptomatic patients did not differ from controls, except significantly better results in Tracking. Across all age groups, data of patients and controls fitted well to the model of negative exponential development.
Conclusions: Dystonic patients predominantly showed motor speed impairment, whereas performance improved with higher cognitive load. Patients without dystonia did not differ from controls. Developmental functions were similar in patients and controls. Cognitive functions seem to be preserved in GA-I, even in patients with striatal degeneration.

13. Carbohydrate disorders

O-031

Transketolase (TKT) deficiency, a novel disease in the non-oxidative part of the pentose phosphate pathway, causing short stature, developmental delay, and congenital heart defects

Wamelink M M C[1], Salomons G S[1], Roos B[1], Boyle L[2], Wynn J[2], Chung W K[2 3]

[1]Metab Unit, Dept Clin Chem, VU Univ Hosp, Amsterdam, Netherlands, [2]Dept of Pediatrics, Columbia Univ, New York, United States, [3]Dept of Medicine, Columbia Univ, New York, United States

Background: Transketolase (TK) is a reversible, thiamine-dependent enzyme in the pentose phosphate pathway (PPP).
Patients and methods: In two sisters currently ages 8 and 5 years with proportional short stature, developmental delay, and congenital heart disease whole exome sequencing was performed and presumed pathogenic mutations in *TKT* were identified. Sugars and polyols in urine and plasma were measured as well as enzymatic activity of TK in lymphoblasts.
Results: Two novel compound heterozygous variants in *TKT* were detected in both siblings. The parents were each carriers of one of the variants. TK activity in lymphoblasts showed a low level of residual activity in both siblings. Elevated urinary excretion of erythritol, arabitol, ribitol,

and pent(ul)ose-phosphates were detected as well as elevated erythritol, arabitol and ribitol in plasma.
Discussion/conclusion: We describe a novel disease in the non-oxidative part of the PPP (TK deficiency). TK catalyses the transfer of a two carbon unit from xylulose-5-phosphate to ribose-5-phosphate or erythrose-4-phosphate. TK is necessary for NADPH synthesis and nucleic acid synthesis and may explain the problems with growth and developmental delay in our patients. These findings warrant polyol testing in urine in patients with short stature, developmental delay, and congenital heart disease of unknown etiology.

O-032

Crystal structure of human galactose-1-phosphate uridylyltransferase (GALT): the molecular basis of classical galactosemia

McCorvie T J[1], Kopec J[1], Pey A L[2], Yue W W[1]

[1]SGC, University of Oxford, Oxford, United Kingdom, [2]Dept Phy Chem, Univ Granada, Granada, Spain

Background: Galactose-1-phosphate uridylyltransferase (GALT) is an essential enzyme in the Leloir pathway of galactose metabolism where it reversibly transfers a uridine monophosphate (UMP) group between galactose-1-phosphate and uridine diphosphate glucose, to generate glucose-1-phosphate. Mutations of the *GALT* gene lead to *type I or classical galactosemia*,withthe most prevalent allele*p.Q188R* known to abolish GALT activity completely.
Methods: To provide a molecular understanding of enzyme mechanism and impact of disease mutations,we determined the 1.8 Å structure of human GALT, in covalent complex with UMP.
Results: The human GALT structure, first for any eukaryotic orthologues, adopts dimer architecture similar to bacterialGALTs (~46% sequence identity), but differs in the nature and stoichiometry of bound divalent metal ions which play a role in the enzyme thermal and kinetic stability. The human structure also provides a molecular template to understand the role of Gln188 within the active site, and rationalizes missense mutations associated with type I galactosemia into molecular defects of stability (p.E291K), catalytic activity (p.C180P), metal binding (p.H319Q, p.H321Y) and ligand binding (p.L74P, p.N97S).
Conclusion: Together our structural and biophysical data establish a starting point for further characterization of four prevalent disease mutations (p.S136L, p.Q188R, p.K285N, and p.D314N) aimed at identifying potential therapy interventions.

14. Disorders of fatty acid oxidation and ketone body metabolism

O-033

Severe neonatal muscular multiple acyl-CoA dehydrogenase-like deficiency due to a novel deletion in *FLAD1* gene, encoding FAD synthetase

Veauville-Merllié A[1], Pichard S[3], Schiff M[3], Rigal O[2], Imbard A[2], Vianey-Saban C[1], Acquaviva C[1]

[1]Service Mal Héréd Métab, CBPE, Lyon, France, [2]Lab Bioch Horm, Hop R Debré, Paris, France, [3]Center Mal Héréd Métab, Hop R Debre, Paris, France

Background: Multiple acyl-CoA dehydrogenase deficiency (MADD) is due to mutations in genes encoding electron transfer flavoprotein dehydrogenase (*ETFDH*) or electron transfer flavoprotein (*ETFA* and *ETFB*), two FAD-dependent proteins. Recently, a disease-causing variation in FAD synthetase gene (*FLAD1*) was reported in two siblings with riboflavin responsive MADD and severe neonatal cardiomyopathy. We present the clinical, biochemical and genetic characterization of a novel patient suffering from MADD-like secondary to *FLAD1* mutation.
Case report: A girl, born from consanguineous Turkish parents, presented with severe neonatal hypotonia and sudden respiratory deterioration and death at 4 months of age. Metabolic investigations (plasma acylcarnitine profile and urine organic acids) were suggestive of MADD, however no mutation was identified in *ETFA*, *ETFB* or *ETFDH* genes. Genes involved in riboflavin metabolism were studied by next-generation sequencing. A novel homozygous small deletion, c.397_400delTTCT (p.Phe134Cysfs*8), was detected in *FLAD1* gene, leading to a premature stop codon in the protein, probably responsible for defective FAD synthetase activity.
Discussion: Riboflavin metabolism abnormalities should be considered in patients with MADD or MADD-like biochemical profile, justifying urgent riboflavin therapeutic trial. It emphasizes the usefulness of targeted next-generation sequencing approach, which allows studying simultaneously all genes involved in MADD and MADD-like patients.

15. Disorders of pyruvate metabolism and the Krebs cycle

O-034

Encephalopathy, combined deficiency of alpha-ketoacid dehydrogenases and hyperglycinemia associated with *LIPT2* mutations: a novel lipoic acid biosynthesis defect

Habarou F[1 2 3], Hamel Y[1 4], Grisel C[1], Boutron A[5], Delahodde A[6], Gazah R[1], Brassier A[1], Arnoux J B[1], Pontoizeau C[1 2], Chadefaux-Vekemans B[1 2 3], Barouki R[1 2 3], Rötig A[7], Ottolenghi C[1 2 3], De Lonlay P[1 4]

[1]IEM Reference Center, Necker Hosp, APHP, Paris, France, [2]Metab Biochemistry, Necker Hosp, APHP, Paris, France, [3]INSERM U1124, Paris Descartes Univ, Paris, France, [4]INSERM U1163, Paris Descartes Univ, Paris, France, [5]Biochemistry, Bicetre Hosp, APHP, Paris, France, [6]Genet Microbio Institute, Paris Sud Univ, Orsay, France, [7]INSERM U781, Paris Descartes Univ, Paris, France

Background: Lipoic acid (LA) is a cofactor of alpha-ketoacid dehydrogenases (PDH, 2-KGDH, BCKDH) and glycine cleavage system (GCS). *De novo* LA mitochondrial synthesis occurs through the sequential actions of octanoyl-ACP, LIPT2, LIAS (an iron-sulfur cluster enzyme) and LIPT1. Defects for LIPT1 and LIAS led to neurological involvement, pulmonary hypertension, cardiomyopathy, and multiple biochemical abnormalities including hyperlactatemia and, in LIAS deficiency, hyperglycinemia.
Results: We identified mutations involving the *LIPT2* gene (c.89T>C ; c.377T>G) by exome sequencing in a 8-year-old boy with encephalopathy, axial hypotonia and spasticity. Brain MRI suggested mitochondrial respiratory chain (RC) deficiency but RC activities were normal in liver and muscle. Blood and CSF lactate and pyruvate concentrations were normal. Plasma aminoacid chromatography found moderately increased glycine, and decreased branched-chain aminoacids. Patient fibroblasts revealed impaired leucine metabolism and severely decreased PDH activity (144 pmol/min/mg; N: 1158-3109 pmol/min/mg). Yeast *lip2* deletion strain did not respond to LA supplementation.
Conclusions: *LIPT2* mutations are a new cause of lipoic acid biosynthesis defects leading to encephalopathy and may help to better understand this pathway, notably the differential lipoylation of GCS vs. the other dehydrogenases. The mild biochemical abnormalities highlight the difficulties of diagnosing LA synthesis defects.

O-035

Exploring stabilizing small molecules as a potential chaperone therapy for pyruvate dehydrogenase complex deficiency

Pavlů-Pereira H[1], Florindo C[1], Silva M J[1], Tavares de Almeida I[1], Leandro P[1], Rivera I[1], Vicente J B[1 2]

[1]Met§Gen, iMed.ULisboa, Fac Pharm Univ, Lisbon, Portugal, [2]ITQB, Univ Nova Lisboa, Oeiras, Portugal

Objectives: We report advances in studying the molecular basis of clinically relevant missense variants identified in pyruvate dehydrogenase complex (PDHc) deficient patients. PDHc deficiency is one of the most common neurodegenerative diseases related to alterations in mitochondrial energy metabolism. Current treatments are limited and poorly efficient. Near 80% of the disease-causing mutations affect the α-subunit of the heterotetrameric E1 enzyme (αα'ββ').
Material & Methods: Since the vast majority of the mutations are missense, inducing decreased stability and/or folding efficiency, we undertook the structural and functional characterization of several E1α variant proteins (p.F205L, p.R253G, p.R378C, p.R378H), and the evaluation of the potential effect of arginine and other small molecular weight (SMW) compounds as protein stabilizers and putative rescuers of enzyme function. Using a bicistronic prokaryotic system, we produced the recombinant $E1\alpha_{wt}/E1\alpha_{mut}$ α-subunits and $E1\alpha_{wt}b_{wt}/E1\alpha_{mut}b_{wt}$ E1 enzyme. Purified variant proteins were characterized at biochemical and biophysical levels. E1 enzyme activity was determined by spectrophotometry, oligomerization profiling was achieved by native gel electrophoresis and conformational stability was evaluated by differential scanning fluorimetry.
Conclusion: We observed a clear beneficial effect of several SMW compounds upon functional and structural properties of most recombinant E1 variants, thus opening new avenues for exploring this new therapeutic approach.
PEstOE/SAU/UI4013/13FCT;SFRH/BD9172/12

16. Mitochondrial disorders: nuclear encoded

O-036

Mutation in mitochondrial ribosomal protein S7 (*MRPS7*) causes congenital sensorineural deafness, progressive hepatic and renal failure and lactic acidemia

Menezes M J[1 4], Guo Y[6], Zhang J[7 14], Riley L G[1 4], Cooper S T[2 4], Thorburn D R[8 9], Li J[7], Dong D[10], Li Z[10], Glessner J[6], Davis R L[11], Sue C M[11], Alexander S I[5], Arbuckle S[3], Kirwan P[13], Keating B J[6 12], Xu X[14], Hakonarson H[6 12], Christodoulou J[1 4 5]

[1]Genetic Metabolic Disorders Research, Sydney, Australia, [2]Institute for Neuroscience and Muscle, Sydney, Australia, [3]Histopathology Department, CHW, Sydney, Australia, [4]Discipline of Paediatrics & Child Health, Sydney, Australia, [5]Discipline of Genetic Medicine, Sydney, Australia, [6]The Center for Applied Genomics, CHOP, Philadelphia, United States, [7]T-Life Research Center, Fudan Uni, Shanghai,

China, [8]Murdoch Children's Research Institute, Melbourne, Australia, [9]Department of Paediatrics, Melbourne, Australia, [10]Department of Chemistry & Biochemistry, Philadelphia, United States, [11]Department of Neurogenetics, Kolling, Syndey, Australia, [12]Department of Pediatrics, Philadelphia, United States, [13]Electron Microscopy Unit, Syndey, Australia, [14]BGI-Shenzhen, Shenzhen, Shenzhen, China

Background: Functional defects of the mitochondrial translation machinery, as a result of mutations in nuclear-encoded genes, have been associated with combined oxidative phosphorylation deficiencies.
Case Report: We report siblings with congenital sensorineural deafness and lactic acidemia in association with combined respiratory chain (RC) deficiencies in fibroblasts and liver. One of the siblings had a more severe phenotype showing progressive hepatic and renal failure.
Methods & Results: Whole exome sequencing revealed a homozygous mutation in the gene encoding mitochondrial ribosomal protein S7 (*MRPS7*), a c.550A>G transition that encodes a substitution of valine for a highly conserved methionine (p.Met184Val) in both affected siblings. MRPS7 is a 12S ribosomal RNA-binding subunit and is required for the assembly of the small ribosomal subunit. Pulse labeling of mitochondrial protein synthesis products revealed impaired mitochondrial protein synthesis in patient fibroblasts. Exogenous expression of wild-type *MRPS7* in patient fibroblasts rescued RC deficiencies, demonstrating the deleterious effect of the mutation on RC function. Reduced 12S rRNA transcript levels observed in the patient's fibroblasts was also restored to normal levels by wild-type *MRPS7*.
Conclusion: Our data demonstrate the pathogenicity of the identified *MRPS7* mutation as a novel cause of mitochondrial RC dysfunction, congenital sensorineural deafness and progressive hepatic and renal failure

O-037

Successful liver transplant in ethylmalonic encephalopathy: a new therapeutic approach for an otherwise fatal disease

Diodato D[1 2], Dionisi-Vici C[1], Torre G[3], Picca S[4], Pariante R[6], Picardo S[6], Zeviani M[7], Tiranti V[7], De Ville de Goyet J[5]

[1]Metab Div, Child Hosp Bambino Gesù, Rome, Italy, [2]Neuromusc Div, Child Hosp Bambino Gesù, Rome, Italy, [3]Hepat div, Child Hosp Bambino Gesù, Rome, Italy, [4]Nephrol Div, Child Hosp Bambino Gesù, Rome, Italy, [5]Liver Transpl, Child Hosp Bambino Gesù, Rome, Italy, [6]Int Care, Child Hosp Bambino Gesù, Rome, Italy, [7]Mol Neurogenetics, Besta Institute, Rome, Italy

Background: Ethylmalonic encephalopathy (EE) is a devastating disorder caused by mutations in *ETHE1*, encoding a mitochondrial sulphur dioxygenase (SDO) involved in hydrogen sulphide (H_2S) catabolism. Accumulation of H_2S and thiosulfate causes the characteristic clinical (rapidly progressive neurological failure, petechial purpura with infantile death) and biochemical (high lactate and C4-/C5-acylcarnitines, ethylmalonic aciduria) features. In EE-mice, AAV-*ETHE1*-gene transfer targeted to the liver markedly improved the clinical and metabolic picture. Based on this successful animal study, we reasoned that a similar result could be obtained in EE patients by liver transplantation (LT).
Case report: An 8-months old EE patient presenting a mild neurological picture with initial involvement of basal ganglia at brain MRI after approval of Ethic Committee, underwent LT having her mother as a living donor.
Results: Eight months after LT the baby showed clear improvement of motor milestones (GMFM score from 12 to 35), stabilization of brain MRI pattern along with normalization of plasma thiosulfate levels and marked reduction of urinary ethylmalonate.
Conclusions: LT is a therapeutic option, increasingly utilized in metabolic disorders to replace missing enzymatic function. Here we report successful LT in EE, providing the proof of principle that this therapeutic approach may clearly improve the disease course.

O-038

Signal transducer and activator of transcription 2 (STAT2) deficiency is a novel disorder of mitochondrial fission

Shahni R[1], Cale C M[2], Anderson G[2], Osellame L D[5], Hambleton S[4], Jacques T S[1 2], Wedatilake Y[1], Taanman J W[6], Chan E[1], Qasim W[1 2], Plagnol V[7], Chalasani A[8], Duchen M R[3], Gilmour K[2], Rahman S[1 2]

[1]UCL Institute of Child Health, London, United Kingdom, [2]Great Ormond Street Hospital, London, United Kingdom, [3]University College London, London, United Kingdom, [4]Newcastle University, Newcastle, United Kingdom, [5]La Trobe University, Melbourne, Australia, [6]UCL Institute of Neurology, London, United Kingdom, [7]UCL Genetics Institute, London, United Kingdom, [8]National Hospital for Neurology, London, United Kingdom

Background: Mitochondrial networks arise from a delicate balance of fusion and fission. Proteins involved in mitochondrial fusion and fission include mitofusins, optic atrophy 1, dynamin-related protein 1 (DRP1), receptor proteins

MiD49/MiD51, mitochondrial fission protein 1 and mitochondrial fission factor (MFF), but the exact cellular mechanisms remain unknown. Unregulated mitochondrial fission may occur in primary mitochondrial diseases (*DRP1* and *MFF* mutations) and neurodegenerative disorders (Alzheimer, Huntington and Parkinson diseases).
Patients and Methods: Whole exome sequencing aimed to identify the underlying defect in two siblings presenting severe neurological deterioration and long mitochondria following viral infection.
Results: Both patients shared a novel homozygous stop mutation (c.1836C>A; p.Cys612Ter) in *STAT2* (a gene involved in innate immunity), which segregated within the family. Both siblings, and a third STAT2 deficient patient, shared a cellular phenotype characterised by DRP1 inactivation leading to impaired mitochondrial fission. SiRNA-mediated *STAT2* knockdown in SHSY5Y cells recapitulated the fission defect, which was rescued in all three patient fibroblasts by lentiviral transduction with wild-type *STAT2*.
Conclusion: STAT2 is a novel regulator of mitochondrial fission. Our findings reveal important interactions between innate immunity and mitochondrial dynamics. Modulation of JAK-STAT activity represents a novel therapeutic target for mitochondrial diseases, the vast majority of which remain incurable.

O-039

A novel mitochondrial tRNA modification defect due to *QRSL1* mutations causes infantile mitochondrial disease

Shimura M[1], Murayama K[1], Fushimi T[1], Ichimoto K[1], Mori M[2], Kishita Y[3], Tokuzawa Y[3], Kohda M[3], Suzuki T[4], Okazaki Y[3], Ohtake A[5]

[1]Dep Metab, Chiba Child Hosp, Chiba, Japan, [2]Dep Pediatr, Matsudo City Hosp, Matsudo, Japan, [3]Res Center Genom Medicine, Saitama Med U, Saitama, Japan, [4]Dep Chem Biot, Grad Sch Engine, Univ Tokyo, Tokyo, Japan, [5]Dept Pediatr, Saitama Med Univ, Saitama, Japan

Background: Defects of tRNA modification (tRNA modopathy) are known as a cause of a variety of diseases. We present two cases of infantile mitochondrial disease with pathogenic mutations in *QRSL1* (hGatA) involved in Glu-tRNA^{Gln} amidotransferase (Glu-Adt), which is composed of three subunits, hGatC, hGatA and hGatB.
Patients and methods: A girl (Pt250) suddenly suffered from hypoglycemia and lactic acidosis on day 1. Subsequently tachypnea, hypertrophic cardiomyopathy, adrenal ?insufficiency and hearing loss appeared. She died of cardiac failure at 5 months. Another girl (Pt860) developed cyanosis and lactic acidosis on day 3. She died of interstitial pneumonia at 2 months. We performed enzyme analysis, whole exome sequencing and their validation tests.
Results: The enzyme analysis showed combined respiratory chain deficiencies (I, III, and IV) in both cases. The whole exome sequencing revealed Pt250 harbored a homozygous mutation and Pt860 harbored a compound heterozygous mutation in *QRSL1*. In vitro reconstitution of Gln-tRNA^{Gln} formation using recombinant hGatCAB showed strongly decreased transamidation activity.
Conclusions: Our study is the first to demonstrate that *QRSL1* mutations lead to a defect in the human mitochondrial translation and are a cause of severe infantile mitochondrial disease.

O-040

CLPB mutations cause 3-methylglutaconic aciduria, progressive brain atrophy, intellectual disability, congenital neutropenia, cataracts, movement disorder

Wortmann S B[1 2], Haack T B[3], Gersting S W[4], Muntau A C[5], Rodenburg R J[2], Rubio-Gozalbo M E[6], Distelmaier F[7], Karnebeek, van C[8], Ounap K[9], Yaplito-Lee J[10], Vaz F M[11], Pronicka E[12], Prokisch H[3], Katsanis N[13], Wevers R A[14]

[1]Dep of Ped, SALK, Salzburg, Austria, [2]Dep of Ped, RadboudUMC, Nijmegen, Netherlands, [3]Inst of Hum Gen, Tech Univ, Munich, Germany, [4]Dep of Mol Ped, Univ Child Hosp, LMU, Munich, Germany, [5]Dep of Ped, Univ Child Hosp, Hamburg, Germany, [6]Dep of Ped, Univ Child Hosp, Maastricht, Netherlands, [7]Dep of Ped, Univ Child Hosp, Dusseldorf, Germany, [8]Div of Bio Dis, Dep of Ped, Child Hosp, Vancouver, Canada, [9]Dep of Gen, United Lab, Univ Hosp, Tartu, Estonia, [10]Met Gen, Child Res Inst, Parkville, Australia, [11]Dep of Clin Chem and Ped, AMC, Amsterdam, Netherlands, [12]Dep of Ped, Univ Child Hosp, Warsaw, Poland, [13]Cent for Hum Dis Modeling, Duke, Durham, United States, [14]TML, Dep of Lab Med, RadboudUMC, Nijmegen, Netherlands

Patients: We studied a group of individuals with elevated urinary excretion of 3-methylglutaconic acid, neutropenia that can develop into leukemia, a neurological phenotype ranging from nonprogressive intellectual disability to a prenatal encephalopathy with progressive brain atrophy, movement disorder, cataract, and early death.
Methods & Results: Exome sequencing of two unrelated individuals and subsequent Sanger sequencing of 16 individuals with an overlapping phenotype identified a total of 14 mutations in *CLPB* in 14 individuals from 9 unrelated families. CLPB encodes caseinolytic peptidase B homolog ClpB, a

member of the AAAþ protein family. To evaluate the relevance of CLPB in the pathogenesis of this syndrome, we developed a zebrafish model and an in vitro assay to measure ATPase activity. Suppression of clpb in zebrafish embryos induced a central nervous system phenotype that was consistent with cerebellar and cerebral atrophy that could be rescued by wild-type, but not mutant, human CLPB mRNA. Consistent with these data, the loss-of function effect of one of the identified variants (c.1222A>G [p.Arg408Gly]) was supported further by in vitro evidence with the mutant peptides abolishing ATPase function.
Conclusion: Taken together, mutations in *CLPB* define a syndrome with intellectual disability, congenital neutropenia, progressive brain atrophy, movement disorder, cataract, and 3-methylglutaconic aciduria.

O-041

Mutations in *TRMT5* cause a defect in post-transcriptional modification of mitochondrial tRNA associated with multiple respiratory chain deficiencies

Kopajtich R[1 2], Powell C A[3], D'Souza A[3], Rorbach J[3], Husain R A[4], Dallabona C[5], Donnini C[5], Alston C L[6], Griffin H[7], Pyle A[7], Chinnery P F[7], Strom T M[1 2], Meitinger T[1 2 8], Rodenburg R J[9], Schottmann G[10], Schuelke M[10], Romain N[11], Haller R G[11], Ferrero I[5], Haack T B[1 2], Taylor R W[6], Prokisch H[1 2], Minczuk M[3]

[1]Inst Hum Genet, Helmholtz Center, Munich, Germany, [2]Inst Hum Genet, Technical University, Munich, Germany, [3]MRC Mitochondrial Biology Unit, Cambridge, United Kingdom, [4]Dept Neuroped, University Hospital, Jena, Germany, [5]Dept Life Science, University of Parma, Parma, Italy, [6]Inst Neuroscience, Newcastle University, Newcastle upon Tyne, United Kingdom, [7]Inst Genet Med, Newcastle University, Newcastle upon Tyne, United Kingdom, [8]DZHK, Munich Heart Alliance, Munich, Germany, [9]Cent Mito Dis, Nijmegen University, Nijmegen, Netherlands, [10]Dept Neuroped, Charité Berlin, Berlin, Germany, [11]Dept Neurol, Texas Hospital Dallas, Dallas, United States

Background: Deficiencies in respiratory chain complexes lead to a variety of clinical phenotypes resulting from inadequate energy production by the mitochondrial OXPHOS system. Defective expression of mtDNA-encoded genes, caused by mutations in either the mitochondrial or nuclear genome, represents a growing group of human disorders.
Methods & Results: By whole exome sequencing we identified two unrelated individuals carrying compound heterozygous variants in *TRMT5*, which encodes a mitochondrial protein with homology to members of the class I-like methyltransferase superfamily. Both individuals presented with lactic acidosis and multiple mitochondrial respiratory chain complex deficiencies in muscle, although the clinical presentation between the two affected subjects was different, one presenting in childhood with failure to thrive and hypertrophic cardiomyopathy and an adult with a history of life-long exercise intolerance. Mutations in *TRMT5* were associated with the hypomodification of G37 of mitochondrial tRNA, which was prominent in muscle. Deficiency of this modification was also detected in human cells subjected to *TRMT5* RNAi. The pathogenicity of the detected variants was confirmed in a heterologous yeast model and by rescue of the molecular phenotype following re-expression of *TRMT5* cDNA in patient fibroblasts.
Conclusions: Our study reports a new mitochondrial disorder, highlighting the importance of post-transcriptional modification of mitochondrial tRNAs for mitochondrial function.

O-042

COQ4 mutations cause a broad spectrum of mitochondrial disorders associated with CoQ_{10} deficiency

Kremer L[1], Brea-Calvo G[2], Haack T B[1], Karall D[3], Ohtake A[4], Invernizzi F[5], Carrozzo R[6], Dusi S[5], Zeviani M[7], Murayama K[8], Ghezzi D[5], Prokisch H[1]

[1]Inst of Hum Gen, TU Munich, München, Germany, [2]Cent Anda Biología, Uni Pablo de Olavide, Sevilla, Spain, [3]Dep of Ped, Paracelsus Med Uni, Salzburg, Austria, [4]Dep of Ped, Saitama Med Uni, Saitama, Japan, [5]Inst of Neuro "Carlo Besta", Milan, Italy, [6]Lab of Mol Med, Bamb Chil Hosp, Rome, Italy, [7]MRC Mit Bio Unit, Cambridge, United Kingdom, [8]Chiba Canc Cen, Chiba, Japan

Background: Primary Coenzyme Q10 (CoQ_{10}) deficiencies are rare, clinically heterogeneous disorders caused by recessive mutations in several genes encoding proteins involved in CoQ_{10} biosynthesis.
Methods & Results: By whole exome sequencing, we identified five individuals carrying biallelic mutations in *COQ4*. The precise function of human COQ4 is not known, but it seems to play a structural role in stabilizing a multiheteromeric complex, which contains most of CoQ_{10} biosynthetic enzymes. The clinical phenotypes of the five subjects varied widely, but four had a prenatal or perinatal onset with early fatal outcome. Two unrelated individuals presented with severe hypotonia, bradycardia, respiratory insufficiency and heart failure; two sisters showed antenatal cerebellar

hypoplasia, neonatal respiratory distress syndrome, and epileptic encephalopathy. The fifth subject had early-onset but slowly progressive clinical course, dominated by neurological deterioration with hardly any involvement of other organs. The pathogenic role of all identified mutations was experimentally validated in a recombinant yeast model: oxidative growth, strongly impaired in strains lacking COQ4, was corrected by expressing a human wild-type *COQ4* cDNA but failed to be corrected by expressing *COQ4* cDNAs with any of the nucleotide variants identified in affected subjects.
Conclusions: *COQ4* mutations are responsible for early-onset mitochondrial diseases with heterogeneous clinical presentations associated with CoQ_{10} deficiency.

O-043

Thioredoxin-2 deficiency impairs oxidative stress defense and leads to early-onset neurodegeneration with severe cerebellar atrophy

Holzerova E[1 2], Danhauser K[3], Haack T B[1 2], Kremer L[1 2], Ingold I[4], Kobayashi S[4], Conrad M[4], Meitinger T[1 2], Prokisch H[1 2], Distelmaier F[3]

[1]Institute of Human Genetics, TUM, Munich, Germany, [2]Institute of Human Genetics, HMGU, Neuherberg, Germany, [3]Dep of Gen Ped, Univ Child Hosp, Düsseldorf, Germany, [4]Ins of Developmental Genetics, HMGU, Neuherberg, Germany

Background: Thioredoxin 2 (TXN2) is a small mitochondrial redox protein, which is ubiquitously expressed with the highest expression levels in brain tissue. It is encoded by a nuclear gene, *TXN2*, containing a mitochondrial targeting sequence. TXN2 plays a crucial role in oxidative stress defense and in the regulation of the mitochondrial apoptotic pathway. Animal studies suggest that TXN2 is essential during embryonic development.
Methods & Resulst: Using exome sequencing in a 15-year-old adolescent suffering from an early onset neurodegenerative disorder with severe cerebellar atrophy, epilepsy, peripheral neuropathy, dystonia and combined respiratory chain deficiency in muscle tissue, we identified a homozygous stop mutation in *TXN2*. TXN2 protein was not detectable in patient fibroblasts, confirming the predicted loss of function. Cellular studies revealed increased reactive oxygen species (ROS) levels, impaired oxidative stress defence and secondary mitochondrial dysfunction with reduced cellular respiration and diminished ATP production. Re-expression of TXN2 restored all these parameters confirming the causal role of the mutation. Supplementations with antioxidants effectively suppressed cellular ROS production, and led to moderate clinical improvement during short-term follow-up of the patient.
Conclusions: Our report highlights the importance of TXN2 for neurodevelopment. Moreover, our results point to a potential role of antioxidant treatment in affected patients.

O-044

Clinical exome sequencing in 650 pediatric cases with mitochondrial disease

Haack T B[1 2], Kopajtich R[1], Ahting U[2], Wieland T[1], Graf E[1], Strom T M[1 2], Taylor R W[4], Karall D[6], Sperl W[3], Distelmaier F[5], Holzerova E[1], Kremer L[1], Hoffmann G F[7], Mayr J A[3], Freisinger P[8], Meitinger T[1 2], Prokisch H[1 2]

[1]Helmholtz Zentrum München, Munich, Germany, [2]Technische Universität München, Munich, Germany, [3]Paracelsus Medical University Salzburg, Salzburg, Austria, [4]Newcastle University, Newcastle, United Kingdom, [5]Universität Düsseldorf, Düsseldorf, Germany, [6]Universität Innsbruck, Innsbruck, Austria, [7]Universität Heidelberg, Heidelberg, Germany, [8]Klinikum Reutlingen, Reutlingen, Germany

Background: Defects of the mitochondrial pyruvate oxidation route comprise a clinically and genetically heterogeneous group of disorders. Exome sequencing has proven an efficient approach to genetically diagnose these patients and is today widely implemented at an early stage in the diagnostic algorithm.
Patients & Methods: In a prospective study over the course of one year, we addressed challenges associated with the diagnostic application of exome sequencing in a clinical context such as turnaround time, and reporting of (negative) results and incidental findings.
Results: Applying exome sequencing to 650 patients with suspected mitochondrial diseases we established firm molecular diagnoses in about half of them with mutations being identified in more than 100 different genes. Clinically relevant variants were in both known disease genes (≈25%) and genes previously not associated with mitochondrial dysfunction (≈25%) including recently published genes such as *COQ4*, *GTPBP3*, and *ECHS1*. Joint analyses of the unresolved cases lead to the identification of novel disease genes involved in tRNA-modification, oxidative stress response, and mitochondrial cofactor metabolism.
Conclusions: The implementation of bioinformatics tools allowing for the detection of CNVs and mitochondrial DNA variants further added to the diagnostic yield. Phenotype-based search algorithms facilitated the identification of (*de novo*) variants associated with reportedly dominant traits mimicking mitochondrial disease presentations.

21. Peroxisomal, sterol and bile acid disorders

O-045

The study of the crosstalk between peroxisomes and other cell organelles is an efficient tool for the diagnosis of peroxisomal disorders with atypical biochemical phenotype

Bravo-Garmendia M M B[1], Matalonga L[1], Diaz H[2], García-Cazorla A[2], Fernández-Marmiesse A[3], Armstrong J[2], Artuch R[4], Fons C[5], Rausell L[6], Vitoria I[6], Tort F[1], Girós M[1]

[1]Hosp Clínic; CIBERER-U737; IDIBAPS, Barcelona, Spain, [2]Dep Neuro, Synaptic Metab, H.SJD CIBERER, Barcelona, Spain, [3]Hosp Clínic Uni Santiago Compostela, Santiago de Compostela, Spain, [4]Mol Gen Med Service. Hosp SJD CIBERER, Barcelona, Spain, [5]Pedia Neuro dep. Hosp SJD CIBERER, Barcelona, Spain, [6]Unit Metabolopat. Hosp La Fe, Valencia., Valencia, Spain

Background: Diseases associated with the malfunction of the peroxisome, PEX deficiencies, generate cellular stress revealing the existence of a cross-talk between this and other cell organelles. The existence of unexpected biochemical phenotypes makes the diagnosis challenging.
Objectives: Our objective was to identify secondary cell organelle-altered patterns in hard to diagnose peroxisomal disorders.
Methods: Exome sequencing enabled the identification of mutations in *DNM1L* and *HSD17B4* in two previously undiagnosed individuals with normal very-long-chain fatty acids. Fibroblasts were analyzed by immunohistochemistry using different organelle specific antibodies: ALDP (peroxisome), PDI (endoplasmic reticulum-ER), GM130 (Golgi) and mitotraker (mitochondria). A PEX1 patient, presenting the typical biochemical phenotype, was used for comparison.
Results: Fibroblasts of *DNM1L* showed beads-on-a-string arranged peroxisomes. In contrast the *HSD17B4* patient cells had fewer and enlarged peroxisomes together with increased ER stress. Our data demonstrate that the combined immunohistochemical pattern of the above mentioned markers are able to distinguish between DNM1L, HSD17B4 and PEX1 deficiencies.
Discussion/Conclusion: The results obtained in our study provide a useful tool to achieve the proper diagnosis of patients with atypical biochemical phenotypes and give new insights into the protocol of metabolic diseases diagnosis, reinforcing the knowledge of the cross-talk between cell organelles in peroxisomal diseases.

O-046

Functional outcome of childhood cerebral adrenoleukodystrophy patients treated with hematopoietic stem cell therapy: a multi-institutional study

Paker A M[1], Aubourg P[4], Baruchel A[8], Fischer A[7], Kurtzberg J[6], Dalle J H[8], Gerard M[9], Escolar M L[5], Bezman L[3], Balser J[10], Paadre S[10], Orchard P[2], Raymond G V[2]

[1]Bluebird Bio Inc, Cambridge, United States, [2]University of Minnesota, Minneapolis, United States, [3]Kennedy Krieger Institution, Baltimore, United States, [4]Hospital Bicêtre-Paris Sud, Paris, France, [5]University of Pittsburgh, Pittsburgh, United States, [6]Duke University, Durham, United States, [7]Hôpital Necker-Enfants Malades, Paris, France, [8]Hôpital Robert Debré, Paris, France, [9]Hôpital de la Timone, Marseille, France, [10]Veristat, Boston, United States

Background: There is limited information about functional outcomes of childhood cerebral adrenoleukodystrophy (CCALD) patients
Methods: We conducted a retrospective study to characterize functional outcomes of untreated and hematopoietic-stem-cell transplanted (HCT) CCALD subjects. Data was collected on 136 cases (72 untreated / 65 HCT) until either 2-years post-diagnosis or death. A severe disability in a Neurological Functional Score (NFS) domain that was identified as critical for independent functioning was labeled as a major functional disability (MFD).
Results: In untreated subjects, the most common MFDs to present first were wheelchair-requirement (70%) and total-incontinence (67%). In subjects with advanced CCALD (NFS>20), total-incontinency, wheelchair-requirement, and loss-of-communication were present in all instances. Other MFDs, including no-voluntary-movement, requirement-for-tube-feeding, and cortical-blindness, were present in 93%, 93%, and 79% of instances, respectively. Although estimated 2-year overall survival rate for untreated subjects with contrast-enhancing-lesions (GdE+) was 64%, only 21% (90%CI: 7.5%, 42%) of subjects were MFD-free 2 years after their first GdE+ MRI. The estimated 2-year overall survival and MFD-free rates for HCT subjects were 82% and 56% respectively. The observed 2-year MFD-free survival of the early CCALD (NFS≤1 & Loes≤9) GdE+ HCT subjects was 81%.
Conclusions: MFDs are generally present in CCALD subjects during the course of progressive disease. HCT improved survival and maintained function of subjects with early CCALD.
Conflict of Interest declared.

O-047

The clinical and biochemical profile of five adult patients with cerebrotendinous xanthomatosis (CTX)

Faghfoury H[2 3], Blaser S[1 3], Semotok J[2 3]

[1]The Hospital for Sick Children, Toronto, Canada, [2]University Health Network, Toronto, Canada, [3]The University of Toronto, Toronto, Canada

Background: Cerebrotendinous xanthomatosis (CTX) is a lipid storage disorder caused by a deficiency of the enzyme sterol 27-hydroxylase leading to decreased primary bile acid synthesis and an increase in plasma cholestanol and urinary bile alcohols.
Case Report: Five male adult patients between the ages of 19-47 years were diagnosed in our centre between 2012-2014. All patients had multiple tendon xanthomas, 3/5 patients had significant neurologic changes, and no patients had findings of cataracts. All patients had biochemical profiles and radiologic findings that were consistent with their diagnosis of CTX. After diagnosis, chenodeoxycholic acid (CDCA) therapy was initiated at a dose of 250 mg PO tid. All patients had appropriate biochemical response to therapy with near or complete normalization of cholestanol and urinary bile alcohols. One patient developed severe GI disruption with diarrhea that was only alleviated with decreasing his CDCA dose to 250 mg PO bid.
Conclusion: While the current literature states that cataracts and neurologic changes are found in the majority of adult CTX patients, we report no patients with cataracts and only 3/5 patients currently suffering from significant neurologic impairment. We recommend patients with multiple tendon xanthomas in the absence of any other symptoms be tested for this treatable disease.

22. Lysosomal disorders: mucopolysaccharidoses, oligosaccharidoses

O-048

Clinical effect of intrathecal enzyme replacement therapy with investigational idursulfase-IT in children with Hunter syndrome

Muenzer J[1], Hendriksz C J[2], Stein M B[1], Fan Z[1], Kearney S[2], Horton J[2], Vijayaraghavan S[2], Santra S[2], Solanki G A[2], Pan L[3], Mascelli M A[3], Sciarappa K[3], Barbier A J[3]

[1]Univ North Carolina, Chapel Hill, NC, United States, [2]Birmingham Child Hosp, Birmingham, United Kingdom, [3]Shire, Lexington, MA, United States

Background: Hunter syndrome (MPSII), iduronate-2-sulfatase (I2S) deficiency, is associated with cognitive decline in the severe phenotype. Intravenous idursulfase is not expected to alter cognitive decline. An extension to a phase I/II trial evaluating the effects of I2S formulated for intrathecal administration (idursulfase-IT) is in progress (up to 6 years).
Patients and Methods: Sixteen cognitively impaired children with MPSII were initially enrolled. Four patients/dose group received either no treatment, 10, 30, or 1 mg idursulfase-IT monthly while continuing intravenous idursulfase. Patients continued into the extension study, receiving either 10 or 30 mg monthly idursulfase-IT. The longest time on treatment is 63 months and currently n=13. Drug safety, change in CSF glycosaminoglycan (GAG) concentrations and neurocognitive function are being assessed.
Results: Mean CSF GAG concentrations reduced by about 80-90% at month 6. Long-term follow up indicates that low CSF GAG values are maintained. Currently, there are 4/16 patients with testable long-term cognitive data. Although data shows large variability, all 4 appear to have experienced stabilization, or decreased rate of decline.
Conclusion: Encouraging signs of clinical efficacy were observed in the moderately affected patients in the 10- and 30-mg groups, warranting further development of idursulfase-IT as a treatment for cognitive impairment in MPSII.
Conflict of Interest declared.

O-049

Intrathecal alpha-L-iduronidase protects from or improves neurodevelopmental decline and neuroimaging abnormalities of children with MPS I below 6 years

Salehpour S[1], Alaei M R[1], Vakili R[2], Rezaei A R[1], Momtazmanesh N[1], Tonekaboni S H[1], Yasaei V R[1], Eshraghi P[2], Setavand S[1], Karimizadeh P[1], Khooshabi K[1], Tavakoli S[1], Sadr S[1], Houshmand M[3], Aryani O[3]

[1]Genomic Res Cent, Mofid Child Hosp, SBMU, Tehran, Iran, Islamic Republic of, [2]Div Metab Dis, Univ Child Hosp, MUMS, Mashad, Iran, Islamic Republic of, [3]National Inst Genetic Engineer & Biotech, Tehran, Iran, Islamic Republic of

Background & Objectives:Enzyme replacement therapy has proven useful in reducing non-neurological symptoms and pain in MPS I. On the other hand, abnormal physical characteristics, except for those affecting the skeleton and eyes, can be

improved, and neurologic degeneration can often be halted by stem cell transplantation. However it has high rates of morbidity and mortality, and takes time to be done. We decide to clarify if intrathecal alpha-L-iduronidase protects or improves mental developmental decline and neural imaging abnormalities of children with MPS I below 6 years.
Patients & Methods:In a multi-centre prospective, randomized controlled clinical trial, 8 genetically proven MPS I children below 6 years of old were treated either with combined intrathecal and intravenous or just intravenous alpha-L-iduronidase (control) weekly for 3 years. Denver developmental screening test and Bayley Scores of Infant Development -Version III were used to compare the development quotient and Magnetic Resonance Imaging (MRI) for neuro-imaging differences between groups.
Results:Combined therapy differed from control in significant protection or improvement both in development quotients and MRI findings ($p< 0.01$).
Conclusion: Intrathecal alpha-L-iduronidase can significantly protect or improve neurodevelopmental or neuro-imaging findings of children with MPS I below 6 years of age.

23. Lysosomal disorders: sphingolipidoses

O-050

Subjects treated with migalastat continue to demonstrate stable renal function and reduced left ventricular mass index over 3 years in a long-term extension study of Fabry disease

Germain D P[1], Bichet D G[2], Giugliani R[3], Hughes D[4], Schiffmann R[5], Wilcox W[6], Skuban N[7], Yu J[7], Castelli J[7], Kirk J[7], Benjamin E[7], Barth J[7]

[1] Hôpital Raymond Poincaré, Garches, France, [2]Hôpital du Sacré-Coeur, Montreal, Canada, [3]HCPA/UFRGS Porto Alegre, Porto Alegre, Brazil, [4]University College London, London, United Kingdom, [5]Baylor Research Institute, Dallas, United States, [6]Emory University School of Medicine, Atlanta, United States, [7]Amicus Therapeutics, Cranbury, United States

Objectives: Migalastat is an orally administered investigational pharmacological chaperone that selectively binds and stabilizes α-Gal A, leading to increased cellular enzyme levels and greater lysosomal activity. The longitudinal effects of migalastat on estimated glomerular filtration rate (eGFR) and left ventricular mass index (LVMi) were assessed in the Phase 3 FACETS study (011) and long-term extension study (041).
Methods: Sixty-seven ERT-naïve patients enrolled in study 011. After completion of 011, 48 patients continued into the 041 extension study. Analyses of eGFR and LVMi (blinded, centrally read) were performed across both studies in 40 subjects with amenable GLA mutations based on the HEK cell assay and who were treated with migalastat for at least 18 months.
Results: Renal function remained stable over an average of 36 months of treatment (range 18-54 months). The annualized rate of change in eGFRCKD EPI was -0.8±0.6 mL/min/1.73m2.
LVMi demonstrated a significant reduction over 30-36 months of treatment (baseline LVMi = 96.50 g/m2, average change from baseline = -17.03, 95% CI = -26.18 to -7.88).
Conclusions: Treatment with migalastat was associated with stable renal function and reduction in LMVi over the course of 36 months in Fabry patients with amenable mutations.
Conflict of Interest declared.

O-051

LC-MS/MS lysosphingolipids measurement in plasma for the screening and follow-up of lysosomal storage diseases

Pettazzoni M[1], Piraud M[1], Froissart R[1], Ruet S[1], Pagan C[1], Vianey-Saban C[1], Latour P[1], Cheillan D[1]

[1]Metab Dis Lab, Hospices Civils, Lyon, France

Background and objectives: Lysosphingolipids have been recently identified as potential biomarkers of several sphingolipidoses and their measurement in plasma was undertaken to evaluate their interest in the diagnosis and follow up of these lysosomal diseases. We have developed and validated a quantitative method (LC-MS/MS) to measure various lysosphingolipids (lysoglobotriaosylceramide, lysohexosylceramide, lysosphingomyelin, lyso-GM1, GM2 and GM3 gangliosides, lysosulfatide) in plasma.
Materials/Patients and Methods: Lysosphingolipids were extracted by a solid-phase extraction method from 200 μL plasma (from about 100 healthy controls and 40 patients affected with various lysosomal disorders, mainly sphingolipidoses). LC-MS/MS analysis, adapted from Boutin *et al.* (2012), was performed after HPLC separation on a C8 column with a gradient elution. Compounds were analysed by MS/MS (multiple reaction monitoring mode) and quantitated using internal standards and external calibration. Method validation was performed.
Results: After MS/MS optimization and quantitative validation for each parameter, reference ranges were determined.

For most of the diseases, we observed an accumulation of specific species of lysosphingolipids.
Discussion/Conclusion: This method, measuring lysosphingolipids in a single run, is suitable for the diagnosis of Fabry, Gaucher, Krabbe, Niemann-Pick A/B diseases, GM1 and GM2 gangliosidosis, which is particularly interesting in a metabolic laboratory. Follow-up of treated patients can be considered.
Conflict of Interest declared.

24. Lysosomal disorders: others

O-052

High prevalence of small fibre neuropathy in patients with Gaucher disease type 1

Devigili G[1], Sechi A[2], Ciana G[2], Deroma L[2], Dardis A[2], Malini E[2], De Filippo M[2], Macor D[2], Lettieri C[1], Rinaldo S[1], Eleopra R[1], Bembi B[2]

[1]Neurol Dep, Univ Hosp of Udine, Udine, Italy, [2]Cent Rare Dis, Univ Hosp of Udine, Udine, Italy

Background: Pain is a disabling symptom in Gaucher disease type 1 (GD1), generally described as secondary to bone involvement. In our clinical practice, we observed the persistence of legs pain in a group of GD1 patients, despite long-term enzyme replacement therapy (ERT).
Objective: to investigate the prevalence of neuropathic pain in our cohort of GD1 patients.
Patients & Methods: 25 adult GD1 patients (13 females, 12 males; 23 on ERT >10 years, 2 untreated) were studied. Retrospective clinical history was collected from patient records. Comorbidity for peripheral neuropathy was excluded. Neuropathic pain features were assessed by Douleur Neuropathique en 4 Questions, Neuropathic Pain Symptom Inventory, Quantitative Sensory Testing and skin biopsy with quantification of intraepidermal nerve fibres at distal leg and proximal thigh.
Results: 12/25 patients complained about chronic pain. All 12 patients complain painful sensation suggestive of neuropathic pain features with proximal patchy distribution (6 presented paroxysmal pain). Epidermal denervation was observed in skin biopsy of 20/21 patients (included the 2 untreated patients). Nerve conduction studies were negative.
Conclusions: Our study demonstrates, for the first time, the high prevalence of small fibre neuropathy in GD1. This could explain the persistence of pain despite ERT in some patients.

O-053

Contribution of plasmatic biomarkers to the diagnosis of Niemann-Pick type C disease

Pagan C[1], Latour P[2], Ruet S[1], Anselmini L[1], Piraud M[1], Pettazzoni M[1], Cheillan D[1], Vanier M T[2], Vianey-Saban C[1]

[1]Inborn Metabolic Errors lab., CBPE, HCL, Lyon, France, [2]Lab. Gillet-Mérieux, CBPE, HCL, Lyon, France

Background: Oxysterols have recently emerged as diagnostic biomarkers of Niemann-Pick type C disease (NPC). In complement to filipin test and gene sequencing, they provide a screening tool for this disease.
Patients and Methods: We implemented a LC-MS/MS-based cholestane-3β,5α,6β-triol and 7-ketocholesterol assay in our clinical laboratory. We assessed oxysterols in 15 patients with confirmed diagnosis of NPC, and confronted our findings with clinical data as well as filipin and genetic results. We assessed oxysterols in other lysosomal disorders (including Niemann-Pick type B, Gaucher, Wolman, Krabbe and Fabry diseases, GM1 and GM2 gangliosidoses, and metachromatic leukodystrophy). Finally, we measured lysosphingomyelin, also proposed as a biomarker of NPC, in some patients.
Results: Oxysterols were elevated in 13 patients with NPC, including those displaying a "variant" filipin staining. However, 2 cases of adult, neurologic forms of NPC with an intermediate filipin staining did not have elevated oxysterol levels. Oxysterols were also elevated in patients with NPB and Wolman diseases. Lysosphingomyelin displayed poor pre-analytical stability and further studies are needed to validate this new biomarker.
Conclusion: Plasma biomarkers make the biological diagnosis of NPC easier. However, oxysterols are not fully specific of NPC, and may not detect some cases of adult form of the disease.
Conflict of Interest declared.

25. Lysosomal disorders: treatment, enzyme replacement therapy

O-054

Efficacy and safety of sebelipase alfa in children and adults with lysosomal acid lipase deficiency: results of a phase 3 trial

Burton B[2], Balwani M[1], Feillet F[4], Baric I[5], Burrow T A[6], Valayannopoulos V[7], Yang Y[3], Eckert S[3], Rojas-Caro S[3], Quinn A G[3]

[1]Mount Sinai School of Medicine, New York, United States, [2]Northwestern Univ Feinberg School of Med, Chicago, United States, [3]Synageva BioPharma Corp., Lexington, United States, [4]CHU Brabois Hôpital d'Enfants, Vandoeuvre-lès-Nancy Cedex, France, [5]Univ Hosp Center Zagreb, Univ of Zagreb, Zagreb, Croatia, [6]Cincinnati Children's Hosp Med Center, Cincinnati, United States, [7]Univ Hosp Necker Enfants Malades, Paris, France

Background: Lysosomal acid lipase deficiency is a progressive multisystem disease that is an underappreciated cause of cirrhosis, severe dyslipidemia and early-onset atherosclerosis.
Methods: A phase 3, double-blind, placebo-controlled trial randomized affected children and adults (N=66) to placebo or sebelipase alfa 1 mg/kg q2wk for 20 weeks. Primary endpoint was ALT normalization. Secondary endpoints included other efficacy assessments, safety and immunogenicity. Baseline abnormalities included fibrosis (100%), bridging fibrosis (47%), and cirrhosis (31%) in biopsied patients (n=32; mean age, 12 y); median LDL was 204.0 mg/dL (range 70-378).
Results: After 20 weeks, ALT normalization (ULN range 34-43 U/L) was achieved in 31% of treated patients and 7% of placebo recipients. Secondary efficacy endpoints included LDL, non-HDL, and triglyceride reductions and HDL increases. After >350 infusions of sebelipase alfa the number of patients with AEs was similar in each arm. Most AEs were mild and unrelated to sebelipase alfa; 6 patients experienced infusion-associated reactions (4 placebo recipients). Dosing was paused in 1 sebelipase alfa recipient after an atypical infusion-related reaction.
Conclusion: Sebelipase alfa provided significant improvements in ALT normalization and improved other important disease-related abnormalities including marked reductions in LDL. The safety profile appears favorable and infusions were generally well tolerated.
Conflict of Interest declared.

O-055

An international, phase 3, switchover study of reveglucosidase alfa (BMN 701) in subjects with late-onset Pompe disease (INSPIRE study)

Geberhiwot T[8], Byrne B[6], Eyskens F[7], Hughes D[9], Kissel J[10], Mengel E[11], Mozaffar T[2], Pestronk A[12], Roberts M[3], Schoser B[5], Sivakumar K[13], Statland J[14], Young P[4], Heusner C[1], Walsh L[1], Dummer W[1]

[1]BioMarin Pharmaceutical Inc., Novato, CA, United States, [2]University of California, Irvine, CA, United States, [3]Salford Royal NHS Foundation Trust, Salford, United Kingdom, [4]University Hospital Munster, Munster, Germany, [5]Ludwig Maximilians University, Munich, Germany, [6]University of Florida School of Medicine, Gainesville, FL, United States, [7]University of Antwerp, Antwerp, Belgium, [8]University Hospital Birmingham, Birmingham, United Kingdom, [9]Royal Free & Univ College Medical School, London, United Kingdom, [10]Ohio State University, Columbus, OH, United States, [11]Johannes Gutenberg University, Mainz, Germany, [12]Washington University School of Medicine, Saint Louis, MO, United States, [13]Neuromuscular Research Center, Scottsdale, AZ, United States, [14]University of Kansas Medical Center, Kansas City, KS, United States

Background: In late-onset Pompe disease (LOPD), a deficiency of the enzyme acid alpha-glucosidase (GAA) results in progressive myopathy that can severely affect mobility and pulmonary function. A novel GILT-tagged recombinant human GAA, reveglucosidase alfa (IGF2-GAA, BMN 701), has recently been developed. This chimeric fusion protein of human IGF2 and GAA is efficiently taken up by myoblasts, and significantly reduced glycogen deposits in the diaphragm, heart, and skeletal muscles in a pre-clinical study. In a Phase 1/2 clinical study, reveglucosidase alfa (BMN 701) was well tolerated and has shown improvements in mobility and respiratory function.
Methods & Results: This Phase 3 clinical trial is an open-label, switchover study of approximately 70 subjects. Dosing is 20 mg/kg by IV infusion every other week. Hypoglycemia is an expected pharmacologic effect and can be managed with caloric supplementation. The primary objective is improvement in MIP (maximum inspiratory pressure). Secondary metrics include MEP (maximum expiratory pressure), FVC (forced vital capacity), 6MWT (6-minute walk test), and safety. Subject eligibility criteria include age ≥18 years, ambulatory, prior treatment with commercial rhGAA (≥48 weeks), MIP ≤60%predicted, and FVC values between 30% and 80%predicted. This trial is currently enrolling patients in Europe and the United States with plans to expand to other countries/regions. (NCT01924845).
Conflict of Interest declared.

O-056

A modified enzyme replacement therapy for adult-onset Pompe disease

Sun B[1], Sun T[1], Armstrong D[2], Yi H[1], Yang C[1], Austin S[1], Kishnani P S[1]

[1]Div Med Genet, Duke Univ Med Ctr, Durham, NC, United States, [2]Valerion Therapeutics, Concord, MA, United States

Background and Objectives: Mannose-6-phophate receptor (M6PR) mediated enzyme replacement therapy (ERT) with recombinant human GAA is unable to digest cytoplasmic glycogen accumulation in skeletal muscle from lysosome rupture in adult-onset Pompe disease. We aimed to test whether the Fab fragment of a highly penetrating mAb 3E10 could deliver GAA into the cytoplasm.
Methods: A Fab-GAA fusion protein was produced in CHO cells. Protein uptake was tested in C2C12, L6 myoblasts, and primary fibroblasts from Pompe patients. Four-week ERT at a weekly dose of 30 mg/kg was conducted in Pompe (GAA-KO) mice.
Results: Immunostaining with anti-Fab antibody revealed strong signals that did not co-localize with the lysosomal marker LAMP2 in all the three cells. Western blot with anti-GAA antibody showed presence of the 150-kDa full-length Fab-GAA form and the 95- and 76-kDa processed forms of GAA. Inhibition of M6PR with M6P markedly reduced the 95- and the 76-kDa forms but not the 150-kDa form. In GAA-KO mice, Fab-GAA treatment reduced glycogen content by 64% in liver, 55% in heart, 40% in diaphragm, 15% in quadriceps, and 38% in gastrocnemius.
Conclusion: Fab-GAA can be efficiently taken up into the cytoplasm and lysosomes, suggesting a modified therapy for adult-onset Pompe disease.
Conflict of Interest declared.

O-057

Intravenous administration of an AAV9-Hexb vector prolongs lifespan and prevents pathology in Sandhoff mice

Niemir N[1], Rouvière L[1], Besse A[2], Vanier M T[3], Dmytrus J[4], Marais T[2], Astord S[2], Cooper J D[4], Barkats M[2], Caillaud C[1]

[1]INSERM U1151, Necker Enfants Malades, Paris, France, [2]INSERM U974, Inst Myologie, Paris, France, [3]INSERM U820, Lyon, France, [4]Dep Neuroscience, James Black Centre, London, United Kingdom

Background: Sandhoff disease is a rare genetic disorder due to mutations in the *HEXB* gene. It is characterized by a Hex A and B deficiency, responsible for GM2 accumulation, mainly in brain. There is no efficient treatment for this disease.
Methods & Results: A scAAV9-Hexb vector was constructed and tested in the Sandhoff murine model mimicking the human disease. Its intravenous administration in neonatal *Hexb*-/- mice significantly prolonged their lifespan (2 years) compared to non-treated Sandhoff mice (4 months). Behavioral tests performed regularly using rotarod and inverted-screen test showed that treated mice had results comparable to controls. Hexosaminidase activities tested at 2 and 4 months were partially restored in treated mice by comparison with naive Sandhoff mice. GM2 storage found in Sandhoff mice was absent in normal as well as in scAAV9-treated Sandhoff mice brain at 2 and 4 months post-injection. Lamp-1 staining on fixed brain showed a significant storage in Sandhoff mice, but not in scAAV9-treated and control mice. A profound thalamic reactive gliosis and a thalamocortical neuron loss were found in naive Sandhoff mice, while they were nearly absent in scAAV9-treated mice.
Conclusions: These results suggest a protective effect of the therapeutic vector administered intravenously in affected mice during the neonatal period.

26. Glycosylation disorders/CDG, protein modification disorders

O-058

A targeted resequencing approach for diagnostics of congenital disorders of glycosylation

Souche E[1], Keldermanns L[1], Rymen D[2], Matthjis G[1], Race V[1]

[1]Center for Human Genetics, Leuven, Belgium, [2]Center for Metabolic Diseases, Leuven, Belgium

Background: Congenital disorders of glycosylation (CDG) are a rapidly growing group of metabolic diseases, comprising almost 100 distinct disorders in protein and lipid glycosylation. Diagnosis of CDG is challenging because of its clinical and genetic heterogeneity. Furthermore, there is no major contribution of one mutation in a single gene, but rather of point mutations distributed all over the affected genes (coding, splicing, intronic...).
Patients & Methods: We designed a capture assay for a panel of 79 genes associated with CDG type I, CDG type II and congenital muscular dystrophy-dystroglycanopathy. A total of 84 CDG patients were Pair-End sequenced on either a Miseq or Hiseq 2500.
Results: A diagnosis was confirmed in 32 of 84 CDG patients. The mean coverage in the target region was about 1,200 and a genotype was called for more than 97 % of the targeted bases.
Discussion & Conclusion: We identified pathogenic mutations in 38 % of our cohort. Interestingly, 8 patients presented mutations in *ALG1*, a gene that could not be assayed by genomic Sanger sequencing due to the abundance of pseudogenes. In addition, 7 patients were picked up with secondary glycosylation defects due to mutations in *GALT*, *GALE* and *ALDOB*.

O-059

Identification of pharmacological chaperones that increase stability and activity of missense folding mutations in PMM2-CDG

Yuste-Checa P[1], Brasil S[1], Gámez A[1], Underhaug J[2], Desviat L R[1], Ugarte M[1], Pérez-Cerdá C[1], Martínez A[2], Perez B[1]

[1]CBMSO, CEDEM, UAM, Madrid, Spain, [2]Bergen University, Bergen, Norway

Background: 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 PMM2-CDG disorder, the most common CDG, for which there is currently 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 approach there were identified some destabilizing mutations such as p.V44A, p.D65Y, p.R162W, p.F207S, p.T237M and p.C241S which are promising candidates to be rescued by pharmacological chaperones.
Methods & Results: A high-throughput screening of a commercial compounds library by differential scanning fluorimetry (DSF) has allowed us to identified fifteen potential pharmacological chaperones for PMM2. Three of these hits have been subsequently evaluated in a prokaryotic system by DSF and a transcription and translation system revealing normal and mutant proteins' stabilization. Furthermore, PMM2 activity has been recovered by these hits in a cellular disease model carrying either the destabilizing mutation p.D65Y, p.R162W or the p.T237M one.
Conclusion: These results are a proof-of-concept of the PMM2-CDG treatment by small stabilizer molecules and pave the way to develop a new promising therapy for PMM2-CDG.

27. Neurotransmitter disorders

O-060

Unraveling secondary neurotransmitter deficiencies in genetic disorders

Horvath G A[1], Van Karnebeek C D[2]

[1]Dept Pediatr, Univ BC Child Hosp, Vancouver, Canada, [2]Dept Pediatr, Ctr Mol Med Ther, Univ BC, Vancouver, Canada

Background and objective: Despite numerous reports of secondary CSF neurotransmitter deficiencies in genetic disorders, pathophysiology is still not fully understood.
Case reports: We reviwed 377 patients for whom CSF neurotransmitter analysis was performed between 2009-2013 in our centre. 70 had abnormal NT values; 2 identified with congenital NT disorders. The majority had primary epilepsy syndromes.
Materials/Methods:15 patients with secondary NT deficiencies and good clinical response to L-dopa/carbidopa and 5-hydroxytryptophan in terms of improvement in seizures, psychiatric and/or movement disturbances were enrolled for whole exome sequencing (Omics2TreatID study, Vancouver). Proteomic, metabolomic, protein phosphorylation studies, intracellular calcium content, full transcriptome analyses were conducted to validate genotypes and reveal mechanism of secondary NT deficiencies.
Results: Pathogenic mutations in genes encoding signal transductions pathways, chanellopathies, lysosomal protein, or splicing coactivator were identified in 10 of 15 patients. *In vitro* experiments showed secondary NT deficiencies due to biogenic amine synthetic enzyme deficiency (inactive form of enzyme due to lack of phosphorylation), intracellular calcium signaling abnormalities, or up- and/or down-regulated genes in pathways related to biogenic amine metabolism.
Discussion/Conclusion: Using a systems biology approach, the complex pathophysiology of secondary neurotransmitter deficiencies was further elucidated. Therapy with dopamine and serotonin precursors is helpful in many cases.

28. Disorders of vitamins, cofactors and trace elements

O-061

Free-thiamine is a potential biomarker of thiamine transporter-2 deficiency: a treatable cause of Leigh syndrome

Molero-Luis M[1], Ortigoza-Escobar J D[2], Arias A[3 4], Oyarzabal A[7], Darin N[5], Serrano M[2], Garcia-Cazorla Á[2], Tondo M[1], Casado M[1], Gort L[3 4], Mayr J A[6], Rodriguez-Pombo P[3 7], Ribes A[3 4], Artuch R[1 3], Pérez-Dueñas B[2]

[1]Laboratory, Hospital Sant Joan Deu, Esplugues Llobregat, Spain, [2]Neurology Dep, Hospital Sant Joan Deu, Esplugues Llobregat, Spain, [3]CIBER-ER, ISCIII, Barcelona-Madrid, Spain, [4]EIM-IBC, Hosptal Clinic, Barcelona, Spain, [5]Dep Ped,Sahlgrenska A, Gothenburg Univ, Gothenburg, Sweden, [6]Dep Ped, Paracelsus Med, Salzburg Univ, Salzburg, Austria, [7]CEDEM, Univ Autonoma Madrid, Madrid, Spain

Background: Thiamine transporter-2 deficiency (THTR2) is a recessive inherited defect due to mutations in the *SLC19A3* gene that leads to acute encephalopathy and brain damage in childhood. An early administration of thiamine and biotin has a dramatic clinical effect. New biochemical markers for an early diagnosis and timely therapeutic intervention are needed.
Materials and Patients: Thiamine isoforms were analyzed by HPLC-fluorescence detection. Different reference intervals were established in whole-blood and CSF. CSF thiamine isoforms were studied in 16 children with Leigh syndrome, six of whom presented with THTR2.
Results: A negative correlation between thiamine derivatives and age in both fluids were observed; thus, different reference intervals were established. CSF free-thiamine was remarkably reduced in all *SLC19A3*-mutated patients before treatment. The other patients without *SLC19A3*-mutations exhibited normal-slightly reduced CSF free-thiamine concentrations. We observed a severe deficiency of free-thiamine and thiamine-monophosphate in fibroblasts from three *SLC19A3*-mutated patients. The mitochondrial substrate oxidation rates in fibroblasts were not different between *SLC19A3*-mutated and control patients.
Conclusions: We found a profound deficiency of CSF free-thiamine in patients with ThTR-2 deficiency that distinguished them from other causes of Leigh syndrome. Low values of CSF free-thiamine and/or good therapeutic response should lead clinicians to consider a genetic analysis of the *SLC19A3* gene.

O-062

Structural and biochemical insights into a human MMAC HC-MMADHC protein complex

Froese D S[1 3], Kopec J[2], Fitzpatrick F[2], Schuller M[2], McCorvie T J[2], Plessl T[1], Fettelschoss V[1], Chalk R[2], Fowler B[1], Yue W W[2], Baumgartner M R[1 3]

[1]Div Metab Dis, Univ Child Hosp, Zürich, Switzerland, [2]Structural Genomics Consort, Univ Oxford, Oxford, United Kingdom, [3]radiz – Rare Disease Initiative Zürich, Zürich, Switzerland

Background: MMACHC and MMADHC are two proteins required for the proper processing of vitamin B_{12} (cobalamin, Cbl) to its cofactor forms, dysfunction of which leads to homocystinuria and/or methylmalonic aciduria, and emerging evidence points to their interaction as key to this function.
Objectives: To better characterize their interaction, we identified minimal regions of MMACHC and MMADHC required, and analyzed the impact of functional and patient missense mutations from both genes, demonstrating interaction disruption from both mutation types.
Methods: To gain structural insight into how this interaction might take place, we generated the X-ray structure of an N-terminally truncated *Mus musculus* (*Mm*)MMADHC construct to 2.2Å.
Results: The *Mm*MMADHC structure shows surprising homology to MMACHC and other members of the dimeric FMN utilizing nitro-reductase protein family, despite poor amino acid conservation. However, it contains a modified NTR-fold such that it does not dimerize or bind Cbl or FMN. We further applied small angle X-ray scattering to generate a low-resolution model of the MMAC HC-MMADHC complex, revealing a 1:1 heterodimeric stoichiometry.
Conclusion: Together this data represents the first structural analysis of the interaction of two proteins required for Cbl cofactor synthesis, and provides a mechanistic framework to understand the function and disease mechanisms of these crucial Cbl processing proteins.

O-063

Genome-wide association study of B6 vitamers in CSF and plasma identifies alkaline phosphatase as a key player in human vitamin B6 metabolism

Albersen* M[2], Olde Loohuis L[3], De Jong S[3], Bosma M[2], Jans J J M[1], Luykx J J[3], Bakker S C[4], Verhoeven-Duif N M[1], Ophoff R A[3 4]

[1]Dept Med Genet, Univ Med Center, Utrecht, Netherlands, [2]Dept Med Genet, Univ Med Center, Utrecht, Netherlands, [3]Center Neurobehav Genet, UCLA, Los Angeles, United States, [4]Dept Psych, Univ Med Center, Utrecht, Netherlands

Background & Objectives: The active form of vitamin B6, pyridoxal phosphate (PLP), is essential for normal brain development and functioning. To obtain insight in the genetic regulation of B6 vitamers and vitamin B6 metabolism and transport, we conducted a genome-wide association study (GWAS).
Materials & Methods: B6 vitamers were quantified by mass spectrometry in CSF and plasma of 493 healthy human subjects. Concentrations and ratios in and between both body fluids were studied for their genetic association with common alleles.
Results: We observed five genome-wide significant associations with single nucleotide polymorphisms (SNPs), all in the same locus near the alkaline phosphatase (*ALPL*) gene on

chromosome 1. The strongest association was found for rs201680459 (p=7.23E-09, MAF=0.47). Subjects homozygous for the minor allele of this SNP showed a 1.6 times higher ratio between PLP and pyridoxal (PL) in CSF than subjects homozygous for the major allele. The PLP:PL ratio and PLP concentrations in plasma were 1.4 times higher.
Conclusion: This GWAS of B6 vitamers in CSF and plasma identifies ALPL as a key player in vitamin B6 metabolism. Our results also demonstrate the usefulness and great potential for studying metabolites in CSF for the discovery of novel genetic factors involved in human metabolism.
**These authors contributed equally*

O-064

CblC and CblG defects of vitamin B12 metabolism lead to endoplasmic reticulum stress and apoptosis that could be rescued by pharmacological activation of SIRT1 by SRT1720 treatment

Wiedemann A[2], Flayac J[2], Ghemrawi R[1], Bassila C[1], Coelho D[2], Guéant J L[2]

[1]Inserm U954, Vandoeuvre-lès-Nancy, France, [2]Nancy Univ Hosp and INSERM U954, Vandoeuvre-lès-Nancy, France

Background: Vitamin B12 or cobalamin (Cbl) metabolism is altered in patients with genetic defects resulting in a wide spectrum of symptoms but little is known about the pathophysiological mechanisms underlying these diseases. Our previous results obtained in a mouse model have shown that Cbl deficit results in SIRT1-mediated endoplasmic reticulum (ER) stress.
Objective: Our aim was to investigate the role of ER stress and apoptosis in fibroblasts from patients with inherited defects of Cbl metabolism.
Methods: Fibroblasts from 8 patients suffering from CblC and CblG inherited defects of Cbl metabolism were incubated with OHCbl or with SRT1720. Apoptosis and ER stress have been measured by western blot of related hallmarks.
Results: Decreased SIRT1 expression and increase of activated forms of PERK, IRE1, ATF6 and caspase-3 indicate the presence ER stress and apoptosis. Vitamin B12 and SRT1720 treatment decreased the expression of ER stress and apoptotic markers.
Conclusions: Both CblC and CblG defects are associated with ER stress and apoptosis, which could be reversed by pharmacological activation of SIRT1. This opens potential benefits for an innovative treatment of patients with acquired or inherited disorders of B12 metabolism.
Acknowledgements: Inserm, Région Lorraine, CHU de Nancy, and Fondation Jérôme Lejeune.

29. Miscellaneous

O-065

Next generation sequencing improves diagnosis in patients with complex neurometabolic phenotypes

Reid E S[1], Drury S[2], Boustred C[2], Lench N[2], Grunewald S[2], Rahman S[1 2], Cleary M A[2], Abulhoul L[2], Gissen P[1 2], Clayton P T[1], Mills P B[1]

[1]UCL Institute of Child Health, London, United Kingdom, [2]Great Ormond Street Hospital, London, United Kingdom

Background: There are more than 600 genes in which mutations are known to cause inborn errors of metabolism (IEMs). Major challenges to accurate diagnosis include non-specific clinical presentation and the broad differential diagnosis of some phenotypes.
Objective: To develop a comprehensive next-generation sequencing panel for IEM diagnosis.
Methods: A panel of 614 genes was designed based on the SSIEM classification. Samples from 13 patients with a known genetic diagnosis and 8 patients with a known biochemical diagnosis were selected for validation. Samples from 21 undiagnosed patients were tested. HaloPlex (Agilent) was used for target capture and MiSeq/HiSeq (Illumina) for sequencing. Data was analysed using an in-house pipeline based on open-source software.
Results: Diagnosis was achieved in 43% of patients who were previously undiagnosed (duration from symptom onset to diagnosis: 2 – 18 years). All diagnosed patients harboured at least one novel mutation and 56% presented with novel phenotypic features. Biochemical findings to support these diagnoses were identified in 56% of patients, either through confirmatory testing or retrospective review of patient notes.
Conclusion: Implementation of a targeted panel sequencing approach for IEM diagnosis facilitates accurate, timely and cost-effective diagnosis for a group of disorders characterised by significant phenotypic, biochemical and genetic heterogeneity.

01. Inborn errors of metabolism in adult

P-001

Glycerol kinase deficiency: a contributory factor in glycogenic hepatopathy in poorly controlled diabetes?

Nevalainen P I[1], Holme E[4], Solakivi T[3], Salo M K[2], Kollberg G[4], Keskinen P[2]

[1]Tampere Univ Hosp, Dept Intern Med, Tampere, Finland, [2]Tampere Univ Hosp, Dept Pediatr, Tampere, Finland, [3]Univ Tampere, Dept Med Biochem, Tampere, Finland, [4]Dept Clin Chem, Sahlgrenska Univ Hosp, Gothenburg, Sweden

Background: Glycerol kinase deficiency is a rare inborn error of metabolism that has not been reported in patients with type 1 diabetes before. Diabetic glycogenic hepatopathy is a rare manifestation of poorly controlled type 1 diabetes. It is characterized by enlarged liver with hepatocytes containing glycogen. The underlying mechanisms are not known.
Case report & results: The patient, a 19-year-old male, presented with coinciding poorly controlled type 1 diabetes, glycerol kinase deficiency and diabetic glycogenic hepatopathy. He has had wide inexplicable fluctuations of plasma glucose levels and a propensity for ketoacidotic episodes due to missed injections or acute illness. Urine organic acids analysis revealed a massive overexcretion of glycerol. Glycerol kinase activity was 0.5 % of the lower limit of normal. A new missense mutation was found in the glycerol kinase gene.
Conclusion: Since only a few patients with poor metabolic control develop diabetic glycogenic hepatopathy a 'second hit' is suggested for the condition to develop. We suggest that in this case glycerol kinase deficiency may be the second metabolic derangement needed for diabetic glycogenic hepatopathy to develop. In diabetic patients with hepatomegaly and/or excessively variable glucose concentrations search for an inborn error of metabolism may be indicated.

P-002 – moved to 29. Miscellaneous

P-003 – moved to 09. Other amino acid disorders

P-004 – moved to 13. Carbohydrates

P-005 – moved to 29. Miscellaneous

P-006

Opinions of patients with inherited metabolic diseases and their families regarding transitional care in Japan

Kubota M[1]

[1]Saitama Children's Medical Center, Saitama, Japan

Background and objectives: The number of children with inherited metabolic diseases (IMD) surviving into adulthood is increasing. Transition of patients with IMD to adult care units has several problems. Many guidelines have been published to resolve these problems, but they do not reflect the opinions of patients and their families.
Materials and Methods: Two patient advocacy groups of IMD were selected as convenience samples, namely the Kanto (close to Tokyo) and the Hokkaido (far from Tokyo) groups. An online or a paper-based survey containing seven questions regarding transition was sent to both group members. Twenty-three and eleven members completed the survey in both the groups, respectively.
Results: Kanto (68%) and Hokkaido (62%) group members thought the adequate age of transition to be up to 22 years of age. In each group, there were four patients who answered transition "impossible," including one and two possibly independent patients in the Kanto and Hokkaido groups, respectively. Only 41% and 36% in the Kanto and Hokkaido groups, respectively, wanted to transit to adult care units; 87% and 91% of members, respectively, expected transitional program in future.
Conclusion: Specialists in IMD and adult practitioners must consider opinions of patients and families to improve transitional care.

P-007

Suitability of sphingomyelinase activity in leucocytes for the diagnosis of late-onset Niemann-Pick type B

Pérez López J[1], Ceberio L[2], Villarrubia J[3], Del Toro M[1], Carnicer C[1]

[1]Hospital Vall d'Hebron, Barcelona, Spain, [2]Hospital de Cruces, Barakaldo, Spain, [3]Hospital Ramón y Cajal, Madrid, Spain

Background: Niemann-Pick type B (NPB) may be an underdiagnosed disease because it is poorly known among physicians who see adult patients. In addition, very mild forms and residual enzymatic activities make the diagnosis difficult when using conventional assays.
Objectives: To study the residual activity of sphingomyelinase in leucocytes in order to evaluate its suitability for the diagnosis of late-onset NPB.
Methods: we enrolled in the study 5 patients from different Spanish hospitals diagnosed with NPB when they were over 30 years old, plus 6 patients from the literature in similar situation.
Results: The average age of patients at diagnosis was 47 years (range 30-61), all of them showed splenomegaly, and 7 presented radiological signs of lung involvement. The most common mutations found were p.G247S and p.R608del. The average residual activity of sphingomyelinase in leucocytes was 12% (range 3-24,4%). Measure of residual activity for this enzyme was performed on fibroblasts only in two patients, and in both cases was less than 0,5%.

Discussion: Sphingomyelinase activity in leucocytes for the diagnosis of late-onset NPB may not be suitable. Enzymatic assay on fibroblast as well as molecular analysis may be more appropriate in this group of patients.

P-008 – moved to 10. Urea cycle disorders

P-009

Ten year specialized adult PKU care in Germany: experience from Leipzig

Mütze U[1], Thiele A G[1], Baerwald C[2], Kiess W[1], Beblo S[1]

[1]Div Metab Dis, Univ Child Hosp, Leipzig, Germany, [2]Div Metab Dis, Depart Int Med, Leipzig, Germany

Background and objectives: Transition and adult care of PKU patients is of increasing importance since their number accumulates. Especially, the care of young female PKU patients represents a challenge with respect to prevention of maternal PKU. Adult outpatient clinics for inborn errors of metabolism can help achieve these goals, but experience is limited. We report a ten year experience in adult PKU care.
Patients and methods: 94 (54f) patients transferred from pediatric to adult medical care between 2005 and 2015 were identified (median age 2015: 32.1 years, 18.3-62.3). Laboratory data and case notes were analyzed retrospectively.
Results: In 2014, 57 patients (29f) had regular contact to the adult outpatient clinic. Median phenylalanine concentration in dried blood was 624μmol/l (177-1218), with no significant differences between women (671μmol/l; 202-1218) and men (615μmol/l; 177-1126). Since 2005, 25 completed pregnancies were supervised, 2 newborns showed characteristic symptoms of maternal PKU.
Conclusion: Metabolic control in adult patients attending the outpatient clinic was mostly good to satisfactory. Maternal PKU is preventable by specialized care of adult PKU patients. However, further effort in adult metabolic care is needed to prevent loss of follow-up and enable the recommended lifelong treatment.

P-010 - moved to 09. Other amino acid disorders

P-011

Dental and periodontal manifestations of glycogen storage diseases

Biosse Duplan M[1 2], Louzoun A[1 2], Perry A[3], Hubert A[3], Labrune P[3], Chaussain C[1 2]

[1]Odont., H. Bretonneau, APHP, Paris, France, [2]Fac. Chir. Dent., U. Paris Descartes, Montrouge, France, [3]CRMHMH, Ped., H. Béclère, APHP, Clamart, France

Background: Glycogen storage diseases (GSDs) are a group of rare inherited diseases affecting the metabolism of glycogen. The most commonly affected organs are the liver and muscle, but GSDs can also lead to neutropenia (as in GSD type Ib). Oral manifestations of GSDs have been reported, including severe periodontitis in GSD Ib.
Case reports: The purpose of this study was to evaluate the dental and periodontal status of patients with GSDs. To this aim, we examined 39 patients (mean age 26.7) diagnosed with 4 types of GSD (Ia, Ib, III, IX), followed in the National Reference Center and compared clinical and radiological indices to those available for the general population.
Results: Delayed tooth eruption, agenesis or tooth shape abnormalities were more frequent, with respectively 21, 24 and 24% of GSD patients affected. These manifestations were different between GSD types, those in type IX patients being the most severe. The amount of dental plaque was similar as in the general population for all types of GSD, but gingival inflammation and alveolar bone loss were increased in all neutropenic type Ib patients.
Conclusion: This study highlights different oral manifestations in patients with GSDs and the need to adapt dental monitoring depending on the type of GSD.

P-012

What should we do to improve adult-oriented biochemical genetic testing?

Ostrozlikova M[1], Behulova D[1], Holesova D[1], Sebova C[1], Brennerova K[2], Bzduch V[2], Hlavata A[3], Rovensky J[4], Saligova J[5], Ostrovsky I[6], Gorova R[6], Skodova J[1], Pereckova J[1], Salingova A[1]

[1]Centre Inherit Met Dis, Univ Child Hosp, Bratislava, Slovakia, [2]1st Dept Pediatr, Univ Child Hos, Bratislava, Slovakia, [3]2nd Dept Pediatr, Univ Child Hosp, Bratislava, Slovakia, [4]National Inst Rheumat Dis, Piestany, Slovakia, [5]Child Fac Hosp, Kosice, Slovakia, [6]Chem Inst Fac Nature Sci, Comenius Univ, Bratislava, Slovakia

Background and objectives: Inherited metabolic diseases (IMD) are lifelong disorders. Advances in diagnosis and therapy resulted in prolonged patient survival and improved prognosis of affected individuals. Our centre for IMD has provided biochemical

genetic testing for majority of pediatric patients (except for phenylketonuria) in Slovakia. The aim of the study was to assess also IMD in adults with a focus on their specific laboratory tasks.
Patients and Methods: All IMD patients older than 19 years investigated in the centre during the last year (2014) were evaluated retrospectively.
Results: The series consisted of 57 monitored and/or detected patients (41 females and 16 males) ranging from 20 to 70 years of age (median 33). Various IMD were included: alkaptonuria (18), classical homocystinuria (9), UCD (5), organic acidurias (2), FAO disorders (2), classical galactosemia (2), GSD (7), MPS (2), mitochondrial disorders (4), biotinidase deficiency (1), GAMT deficiency (1) and mild hyperhomocysteinemia (4). Predominance of affected women was disclosed. Approximately 80 % of them were at a reproductive age (3 pregnancies).
Conclusion: High number of adult patients in the centre requires the extension of the laboratory capacity. Reference values for age and gender categories and specific conditions (pregnancy) should be stated more precisely.
ERDF project ITMS26240220071

P-013 - moved to 09. Other amino acid disorders

P-014

Glycogen storage disease type I, intestinal inflammatory disease and ankylosing spondylitis: unusual complications of a rare disorder

Castro-Chaves P[1], Teresa Cardoso M[1], Rodrigues E[2], Vasconcelos C[2], Vaz C[3], Vilarinho L[4], Leão Teles E[2]

[1]Metab Dis Un, Int Med Depart. CH S Joao, Porto, Portugal, [2]Metab Dis Unit, Paed Depart, CH S Joao, Porto, Portugal, [3]Rheumatology Department, CH S Joao, Porto, Portugal, [4]Newb Screen, Met and Gen Unit, INSA, Porto, Portugal

Background: Glycogen Storage Disease (GSD) type I is the most severe liver glycogenosis but many affected individuals live into adulthood and have protean manifestations.
Case report: We describe a 31 years-old male patient who is a compound heterozygous for glucose-6-phosphatase gene mutations (p.Q54P plus p.G188R of the exons 1 and 4). He was diagnosed in the first trimester of life due to hypoglycemia and convulsions. During follow-up he developed growth delay, hypertriglyceridemia, hepatic steatosis, osteopenia and renal calculi. When first seen at the adult clinic the patient had poor metabolic control due to lack of adherence to diet and long working night shifts. These factors were corrected. Around 20 years-old the patient developed low back pain of inflammatory characteristics. After an episode of complicated acute appendicitis with fistulae, intestinal inflammatory disease was diagnosed. No neutropenia was present. Later on HLA-B27 ankylosing spondylitis was also diagnosed. He is presently under therapy with infliximab with good response, both gastrointestinal and rheumatologic.
Conclusion: This case describes an unusual association of complications and highlights that, although medical nutritional therapy is critical for a good control, a multidisciplinary approach is necessary in order to deal with the multiple long term manifestations of this disease in adults.

P-015

Bone profile and metabolic control in adults with cystathionine β-synthetase (CBS) deficiency

Estrella J F G[1], McLean M[1], Tchan M[2], Bhattacharya K[3], Wilcken B[3 4], Munns C F[5 6]

[1]Uni West Syd Bltwn-MtDruitt Clin School, Blacktown, Australia, [2]Dept Gen Med, Westmead Hosp, Westmead, Australia, [3]WSGP, Child Hosp Westmead, Syd Uni, Westmead, Australia, [4]Dept Newbrn Screening, Child Hosp Westmd, Westmead, Australia, [5]Dep Endocrinology, Child Hosp Westmead, Westmead, Australia, [6]Paed Child Hlth, Syd Med School,Syd Uni, Sydney, Australia

Background: Osteoporosis in homocystinuria secondary to cystathionine β-synthetase (CBS) deficiency is attributed to impaired collagen cross-linking. Limited adult data on metabolic control and bone health exist. We describe bone profile and metabolic control in 9 cases.
Methods: Clinical data, plasma amino acids, gonadotropins, 25-hydroxyvitamin D and parathyroid hormone (PTH) levels were collected. Dual energy x-ray absorptiometry (DXA) and peripheral quantitative computer tomography (pQCT) were performed; T-tests compared with normative data.
Results: Median age at review and diagnosis were 29 years (25-69) and 7 years (1-29). Four patients were B6-responsive. All received B6, B12, folate and betaine. Biochemical control was moderate: total homocysteine usually < 100 μmol/L (39-176). Vitamin D was > 50nmol/L ; PTH was normal. Two patients had fracture histories. DXA demonstrated decreased lumbar spine BMD (Median Z-score – 1.39; p=0.03), and increased fat mass (Median Z-score +1.70 ; p=0.001). Trabecular pQCT vBMD (radius and tibia) was reduced (Median Z-score -1.87; p=0.01 and -0.95;p=0.01). Tibial cortical pQCT vBMD was increased (Median z-score +0.58; p=0.03).
Conclusions: Our cohort had increased fat mass, decreased trabecular BMD and increased cortical BMD, suggesting

increased material bone density. The aetiology is unclear, possibly reflecting reduced bone turnover. It is unclear if this cohort had increased fracture risk.

P-016 – Moved to 11. Organic acidurias: branched-chain

P-017

Lysosomal storage diseases in a neurologic adult outpatient clinic

Gouveia A[1], Duque C[1], Garcia P[2], Diogo L[2], Lacerda L[3], Macário M C[1]

[1]Neurol Dep, Coimbra Univ Hosp Centre, Coimbra, Portugal, [2]Pediat Hosp, Coimbra Univ Hosp Centre, Coimbra, Portugal, [3]National Inst of Health Dr Ricardo Jorge, Porto, Portugal

Background: Lysosomal storage diseases (LSD) are a heterogeneous group of inherited disorders. Adult-onset forms usually have a milder phenotype.
Objective: To characterize the population of patients with LSD of our adult's neurometabolic outpatient clinic.
Methods: We analysed all patients followed in our neurometabolic clinic since its creation in 2003, with the diagnosis of LSD. We reviewed age of symptom onset, age of diagnosis and phenotypes.
Results: Nineteen patients with LSD were observed. Most (14) were women. In 7 patients, the diagnosis was made during childhood; 7 patients had an adult-onset LSD; and in 5 patients the diagnosis was only possible in adulthood, despite the first clinical signs in paediatric age. Regarding the specific diagnosis, we found 5 sphingolipidoses (Gaucher disease types I and III, Krabbe disease and Progressive Myoclonic Epilepsy), 5 mucopolysaccharidoses (types I, III and IV), 4 Pompe disease, 2 oligosaccharidoses (α-mannosidosis and sialidosis type I), 2 neuronal ceroid lipofuscinoses (types 3 and 11) and 1 Niemann-Pick type C. In the group with early-onset disease, the presentation with developmental delay and encephalopathy was more common, while for patients with an adult-onset form, ataxia was more frequent.
Conclusion: In our sample, late-onset forms of LSD exhibit a more neurodegenerative phenotype.

P-018 - Moved to 14. Disorders of fatty acid oxidation and ketone body metabolism

P-019

The outcomes of liver transplantation in adults with inborn errors of metabolism

Morel C[1 2], Bell H[1 2], Semotok J[1 2], Faghfoury H[1 2]

[1]University Health Network, Toronto, Canada, [2]The University of Toronto, Toronto, Canada

Background: Hepatic transplantation has been increasingly used in the management of inborn errors of metabolism. There is a paucity of literature regarding clinical and biochemical outcomes of this procedure in adult metabolic patients.
Case Report: We report 7 adult patients with inborn errors of metabolism (ages of 19-47) who underwent deceased donor liver transplantation at the Toronto General Hospital between 2013-2015. Patients had a diagnosis of citrullinemia, arginase deficiency, MSUD, GSD1b, and primary hyperoxaluria.
Results: The patients with urea cycle disorders, GSD1b all had sustained normalization of their biochemical values post-transplantation. MSUD patients still had minor elevations in both leucine and allo-isoleucine post transplantation. There were no further metabolic decompensations in any patient. Transient and mild post-operative complications included CMV viremia, medication-induced insulin dependent diabetes mellitus, and unilateral toe numbness. Our patient with citrullinemia had a severe complication of a hepatic artery thrombosis with impaired blood flow requiring the patient to be relisted.
Conclusion: Liver transplantation has been performed in adult patients with inborn errors of metabolism to achieve metabolic stability. The procedure has been well tolerated in our patients except one who had a severe complication. Long-term outcomes in this population will require further study.

02. Novel diagnostic/laboratory methods

P-020

A liquid chromatography tandem mass spectrometry (LC-MS/MS) method for contemporary measurement of aminolevulinic acid (ALA) and porphobilinogen (PBG) levels in plasma and urine

Turgeon C[1], Ahlman H[2], Sardh E[2], Raymond K M[1], Tortorelli S[1]

[1]Biochemical Genetics Lab, Mayo Clinic, Rochester, MN, United States, [2]Porphyria Centre, Karolinska Institutet, Stockholm, Sweden

Background and objectives: Diagnosis and therapeutic management of acute porphyrias require accurate analysis of ALA and PBG. Traditionally ALA and PBG are analyzed in urine separately, with laborious methods prone to interferences. Plasma PBG concentration and the PBG/ALA ratio appeared

more sensitive markers in monitoring acute attacks than urinary ALA or PBG (Sardh et al., 2009). Given these findings, we developed a new method for the simultaneous analysis of ALA and PBG levels in plasma and urine.
Method: Plasma and urine concentrations of PBG and ALA in patients with AIP and normal individuals were simultaneously measured using LC-MS/MS. Plasma specimens undergo cation exchange solid phase extraction. Random urine specimens are diluted with acetonitrile after filtration. Total run time is 3 minutes.
Results: Pre-analytical and analytical factors were studied and met predetermined acceptance criteria for clinical testing. ALA and PBG ranges were measured in 180 plasma and 241 random urine controls and 6 plasma and 39 urine AIP patients, showing a clear separation between controls and affected patients.
Discussions: This method shows promise in helping to increase sensitivity of detecting PBG and ALA, and being a more efficient method for clinicians for diagnosis and management of patients with acute porphyria.

P-021

Flow injection liquid chromatography mass spectrometry (FIA-MS/MS) analysis for lysosomal and peroxisomal disorders in dried blood spots (DBS)

Tortorelli S[1], Turgeon C[1], Gavrilov D[1], Oglesbee D[1], Raymond K M[1], Rinaldo P[1], Matern D[1]

[1]Biochemical Genetics Lab, Mayo Clinic, Rochester, MN, United States

Background and objectives: Lysosomal and peroxisomal disorders are slowly being added to newborn screening programs in the USA. Therefore, a screening method that can identify several conditions with a single high-throughput test is compelling. We developed such a method for the simultaneous analysis of six lysosomal enzymes activities and lysophosphatidylcholine (LPC) concentrations in DBS for screening of at risk patients.
Method: DBS are extracted in buffer for enzyme activity determinations (after overnight incubation) and methanol for LPC analysis. Using FIA-MS/MS, concentrations of LPC (C26:0, C24:0, C22:0, C20:0) and reaction products of acid sphingomyelinase, β-glucocerebrosidase, α-glucosidase, α-galactosidase, galactocerebrosidase and α-L-iduronidase are measured. Total FIA-MS/MS run time is 1 minute/sample.
Results: Specimens from subjects with MPS I (N=5), Gaucher disease (N=5), Niemann-Pick A/B (N=2), Pompe disease (N=5), Krabbe disease (N=5), Fabry disease (N=11), ALD (N=8), heterozygous ALD (N=5) and Zellweger-spectrum disorders (N=5) were correctly identified by the simultaneous analysis of the enzyme activities as well as C20 to C26 LPC concentrations in DBS by FIA-MS/MS.
Conclusions: This method is a rapid, effective and high-throughput screening assay for six lysosomal diseases, and peroxisomal disorders using FIA-MS/MS.

P-022

Anthropologist's contribution supporting diagnosis of rare diseases

Różdżyńska-Świątkowska A[2], Tylki-Szymanska A[1]

[1]Dept Ped Nutr & Metab Dis, Child Mem He, Warsaw, Poland, [2]Ant Lab, Child Mem Health Inst, Warsaw, Poland

Background: Rare diseases are life-threatening or chronically debilitating diseases which are of such low prevalence that special combined efforts are needed to address them. Most rare diseases are genetic, and thus are present throughout the person's entire life, even if symptoms do not immediately appear. Many of these syndromes involve body stature and craniofacial abnormality and dynamically change during development. Assessment of body proportion, some dysmorphic characteristics and other morphological parameters must be determined precisely. This goal can be achieved only with the help of an anthropologist having adequate tools.
Obiective. To show contribution of a clinical anthropologist in the diagnostic process of rare diseases.
Methods. Examples of importance of anthropometric techniques and methods in diagnostic of rare disease such as analysis of demographics, birth date, percentile charts, growth patterns, bioimpedance analysis, somatometric profile, craniofacial profile, body proportion's indexes was shown based on rare diseases examples.
Results and conclusions. All methods were used in rare disease diagnosis and showed that anthropology is an important tool supporting diagnosis and also enables the description of the natural history of a given rare genetic disease.

P-023

Knowledgebase and mini-expert platform for Inborn Errors of Metabolism (IEMBASE)

Lee J[1], Wasserman W[1], Hoffmann G F[4], Van Karnebeek C[2], Blau N[3]

[1]Ctr Mol Med Ther, Univ British Columbia, Vancouver, Canada, [2]Dept Pediatr, Ctr Mol Med Ther, Univ BC,

Vancouver, Canada, [3]D. Hoppe Matabol Ctr, Univ Child Hosp, Heidelberg, Germany, [4]Dept Pediatr, Univ Child Hosp, Heidelberg, Germany

Objectives: Timely and accurate diagnosis of IEM is essential, yet poses a challenge given clinical heterogeneity and required knowledge on hundreds of IEMs. To overcome this, we have now developed an online platform, which combines a comprehensive expert knowledgebase and a smart system to support efficient diagnosis and management for clinicians.
Design and Methods: We extracted disease-characterizing profiles of clinical and biochemical markers (n=3140) from an expert-generated database of 529 IEMs. These profiles were then mapped to the HPO and LOINC in order to exploit the semantic relationships of symptoms from the profiles. This, in turn, allows the expert system to algorithmically determine a tiered list of possible IEMs, which match the user-provided symptoms.
Results: The IEMBASE accepts an array of clinical and biochemical markers from a user and returns to the user a ranked list of possible IEM disorders that match the input profile. In addition, the system can explain the rationale of its results, suggest additional tests to narrow down the differential diagnosis, lists possible treatment options and provide access to external resources.
Conclusions: We expect that this unique system dedicated to IEMs will significantly improve clinical practice to benefit patients and families suffering these rare diseases.

P-024

Targeted analysis in urine by high resolution nuclear magnetic resonance spectroscopy (NMRS) and gas chromatography/mass spectroscopy (GC/MS) in the selective screening program for inborn errors of metabolism

Cannet C[1], Beedgen L[2], Trefz F K[2], Okun J G[2], Langhans C D[2], Schäfer H[1], Spraul M[1], Hoffmann G F[2]

[1]Bruker BioSpin GmbH, Rheinstetten, Germany, [2]Dep Ped, Div Neuroped Metab Med,Univ Heid, Heidelberg, Germany

Introduction: NMRS has shown to be a highly reproducible and quantitative method capable of high throughput analysis. Lower sensitivity and higher instrument costs may have limited the widespread introduction into the metabolic laboratory. GC/MS is more sensitive but less quantitative, sample preparation and analysis time is longer. The purpose of this study was a comparison of both methods in view of quantification of relevant metabolites and of general efficiency to correctly diagnose disorders in the routine metabolic laboratory.
Method: 600 urine samples which were sent to exclude metabolic disorders were split for GC/MS and NMRS analysis using a quadrupol GCMS Trace/DSQ II (ThermoScientific) and a Bruker Avance IVDr 600 MHz system respectively.
Results: Correlation was calculated for 28 substances. Detection limit was higher for most substances in NMRS than in GC/MS. However, in some pathologic conditions quantitative results are 2-5 times higher in GCMS than in NMRS. Diagnoses of Isovaleric, Propionic, Methylmalonic acidemia and MCAD deficiency could be confirmed in the patient urines by both methods.
Conclusion: NMRS is similar efficient as GC/MS but is more quantitative for higher concentrations. However, for some metabolites GC/MS or HPLC/MS methods are necessary to quantify low concentrations.

P-025

A novel plasma biomarker for Snyder Robinson Syndrome (X-linked spermine synthase deficiency)

Simmons L[1], Abela L[1], Steindl K[2], Schmitt B[1], Joset P[2], Papuc M[2], Crowther L[1], Rauch A[2], Plecko B[1]

[1]Div Neuro, Univ Child Hosp, Zurich, Switzerland, [2]Inst Med Genetics, Univ Hosp, Zurich, Switzerland

Background: Snyder-Robinson syndrome (SRS) is a rare X-linked mental retardation syndrome caused by mutations in the spermine synthase (*SMS*) gene. SMS dysfunction causes a reduction in intracellular spermine, a polyamine involved in various cellular processes. To date, the diagnosis of SRS has relied on an elevated spermidine/spermine ratio in lymphoblasts and/or sequencing of the *SMS* gene.
Case: We report male monozygotic twins who presented with dysmorphic features, developmental delay and progressive microcephaly. At the age of 15 months they developed a severe encephalopathy with frequent seizures, loss of milestones and impairment of visual interaction.
Methods: An untargeted plasma metabolomics analysis was performed by liquid chromatography−high-resolution mass spectrometry (LC-MS). Genetic analysis was performed by whole exome sequencing (WES).
Results: Statistical analysis of LC-MS data revealed a distinct plasma metabolomics profile when compared to controls. Concurrent WES analysis showed a novel *SMS* missense mutation. Among the discriminating metabolites, we identified N-acetylspermidine, which showed significant relative increase versus age matched controls.
Discussion: In a combined metabolomic-genetic approach, we identified elevated N-acetylspermidine as a novel plasma biomarker in twin patients with a pathogenic mutation of the *SMS*

gene. This discovery will facilitate recognition of SRS and will allow monitoring of potential therapeutic interventions.

P-026

Vacuolated lymphocytes – a biomarker for disease severity in neuronal ceroid lipofuscinosis type 3?

Oostendorp M[1], Van Alfen C[3], Nierkens S[2], Huisman A[1], Van Hasselt P M[4]

[1]Lab Clin Chem Haematol, UMCU, Utrecht, Netherlands, [2]Dept Immunology, UMCU, Utrecht, Netherlands, [3]Bartimeus institute, Doorn, Netherlands, [4]Dept Met Dis, Wilhelmina Child Hosp,UMCU, Utrecht, Netherlands

Background Lymphocyte vacuolization provides an important diagnostic clue for neuronal ceroid lipofuscinosis type 3 (NCL3) in children presenting with rapid loss of vision around 6 years of age. We hypothesized that the extent of lymphocyte vacuolization could also serve as a biomarker for disease severity in NCL3.
Methods Peripheral blood was obtained at follow up visits from 12 NCL3 patients (ages 6–19 years, 24 measurements in total). Blood smears were scored by 4-5 experienced laboratory technicians. We determined a) the percentage of vacuolated lymphocytes and b) the number of vacuoles per lymphocyte. These indices were correlated with age and the Universal Batten Disease Rating Score (UBDRS) summary score, available for 15/24 measurements.
Results A clear association was found between the percentage of vacuolated lymphocytes as well as the average number of vacuoles per lymphocyte and age (R^2=0.28, P=0.008 and R^2=0.71, P< 0.0001, respectively). Additionally, a significant correlation was found between the number of vacuoles per lymphocyte and the UBDRS summary score (R^2=0.62, P=0.0005), but not with the percentage of vacuolated lymphocytes.
Conclusion Quantitative microscopic analysis of vacuolated lymphocytes, especially the average number of vacuoles per lymphocytes, in peripheral blood smears may serve as a biomarker for disease severity in NCL3.

P-027

Development of a dried blood spot method for amino acid analysis in MSUD using the Waters Acquity UPLC with UV detection

Horman A J[1], Smith E[1], Wu H Y[1], Cooper J A[1], Henderson M J[1]

[1]Willink Laboratory, St. Mary's Hospital, Manchester, United Kingdom

Objective: Amino acid monitoring using dried blood spots offers certain advantages over plasma but can be limited by sample quality and analytical sensitivity/robustness. The aim of this study was to develop a fast and robust assay to determine amino acid concentrations in dried blood spots for MSUD monitoring.
Methods: A 10 minute separation program was designed to focus on the separation of branched chain and phenolic amino acid AQC derivatives using the Waters Acquity UPLC-TUV system and extraction efficiency was examined.
Results: A simple method was created to quantify blood spot solvent extracts and resolve Ile, Leu, allo-Ile, Phe, Tyr and Met. Quantitation was linear up to 8 mM Leucine, between-batch CVs were typically < 5%, and retrospective comparison of UK-NEQAS material for Tyr, Phe and Met demonstrated overall good performance although some biases existed which may reflect extraction methodologies using typical newborn screening methods.
Conclusion: This method is suited to MSUD monitoring and newborn screening follow up. Blood spot extraction conditions must acknowledge the effect of potential cell disruption when used for the purpose of monitoring over screening. Inclusion of Tyr, Phe and Met to the profile provides additional information which may be helpful in judging protein intake or blood spot quality.

P-028

Acylglycine profiling: a new liquid chromatography-tandem mass spectrometry (LC-MS/MS) method, applied to disorders of organic acid, fatty acid and ketone metabolism

Bherer P[1], Cyr D[1], Buhas D[2], Al-Hertani W[2 3], Maranda B[1], Waters P J[1]

[1]Dept Peds, Sherbrooke Univ Hosp Centre, Sherbrooke, Quebec, Canada, [2]Dept Med Genet, McGill Univ Health Ctr, Montreal, Quebec, Canada, [3]Depts Peds and Med Genet, Univ Calgary, Calgary, Alberta, Canada

Background: Acylglycine profiles are complementary to organic acid and acylcarnitine profiles. Traditional methods have limitations of sensitivity and specificity. Metabolomic studies indicate many acylglycines present in urine at low concentrations.

Objectives: We developed an LC-MS/MS method suitable for follow-up of newborn screening, investigation of patients with clinical suspicion of inborn errors of metabolism (IEM), and management of patients with confirmed IEM.
Method: The panel includes 21 acylglycines; some established IEM markers plus other molecules considered potentially informative. Butylated acylglycines are gradient separated. MS/MS analysis with multiple reaction monitoring is fully quantitative for 15 acylglycines (linearity 0.01-100 micromol/L), semi-quantitative for 6 others. Age-related reference ranges were established (282 samples). Validation included > 200 samples from ~ 100 patients with various IEM.
Results: Examples of applications include: 1. medium-chain acyl-CoA dehydrogenase (MCAD) deficiency: our method is now integrated into the confirmatory testing algorithm for newborn screening cases in Quebec. 2. Rare disorders, challenging to diagnose: abnormal acylglycine profiles associated with 3-hydroxy-3-methylglutaryl-CoA synthase deficiency and glutaric acidemia type 3, among others, will be presented.
Conclusions: We have established a new LC-MS/MS method for urine acylglycine profiling, which is proving valuable for investigation of disorders of organic acid, fatty acid and ketone metabolism

P-029

Determination of urinary glycosaminoglycan levels in mucopolysaccharidosis type VII by colorimetric (DMB) and two liquid chromatography/mass spectrometry (LC/MS) methods; methanolysis and Sensi-Pro® non-reducing ends assay

Taylor J E[1], Haller C[1], Wood T[2], Pasquali M[3], Kakkis E D[1]

[1]Ultragenyx Pharmaceutical, Novato, United States, [2]Greenwood Genetic Center, Greenwood, United States, [3]Univ of Utah/ARUP Labs, Salt Lake City, United States

Background: Currently available methodologies were compared for their ability to detect pathologic urinary accumulation of glycosaminoglycans (chondroitin, dermatan and heparan sulphate; CS, DS and HS respectively) in patients with MPS VII, and pharmacodynamic response to rhGUS enzyme infusion.
Patients and Methods: Pre- and post-treatment urine samples were obtained from four MPS VII patients in rhGUS clinical studies. Urinary glycosaminoglycans (uGAGs) were analyzed by DMB (total uGAG), LC-MS/MS (CS/DS and HS Sensi-Pro® Non-Reducing Ends Assay; NRE) and LC-MS/MS (CS, DS and HS).
Results: All three assays detected high baseline: normal uGAG ratios, with DMB showing a mean 2.1-fold higher uGAG, LC-MS/MS detecting mean 10.1-, 14.0- and 0.3–fold higher CS, DS and HS, and NRE showing mean 56.7-fold higher CS/DS and 1.5-fold higher HS ratios. Following rhGUS infusions at 4 mg/kg, DMB showed a 37.5% reduction in total uGAG; LC-MS/MS showed 55.8, 59.5 and 47.6% reduction of CS, DS and HS; and NRE showed 55.6% and 53.3% reduction of CS/DS and HS respectively.
Discussion: LC-MS/MS and the NRE assay showed clear advantages over DMB in differentiating and quantifying individual GAGs and identifying much higher baseline: normal urine ratios, particularly of CS and DS. Notably, HS was not elevated to a significant extent.

P-030

Urine keratan sulfate (uKS) in Morquio A patients measured via LC-MS/MS method: improved KS detection as compared to dye-based methods and report of age-specific uKS reference ranges

Pasquali M[1], Auray-Blais C[2], Ellsworth K[3], Fietz M[4], Giugliani R[5], Harmatz P[6], Izzo E[7], Lavoie P[2], La Marca G[8], Millington D[9], Trinh M[4], Van Vlies N[10], Varfaj F[7], Wijburg F A[10], Wood T[3], Zhang H[9], Miller N[7]

[1]University of Utah School of Medicine, Salt Lake City, United States, [2]Université de Sherbrooke, Sherbrooke, Quebec, Canada, [3]Greenwood Genetic Center, Greenwood, South Carolina, United States, [4]Women's and Children's Hospital, North Adelaide, South Australia, Australia, [5]Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil, [6]UCSF Benioff Children's Hospital Oakland, Oakland, California, United States, [7]BioMarin Pharmaceutical Inc., Novato, California, United States, [8]Meyer Children's Hospital, Florence, Italy, [9]Duke University Medical Center, Durham, North Carolina, United States, [10]Academic Medical Center, Amsterdam, Netherlands

Background/Objectives: Demonstration of deficient GALNS enzyme activity is the gold standard for diagnosis of Morquio A. However, detection of elevated urinary GAG (uGAG) levels by dye-based methods is often used as a screening test despite its poor sensitivity. To improve screening for Morquio A, two international data steering committees were organized by BioMarin in 2014 to evaluate the LC-MS/MS–based uKS test.
Methods: Seven independent international laboratories assessed creatinine-normalized uKS levels (by LC-MS/MS) and uGAG levels (by the dye-based test) in unaffected individuals and those with an established diagnosis of Morquio A.

Results: Reference ranges confirmed previous findings that creatinine-normalized uKS levels are highest in children < 1 year of age in both unaffected and Morquio A individuals and then decrease rapidly. Additionally, age-specific uKS measurements for individuals with Morquio A were significantly elevated and did not overlap with the corresponding values of unaffected individuals.We highlight multiple cases where the superior sensitivity of the LC-MS/MS uKS test would result in identification of individuals with Morquio A while the dye-based test would not.
Conclusions: The LC-MS/MS-based uKS test provides a highly sensitive, specific, and quantitative measure of elevated KS. Use of age-based reference ranges is crucial for appropriate interpretation of uKS data.
Conflict of Interest declared.

P-031

Urine keratan sulfate (uKS) elevation in lysosomal storage disorders (LSDs): comparison of uKS levels in Morquio/mucopolysaccharidosis (MPS) IV versus non-Morquio LSDs

Wijburg F A[7], Auray-Blais C[1], Ellsworth K[2], Giugliani R[3], Harmatz P[4], Izzo E[5], Lavoie P[1], Millington D[6], Wood T[2], Van Vlies N[7], Zhang H[6], Miller N[5]

[1]Université de Sherbrooke, Sherbrooke, Quebec, Canada, [2]Greenwood Genetic Center, Greenwood, South Carolina, United States, [3]Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil, [4]UCSF Benioff Children's Hospital Oakland, Oakland, California, United States, [5]BioMarin Pharmaceutical Inc., Novato, California, United States, [6]Duke University Medical Center, Durham, North Carolina, United States, [7]Academic Medical Center, Amsterdam, Netherlands

Background/Objectives: Demonstration of deficient enzyme activity is the gold standard for diagnosis of Morquio/MPS IV; however, detection of elevated urine keratan sulfate (uKS) is being utilized as a positive screening biomarker. A prior study showed that KS levels were elevated in several non-Morquio LSDs. To examine the specificity of uKS levels as a screening biomarker for Morquio disease, an international data steering committee was organized by BioMarin in 2014.
Methods: Five independent international testing laboratories evaluated creatinine-normalized uKS levels by LC-MS/MS in individuals with an established diagnosis of Morquio A or another LSD and compared with levels in unaffected controls.
Results: While the highest mean elevations in uKS levels were detected in individuals with Morquio A, elevated uKS levels were detected in individuals with other LSDs: MPS I, MPS II, MPS III, MPS VI, ML II/III, ML III, GM1, galactosialidosis, and α-fucosidosis. To date, elevated uKS levels in individuals with α-mannosidosis, Sandhoff, Pompe, Fabry, or sialuria have not been detected.
Conclusions: Although the sensitive LC-MS/MS uKS test confirmed that mean uKS levels were higher in Morquio disorders than non-Morquio LSDs, elevations in uKS levels were also observed in multiple non-Morquio LSDs. Therefore, caution is advised when interpreting elevated uKS levels.
Conflict of Interest declared.

P-032

Determination of S-adenosylmethionine and S-adenosylhomocysteine in human plasma using LC-MS/MS for differential diagnosis of hypermethioninemia

Kozłowski D A[1], Bogdańska A[1], Pajdowska M[1], Nowicki L[2]

[1]Dept Bioch, Child Mem Health Inst, Warsaw, Poland, [2]Perlan Technologies Polska Sp. z o.o., Warsaw, Poland

Background: S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) are transmethylation pathway intermediates that are clinically relevant to wide variety of disease conditions. Their plasma levels could be helpful in differential diagnosis of hypermethioninemia.
Objective: To develop and validate a stable isotope dilution liquid chromatography-mass spectrometry (LC-MS/MS) method for the quantification of SAM and SAH in human plasma.
Methods: Previously acidified plasma was spiked with deutered internal standard solution and then deproteinised by centrifugal ultrafiltration. 2 μL of filtrate was injected on Zorbax SB-Aq column and the analytes were separated using a gradient elution ion-pair reversed-phase high-performance liquid chromatography. Detection was performed using a triple quadrupole mass spectrometer operating in electrospray positive, MRM mode. Quantitation was performed using internal standard calibration.The analysis time remains relatively short with 20 min per sample. The assay was linear up to 2000 nM with the lower limit of quantitation (LLOQ) of 5 nM for both analytes. The precision (in terms of coefficient of variation) was 4.6 and 2.4% for SAM and SAH, respectively.
Conclusion: This metod fullfils all the regulatory requirements for specifity, linearity, LOQ, precision and recovery for the determination of SAM and SAH in human plasma.
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P-033

Validation of different blood collection methods for Fabry Disease screening

Pereira V G[1], Braga M C[1], Yamamoto J U S[1], Esteves A R[1], Martins A M[1], D'Almeida V[1]

[1]Univ Federal de Sao Paulo, Sao Paulo, Brazil

Background and objectives: The aim of this study was to compare two different types of blood collection and two different types of filter paper used for the analysis of alpha-galactosidase A activity as a screening tool for Fabry Disease diagnosis.
Methods: Samples from eighteen healthy volunteers were collected either by venipuncture into heparin tubes and spotted on Whatman® 903 and Whatman® FTA filter papers, or by a lancing device (BD Microtainer® Contact-Activated Lancet) and spotted on both filter papers. alpha-galactosidase A activity was determined through a fluorometric assay, routinely used in our lab for Fabry Disease screening and diagnosis.
Results: Intra-assay coefficients of variation were adequate for all samples (below 20%). Regardless of the blood collection method, alpha-galactosidase A activity on FTA paper was lower than those on 903; some samples showed activities below our normal range. Although mean values were statistically different (5.46±1.82 and 6.06±1.97 for venous puncture and lancet, respectively) depending on the collection method, alpha-galactosidase A activity on 903 remained inside our reference range.
Discussion/Conclusion: Both blood collection methods are suitable for Fabry screening, with DBS prepared on 903. The use of FTA for screening/diagnosis purposes would require determination of specific reference values.
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P-034

Metabolic investigations of intact fibroblasts by ^{1}H HR-MAS NMR Spectroscopy

Eggimann S[1], Diserens G[2], Hertig D[3], Vermathen M[3], Furrer J[3], Vermathen P[2], Nuoffer J M[1]

[1]Univ Inst of Clin Chem, Bern Univ Hosp, Bern, Switzerland, [2]Depts Radio and Clin Research, Univ Bern, Bern, Switzerland, [3]Dept of Chem & Biochem, Univ Bern, Bern, Switzerland

Background: Determination of small molecules in primary fibroblasts in cell culture (FB) may be clinically interesting. In order to use proton high-resolution magic-angle-spinning (^{1}H HR-MAS) NMR for metabolic profiling, the variability of metabolite estimation must first be established with a robust measurement protocol.
Methods: The variability of metabolites between control FB cell-lines (n=7), the effect of cell storage (fresh and frozen under cryoprotective conditions), and the viability during the measurement were examined. 1D-NMR spectra were acquired at 3kHz spinning rate at 279K.
Results: Approximately 30 small molecules were detected and analyzed. Overall metabolite variability between cell-lines excluding the aromatic region was 20%. Variability between fresh and frozen was generally similar to that between cell-lines. However, the variability of aromatic metabolites was significantly lower ($p < 0.0001$) between fresh/frozen pairs than between cell-lines. Fresh and frozen pairs could be discriminated by chemometric analysis where the main discriminative metabolites were lactate and proline. Cell viability decreased slowly during ^{1}H HR-MAS NMR-measurements (before: 95%, 2.5h: 74%; 4h: 66%, 10h: 46%).
Conclusion: The results demonstrate that ^{1}H HR-MAS NMR allows for non-destructive metabolic profiling of frozen FB and the protocol can be used to gain further insights in the metabolism of FB in cell culture.

P-035

Simultaneous measurement of glycolytic intermediates using liquid chromatography tandem mass spectrometry

Wang B T[1], Schmidt K[1], Okun J[1], Sauer S W[1]

[1]Inborn Error of Metab, Univ Hosp, Heidelberg, Germany

Background: Glycolysis is essential for maintaining cellular bioenergetics states in organisms. In addition to produce pyruvate, the synthesized glycolytic intermediates during glycolysis provide the source for the detoxification of reactive oxygen species in pentose phosphate pathway, the regulation of protein glycosylation post-modification in hexosamine biosynthesis pathway, and the biosynthesis of amino acids, lipid and nucleotides. The disturbance of glycolytic flux and mitochondrial bioenergetics has been indicated in several acquired and inborn diseases such as cancer and metabolic disorders.
Methods: Our study aimed to establish a method for simultaneous measurement of the main glycolytic intermediates by a liquid chromatography-tandem mass spectrometry. For multiple reaction monitoring (MRM) analysis, the precursor/product mass-to-charge ratio of glucose/fructose 6 phosphate, fructose 1,6 bisphosphate, glyceraldehyde-3-phosphate, 2-phosphoglycerate,

phosphoenolpyruvate, pyruvate, lactate and ribulose-5-phosphate were -258.7/-96.7, -338.9/-96.8, -169/-97, -84.8/-78.8, -166.7/-78.8, -86.8/-43.2, -88.7/-43 and -229/-97, respectively. Lactate-$^{13}C_3$ (m/z -89.9/-44.1) was used as internal standard.
Results: The standard curves of glycolytic intermediates were performed from 2 μM to 25 μM. The impression (CV%) of the metabolites were < 10%. The established method is suitable for different sample matrices such as body fluids, cell lysates and organ homogenates.
Conclusion: Our study provides a fast and convenient method to study glucose metabolism on high-throughput scale.

P-036

Comparison of tandem mass spectrometry and amino acid analyzer for phenylalanine and tyrosine monitoring–implications for clinical management of patients with hyperphenylalaninemia

Groselj U[1], Murko S[1], Zerjav Tansek M[1], Kovac J[1], Trampus Bakija A[1], Repic Lampret B[1], Battelino T[1]

[1]University Children's Hospital Ljubljana, Ljubljana, Slovenia

Background: We aimed to compare the tandem mass spectrometry (MS/MS) and the amino acid analyzer (AAA) as methods to measure blood Phe and Tyr levels and Phe/Tyr ratio.
Methods: Venous blood samples were collected for the AAA analysis, using Pinnacle PCX (Pickering Laboratories), with HPLC Series 1200 (Agilent). Capillary blood was spotted directly on filter paper (Whatman 903) for the MS/MS analysis, using 3200 QTrap AB SCIEX and Perkin Elmer Series 200 HPLC system. The Bland-Altman test was used to compare agreement between the methods and Pearson correlation coefficient to assess the association between the methods.
Results: 207 pairs of measurements were performed. The Phe levels (range 0-2500μM) obtained by the MS/MS were on average 26.1% (SD 13.9%) lower compared to those obtained by the AAA. The Tyr levels by the MS/MS were on average 15.5% (SD 20.6%) lower. The Phe/Tyr ratio by the MS/MS was on average 10.6% (SD 15.9%) lower.
Conclusions: Due to the considerable inter-assay variability, a single method is preferable for long-term follow-up of patients. When using MS/MS, on average 26% lower blood Phe levels were obtained as compared to the AAA. The guidelines on HPA management should take into consideration the differences in laboratory methods.

P-037

Performance of a 6-plex enzyme assay for the diagnosis of Gaucher disease, Fabry disease, Krabbe disease, Niemann-Pick disease A/B, Pompe disease and mucopolysaccharidosis type I from dried blood spots

Gelb M H[2], Ranieri E[1], Stark S[1]

[1]SA Pathology, Women's Children's Hosp, Adelaide, Australia, [2]Dept Chemistry, Univ Washington, Seattle, United States

Introduction: Lysosomal storage disorders (LSDs) are inherited disorders caused by the deficiency of specific enzymes within the lysosome. The lysosome is unable to break down certain lipids, glycoproteins or mucopolysaccharides, which results in accumulation, cell dysfunction and often death without early intervention. Early detection and treatment (e.g. enzyme replacement therapy) can greatly increase the chances of survival and life expectancy. Therefore, newborn screening is becoming an important approach for early diagnosis of these disorders.
Methods: Single 3.2 mm blood spots from normal controls and patients with diagnosed LSDs were incubated with a substrate/internal standard mixture, quenched and analysed using a LC-MSMS system. Enzyme activity was determined by measuring product formation relative to internal standard concentration.
Results: Using dried blood spots, the 6-plex assay demonstrates similar diagnostic capacity compared to leucocyte-based assays currently performed in the National Referral Laboratory to diagnose Gaucher disease, Fabry disease, Krabbe disease, Niemann-Pick disease A/B, Pompe disease and mucopolysaccharidosis type I.
Conclusion: The simplicity and accuracy of 6-plex enzyme assay provides a valuable alternative to the labour intensive leucocyte-based assays used to diagnose these LSDs and could be implemented by laboratories performing newborn screening for these disorders.
Conflict of Interest declared.

P-038

Urine sepiapterin excretion as a new diagnostic marker for sepiapterin reductase deficiency

Carducci C L[1], Santagata S[1], Friedman J[2], Pasquini E[3], Tolve M[1], Carducci C A[1], Angeloni A[4], Leuzzi V[5]

[1]Dep Exp Medicine, Univ of Rome Sapienza, Rome, Italy, [2]Dep Neur Ped, Univ of San Diego, San Diego CA, United

States, [3]Metab Unit, Meyer Children's Hospital, Florence, Italy, [4]Dep Mol Med, Univ of Rome Sapienza, Rome, Italy, [5]Dep Ped Neurol, Univ of Rome Sapienza, Rome, Italy

Background: Sepiapterin reductase deficiency (SRD) is an inherited disorder of tetrahydrobiopterin biosynthesis which results in early onset movement disorders and intellectual disability. While potentially treatable, the disorder is under- and late-diagnosed given the nonspecific clinical presentation and the invasive diagnostic procedures, which implies the determination of pterins and neurotransmitter metabolites in CSF.
Methods: We set up a reliable method for the determination of Sp in urine by HPLC. The separation was accomplished in 9 minutes, the within-run and between-run CVs were all less than 4.1%, the response was linear over the range of 100-2000 nmol/L, and the mean recovery was 96%. We analyzed urine from four patients affected by SRD, four carriers of *SR* mutations, and 43 normal subjects.
Results: In urine of patients Sp exceeded more than 5 times the values of controls and was not influenced by the patients' age and treatment. No altered Sp excretion was found in the urine from four heterozygotes.
Conclusion: This test may be proposed as a first line diagnostic tool for patients presenting with early onset development delay and/or movement disorders. The increase of Sp in the urine of the SRD subjects opens new questions on the role of SR in BH_4 homeostasis.

P-039

Development of a LC-MS global metabolomics method for dried blood spots

Østeby A[1], Skogvold H[1], Arnesen C[1], Rowe A[1], Rootwelt H[1], Woldseth B[1], Sæves I[2], Elgstøen K[1]

[1]Dept Med Biochem Oslo Univ. Hospital, Oslo, Norway, [2]Neonatal Screening Oslo Univ. Hospital, Oslo, Norway

Background and objectives: Global metabolomics is the analysis of all metabolites in a biological sample like blood or urine. We have previously developed a LC-MS QTOF metabolomics method for urine. Dried blood spot (DBS) is used in newborn screening and also increasingly in biochemical diagnostics. The aim of the present work was to optimize the method for analysis of the DBS-metabolome.
Materials and Methods: The method was developed using DBS from controls and a patient on known medication. 1-6 punches were analyzed using different extraction procedures and metabolites separated on a C18-ether column coupled to an Agilent 6520 Q-TOF.
Results: Optimal extraction was achieved using 80% methanol + 0,1% formic acid, heating for 45 min, centrifugation at 45°C at 700 rpm, followed by evaporation to dryness and reconstitution in 5% methanol +0,1% formic acid. The number of molecular features (MF) found on the QTOF was optimal using 4 punches (typically 2000 MF). The known drug metabolites were identified in the patient sample. Reproducibility for selected metabolites was 5-15%.
Conclusion: Using our method about 2000 MF in DBS can be extracted and analyzed. Future work includes use of MS with higher mass resolution and sensitivity and analysis of DBS from patients with known IEMs.

03. Newborn screening

P-040

Newborn screening for lysosomal storage diseases using tandem mass spectrometry

Gelb M H[1], Scott C R[1], Turecek F[1], Spacil Z[1], Yi F[1], Masi S[1], Kumar A[1], Chennamaneni N[1]

[1]Univ. of Washington, Seattle, United States

Background: We are developing tandem mass spectrometry assays for several lysosomal storage diseases (LSDs).
Methods: Synthetic chemistry and tandem mass spectrometry are used to develop novel multiplex assays of lysosomal enzymes. We are also conducting pilot studies in the WA newborn screening lab to test the ability of the assays to detected LSD newborns.
Results: We have developed assays for Gaucher, Niemann-Pick-A/B, Fabry, Pompe, Krabbe, MPS-I, -II, -IIIA, -IIIB, -IVA, -VI, and -VII. The assays greatly outperform fluorimetric assays with 4MU-substrates in terms of analytical range and reduction of false positives. The assays are simple to execute, inexpensive, and highly robust.
Conclusions: We have developed new tandem mass spectrometry assays of several lysosomal storage diseases. We will report the results of large scale pilot studies in a newborn screening lab (up to 100,000 dried blood spots) and compare the results to those obtained with fluorimetric assays. We will also report on the commercialization of the assays and worldwide distribution of reagents.
Conflict of Interest declared.

P-041

Potential pitfall in screening for argininosuccinic acidemia by tandem mass spectrometry (MS/MS)

Lim J S[3], Goh D L M[2], Poh S[3], Jamuar S S[1], Tan E S[1]

[1]Div Paed Genetic Serv, KK Hospital, Singapore, Singapore, [2]Dept Paed, National University Hospital, Singapore, Singapore, [3]DPLM, BG/NENS Lab KK Hospital, Singapore, Singapore

Background (Case report): We report two neonates with argininosuccinic acidemia (ASA) detected in newborn screening by MS/MS. Citrulline levels were within the reference range but their argininosuccinic acid levels were abnormally raised. Confirmation of diagnosis was achieved through enzyme assay.
Methods: Blood collected on a Guthrie card at 24-72 hours of life is processed and analyzed by MS/MS. Abnormal levels of citrulline can identify newborns at risk of having citrullinemia type I and ASA. Increased specificity for ASA can be achieved by including argininosuccinic acid as a marker.
Results: Neonate A: Citrulline=21 μmol/L (normal< 40), Cit/Phe = 0.41 (normal< 0.55), Cit/Arg = 7.0 (normal < 5.0), ASA=5.06 μmol/L, (normal< 1.3).Neonate B: Citrulline=13μmol/L, Cit/Phe = 0.29, Cit/Arg = 1.3, ASA=3.61 μmol/L.
Discussion: Both neonates were clinically well at admission for follow-up investigations. Plasma citrulline levels were within reference range but argininosuccinic acid in the urine were elevated (883 and 216 μmol/L for neonate A and B respectively). Argininosuccinate lyase activity tests showed 0.0 and 0.5 μmol/hr/gm Hb (normal 3-13) for neonate A and B respectively.
Conclusion: Neonates with ASA may screen negative if citrulline and its related ratios were the only markers used. Inclusion of argininosuccinic acid as a marker could correct potential pitfall.

P-042

Second-tier LC-MS/MS analysis using dried blood spots of C5-OH-acylcarnitine-positive cases in newborn screening and high-risk screening

Shigematsu Y[1], Hata I[1], Nakajima H[2], Tajima G[4], Bijarnia-Mahay S[3], Puri R D[3], Saxena R[3], Verma J[3], Verma I C[3]

[1]Dep Pediat, Univ Fukui, Fukui, Japan, [2]Scr Lab, Nat Res Ins Child Health Dev, Tokyo, Japan, [3]Center Med Genet, Sir Ganga Ram Hosp, New Delhi, India, [4]Dep Pediatr, Hiroshima Univ, Hiroshima, Japan

Background: In newborn screening or high-risk screening for symptomatic infants using tandem mass spectrometer (MS/MS), increased levels of C5-OH acylcarnitines in dried blood spots (DBS) suggest several organic acidemias or biotin deficiency, and additional analysis of organic acids in urine is necessary for chemical diagnosis, as usual. Thus, for quick differential diagnosis using the DBS, LC-MS/MS methods to determine the marker metabolites have been developed.
Methods: Using AB Sciex API-4000 LC-MS/MS equipped with an Imtakt multi-mode column and stable-isotope dilution methods, short-chain acylcarnitines, acylglycines, and organic acids in DBS were analyzed.
Results: In 3-methylcrotonyl-CoA carboxylase deficiency, increased levels of 3-methylcrotonylglycine (3MCG) and 3-hydroxyisovaleric acid (3HIVA), in HMG-CoA lyase deficiency, those of 3-hydroxy-3-methylglutaric acid and 3HIVA, in beta-ketothiolase deficiency, those of tiglylcarnitine and tiglylglycine, and in multiple carboxylase deficiency, those of propionylglycine, 3MCG and 3HIVA were characteristic, respectively.
Conclusion: The present methods are very useful for quick diagnosis for C5-OH-positive cases with typical organic acidemias. Further investigations are needed for increased C5-OH cases with biotin-deficiency in preterm babies or those with maternal 3-methylcrotonyl-CoA carboxylase deficiency in newborn screening, and cases with mildly elevated C5-OH in the DBS from foreign countries for high-risk screening.

P-043

A novel *ETHE1* mutation identified in a First Nations Canadian patient, ascertained following a positive newborn screen for isovaleric acidemia

Sosova I[1], Jackson S[2], Reeves M[2], Bamforth F[1], Rinaldo P[3], Sinasac D[2 5], Casey R[2 4 5], Al-Hertani W[2 4 5]

[1]Dept Lab Med and Path, Univ of Alberta, Edmonton, Canada, [2]Alberta Children's Hospital, Calgary, Canada, [3]Dept Lab Med and Path, Mayo clinic, Rochester, United States, [4]Dept of Peds, Univ of Calgary, Calgary, Canada, [5]Dept of Medical Genet, Univ of Calgary, Calgary, Canada

Background (Case Report): a male child born to consanguineous First Nations parents was referred for a positive newborn screen for isovaleric acidemia (IVA). At 1.5 months of age, the patient was admitted to ICU for poor feeding, necessitating nasogastric feeds. He was subsequently re-admitted at 4 months with tachypnea and lactic acidosis, and passed away 3 days later.
Results: Initial investigations revealed: markedly elevated urine ethylmalonic (EMA) and glutaric (GA) acids; elevated urine methylsuccinic (MSA), 2-hydroxyglutaric, malic and fumaric acids, and butyryl- and isovalerylglycines; and mildly elevated plasma C4 and C5 acylcarnitines. Newborn screen dried blood spots were also analyzed at the Mayo Clinic and showed significantly elevated EMA with elevations of GA and MSA. A working diagnosis of glutaric aciduria type II was suspected but not

confirmed by the time of death. Subsequent sequencing of the *ETHE1* gene revealed a novel homozygous c.423delC frameshift mutation.
Discussion: We report a novel *ETHE1* mutation in the first case of EE reported in a patient of First Nation ancestry. Furthermore, the false positive newborn screen for IVA in this case, raises the question whether other patients with EE may be ascertained through false positive C5 newborn screens.

P-044

Early developmental outcome in glutaric aciduria type 1 following diagnosis on UK newborn screening

Hart A R[1], Bonham J[1], Yap S[1]

[1]Sheffield Children's Hospital, Sheffield, United Kingdom

Background: UK newborn screening was extended to include GA1 in January 2015. Presymptomatic diagnosis and treatment aims to improve developmental outcome. Long-term developmental data is limited. We present data from 3 UK children.
Methods: Three children with GA1 were identified on UK newborn screening in our region. All children were referred to a metabolic team and commenced on appropriate treatment. Developmental assessment was performed between 14 and 23 months of age.
Results: One child experienced metabolic decompensation with MRI abnormalities. He developed four-limb dystonia and cognitive, motor and language scores below 2 standard deviations. Two children remained well on treatment. One had composite scores of 80 for cognition, 60 for language and 85 for motor skills. Receptive communication was particularly affected. The second child had composite scores of 100 for cognition, 89 for language and 107 for motor skills.
Conclusions: Children with GA1 can have normal developmental scores if treated appropriately, but decompensation and dystonia are not precluded. Early language skills may be disproportionately lower than other developmental areas. A better understanding of the long-term effects of GA1, markers for poor developmental outcome, and alternative treatment strategies to improve outcome are required.

P-045

Follow up of B_{12} deficient infants detected through the newborn screening program of Catalonia

Navarro-Sastre A[1], López R M[1], Arranz J A[3], Artuch R[2], Del Toro M[4], García-Villoria J[1], Ormazabal A[2], Pajares S[1], García-Cazorla A[2], Meavilla S[2], Castejón E[2], Marín J L[1], Ribes A[1]

[1]ECM-BGM-Hospital Clínic, IDIBAPS, Barcelona, Spain, [2]In Err Metab Unit and CIBERER, HSDJ, Barcelona, Spain, [3]Lab Metab Dis, Hospital Vall d'Hebrón, Barcelona, Spain, [4]Metab Unit, Hospital Vall d'Hebrón, Barcelona, Spain

Background: Maternal vitamin B_{12} deficiency is the most common cause of newborn vitamin B_{12} deficiency and leads to the accumulation of the main metabolites used in the detection of propionic and methylmalonic acidemias in newborn screening programs. These metabolites are altered in B_{12} deficient newborns and prompted us to study and follow up all these patients.
Methods: A total of 1200 samples of 132370 newborns showed elevation of C3, C3/C2, C3/Met or C4DC. A second sample of blood and urine dried on filter paper was requested and analyzed.
Results: The organic acid analysis of 33 urines showed elevation of methylmalonic acid and patients were referred to the hospital for further studies. Methylmalonate levels during admission normalized completely in 4 newborns, but remained elevated in 28 (9.9-1382 mmol/mol creatinine). Most of them normalized after B_{12} treatment except for one patient, whose levels of methylmalonate never normalized (184-1053 mmol/mol creatinine-molecular studies ongoing). One newborn was diagnosed of cblC deficiency, with methylmalonate levels in urine of 808 mmol/mol creatinine.
Conclusions: The results obtained in this study reveal the importance of the detection and treatment of B_{12} deficient infants, whose methylmalonate levels may be similar or even surpass the levels of newborns affected by a genetic methylmalonic aciduria.

P-046

Role of expanded newborn screening in post-mortem diagnosis of three cases of glutaric aciduria type 2

Maines E[1], Vincenzi M[2], Camilot M[2], Teofoli F[2], Ionpopa F[2], Wanders R J A[3], Waterham H R[3], Funghini S[8], Burlina A B[4], Ghizzi C[5], Zaglia F[6], Degani D[1], Lauriola S[1], Zaffanello M[1], Ganzarolli S[1], Dianin A[1], Lamantea E[7], Ghezzi D[7], Bordugo A[1]

[1]Inherit Metab Dis Unit, Dept of Ped, Verona, Italy, [2]Newborn Screening Centre, Dept of Ped, Verona, Italy, [3]Lab Gen Metab Dis, Univ of Amsterdam, Amsterdam, Netherlands, [4]Inherit Metab Dis Div, Dept of Ped, Padua, Italy, [5]Ped Int Care Unit, Hospital of Verona, Verona, Italy, [6]Ped and Neonat Unit, Hospital of Legnago, Verona, Italy, [7]Mol Neurogen Unit,

Besta Neur Found Inst, Milan, Italy, [8]Newborn Screening Lab, Meyer Hospital, Florence, Italy

Background: Glutaric aciduria type 2 (GAII; OMIM#231680) is caused by defects in the electron-transfer flavoprotein (ETF) or its electron acceptor, ETF dehydrogenase (ETF-QO). The clinical phenotype is heterogeneous and inherited as autosomal recessive trait. Expanded newborn screening (ENS) has provided the possibility to detect GAII in asymptomatic newborns, allowing to start a prompt treatment. Here we describe a case of GAII, identified by ENS, who allowed the definition of the cause of death of three unscreened siblings.
Case Report: The proband is the fifth born of consanguineous parents, three siblings died during the first year of life for undiagnosed progressive encephalomyopathy. A newborn screening sample obtained at 48 h of age was positive for GAII. Urine acid organic analysis and plasma acylcarnitine profile confirmed the diagnosis. The molecular analysis performed in the DNAs of proband and of two of his affected siblings stored in biobank revealed the homozygous predicted damaging missense mutation c.1387G>C of *ETFDH* gene affecting an highly conserved aminoacid residue (p.Gly463Arg).
Conclusions: Our experience stresses the importance of collecting tissue/blood samples before death when a genetic basis of disease is suspected and highlights the utility of ENS program to help the diagnosis in some familial unexplained cases.

P-047

Differential diagnoses of hypergalactosemia by newborn screening: Value of the Gal-1-P/Gal ratio and the serum total bile acid concentration in detecting congenital portosystemic shunts

Matsuoka R[1 3], Kubota M[1], Mochizuki H[2], Kagimoto S[1], Ida H[3]

[1]Div General Pediatr, Saitama Child Hosp, Saitama, Japan, [2]Div Metab & Endo, Saitama Child Hosp, Saitama, Japan, [3]Dept of Pediatr, Jikei Univ Med, Tokyo, Japan

Background: Hypergalactosemia determined by newborn screening (NBS) has many differential diagnoses; however, congenital portosystemic shunts (CPSS) are at times especially difficult to detect.
Objective: To detect CPSS based on the galactose (Gal) and galactose-1-phosphate (Gal-1-P) levels determined by NBS and the total bile acid (TBA) concentration at first close investigation.
Methods: We reviewed the medical records of patients with hypergalactosemia determined by NBS who visited the Saitama Children's Medical Center between 2001 and 2015.
Results: In total, 53 patients visited our department, of which 9 were diagnosed with CPSS, 3 with neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD), 7 with galactosemia type III, and 34 with transient galactosemia. For CPSS diagnosis, sensitivity of 57.1% and specificity of 97.4% were obtained by assuming a Gal-1-P/Gal ratio less than or equal to 1, and a TBA concentration of more than 20 μmol/l. Unfortunately, NICCD showed similar values for these tested parameters.
Conclusion: CPSS can be detected by abdominal imaging studies; but is sometimes difficult to detected at the first examination. When a patient with hypergalactosemia showed the index and no shunts are detected, they may need to be evaluated repeatedly on several occasions.

P-048

Lysosomal acid lipase activity in dried blood spots; from newborns to adults

Jans J J[1], Van Aalderen M[1], Schielen P C[2], Prinsen H C M[1], Verhoeven-Duif N M[1], Sain-van der velden M[1], Van Doorn J[1]

[1]Dep Med Genet UMC, Utrecht, Netherlands, [2]Natl Inst Pub Health Env (RIVM), Bilthoven, Netherlands

Background: Cholesteryl ester storage disease (CESD) is caused by a deficiency of lysosomal acid lipase (LAL). Since treatment is available, early diagnosis is desirable. Little is known about reference values. We established reference values for newborns of 3-5 days old and compared the LAL activity to that of older subjects.
Methods: A method using the fluorimetric substrate 4-methylumbelliferyl-palmitate was adapted based on the methods of Hamilton et al. (2012) and Dairaku et al., (2014).
Results: The fluorescence intensity was unstable after termination of the reaction and increased non-linearly with time (after 15 min: ~ 38%). Intra- and interassay CV's were 6.4 and 13%, respectively. While heparin as anticoagulant did affect LAL activity, EDTA did not. The mean reference value of LAL activity in DBS from subjects aged 1-65 yr (174 μmol/h.L, n=38) and from newborns (132 μmol/h.L, n=50) were significantly different ($p < 0.05$). LAL activities for CESD patients (n=7) were between 0 and 3.7 μmol/h.L blood.
Conclusion: Fluorescence should be measured immediately after terminating the reaction. LAL activities in DBS of newborns of 3-5 days old are significantly lower compared to older subjects, implying the need for age-related reference values.

P-049

Newborn screening for fatty acid oxidation disorders: effects on population frequency and clinical outcome in the Czech Republic (CZ)

Jesina P[1], Chrastina P[1], Pavlikova M[1], Hruba E[1], Vlaskova H[1], Honzik T[1 2], Sokolova J[1], Hnizda A[1], Smolka V[3], Friedecky D[4], Adam T[4], Kozich V[1]

[1]Inst Inher Met Dis, Charles Univ, Prague, Czech Republic, [2]Dept Pediatr, Gen Fac Hosp, Charles Univ, Prague, Czech Republic, [3]Dept Pediatr, Univ Hosp, Olomouc, Czech Republic, [4]Dept Clin Biochem, Univ Hosp, Olomouc, Czech Republic

Background: Newborn screening (NBS) in CZ was expanded from three to 13 disorders in 10/2009 and tests also for the mediumchain acyl-CoAdehydrogenase (MCADD) and longchain 3-hydroxyacyl-CoAdehydrogenase deficiency (LCHADD). We examined the effect of NBS on detection rate and clinical outcome compared to the pre-NBS period.
Patients/Methods: In the pre-NBS cohort 21 MCADD and 12 LCHADD patients were ascertained on clinical basis and followed for 236 and 73 patient-years, respectively. In the NBS cohort 29 MCADD and 10 LCHADD patients were detected among 661,000 newborns with follow-up of 74 and 26 patient-years, respectively. A severity scoring index (SSI) was developed to assess the clinical outcome.
Results: The NBS increased significantly the frequency of ascertained patients with MCADD from 1:211,300 to 1:22,800 and with LCHADD from 1:141,300 to 1:66, 100. A total of 12 and 8 variants in the *ACADM* and *HADHA* genes were detected and different spectrum of mutations was observed between the NBS and clinically ascertained cohorts. The age-adjusted clinical SSI in the pre-NBS cohort of MCADD (median 0.8 versus 0.0) and LCHADD (median 3.5 versus 0.4) were significantly higher compared to NBS cohort.
Conclusions: Five years of NBS for MCADD and LCHADD in CZ significantly increased detection rate and improved clinical outcome in patients.
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P-050

Myopathic form of carnitine palmitoyl transferase type II deficiency detected by newborn screening

Tangeraas T[1], Hoff Backe P[2], Woldseth B[1], Gaup H J[1], Navarrete D[1], Stray-Pedersen A[1], Jorgensen J V[1], Lilje R[3], Pettersen R[1]

[1]NBS and Lab Diag of IEM, Oslo Univ Hosp, Oslo, Norway, [2]Dept of Med Biochem Univ of Oslo, Oslo, Norway, [3]Dept.of Paed Med Oslo Univ Hosp, Oslo, Norway

Background: In Norway, newborn screening (NBS) was expanded from 2 to 23 conditions in March 2012, including carnitine palmitoyl transferase-type II deficiency (CPT-II). However, the detection of myopathic form of CPT-II by NBS, has been questioned.
Methods: Acylcarnitines were quantified using LC-MS/MS. CPT-II activity was measured in leukocytes using palmitoyl-^{3}H-carnitine as substrate. DNA analysis was performed by PCR amplification and sequence analysis of all CPT-II coding exons/exon-intron junctions. The CPT-II protein was investigated by 3D- structural analysis.
Results: Of 180,000 babies screened, n=3 male newborns were flagged with elevated C16+C18:1/C2; median 1.4 (1.27-1.45, cut-off < 0.52). C16 was median 4.9 μmol/l (4.6-5.3,cut-off < 5.5μmol/l). Long-chain plasma acylcarnitines were elevated in all three cases. CPT-II enzyme analysis in leukocytes from the patients revealed severely reduced activity (0.1-0.4 nmol/mg prot./min) compared to healthy adult controls (reference ranges 7.0-17.2). However this assay does not discriminate between myopathic and severe forms of CPT-II deficiency. DNA analysis (filtercards) showed at least one allele associated with a myopathic phenotype; p.Pro50His/p.Pro50His, p.Pro50His/p.Lys457X, p.Ser113Leu/p.Thr482Trpfs*49, respectively.
Conclusion: Based on the mutational findings and hitherto asymptomatic clinical course of the children, we conclude that myopathic CPT-II deficiency may be detected by NBS. C16+C18:1/C2 may be the only informative marker in the bloodspot.

P-051

Inborn errors of metabolism causing Reye syndrome and sudden infant death syndrome: a systematic review with implications for population neonatal screening programs

Van Rijt W J[1], Koolhaas G D[2], Bekhof J[2], Heiner-Fokkema M R[3], De Koning T J[1], Visser G[4], Schielen P C[5], Van Spronsen F J[1], Derks T G J[1]

[1]Sect Metab Dis, Beatrix Child Hosp, Groningen, Netherlands, [2]Princess Amalia Child Clin, Isala Zwolle, Zwolle, Netherlands, [3]Lab Metab Dis, Dept Lab Med, Groningen, Netherlands, [4]Dept Metab Dis, Wilhelmina Child Hosp, Utrecht, Netherlands, [5]Lab Infect Dis and Screening, RIVM, Bilthoven, Netherlands

Background: Many inborn errors of metabolism (IEM) may present as either Reye syndrome (RS) or sudden infant death

syndrome (SIDS). Nowadays many patients are identified presymptomatically by population newborn screening (NBS) programs. Some patients escape early detection because their symptoms and signs start before NBS test results become available.
Methods: A comprehensive systematic literature review to identify all IEMs associated with RS and SIDS, including their treatability and detectability by NBS technologies.
Results: 42 IEMs were identified in the literature that were associated with either RS or SIDS of which (a) 25 can already present during the neonatal period, (b) treatment is available for at least 32, and (c) 24 can be identified by analysis of acylcarnitines and amino acids in dried bloodspots.
Discussion: We advocate extensive dried blood spot analysis of acylcarnitines and amino acids as a minimal recommended test after both RS and each (unexpected) neonatal death, especially when a dried blood spot for the NBS program has not yet been drawn.

P-052

The use of tandem mass spectrometry (MSMS) based method for determining enzyme activities for six lysosomal storage diseases (LSD) from a single dried blood-spots for use in newborn screening

Ranieri E[1], Stark S[1], Fletcher J M[1], Gelb M H[2]

[1]Genetics & Molecular Path., SAPathology, Adelaide, Australia, [2]Dept of Chemistry, Univ Washington, Washington, United States

Background: The use of an MSMS method for measuring the activity of six LSD enzymes from a single 3mm dried blood spot (DBS) for newborn screening. Included are; galactocerebroside β-galactosidase (GALC, Krabbe disease), acid α-galactosidase A (GLA, Fabry disease), acid sphingomyelinase (ASM, Niemann Pick A/B disease), acid α-glucosidase (GAA, Pompe disease), α-L-iduronidase (IDUA, mucopolysaccharidosis type I and acid β-glucocerebrosidase (ABG, Gaucher disease).
Methods: De-identified DBS were sourced from the SA newborn population (n~1,000) and DBS from positive LSD cases (n=75) obtained from the National Referral Laboratory (NRL); GALC (5), GLA and carriers (14 & 28), ASM A/B (1), IDUA (6), GAA (20) or ABG (20). LSD reagents were obtained from PerkinElmer (Waltham, MA, USA). Enzyme activities were determined against stable isotopes by MRM after a single organic solvent extraction. Analysis by FIA-API5000 with settings: IS voltage 5000, DP 95, CE 55 and CXP 15
Results and discussion: The 1st centile determined from 592 DBS as IU/hr/L wb of ABG 2.2, IDUA 1.3, GAA 4.1, ASM 0.8, GALC 1.8 and GLA 1.3. All LSD cases had activities below the 1st centile, GLA showed some overlap, due to in-source fragmentation, but not seen on API4000/3200 instruments. Each assay showed > 3 orders of magnitude in dynamic range with excellent lower end sensitivity.

P-053

High incidence of heterozygotes of Pompe disease: 2-year experience of newborn screening in Japan

Momosaki K[1], Yoshida S[1 2], Matsumoto S[1], Nakamura K[1], Tasaki R[2], Okumiya T[3], Endo F[1]

[1]Dept Pediat, Kumamoto University, Kumamoto, Japan, [2]Kaketsuken, Research Institute, Kumamoto city, Japan, [3]Analytical Biochemistry, Kumamoto Univ., Kumamoto city, Japan

Background: We started population-based newborn screening (NBS) of Pompe disease in Apr 2013. We also examined the incidence of *GAA* mutation among newborns with low enzyme activities.
Method: We screened 31,274 DBSs from the NBS program in Kumamoto, Japan from April 2013 to January 2015. We assayed acid α-glucosidase (AαGlu) activity in DBS with two different methods; 4-methylumbelliferone (4-MU) method and Ba/Zn method. Definitive diagnosis was done by the measurement of AαGlu activity in fibroblasts and *GAA* mutation analysis using next-generation sequencing technology. Genetic analysis was also performed by Sanger methods.
Results: A total of 91 (0.29%) newborns were recalled for second DBSs with low enzyme activity. AαGlu activity in fibroblasts, carried out for 15 samples with abnormal low enzyme activity (GAA< 4.0), revealed 8 subjects with remarkable low GAA activity at levels seen in patients. 6 of 8 subjects had heterozygous mutations; 4 pathogenic mutations and 2 new mutations.
There were no subjects with 2 independent pathogenic mutations. All 8 screening positive newborns were asymptomatic.
Conclusions: High incidence of heterozygotes of *GAA* mutation among newborns with low enzyme activity was revealed. We need to investigate genotype-phenotype correlation and effect of pseudodeficiency allele on GAA activity.

P-054

Identification of *G6PD* common mutations using a multiplex primer extension-based method

Chiu Y H[1], Liu Y N[1], Chang Y C[2], Liu T T[1 3], Hsiao K J[1 4]

[1]Dept Edu Res, Taipei City Hosp, Taipei, Taiwan, [2]Excelsior Pharmatech Labs, Taipei, Taiwan, [3]VYM Genome Res Center, Natl Yang-Ming, Taipei, Taiwan, [4]Prev Med Found, Taipei, Taiwan

Background: Glucose-6-phosphate dehydrogenase (G6PD, E.C. 1.1.1.49; gene symbol: *G6PD*) deficiency is highly prevalent in Southeast Asians and may lead to extreme hyperbilirubinemia and/or life-threatening bilirubin encephalopathy. Neonatal screening for G6PD deficiency has long been established to provide important information for genetic counseling and to prevent the occurrence of hemolytic crisis in affected populations. However, heterozygous females are difficult to be identified reliably because their enzyme activities may range from deficiency to normal.
Methods: A multiplex polymerase chain reaction coupled with a single-base primer extension method was applied to detect 22 *G6PD* mutations found in Taiwanese population using dried blood spots of 483 male patients.
Results: Four hundred and fifty out of 484 mutant alleles (93.0%) were determined in our assay, among which the c.1376G>T and c.1388G>A mutations of *G6PD* gene were the most frequent ones comprising 47.9% and 21.1% of the mutated alleles, respectively.
Conclusion & Discussion: Our assay is a rapid and precise method for detecting known *G6PD* mutations and can be directly applied to dried blood spot used in newborn screening. This method may be useful to detect female carrier of the border-line G6PD activity with 93% of detection sensitivity in Taiwan.

P-055

Pilot newborn screening for lysosomal disorder by tandem mass spectrometry based method

Polo G[1], Furlan F[1], Kolamunnage T B[1], Del Rizzo M[1], Celato A[1], Giordano L[1], Burlina A P[2], Burlina A B[1]

[1]Div Metab Dis, Dep Ped, Padua Univ Hosp, Padua, Italy, [2]Neurol Unit, St. Bassiano Hospital, Bassano del Grappa, Italy

Background: Several lysosomal storage disorders (LSD) have been recently proposed for inclusion in screening programs because of increasing availability of treatment options and relative prevalence of these conditions. The primary objective of this study was to evaluate the feasibility of a screening population for Pompe disease, Fabry disease, Gaucher disease and MPS I disease using a LC-MS/MS based method.
Methods: Enzyme activities of α-galactosidaseA, α-glucosidase, α-L-iduronidase, β-glucocerebrosidase were determined in dried blood spot (DBS) by stable isotope dilution flow injection analysis MS/MS(FIA-MS/MS) using Perkin Elmer Six-Plex LSD reagents kit.
Results: The performance of each LSD enzyme assay was determined from repeated measurement of 4 levels CDC quality controls (CV intra and inter assay < 15%). Reference ranges for each enzyme were defined from analysis of healthy newborn DBS (n=1000). Enzyme activity in DBS from patients affected by the above mentioned disease were used to have a low enzyme activity in order to test specificity and sensitivity.
Conclusions: The results of this study showed that Perkin Elmer LSD kit perform well on DBS screening allowing simultaneous detection of the 4 enzymes tested. The method was able to distinguish LSD patients from the normal population.

P-056

Free carnitine (FC) levels pattern in a group of very low birth weight newborns identified by a regional screening program

Righi B[1], Bettocchi I[1], Righetti F[1], Baronio F[1], Incorvaia L[1], Vitali A[1], Pession A[1], Cassio A[1]

[1]Dept of Paed, Univ Bologna, Bologna, Italy

Background: There are few and controversial data about the pattern of FC levels measured on dried blood spot (DBS) samples in very low birth weight neonates (VLBW), who were at risk for carnitine depletion compared to full-term neonates.
Methods: In our regional screening program the collection of DBS samples is recommended at 48-72 hours of life. Additional specimens are routinely collected in VLBW (BW < 1800gr) on days 14 and 30. We evaluated 156 VLBW divided into two groups according to FC levels ≥ (Group 1) or < (Group 2) cut-off value on the third sample and 84 neonates with BW > 1800gr (Group 3).
Results: Birth weight (BW) and gestational age (GA) were significantly lower in group 2 than in groups 1 and 3. On day 14, FC levels decreased significantly both in group 1 and 2; on day 30 only in Group 1 there was a significant increase of these levels. In Group 2, newborns with 23-27 weeks GA and BW < 1000gr showed FC levels on day 30 significantly lower than newborns with greater GA and BW.
Conclusions: We identified a subgroup of VLBW particularly at risk for carnitine depletion, whose outcome might be improved by precocious carnitine supplementation.

P-057

The expanded newborn screening in Estonia using tandem mass-spectrometry

Reinson K[1 2], Künnapas K[1], Kriisa A[1], Vals M - A[1], Muru K[1], Ounap K[1 2]

[1]Tartu Univ Hosp, Dep of Genetics, Tartu, Estonia, [2]Univ of Tartu, Dep of Pediatrics, Tartu, Estonia

Background: Expanded newborn screening using tandem mass-spectrometry allows to screen for several metabolic disorders and this is introduced already in many developed countries.
Methods: Since the beginning of 2014, in addition to congenital hypothyroidism and phenylketonuria, 18 new treatable congenital metabolic diseases were included in the Estonian newborn screening program (maple syrup urine disease, tyrosinemia type I, homocystinuria, argininemia, citrullinemia type I, isovaleric aciduria, methylmalonic aciduria, propionic aciduria, glutaric aciduria type I and II, carnitine uptake defect, and vitamin B_{12}, medium-chain acyl-CoA dehydrogenase, long-chain 3-hydroxy acyl-CoA dehydrogenase, very long-chain acyl-CoA dehydrogenase, carnitine-acylcarnitine translocase andcarnitine palmitoyltransferase I and II deficiency).
Results: During 15 months, we screened 16,938 newborns, and confirmed diagnosis in nine patients. After introducing our own reference intervals and Mayo Clinic post-analytical interpretive tools, false-positive rate decreased from 0.21% to 0.09%. Six patients had acquired vitamin B_{12} deficiency, while their mothers showed values within the reference interval. The rate of nutritional vitamin B_{12} deficiency was 35.4/100 000 births. One newborn had low free carnitine, so had her vegetarian mother. One patient has biopterin responsive phenylalanine hydroxylase deficiency and one glutaric aciduria type I.
Conclusion: our own reference intervals and use of Mayo Clinic database improved the quality of screening expressively.

P-058

Newborn screening in southeastern Europe

Groselj U[1], Zerjav Tansek M[1], Smon A[1], Angelkova N[2], Anton D[3], Baric I[4], Djordjevic M[5], Grimci L[6], Ivanova M[7], Kadam A[8], Kotori V[19], Maksic H[9], Marginean O[10], Margineanu O[11], Milijanovic O[12], Moldovanu F[13], Muresan M[14], Nanu M[13], Samardzic M[12], Sarnavka V[4], Savov A[7], Stojiljkovic M[15], Suzic B[16], Tincheva R[8], Tahirovic H[17], Toromanovic A[17], Usurelu N[18], Repic Lampret B[1], Battelino T[1]

[1]University Children's Hospital Ljubljana, Ljubljana, Slovenia, [2]University Children's Hospital Skopje, Skopje, The F.Y.R of Macedonia, [3]Clinical Hospital "Sfanta Maria", Iasi, Romania, [4]Department of Pediatrics, CMC Zagreb, Zagreb, Croatia, [5]Mother and Child Health Care Institute, Belgrade, Serbia, [6]Univ. Hospital Centr. "Mother Teresa", Tirana, Albania, [7]National Genetics Laboratory, Sofia, Bulgaria, [8]University Pediatric Hospital Sofia, Sofia, Bulgaria, [9]University Clinical Center Sarajevo, Sarajevo, Bosnia and Herzegovina, [10]Clinical Hospital of Targu Mures, Targu Mures, Romania, [11]Clinical Hospital "Luis Turcanu", Timisoara, Romania, [12]Institute for Sick Children, Podgorica, Serbia, [13]Mother and Child Health Care Institute, Bucharest, Romania, [14]Clinical Hospital "Iuliu Hateganu, Cluj-Napoca, Romania, [15]IMMGE, University of Belgrade, Belgrade, Serbia, [16]Children Hospital Banja Luka, Banja Luka, Bosnia and Herzegovina, [17]University Clinical Center Tuzla, Tuzla, Bosnia and Herzegovina, [18]Institute of Mother and Child, Chisinau, Moldova, Republic of, [19]Pediatric Clinic, Univ. Clinical Center, Pristina, Kosovo

Background: Aim was to assess the current state of newborn screening (NBS) in the region of southeastern Europe, as an example of a developing region, focusing also on future plans.
Methods: Questionnaire based survey was performed and the responses were obtained from 11 countries (Albania, Bulgaria, Bosnia and Herzegovina, Croatia, Kosovo, Macedonia, Moldova, Montenegro, Romania, Serbia and Slovenia), with a cumulative population of 52.5 million.
Results: Phenylketonuria (PKU) screening was not introduced in four of 11 countries (Albania, Kosovo, Macedonia, Montenegro), while congenital hypothyroidism (CH) screening was not introduced in three of 11 countries (Albania, Kosovo, Moldova). A few other diseases were screened for - congenital adrenal hyperplasia in Bulgaria and cystic fibrosis in Moldova. The costs of NBS per newborn varied widely, from 1 to 49 EUR. No country had introduced expanded NBS program, while a few were planning to introduce it in the following years. As a main obstacle perceived in expanding NBS was lack of financial resources. The mean perceived urgency of expanding NBS program was 4.0 (on a scale from 1 to 5, 5 meaning the highest urgency).
Conclusions: The primary challenges were identified. Implementation of NBS to developing countries worldwide should be considered as a priority.

P-059

A newborn screening method for cerebrotendinous xanthomatosis: quantification of bile alcohols in dried blood spots

Vaz F M[1], Kulik W[1], Verrips A[3], Huidekoper H H[2 4]

[1]Dept Clin Chem Ped, Lab Genet Metab Dis, Amsterdam, Netherlands, [2]Dept Ped, Acad Med Center, Amsterdam, Netherlands, [3]Dept Neurol, Can Wilhelm Hosp, Nijmegen, Netherlands, [4]Dept Ped, Erasmus Med Center, Rotterdam, Netherlands

Background: Cerebrotendinous xanthomatosis (CTX) is caused by the deficiency of 27-sterol hydroxylase (CYP 27), resulting in a reduced production of cholic acid (CA) and chenodeoxycholic acid (CDCA), and the accumulation of cholestanol and cholesterol. Besides bilateral cataract, neonatal cholestasis, chronic diarrhoea during childhood and tendon xanthomas, progressive neurological symptoms including pyramidal and cerebellar signs, peripheral neuropathy and dementia develop from the second decade onward. These symptoms can be halted or prevented by supplementation of CDCA, reducing bile acid synthesis through negative feedback. Prognosis is good when CDCA is started at an early age, but is less favourable when initiated later. This makes CTX a good candidate for implementation in newborn screening programmes. However, a validated screening method is not yet available.
Methods: We developed a screening method for CTX, based on the quantification of glucuronic acid conjugates of bile alcohols in dried blood spots (DBS) using UPLC/MS after standard methanol extraction without the need of derivatization.
Results: Our pilot data show a clear discrimination between patients with CTX and control subjects. Currently, we are establishing ratios between different bile alcohols to make the method more specific for CTX.
Conclusions: We are planning a pilot study on blinded DBS material to validate our method.

P-060

Congenital hypothyroidism: data linkage of neonatal thyroid stimulating hormone levels and measures of school attainment

Lain S[2], Wilcken B[1 3], Nassar N[2], Bentley J[2], Roberts C[2], Wiley V C[1 3]

[1]Univ Sydney, Sydney, Australia, [2]Kolling Inst, Uni Sydney, Sydney, Australia, [3]Childrens Hosp Westmead, Sydney, Australia

Background: Congenital hypothyroidism causes intellectual delay unless treated soon after birth. Newborn screening (NBS) of thyroid stimulating hormone (TSH) levels enables early detection. It is unclear whether untreated transient or mild hypothyroidism adversely affects intellectual development.
Methods: We performed a population-based record-linkage cohort study of all children born in New South Wales with both newborn TSH results and subsequent school performance results based on the National Assessment Program of Literacy and Numeracy (NAPLAN). This tests children aged 8,10,12,and 14 years over five domains: numeracy, reading, writing, spelling and grammar.
Results: There were 370,377 eligible children with a linked TSH and NAPLAN record attending public schools (linkage >95%). For those with TSH results in the 95-99.95th centiles the likelihood of a result below the national minimum standard (band 1 of 6) was significantly increased in a dose-response pattern for all domains compared to those < 95th centile. For children with TSH > 99.95th centile, with early-treated congenital hypothyroidism, the results were not significantly different from those with TSH < 95th centile.
Conclusion: Neonatal TSH levels above the population 95th centile at 48-72 hours are associated with poorer school performance on NAPLAN testing. The management of neonatal transient/mild hypothyroidism needs further exploration.

P-061

Inborn errors of metabolic screening in Qatar: a successful journey

Mitri R[1], Younes N[1], Abdien L[1], Skrinska V[1]

[1]Hamad Medical Corporation, Doha, Qatar

Background: Neonatal screening is the most important preventive public health program of the 21st century. It is implemented in majority of the developed countries. The screening of newborns in Qatar was previously very limited; specimens were sent abroad for detection of metabolic disorders. This process delayed treatment of affected babies.
Methods: The Department of Laboratory Medicine and Pathology moved forward to establish a comprehensive newborn screen laboratory at Hamad Medical Corporation, with purchase of instrumentation and recruitment of personnel. Personnel underwent training in recognized world class newborn screening laboratories including University Clinics Heidelberg and Mayo Clinic. The Metabolic Screening Laboratory systematically developed, validated, and introduced newborn screening tests on dried blood spots DBS.
Results and discussion: In 2014, the metabolic laboratory performed newborn screening for amino acids/acylcarnitines using 104 primary markers and ratios for over 55 disorders. A total of 24, 875 newborn babies were screened compared to 20,506 in 2013. The false positive rate was 0.19% compared

to 0.16% in 2013 with 0 % false negative. The true positive rate was 0.064% or 1/1554 compared to 0.098% or 1/1025 in 2013. Over the past 12 months 100% of the external proficiency test results were acceptable for all tests and specimens.

P-062

A case of cobalamine C deficiency detected by newborn screening

Lemes A[1], Zabala C[1], Machado M[1], Gonzalez M F[1], Cabrera A[1], Queijo C[1]

[1]Laboratorio de Pesquisa Neonatal, Montevideo, Uruguay

Background: CblC deficiency produces a combination of methylmalonic aciduria (uMMA) and hyperhomocysteinemia (pHcty), and is the most common inborn error of cobalamin metabolism. Is panethnic disease, with an estimated incidence of 1:100,000 live births. It seems that the treatment with high dose of hydroxocobalamin (OHCbl) has better results than cyanocobalamin (CNCbl). Long-term follow-up of early-onset patients is unsatisfactory with neurological and ocular progression.
Objectives: To present our first case of CblC deficiency detected by NBS and treated with CNCbl.
Case report: 7 months of age male patient. 40 hours of life NBS showed high C3 and ratios: C3/C2 and C3/C16. Basal: elevated uMMA and pHcty, with normal plasma vitamin B12 and folic acid. Mother had normal plasma B12. After the administration of 1 mg intramuscular of CNCbl (two consecutive days), uMMA reduced 69% from the basal sample and 49% the pHcty. From the clinical point of view, before B12 injection the patient presented generalized hypotonia that improves after started 1mg once a week (4 months of age) but he still has slight hypotonia. He is not receiving betaine yet. Two pathogenic mutations were found in MMACHC gene.
Conclusion our patient improved biochemical and clinically after once a week CNCbl intramuscular treatment.

P-063

Five years of newborn screening of inherited metabolic disorders in the Czech Republic

Bártl J[1], Chrastina P[1], Friedecký D[2], Hlídková E[2], Pinkasová R[1], Vlášková H[1], Pešková K[1], Pazdírková R[3], Procházková D[5], Ješina P[1], Hrubá Z[4], Adam T[2], Kožich V[1]

[1]Inst Inher Metab Dis, Gen Univ Hosp, Prague, Czech Republic, [2]Lab Inher Metab Dis, Univ Hosp Olomouc, Olomouc, Czech Republic, [3]Depart Ped, Univ Hosp Kral Vinohrady, Prague, Czech Republic, [4]Cen Molec Biol Gene Ther, Univ Hosp, Brno, Czech Republic, [5]Depart Ped, Facul Med Univ Hosp, Brno, Czech Republic

Background: Early diagnostic of inherited metabolic disorders (IMD) enables better prognosis, treatment and quality of patient's life. After five year, we present the results of newborn screening program in the Czech Republic including 10 IMDs.
Methods: Amino acids and acylcarnitines were extracted from dried blood spot samples and analyzed by tandem mass spectrometry.
Results: Between October 2009 and December 2014 we analyzed samples from 577,756 newborns. We detected 165 patients with subsequently confirmed IMD. One patient with intermittent MSUD was not recognized. The positive predictive value was 26.2 % and false positive rate 0.08 %. The total detection rate was 1:3,500. For the most frequent diseases (PKU/HPA 1:5,200; MCADD 1:19,900 and LCHADD 1:64, 200) frequency of pathological alleles and its geographical distribution in regions were evaluated.
Conclusion: The results are in agreement with Region4Screening target performance. Nevertheless, our laboratory algorithms are still being optimized in order to reduce number of false positive cases. Nowadays, we propose to extend the newborn screening panel from 10 to 15 IMDs by adding citrullinemia type I, argininemia, CBS/methylenetetrahydrofolate reductase deficiency and biotinidase deficiency. Other 10 IMDs could be detected secondarily.
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P-064

Clinical course of six patients with congenital portosystemic shunt detected in newborn screening for galactosemia

Yokoi K[1], Yokoi K[1]

[1]Dept.Pediatr ,Toyohashi Hosp,Fujita Hosp, Aichi prefecture, Japan

Background: Congenital portosystemic shunt (CPS) is a vascular anomaly and can be suspected by the presence of galactosemia in newborn screening (NS). Here, we report clinical course of 6 patients with CPS detected in NS.
Patients: Patient 1; patent ductus venosus with absence of intrahepatic portal vein, patient 2; portacaval shunt, patients 3 and 4; portal-renal shunt, patient 5; portacaval shunt with

aplasia of intrahepatic portal vein, and patient 6; patent ductus venosus. In all patients, blood levels of galactose, galactose-1-phospate and total bile acids (TBA) were elevated (TBA: 50-200 umol/L). Diagnosis of CPS was made by ultrasonography and/or abdominal contrast 3D-CT. Head MRI of patient 2, 5 and 6 showed high signal in the basal ganglia and anterior pituitary gland on T1-weighted image.Patient 1 and 5 underwent liver transplantation, surgical ligation was performed for patients 2 and 3, and transcatheter embolization for patients 4 and 6. Blood galactose and TBA levels in all patients became normal after surgical shunt closure.
Conclusion: CPS may cause portosystemic encephalopathy by hyperammonemia and/or manganese toxicity, and pulmonary hypertension in long-term. It is important not to overlook patients with CPS when galactosemia of unknown etiology was pointed out by NS.

P-065

The first 21 months experience of a pilot expanded newborn metabolic screening programme in Hong Kong

Hui J[1], Yuen Y P[2], Law L K[2], Leung T Y[3], Chong S C[1], Lai C Y[5], Yip K S[5], Chan Y M[1], Lee L K[1], Yau P[4], Ng P C[6], Li C K[6]

[1]Dept of Paed&Ctr of IEM, PWH, CUHK, Shatin, Hong Kong, China, [2]Dept of Chem Path&Ctr of IEM, PWH, CUHK, Shatin, Hong Kong, China, [3]Dept of O&G&Ctr of IEM, PWH, CUHK, Shatin, Hong Kong, China, [4]Dept of Dietetics&Ctr IEM, PWH, CUHK, Shatin, Hong Kong, China, [5]Dept. of O&G, PWH, CUHK, Shatin, Hong Kong, China, [6]Dept. of Paed, PWH, CUHK, Shatin, Hong Kong, China

Background: Newborn screening by MS/MS is now the standard of newborn care in most developed countries. The implementation of similar screening programme in Hong Kong is under consideration by the Hong Kong Government. Our objective was to gather local experiences and examine the feasibility of running an expanded newborn metabolic screening programme in Hong Kong.
Methods: Since July 2013, a voluntary participation fee-for-service screening programme for 30 IEMs was initiated. DBS cards were collected from newborn babies (more than 34 weeks gestation) from fifteen public, private maternity and paediatric units in the territory.
Results: 14,008 newborn babies were screened. 28 babies were called back for repeat DBS +/- additional metabolic investigations. The re-call rate was 0.200%. There were five true positive cases. One baby was confirmed with Medium-chain acyl-CoA dehydrogenase deficiency. The second case had 2-methylbutyryl-Co A dehydrogenase deficiency. The third case had Methionine adenosyltransferase deficiency. The fourth case had Carnitine acylcarnitine translocase deficiency. Pending genetic confirmation, the fifth case may have Carnitine transporter defect.
Conclusion: Our pilot programme confirms the feasibility of its future application and expansion into a territory wide universal mandatory programme for all newborn babies in Hong Kong.

P-066

Riboflavin responsive MADD presenting with a VLCADD like profile at newborn screening

Santos H[1], Vieira A[1], Rocha H[2], Nogueira C[2], Vilarinho L[2], Sales Marques J[1]

[1]Div Metab Dis, Pediatr Dep, VNGaia Hosp, Vila Nova de Gaia, Portugal, [2]Newb Scr Unit, Nat Inst Health, INSA, Porto, Portugal

Background: Multiple acyl-CoA-dehydrogenation-deficiency (MADD) is a disorder of fatty acid, amino acid and choline oxidation caused by defects in electron transfer flavoprotein (ETF) or ETF:ubiquinone oxidoreductase (ETF-QO).
Case report: Male newborn referred by national neonatal screening program at day 13 for VLCADD suspicion. Born after uneventful pregnancy, he had no malformations or analytical alterations. He initiated MCT formula on day 16 and was admitted on day 28 for vomiting, elevated liver enzymes and hypoglycemia. Elevated C8 was found and supplement was stopped. He was kept on IV 10% glucose, with liver enzyme improvement. Acylcarnitine profile had elevation of C4, C5, C6, C8, C10, C12, C14, so MADD was considered. Very low lipid formula was started, supplemented with essential fatty acids with analytical normalization. Genetic study showed two riboflavin responsive mutations (p.P456S and p.P534L) in *ETFDH* gene confirmed MADD. Riboflavin 100 mg/day was started and dietary fat were progressively increased. He is now 24 months, has normal growth, development and tonus, and is on a mildly fat-restricted diet and riboflavin 200mg/day.
Conclusion: Acylcarnitine analysis may not provide definitive diagnosis in MADD, and can show a profile similar to VLCADD, leading to the initiation of MCT enriched diet, which is deleterious to MADD patients.

P-067

Rapid screening of classical galactosemia patients: a proof-of-concept study using high-throughput FITR analysis of plasma

Garnotel R[1], Untereiner V[2], Lacombe C[2], Gobinet C[2], Zater M[3], Sockalingum G D[2]

[1]Pediatrics Lab, CHU Reims, Reims, France, [2]MéDIAN, CNRS UMR 7369 MEDyC, Reims, France, [3]Biochemistry Lab, CHU Bicetre, Paris, France

Background: Classical galactosemia is an autosomal recessive metabolic disease involved in the galactose pathway and results in an accumulation of galactose and its derived products (Gal-1-P and galactitol) in blood. The aim of this feasibility study is to assess, using plasma samples, the potential of FTIR spectroscopy for screening patients with classical galactosemia and to differentiate them from healthy and from diabetic patients.
Methods: High-throughput FTIR spectroscopy was investigated as a potential to the current screening methods. IR spectral data were exploited on the carbohydrate region (1200-900 cm-1) by using principal component analysis (PCA), an unsupervised classification, and support vector machine leave-one-out cross-validation (SVM-LOOCV), a supervised classification model.
Results: The plasma analysis shows significant differences in the carbohydrate region when mean spectra of healthy, diabetic, and galactosemic patients were compared. The PCA classification partially separated the three plasmatic groups with however some overlapping. By using SVM-LOOCV, FTIR spectroscopy was able to discriminate healthy from diabetic and galactosemic patients with sensitivity between 80 and 95% and specificity between 87 and 94%.
Conclusions: High-throughput FTIR spectroscopy combined with SVM-LOOCV classification procedure appears to be a promising tool in the screening of galactosemia patients, with good performance, in terms of sensitivity and specificity.

P-068

Pilot study of IEM by MS/MS in Lebanon: An additional step towards a national registry

Khneisser I[1], Mansour H[2], Karam P[3]

[1]newborn screening lab UGM-FM-USJ, Beirut, Lebanon, [2]Dept of Ped, SGUMC, balamand university, Beirut, Lebanon, [3]Dept of Ped, AUBMC, Beirut, Lebanon

Background: Early detection of Inborn Errors of Metabolism (IEM) improve the quality of life. Therefore many countries had been including IEM newborn screening with variability about the number diseases. In Lebanon, there is a lack of information on incidence of IEM.
Methods and results: To address this, we launched in 2007 a pilot private newborn screening by MS/MS, with diseases panel around 25, in collaboration with 50 hospitals. Among 150,000 newborns analyzed till end 2014, 130 (12 per 10,000) were recalled. Of those, 78 were confirmed with IEM, giving a positive predictive value (PPV) of 60%. At least one case of each IEM screened was found with remarkably 15 cases of MSUD, 10 cases of PKU, etc... The MSUD high incidence etiology was related to founder effect in a village of 40,000 residents ethnically isolated (14 of the 15 cases were from this village). An estimated 40% of all annual live births in Lebanon are not currently screened. A cost benefit study was conducted and a national committee should decide to maintain or to limit the number of diseases screened.
Conclusions: Efforts should continue to reach a national neonatal screening. The creation of IEM national registry is needed for immediate public health actions and diet management.

P-069

Continuous, covariate-corrected two-dimensional reference surfaces. Theoretical considerations and practical solutions

Mørkrid L[1], Rowe A D[1 2], McHugh D[3], Stoway S D[3], Rinaldo P[3]

[1]Div Med Biochem, Oslo Univ Hosp, Oslo, Norway, [2]Unit National NBS, Olo Univ Hosp, Oslo, Norway, [3]Biochem Gen Lab, Mayo Clinic, Rochester MN, United States

Background: Disease markers in newborn screening (NBS) often exhibit significant dependence on the continuous covariates age and body weight (BW) and the categorical variables gender and ethnicity.
Objectives: To develop 2-D percentile surfaces, where the covariate axes are x=age and y=BW, and the 3rd axis represents the marker value.
Material & Methods: Large datasets (n~10^3–10^6) were obtained from laboratory productions with low prevalence of the relevant diseases. True positives were

removed. Data were partitioned into bins covering rectangular xy-grids. Bin specific median μ and dispersion s were obtained by regression analysis: z-scores calculated from ranks vs. transformed marker values. A final multiple regression analysis estimated μ and s surfaces, from which marker values were converted to z-scores.
Results: Age affected μ and s for most markers. For some BW also had a substantial contribution, and in several cases there was an interaction between age and BW that needed correction, e.g. for 17-OH-progesterone, where premature babies has a much lower decline with age than full term babies.
Conclusion: The percentiles of marker values determined by two continuous covariates can be represented by 2-D z-score surfaces. The effect of categorical variables can subsequently be examined by comparing z-score subsets.

P-070

Newborn screening for severe primary immunodeficiencies in Spain

Delgado-Pecellin C[1], Olbrich P[1], De Felipe B[1], Lucenas J M[1], Sanchez B[1], Borte S[2], Neth O[1]

[1]Hospital Universitario Virgen del Rocio, Sevilla, Spain, [2]Immunodeficiency Center Leipzig at Hospi, Leipzig, Germany

Background: Early diagnosis of severe combined immunodeficiency (SCID) and X-linked agammaglobulinemia (XLA) improves outcome of affected children. The measurement of T-cell-receptor-excision circles (TRECs) and kappa-deleting-recombination-excision circles (KRECs) can identify neonates with severe T and/or B lymphopenia. Data regarding the impact of variables such as gestational age (GA) and birth weight (BW) on the values of TRECs and KRECs is conflicting.
Objective: Quantify levels of TRECs and KRECs prospectively in dried blood spots (DBS). Describe the possible influences of the variables GA and BW on the values of TRECs and KRECs.
Material and Methods: Prospective study with determination of TRECs and KRECs by multiplex PCR from DBS of neonates. The PCR cut-off levels were: TRECs 1000 copies/ml).
Results: 4277 samples were tested. Sixty-nine samples (1.61%) were insufficient (beta actin) for analysis. The mean GA was 38.6 weeks (2.47) and mean BW 3151g (+/-928.1). Mean (min-max) copies/ul obtained were: TRECs 108 (0-463), KRECs 59 (2-341) and beta-actin 1910 (82-7554). Preterm neonates and low weight showed different TRECs levels but similar KRECS.
Conclusions: Prematury and BW are associated with lower TRECS levels. No such association was found for KRECS, indicating simultaneous determinations doesn't increase the rate of false positive.

P-071

Outcome of 3-methylcrotonyl-CoA carboxylase deficiency (3-MCCD) in Austria – a retrospective data analysis

Goeschl B[1], Moeslinger D[1], Greber-Platzer S[1], Karall D[2], Scholl-Buergi S[2], Maier E[3], Brunner-Krainz M[4], Konstantopoulou V[1]

[1]Dep Paed Med AKH, Med Univ Vienna, Vienna, Austria, [2]Med Univ Innsbruck, Clin Paed I, IEM, Innsbruck, Austria, [3]Dep Paed, Paracelsus Med Univ Salzburg, Salzburg, Austria, [4]Dep Paed, Med Univ Graz, Graz, Austria

Background: 3-MCCD is a lack of the enzyme catalyzing the leucine metabolism. The first cases described patients with Reye-like syndrome. After the introduction in NBS a lot of affected, but asymptomatic family members have been diagnosed. 3-MCCD is an autosomal recessive disorder with a low clinical expressivity and penetrance, deducing that the genetic mutation and the residual enzyme's activity do not stand in any relation with the clinical appearance. The incoherent clinical picture and the inconclusive genetic mutations led to the assumption that the causes for symptoms are different. Recently published, environmental triggering factors or/and consanguinity should be responsible for the symptoms presented by 3-MCCD-individuals.
Methods: The aim of this study is to compare the outcome of selective and through NBS diagnosed 3-MCC-individuals in Austria until 2012.As parameter for the clinical outcome, events of metabolic derangement and/or impaired development have been surveyed.
Results: The study population consisted of (n=33) 3-MCCD individuals, where from (n=18) selective diagnosed and (n=15) through NBS with a calculated incidence of 1:45, 135. The results showed that 3-MCCD-individuals did not benefit from an early detection in NBS (p=0.2), since the individuals did not develop or present clinical symptoms.
Conclusions: Therefore 3-MCCD was excluded of the Austrian NBS panel in January 2015.

P-072

Outcome of medium-chain-acyl-CoA Dehydrogenase deficiency (MCADD) in Austria – a retrospective data analysis

Goeschl B[1], Moeslinger D[1], Greber-Platzer S[1], Karall D[2], Scholl-Buergi S[2], Maier E[3], Brunner-Krainz M[4], Konstantopoulou V[1]

[1]Dep Paed Med AKH, Med Univ Vienna, Vienna, Austria, [2]Med Univ Innsbruck, Clin Paed I, IEM, Innsbruck, Austria, [3]Dep Paed, Paracelsus Med Univ Salzburg, Salzburg, Austria, [4]Dep Paed, Med Univ Graz, Graz, Austria

Background: MCADD is the most common inborn error of fatty acid oxidation. Before screening, patients presented Reye-like syndrome. Since the possibility of early diagnosis, those metabolic events can be prevented by avoiding fasting periods and by applying high carbohydrates in situation at risk. K329E is the most common and severe mutation, being prevalent in the Caucasian population and being associated with higher C_8 concentration in NBS and a higher risk of clinical manifestation than others mutations.
Methods: The aim of this study is to compare the outcome of selective and through NBS diagnosed MCADD-patients in Austria until 2012. As a parameter for the clinical outcome, events of metabolic derangement and/or impaired development have been surveyed. The study population consisted of (n=45) MCADD-patients, wherefrom (n=9) were diagnosed selectively and (n=36) in NBS, deducing an incidence of 1:25, 987.
Results: The results showed that MCADD-patients diagnosed through NBS had a significant (p=0.0018) better outcome than selectively diagnosed patients, wherefrom 66.7% presented a bad outcome. Moreover, 73.7% of the patients genetically tested carried the homozygote mutation K329E, which was associated with a higher level of C_8 concentration in NBS
Conclusion: MCADD being a severe disease, with its patients having a benefit from an early detection in NBS.

P-736

Performance of an FIA MS/MS method to simultaneously measure ABG, ASM, GAA, GALC, GLA and IDUA activities from a single dried blood spot

Kuracina M[1], Cournoyer J[1], Potier A[1], Trometer J[1], Rehnberg J[2], Schermer M[1], Gelb M[3]

[1]PerkinElmer, Waltham, United States, [2]PerkinElmer, Turku, Finland [3]University of Washington, Seattle, United States

Background: Most of the MS and fluorescent methods to measure lysosomal enzymes activities require six separate incubations cocktails.
Methods: We describe the performance of a multiplexed flow injection analysis mass spectrometry (FIA-MS/MS) method that simultaneously measures 6 lysosomal enzyme activities from a single 3.2 mm punch from a dried blood spot (DBS); beta-glucocerebrosidase (ABG), acid sphingomyelinase (ASM), acid alpha-glucosidase (GAA), beta-galactocerebrosidase (GALC), alpha-galactosidase (GLA) and alpha-L-iduronidase (IDUA).
Results: Compared to currently available MS reagents, structural modifications made were; ABG and GALC substrates (S) were altered to improve solubility, IDUA-S is now similar to GAA-S and GLA-S, and internal standards (IS) for ABG, ASM, GALC and IDUA are deuterium-labeled versions of the enzymatic product. The reagent buffer was optmized for a single cocktail six-plex format. After overnight incubation, sample processing takes less than 30 minutes per 96-well plate. Sample-to-sample analysis time is 1.35 minutes. Method performance studies show good linear range and precision for all six enzymes. The six-plex method demonstrated a clear distinction between the activities in DBS from healthy newborns (n=1700) and DBS from subjects with confirmed low lysosomal enzyme activity.
Conclusions: The six-plex method and the established MS method produced comparable enzyme activities. The six-plex assay showed better signal-to-noise and linearity than fluorescent assays.

04. Dietetics and nutrition

P-073

Does long-term treatment for hyperphenylalaninemia and phenylketonuria create a risk of vitamin B12 nutritional deficiency?

Prochazkova D[4], Jarkovsky J[1], Konecna P[4], Benakova H[2], Vinohradska H[3]

[1]Ins Biostat Anal, Med Fac, Masaryk Univ, Brno, Czech Republic, [2]Inst Med Bioch Lab Diag, Gen Hosp, Prague, Czech Republic, [3]Dept Biochem, Univ Hosp, Brno, Czech Republic, [4]Dpt Pediat, Med Fac, Masaryk Univ, Brno, Czech Republic

Background: The study's aim was to determine occurrence of vitamin B12 deficiency in patients under long-term treatment for phenylketonuria (PKU) and hyperphenylalaninemia (HPA), as well as that treatment's associations with B12 vitamin parameters.

Methods: The cohort consisted of 51 patients with PKU (29 subjects) and HPA (22 subjects).
Results: We determined a statistically significant difference in serum folate levels between PKU patients and HPA patients ($p = 0.046$, Mann–Whitney U test). This difference was also statistically significant between adult HPA patients and PKU patients ($p = 0.004$, Mann–Whitney U test). We demonstrated a statistically significant difference in the proportions of patients having plasma homocysteine concentrations within the normal levels during overall evaluation of the two examined groups (p = 0.023, chi-square test). This difference was also statistically significant for adult HPA patients and PKU patients ($p = 0.032$, chi-square test). In the group of adults, we detected a statistically significant differences in serum holotranscobalamin concentrations regarding both concentration levels and proportion of patients having concentrations within the normal levels ($p = 0.031$, Mann–Whitney U test; $p = 0.006$, chi-square test).
Conclusion: The present study suggests that adult PKU and HPA patients were at risk of developing vitamin B12 deficiency.

P-074

Disordered eating in inborn errors of metabolism, a single centre's experience

Carruthers R B[1], Ellerton C E[1], Hansen K K[1], Cloherty M E[1], Freedman F[1], Murphy E M[1], Lachmann R H[1]

[1]Charles Dent Metabolic Unit, London, United Kingdom

Many IEM require strict dietary management, ideally from an early age, to maintain optimal health and well-being. Inability to adhere to dietary guidelines can risk long term neurological and physiological damage ranging from cognitive under functioning to metabolic decompensation, coma and death. While many patients adjust well to a diet for life, little is known about how food restriction affects long-term eating behaviours.
In our unit 20% of psychology referrals in the last year were for difficulties in eating accompanied by impairment in at least one aspect of functioning (social or psychological) and by physical and/or metabolic complications. There appeared to be a psychological component to the eating difficulties but not necessarily the overvaluation of weight and/or shape characteristic of anorexia nervosa and bulimia nervosa.
9 patients referred including 3 UCD, 2 PKU, 1 tyrosinaemia type 1, 1 leukodystrophy, 1 TMAU and 1 GSD IX.
Reasons for referral included selective eating, restricted eating (with and without anorexic cognitions, associated sensory sensitivities or to control symptoms of TMAU), binge eating, fear of eating socially and bulimia nervosa.
For effective dietetic management consideration of many and varied psychological factors in the development and maintenance of disordered eating is warranted.

P-075

Dietary analysis of PKU diets in adults

Robertson L V[1], Boocock S[1], Palermo L[2], Gerberhiwot T[1], MacDonald A[3]

[1]Univ Hosp Birmingham NHS F Trust, Birmingham, United Kingdom, [2]Aston Univ, Birmingham, United Kingdom, [3]Birmingham Child Hosp NHS F Trust, Birmingham, United Kingdom

Background & Objectives: There is increasing concern that high numbers of PKU adults are overweight and obese but similar to the UK population. Contributing factors are ill defined but the composition of the low phe diet could be a cause. The aim of this study is to compare the diet components in adult PKU patients on diet compared with controls.
Patients & Methods: Twenty six PKU adults (18-41y; 10 males, 16 females) on diet and 30 age/gender/education matched controls completed a Food Frequency Questionnaire (130 foods) to determine weekly food intake.
Results: PKU adults consumed more carbohydrate (p=0.001), energy, sugar, protein, fruit and vegetables than controls, but significantly less fat (p=0.020). They consumed less measures of milk / milk alternatives (p=0.006), more low protein meals (p=0.000) and more snack foods, especially savoury carbohydrate based snacks.
Conclusion: The diets of this group of adult PKU patients on diet were higher in carbohydrate and lower in fat, smilar to reports in other studies. Diets higher in carbohydrates but lower in fat and natural protein may reduce satiety, resulting in frequent intake of high carbohydrate snacks and thereby increasing total calorie intake.
Conflict of Interest declared.

P-076

Dietary compliance in PKU adults on dietary treatment

Robertson L V[1], Boocock S[1], Palermo L[2], MacDonald A[3], Gerberhiwot T[1]

[1]Univ Hosp Birmingham NHS F Trust, Birmingham, United Kingdom, [2]Aston Univ, Birmingham, United Kingdom, [3]Birmingham Child Hosp NHS F Trust, Birmingham, United Kingdom

Background & Objectives: There is little in the literature about how adults with PKU manage their diets on a day to day basis. We aimed to study their practical management and how well they perceive their adherence and their feelings about non-diet adherence.
Patients & Methods: Twenty four PKU adults (aged 18-41y; 9 males 15 females), following a low phenylalanine (phe) diet, completed a questionnaire regarding their dietary management.
Results: Sixty three percent use liquid phe-free protein substitutes (PS), 79% receive 60g protein equivalent from PS, the rest more. 88% report they take PS every day. 67% report to count protein exchanges with only 21% weighing these regularly and 92% avoiding high protein foods. 54% considered it was very important to follow their PKU diet. 67% reported negative feelings (e.g. guilty, tired, unable to concentrate, naughty) if they consumed high protein foods.
Conclusion: PKU adults who choose to follow a low phe diet usually follow a relaxed regimen. Most take their PS everyday and avoid high protein foods. They do this as they feel it is important to them and report negative feelings associated with eating high protein foods.
Conflict of Interest declared.

P-077

Glycemic index (GI) of frequently consumed bread types used for patients with glycogen storage disorders (GSD)

Gokmen Ozel H[1], Ergun R[1], Buyuktuncer Z[1], Koksel H[2]

[1]Hacettepe Univ, Dept Nutrition and Diet, Ankara, Turkey, [2]Hacettepe Univ, Dept Food Eng, Ankara, Turkey

Background: In GSDs, ingestion of slowly digested carbohydrates is recomended to maintain normoglycemia. Bread is the staple food in Turkish population. The aim of this study was to assess GI of frequently consumed bread types used for patients with GSD.
Methods: Ten healthy volunteers aged 19-35 years were included. Capillary blood glucose (BG) was measured after 12 hours starvation and at 15, 30, 45, 60, 90 and 120 minutes. Each of the 10 subjects consumed portions of the ten types of breads and the reference food (glucose powder), containing 50 g of available carbohydrate on separate mornings over a-11-week period. GI were calculated using WHO incremental area under the BG response curve.
Results: The lowest GI were found in village bread (VB) rich in whole wheat (42±4). The GI values of other bread types were as follows: Stone mill (46±5), wheat bran (51±3), rye (52 ±7), ciabatta (54±6), germ (54±6), bagel (60.0±3), white (65 ±7), pita (70±8) and hazelnut (75±9). BG after VB consumption was significantly less than after hazelnut ($p< 0.01$), pita and white breads at 90 minutes ($p< 0.05$).
Conclusion: Since low GI breads may control postprandial BG and insulin responses better, GI can be used to guide bread choices in GSDs.

P-078

Use of a very high protein diet for muscular symptoms in GSD IIIa: a case report

Ellerton C E[1], Kaalund Hansen K[1], Carruthers R B[1], Murphy E[1], Lachmann R[1]

[1]CDMU, Univ College London Hospitals, London, United Kingdom

There are anecdotal reports that high protein diets can help ameliorate the muscular symptoms associated with glycogen storage disease (GSD) type III, although there is no published literature to support this at present. A 21 year old male with GSD IIIa wanted to trial a high protein diet due to symptoms of fatigue and muscle weakness. His protein intake at baseline was 1.6 g/kg/day. With this protein intake, urea and creatinine were within normal parameters (Ur 6.2 mmol/L, Cr 88 μol/L; eGFR > 90ml/min). His CK at baseline was 3557 IU/L. Dietary advice was provided to increase to a total of 240g protein/day (3g/kg/day) through a combination of food and protein supplement powder. Baseline dynamometry showed good muscle strength.The patient reported improved energy and exercise recovery with dietary changes and therefore chose to continue. Repeat dynamometry after 11 months showed no significant improvement in muscle strength, and CK did not improve. After 18 months on diet there was a 48% increase in urea and 20% increase in creatinine compared to baseline. Urate was also elevated at 584 μmol/L [266-474 μmol/L] and advice was given to reduce to 2g protein/kg/day. Follow up is awaited to reassess dietary intake and biochemistry.

P-079

Breast feeding (BF) management of infants with glutaric aciduria type 1 (GA1)

Fitzachary C[1], Grunewald S[1], Cleary M[1], Dixon M[1]

[1]Gt Ormond St Hosp for Children, NHS FT, London, United Kingdom

Background There are no published dietetic reports of BF in GA1. We report experiences of BF two infants, diagnosed by

expanded newborn screening. Both were treated with low lysine, low tryptophan diet and carnitine, according to published guidelines. Lysine intake was limited by giving a measured volume of lysine free, low tryptophan formula (LFF) pre breast feeds.

Methods A retrospective review of patient records from diagnosis to 6 months. Data was collected on LFF intake, BF, growth, plasma lysine, arginine and tryptophan, free and glutaryl carnitine levels. Breast milk volume was estimated as 170ml/kg bodyweight/day minus prescribed daily volume of LFF.
Results Both fed well and grew normally. LFF provided 0.8-1.3g L-amino acids/kg/day. Lysine intake from breast milk ranged: case 1, 104-113mg/kg/day (median 107mg); case 2, 107-111mg/kg/day (median 110mg). Plasma lysine levels ranged: case 1, 99-191umol/L; case 2, 58-84umol/L (normal reference range 100-300umol/L). Plasma arginine and free carnitine were in the normal reference range for both. Plasma glutaryl carnitine remained above normal reference range for case 1 and normalised in case 2.
Conclusion Infants with GA1 can be successfully managed with a LFF and BF regimen. Plasma lysine should be regularly monitored to enable feed adjustment and avoid deficiency.

P-080

Experience of breastfeeding an infant with glucose transporter type 1 deficiency syndrome (GLUT1 DS) on the ketogenic diet – a case study

Skeath R[1], Grunewald S[1]

[1]Great Ormond St Hosp for Child NHS FT, London, United Kingdom

Background: Ketogenic diet (KD) is a recognised treatment for GLUT1 DS. Although in children use of KD is well established, experience with infants is limited, particularly incorporating breastfeeding.
Case: A 6 month old female diagnosed with GLUT1 DS. Diagnosis was made on biochemistry and clinical presentation (apnoeas and seizures), confirmed by *SLC2A1* mutation. At diagnosis she was exclusively breast feeding but was ready for solids.
Dietary treatment: A classical KD was calculated based on estimated fluid, energy and protein requirements. A 3-hourly feed plan was devised giving a measured amount of Ketocal 4:1® (Nutricia) followed by a breast feed to appetite. Breast feed volume was estimated as total fluid requirement minus volume of Ketocal 4:1® given. The initial fat:carbohydrate and protein ratio was 1.5:1 and then increased until blood ketones of 2 – 5 mmol/L were attained. Ketosis was maintained with an estimated 2.5:1 ratio KD. For the next stage, solids were introduced by exchanging an isocaloric meal (2.5:1 ratio) for a Ketocal 4:1® and breast feed. Seizures were controlled and breast feeding continued until fully weaned onto solids.
Conclusion: This case demonstrates it is possible to incorporate breast feeding into a KD to maintain ketosis for treatment of GLUT1 DS.

P-081

The challenges of managing co-existent disorders with phenylketonuria: 32 cases

MacDonald A[1], Ahring K[2], Almeida M F[3], Belanger-Quintana A[4], Blau N[22], Burlina A B[5], Cleary M A[6], Coskun T[7], Dokoupil K[11], Evans S[1], Feillet F[21], Gizewska M[9], Gokmen Ozel H[10], Lammardo A M[14], Lotz-Havla A S[11], Kamieńska E[12], Maillot F[13], Muntau A C[20], Puchwein-Schwepcke A[8], Rocha J C[17], Robert M[15], Santra S[16], Skeath R[6], Strączek K[9], Trefz F K[19], Van Dam E[18], Van Rijn M[18], Van Spronsen F J[18], Vijay S[16]

[1]Dietetic Dept, The Child Hosp, Birmingham, United Kingdom, [2]The Kennedy Centre, University Hospital, Copenhagen, Denmark, [3]C. Gen Méd Doutor Jacinto de Magalhães, Porto, Portugal, [4]Hospital Ramon y Cajal, Madrid, Spain, [5]Div of IMD, University Hospital, Padua, Italy, [6]GOSH, London, United Kingdom, [7]Dept IMD, Hacettepe University, Ankara, Turkey, [8]Dr. von Hauner Child Hosp, Univ Munich, Munich, Germany, [9]Dep Met Dis & Card, Pomeranian Med Univ, Szczecin, Poland, [10]Dept Nutr & Diet, Hacettepe University, Ankara, Turkey, [11]Dr. von Hauner Child Hosp, Univ Munich, Munich, Germany, [12]Dept Paeds/Haem, Pomeranian Med Univ, Szczecin, Poland, [13]Malad Her Metab, Univ Franç Rabelais, Tours, France, [14]Dept Ped, San Paolo Hosp Univ Milan, Milan, Italy, [15]Hôp Univ des Enfants Reine Fabiola, Brussels, Belgium, [16]IMD Dept, Birmingham Child Hosp, Birmingham, United Kingdom, [17]Central Hospital of Porto EPE, Porto, Portugal, [18]UMCG, Beatrix Child Hosp, Div Metab Dis, Groningen, Netherlands, [19]Dep Ped,Div Neuroped Metab Med,Univ Heid, Heidelburg, Germany, [20]Univ Childs Hos, Univ Med Center, Hamburg Eppendorf, Germany, [21]Hôpital d'enfants Brabois, Vandoeuvre lè, Nancy, France, [22]D. Hoppe Matabol Ctr, Univ Child Hosp, Heidelburg, Germany

Introduction: There are few published case reports of co-existent disease with PKU. The clinical and demographic features of PKU patients with co-existent conditions from a multi-centre, retrospective cohort study are described.

Methods: Diagnostic age of PKU and co-existent condition, mutation analysis, treatment regimen, and impact of co-existent condition on blood phenylalanine control and PKU management were collected.
Results: There were 32 PKU patients (12 males/20 females) with a co-existent condition (current median age: 14 years; range 0.3 months to 40 years) from 15 treatment centres across Europe/Turkey. They had 23 different co-existent conditions; 9 were autoimmune; 6 gastrointestinal, 4 chromosomal abnormalities and 4 inherited metabolic diseases. There were 5 cases of parental consanguinity; delayed diagnosis of co-existent conditions (n=3) and delayed treatment of PKU (n=2) (including amenorrhoea associated with Grave's disease that masked a PKU pregnancy for 12 weeks). Co-existent conditions adversely affected blood Phe control in 44% (n=14) of patients with conflicting diet therapy (n=5) and nutritional support (n=7) required.
Conclusions: Occurrence of co-existent disease is not uncommon in patients with PKU and investigation for co-existent disorders is essential when the clinical history is inconsistent with PKU. Co-existent conditions increase disease management burden for PKU patients, caregivers, and professionals.

P-082

The challenge of vitamin and mineral supplementation in children with inherited metabolic disorders: a prospective study

Daly A[1], Evans S[1], Chahal S[1], Surplice I[1], Ashmore C[1], MacDonald A[1]

[1]Birmingham Children's Hospital, Birmingham, United Kingdom

Introduction: Supplementary vitamins and minerals are essential for children with inherited metabolic disorders (IMD) on restrictive diet therapy. The range of comprehensive preparations are limited, commonly unpalatable, or contain incomplete micronutrients to meet reference nutrient intakes. In an open prospective, longitudinal, interventional study in children with IMD, our aim was to investigate nutritional biochemistry and haematological status and dietary adherence with a comprehensive vitamin/mineral preparation (Fruitivits; Vitalflo International).
Methods: Fifteen subjects (OA 4, UCD 5, GSD 4, CPTI 1, HIHA 1), median age 8.4y (range 3-15y) were recruited. One daily sachet (6g) of vitamin/mineral powder (mixed with 60ml of water) was taken. No other concurrent vitamin/mineral supplementation was given. Anthropometry, nutritional blood samples and food frequency diary was completed at baseline, week 12 and 24. Supplement adherence was checked monthly.
Results: There was a significant improvement from baseline to 24 weeks for serum folate, vitamin E, selenium, whole blood selenium, vitamin D ($p=< 0.05$). No significance was reported for anthropometry or dietary food patterns. A median (range) of 11 (4-16) monthly doses (37%) of vitamin/mineral powder remained unused.
Conclusions: Although this vitamin/mineral supplement improved nutritional status, adherence was poor. Gaining adherence with separate vitamin/mineral supplements in IMD children is challenging.
Conflict of Interest declared.

P-083

Serum vitamin D levels in patients with phenylketonuria

Rocha J C[1 2 3], Silva N[4 5 6], Van Spronsen F J[7], Almeida M F[1 8], Borges N[3 9], Guimarães J T[4 5 10]

[1]Centro de Genética Médica JM, CHP EPE, Porto, Portugal, [2]Faculdade de Ciências da Saúde, UFP, Porto, Portugal, [3]Cent Health Technol Serv Res (CINTESIS), Porto, Portugal, [4]Biochemistry, Fac Med, Univ Porto, Porto, Portugal, [5]Clinical Pathology, Sao João Hosp Center, Porto, Portugal, [6]Cardiovascular Research Unit, FMUP, Porto, Portugal, [7]Beatrix Children's Hospital, UMCG, Groningen, Netherlands, [8]UMIB/ICBAS/UP, Porto, Portugal, [9]Faculty Nutrition and Food Sciences, UP, Porto, Portugal, [10]EPIUnit - Institute of Public Health, UP, Porto, Portugal

Background: Doubts persist about vitamin D (VitD) adequacy and VitD insufficiency (VitDi) in patients with PKU.
Objective: To determine the prevalence of VitDi in early treated patients with PKU under dietary treatment and controls.
Patients and methods: A sample of 81 PKU patients (3-29 y; 14.2±6.5 y) and 75 controls (3-47 y; 16.5±8.0 y) was studied. Fasting serum VitD concentration was measured. VitDi was considered with [25(OH)VitD] below 29 ng/ml.
Results: VitD levels were higher in patients than in controls (25.6±8.7 vs. 17.9±7.3 ng/ml; $p< 0.001$). The prevalence of VitDi was higher in controls compared to patients (90.7% vs. 70.4%; $p=0.003$). Patients with VitDi (N=57) revealed reduced amino acid mixture intake compared with other patients (1.21±0.50 vs. 1.44±0.36 g aa/kg; $p=0.038$), while showing no relation with natural protein intake (0.70±0.42 vs. 0.74 ±0.52 g/kg; $p=0.660$), median phenylalanine concentrations ($p=0.333$) or age (14.8±6.4 vs. 12.7±6.8 y; $p=0.197$). Patients taking multivitamin supplements had higher VitD levels (28.0±8.3 vs. 21.1±7.8 ng/ml; $p< 0.001$).
Conclusion: PKU patients had a better VitD status compared to controls. Dietary amino acid and vitamin supplementation intake, rather than metabolic control,

seems to be the major determinant for serum VitD concentration in patients with PKU.
Conflict of Interest declared.

P-084

Nutritional management in patients with phenylketonuria undergoing sports

Rocha J C[1 2 3], Van Dam E[4], Ahring K[6], Almeida M F[1 5], Bélanger-Quintana A[7], Dokoupil K [8], Gokmen Ozel H[9], Lammardo A M[10], Robert M[11], Heidenborg C[12], MacDonald A[13]

[1]Centro de Genética Médica JM, CHP EPE, Porto, Portugal, [2]Faculdade de Ciências da Saúde, UFP, Porto, Portugal, [3]Cent Health Technol Serv Res (CINTESIS), Porto, Portugal, [4]Beatrix Children's Hospital, UMCG, Groningen, Netherlands, [5]UMIB/ICBAS/UP, Porto, Portugal, [6]Department of PKU, Kennedy Centre, Glostrup, Denmark, [7]Unid Enf Met Serv Ped, Hosp Ramon y Caj, Madrid, Spain, [8]Dep Met Nut Dr von Haun Child Hosp Un Mu, Munich, Germany, [9]Fac Heal Scien, Dep Nutr Diet, Hacet Uni, Ankara, Turkey, [10]Depart Ped, San Paolo Hosp Univ Milan, Milan, Italy, [11]Nutr Metab Unit, Hôp Univ Enf Rein Fab, Brussels, Belgium, [12]Depart Clin Nutr Diet, Karol Univ Hosp, Stockholm, Sweden, [13]The Children's Hospital, Birmingham, United Kingdom

Background & Objectives: Early treated patients with PKU perform sport to the same level as the general population. No studies describe appropriate dietary management for the active patient with PKU. We describe three case reports with PKU illustrating the need to adapt general sports nutrition guidelines.
Case reports: Case report 1 describes an adult 24 year old male practicing resistance exercise, 4 times a week, aiming to increase fat free mass. Case 2 illustrates a 19 year old girl, member of the England ladies rugby team, who has a 2.5 hour daily training programme. In case 3, a 24 year old girl taekwondo champion struggles with the body weight fluctuations according to different exercise workloads. Their main nutrition objectives were to maintain a high carbohydrate diet, carefully maintain hydration status and give attention to the timing of ingestion of phe-free L-amino acid supplements in the immediate post exercise recovery phase. Optimal energy intake was given prior, during and post exercise training sessions or competition.
Discussion & Conclusion: In PKU, the goal of sports nutrition should be to achieve maximal athletic performance as well as maintain acceptable blood phenylalanine control. Studies are necessary to define impact of exercise on phenylalanine tolerance in PKU.
Conflict of Interest declared.

P-085

Overweight in patients with inborn errors of protein metabolism

Luz R[1], Almeida M F[1 4], Rocha S[5], Guimas A[5], Ribeiro R[5], Martins E[4 5], Bandeira A[5], Rocha J C[1 2 3]

[1]Centro de Genética Médica JM, CHP EPE, Porto, Portugal, [2]Faculdade de Ciências da Saúde, UFP, Porto, Portugal, [3]Cent Health Technol Serv Res (CINTESIS), Porto, Portugal, [4]UMIB/ICBAS/UP, Porto, Portugal, [5]Unid Doen Heredit Metab, CHP, EPE, Porto, Portugal

Background: Patients with inborn errors of protein metabolism (IEPM) are treated with natural protein restricted diets, supplemented with amino acids and energy. Little is known about its impact on overweight prevalence.
Objective: To investigate the prevalence of overweight in patients with IEPM.
Patients & Methods: A sample of 19 patients (63% females), aged 11.7±7.6 y (2-35y), with diagnosis of urea cycle disorders (n=6), maple syrup urine disease (n=3), homocystinuria (n=2), type 2 tyrosinemia (n=2) and organic acidurias (n=6) was studied. Natural protein and amino acid intakes were collected on the last nutrition appointment during 2013. Body mass index (BMI) was classified according with WHO criteria.
Results: Mean natural protein and amino acid intakes were 0.62±0.33 g/kg and 0.89±0.30 g aa/kg, respectively. Prevalence of overweight was 36.8% (33.3% in females and 42.9% in males). Overweight was only present in patients < 19 y (N=17). Natural protein and amino acid intakes were similar in overweight and non-overweight patients (0.50 ±0.17 vs. 0.70±0.38 g/kg, p=0.218; 0.97±0.25 vs. 0.85 ±0.34 g aa/kg, p=0.452, respectively).
Conclusion: Although the majority of patients manifested normal BMI, careful nutritional management is necessary in order to prevent overweight.

P-086

Evidence of underestimated natural protein tolerance in patients with phenylketonuria

Rocha J C[1 2 3], Pinto A[1], Ramos P C[1], Rocha S[6], Guimas A[6], Ribeiro R[6], Martins E[5 6], Bandeira A[6], Van Spronsen F J[4], Almeida M F[1 5]

[1]Centro de Genética Médica JM, CHP EPE, Porto, Portugal, [2]Faculdade de Ciências da Saúde, UFP, Porto, Portugal, [3]Cent Health Technol Serv Res (CINTESIS), Porto, Portugal,

[4]Beatrix Children's Hospital, UMCG, Groningen, Netherlands, [5]UMIB/ICBAS/UP, Porto, Portugal, [6]Unid Doen Heredit Metab, CHP, EPE, Porto, Portugal

Background: Natural protein (NP) tolerance may be underestimated in continuously treated patients with phenylketonuria (PKU). Before BH4 loading test, NP tolerance should be optimized.
Objective: To study possible changes in NP tolerance in diet treated PKU patients.
Patients and Methods: In 60 early diagnosed, diet-treated, well-controlled PKU patients (1-29 y; 14.0±6.2 y) NP tolerance was compared with 4-6 months in between. Anthropometry and dietary intake [NP (g/kg) and protein substitute (g/kg)] was recorded at each appointment. Good metabolic control was defined as median blood phenylalanine [PHE] < 6 mg/dL or < 8 mg/dL in patients < or > 12 y, respectively.
Results: NP ingestion increased from first to second appointment (0.84 [0.46-1.5] vs. 0.92 [0.53-1.65]; p=0.002) while protein substitute intake decreased (0.77 [0.49-1.1] vs. 0.75 [0.36-1]; p< 0.001). Although medians of [PHE] increased (p=0.014), final metabolic control was still within target range. NP increased in 72.7% of hyperphenylalaninemia, 61.3% of mild PKU and 57% of classical PKU. Out of the 60 patients, 22 (36.7%) increased their NP intake ≥ 25% (40.9% hyperphenylalaninemia, 45.5% mild PKU and 13.6% classical PKU).
Conclusion: All forms of PKU may tolerate higher intakes of NP. Careful titration of NP intake is advisable before BH4 loading test.
Conflict of Interest declared.

P-087

Is BMI enough to assess body composition in phenylketonuric patients?

Mexia S[1], Ferreira M[4], Jardim I[1], Asseiceira I[1], Rodrigues T[2], Nunes P A[1], Costa C[3], Janeiro P[3], Gaspar A[3]

[1]Diet and Nutr Dep, Santa Maria Hospital, Lisbon, Portugal, [2]Biomath Lab, Medical University, Lisbon, Portugal, [3]Metab Dis Unit, Ped Dep, Santa Maria Hos, Lisbon, Portugal, [4]Health Tec School/Medical University, Lisbon, Portugal

Background: Phenylketonuria was the first error of metabolism successfully treated with a nutritional approach. A lifelong restriction of natural protein is required, which may compromise the nutritional status. The aim of this study was to assess body composition in phenylketonuric paediatric patients.
Methods: A cross-sectional, observational study of 30 phenylketonuric patients followed in the inherited metabolic diseases unit was performed. Demographic, clinical and body composition data: sex, age, nutritional and metabolic parameters, weight, height, body mass index (BMI), fat mass index (FMI) and fat free mass index (FFMI) were collected. Statistical analysis: IBM®SPSS® 22.
Results: The mean age was 12.2±3.6Y and 57% were female. Twenty (66.6%) patients had mild PKU and 50% of the patients had good metabolic control (majority females). According to BMI, 3.3% were underweight, 83.3% were eutrophic and 13.3% had overweight. Most patients exhibited significant changes in body composition (excess of FMI (57.6%) and a deficit of FFMI (30.8%)).
Conclusion: In this study the patients exhibit significant changes in body composition characterized by an excess of FM and a deficit of FFM even though the BMI showed they were eutrophic. In conclusion, the evaluation of these patients should include other parameters of body composition in order to make a more effective nutritional intervention.

P-088

Importance of early diagnosis of homocystinuria and free breastfeeding initiation

Aktuglu Zeybek A C[1], Cakir Bicer N[3], Kiykim E[1], Zubarioglu T[1], Cansever M S[2], Aydin A[1]

[1]Div Nutr Metab Dis, Ist Univ Cer Med Fac, Istanbul, Turkey, [2]Cent Lab, Ist Univ Cer Med Fac, İstanbul, Turkey, [3]Arel Univ, Dep Nutrition and Dietetic, Istanbul, Turkey

Background: Homocystinuria (HCU) is an autosomal recessive disorder of methionine catabolism. Without early diagnosis and treatment there is a progressive onset of the multisystem clinical features of HCU.
Objective: Our aim is to emphasize the importance of early diagnosis of HCU and free breast feeding practice in dietary management in a 3 months age baby detected through her sister with HCU.
Case report: After the diagnosis and elimination diet, essential amino acid mixture free from methionine (eaa) and energy support were initiated average 0,9 g/kg/day and 55 kkal/kg/day with free breastfeeding. Complementary feeding education was given at the 6th months and natural protein (np) intake was added to the diet. She was followed with average 0,5 g/kg/day np, 1,4 g/kg/day eaa, 95 kkal/kg/day containing diet for 18 months and also she is still breastfed. Average blood methionine and homocysteine levels were 93 μmol/L and 21 μmol/L under treatment, respectively (reference range 9-44 μmol/L and 5-14 μmol/L).

Conclusion: Early diagnosis is important in all metabolic diseases as well as HCU and free breast feeding practice is a successful way to follow up HCU patients.

P-089

Arginine levels determine height in patients with an urea cycle defect (UCD) or organic acidemia (OA)

Williams M[1], Wensink D[1], Verhagen M[3], Ruijter G[2], Huijmans J G M[2]

[1]Erasmus Medical Center-Dept Pediatrics, Rotterdam, Netherlands, [2]Erasmus Medical Center-Dept Clin Genetic, Rotterdam, Netherlands, [3]Erasmus Medical Center-Dept of Dietetics, Rotterdam, Netherlands

Introduction: Growth restriction occurs in UCD and OA patients. The cause of this growth restriction remains unclear. Arginine is related to growth-velocity. This study examines the influences of protein restriction, arginine and branched chain amino acids levels on growth in these patients.
Methods: Retrospective data from 28 patients, during childhood, were studied: body height, weight, amino acids, IGF-BP3 levels, bone density and dietary intake.
Results: A height of SD (-0.5 to -2) compared to target height was found. Plasma area under curve (AUC) arginine and valine levels were significantly higher ($P< 0.05$) in the UCD than OA group. AUC Arginine correlated to height SD between age 5-10 years in the OA group. Valine plasma levels were far below reference values in the OA group. Other correlations were found between valine and growth velocity, natural protein and growth velocity and isoleucine and height. Total protein intake was higher in the OA versus UCD group in the first 10 years, but no differences were found in natural protein intake.
Conclusions: Arginine and BCAA levels affect growth in these patients. Natural protein intake is correlated with growth spurt. The quality of amino acid supplements needs to be tailored to individual patients.

P-090

Nutritional issues and growth in a single centre cohort of children with mitochondrial disease

Stafford J[1], Chentouf L[2], Dixon M[1], Rahman S[2]

[1]Gt Ormond St Hosp for Children, NHS FT, London, United Kingdom, [2]UCL Institute of Child Health, London, United Kingdom

Background: Childhood-onset mitochondrial disease is frequently complex and multisystem. Poor growth has been reported anecdotally; it is unknown if nutritional intervention improves outcomes.
Objectives: To assess growth and nutritional interventions in children attending a mitochondrial disease clinic.
Methods Retrospective case-notes review of 45 children (25 male), 30 with confirmed genetic diagnosis. Data collected on longitudinal growth, early feeding history, feeding problems, gastrointestinal symptoms and nutritional interventions. Height and BMI SDS calculated using LMS growth.
Results: Cohort aged 0.16-19.4y (median 6.19y). Growth data SDS (n=40): height median -1.75 (range -3.88-1.9), BMI median -0.43 (-4.69-2.96). Faltering growth occurred in 23/40, often by 4 years. Early feeding problems in 71%, commonly reflux (n=21); slow to feed (n=12). From 1 year, 17 had dysphagia and/or anorexia. Forty reported ≥1 gastrointestinal symptom: nausea/vomiting (n=23), diarrhoea (n=15), constipation (n=22), abdominal pain (n=12). Nutritional interventions: 24 received tube feeds; high-energy (n=17), hydrolysate/elemental formula (n=12). Median height SDS improved (0.61) in 13/40; 61%(n=8) receiving tube feeds. Of 27, median height SDS decreased (-0.80), 26%(n=7) receiving tube feeds.
Conclusion: Children with mitochondrial disease are at risk of growth faltering. Early intervention, tube feeding and dietetic support may help optimise nutritional status and growth.
Conflict of Interest declared.

P-091

Vitamin B12 deficiency and homocysteine increase in juvenile idiopathic arthritis

Cakir Bicer N[3], Aksoydan E[4], Aktuglu Zeybek A C[1], Eroglu H[5], Barut K[2], Kasapcopur O[2]

[1]Div Nutr Metab Dis, Ist Univ Cer Med Fac, Istanbul, Turkey, [2]Div Rheum, Ist Univ Cer Med Fac, Istanbul, Turkey, [3]Arel Univ, Dep Nutrition and Dietetic, Istanbul, Turkey, [4]Baskent Univ, Dep Nut and Dietetic, Ankara, Turkey, [5]Biochemistry Lab, Baskent Edu Res Hosp, Istanbul, Turkey

Background: Vitamin B12 (B12) deficiency is a widespread health problem and recent studies point to functional deficiencies below blood B12 levels of 400 pg/ml. Juvenile idiopathic arthritis (JIA) is a systemic in?ammatory disease requiring medical treatments such as methotrexate, leading to folate and B12 depletion.
Objective: Our aim is determine the frequency of B12 deficiency and homocysteine increase in JIA patients.

Method: 72-h dietary records and blood B12, homocysteine levels were evaluated in 101 JIA patients (40 male, 61 female; mean age 9,8±4,3 years; 26 polyarticular, 40 oligoarticular, 17 systemic JIA, 9 JSPA, 9 psoriatic arthritis). Patients who have used vitamin supplements in last 3 months excluded.
Results: Average B12 consumption was 2±1,1 μg/day and dietary intake was under RDA in 43,5% of the patients. 60% of patients' B12 levels were below 400 pg/ml (mean 385±157 min-max: 93-766 pg/ml) and mean homocysteine level was 13,7±5,9 μmol/L (min-max: 5,5-40,1 μmol/L).
Conclusion: B12 deficiency and homocysteine increase are common in JIA patients. Medical treatment can affect intestinal absorption of B12 even if daily intake is adequate. Evaluation of B12 intake and blood levels in routine clinic control and developing recommendations about healthy nutrition can avoid B12 deficiency.

P-092

Relationship between fructose consumption and obesity in adolescent phenylketonuria patients and their healthy siblings

Cakir Bicer N[1], Aktuglu Zeybek A C[3], Balci M C[2], Gokcay G[4], Demirkol M[2]

[1]Arel Univ, Dep of Nutrition and Dietetic, Istanbul, Turkey, [2]Ped Nutr Metab, Ist Med Faculty Ist Uni, Istanbul, Turkey, [3]Div Nutr Metab Dis, Ist Univ Cer Med Fac, Istanbul, Turkey, [4]Ped Nutr Metab, Ist Med Faculty Ist Univ, Istanbul, Turkey

Objective: Determining relationship between dietary fructose consumption and obesity in adolescent phenylketonuria (PKU) patients, their healthy siblings and healthy adolescents.
Method: 72-h dietary records and anthropometric measurements were assessed in 17 PKU patients, 19 siblings and 40 healthy adolescents (ages 12,8±2,1, 14,1±3,4 and16,4±1 years), respectively.
Results: The numbers of overweight/obese cases in PKU, siblings and healthy group were 3/0, 5/1, 10/7, respectively. PKU group consumed significantly lower daily intake of phenylalanine, fat, fiber and had lower protein-higher carbohydrate intake of the daily energy with higher fructose ratio in the daily carbohydrate intake than siblings and healthy adolescents. Healthy group consumed significantly higher daily intake of energy and had higher fat ratio in the daily caloric intake than PKU group. Intake of added sugar and fructose in all groups were higher than WHO recommendations. Fruit consumption in PKU group was significantly higher than others. In healthy adolescents' weight, BMI, waist-, upper-middle arm circumferences, triceps-subscapular-skinfold thickness Z-scores were higher than others; height, weight-for-age and neck-circumference Z-scores were higher than PKU group. Increasing fructose ratio of the carbohydrate intake, subscapular-skinfold thickness was increased.
Conclusion: Consumption of added sugar higher than recommendations increases the risk of non-communicable disease in all groups.

P-093

Dietary management and outcome of newly diagnosed pregnant woman with citrullinemia type 1

Uroić V[1], Mesarić N[1], Pavić E[1], Petković Ramadža D[2], Sarnavka V[2], Fumić K[3], Zekušić M[3], Škaričić A[3], Grgić Medić M[5], Zlopaša O[5], Gašparović V[4 6], Elveđi Gašparović V[7], Barić I[2 6]

[1]Dept of Diet&Nutr, Univ Hosp Cent Zagreb, Zagreb, Croatia, [2]Dept of Pediatr, Univ Hosp Cent Zagreb, Zagreb, Croatia, [3]Clin Ins Lab Diag, Univ Hosp Cent Zagreb, Zagreb, Croatia, [4]Dept of Int med, Univ Hosp Cent Zagreb, Zagreb, Croatia, [5]Div Int Care Med, Univ Hosp Cent Zagreb, Zagreb, Croatia, [6]Univ of Zagreb, School of Med, Zagreb, Zagreb, Croatia, [7]Dep Obst and Gyn, Univ Hosp Cent Zagreb, Zagreb, Croatia

Background and objective: Citrullinemia type 1 (CTLN 1) is a rare autosomal recessive disorder caused by deficiency of argininosuccinate synthetase. CTLN1 presents as a clinical spectrum ranging from classic neonatal form to late-onset and even asymptomic form. Some women remain undiagnosed until acute deterioration during pregnancy or post partum period. There are no clear recommendations for management of newly diagnosed illness during pregnancy (pregnant women).
Case report: A 20-year old women presented at 27th week of gestation with acute liver failure and encephalopathy. As plasma ammonia was high (220 μmol/L) metabolic work-up was performed. Plasma citrulline (921 μmol/L; N 10-55 μmol/L) and glutamine (1141 μmol/L; N 340-740 μmol/L) were high, arginine and argininosuccinate were unmeasurably low. Orotic acid excretion in urine was elevated. Treatment with sufficient caloric intake and low protein diet, arginine supplementation and sodium benzoate resulted in complete recovery. Patient tolerated 1.1g protein/kgBW, and probably could more. Plasma ammonia remained normal and amino acids in acceptable range. In 39th week the patient delivered a healthy baby.
Conclusion: Dietary management is an essential part of treatment of pregnant women with citrullinemia type I and contributes to good outcome both for the mother and baby.

P-094

Evaluation of quality of life in patients with GLUT1 deficiency treated with a ketogenic diet (KD)

Castejón E[1], Egea N[1], Garcia D[1], Gutierrez A[1], Meavilla S[1], De Los Santos M[1], Martin de Carpi J[1]

[1]Gastro and Nutr Dep, St.Joan de Déu Hosp, Barcelona, Spain

Objectives: To determine if our patients with GLUT1 deficiency have improved their behaviour, school assistance, learning, autonomy, relationship, frequency and intensity of the seizures, since the KD began.
Methods: Retrospective study of our GLUT1 patients diagnosed in the last 10 years and treated with a KD.We evaluated the quality of life with a specific test for children with epilepsy (*Herranz JL, Casas C. Escala de Calidad de Vida del niño con epilepsia* (*CAVE*). *Rev Neurol 1996*; *24*: *28-30.* Patient Weighted Quality Of Life In Epilepsy: QOLIE-10-P, Epilepsy Foundation).
Results: 7 patients with GLUT1 deficiency (6 girls, ages 6-18). All of them were treated with a classical KD, for more than 2 years, 5 patients with 3:1 ratio, 1 patient with 3.5:1 ratio and 1 with 4:1 ratio. The quality of life of these patients after the diet was: 86% declared an improvement (3/7 very good, 3/7 good), only one patient preferred to be on a normal diet
Conclusions: The majority of our patients improved their quality of life after they started the KD. It is very important to give the families with KD diet correct education and training in order to improve the adherence and the quality of life in patients with GLUT1 deficiency.

P-095

Glycogen storage disease type VI growth and feeding outcomes

Kitchen S M[1], Daly A[1], MacDonald A[1]

[1]Birmingham Childrens Hospital, Birmingham, United Kingdom

Background: There is little information about feeding patterns of patients with glycogen storage disease (GSD) type VI. We analysed growth, dietary and feeding practices of 7 British Pakistani children with GSD type VI diagnosed at a median age 4 years (range 2-5y).
Methods: Open/multiple choice questions were conducted with parents/caregivers to assess early/current feeding practices. Energy intake and anthropometric measurements from the IMD database and Child Health Records were collated.
Results: Pre diagnosis, 57% (n=4) were bottle fed, 43% (n=3) breast fed, and all patients were fed at least 3 hourly with 5 (71%) continuing with frequent breast/bottle feeds post 1 year of age. In 57% (n=4) weaning commenced between 17 weeks and 6 months with the remainder weaned at 6-8 months. Only one infant experienced gagging/food refusal. There was minimal dietary change post diagnosis with 3/7 prescribed one dose of bedtime cornstarch at 0.7 to 1.5g CHO/kg. Energy intake met estimated average requirements. Normal growth patterns were observed with mean BMI (-0.05 SDS, ± 0.34), height (-0.08 SDS, ± 0.45) and weight (-0.13 SDS, ± 0.33) marginally below average.
Conclusion: In this cohort, children had normal feeding patterns, with few feeding difficulties and minimal dietary intervention.

P-096

Development of educational teaching packages on inherited metabolic diseases for parents and health professionals in the United Kingdom

Webster D[1], MacDonald A[2], O'Hara P[5], Skeath R[3], Van Wyk K[4], Forbes E[6]

[1]Freelance IMD dietitian, Bridgwater, United Kingdom, [2]IMD Dept, Child Hosp, Birmingham, United Kingdom, [3]Dietetics, GOSH, London, United Kingdom, [4]Willink Unit, Manchester, United Kingdom, [5]Galactosaemia Society, Birmingham, United Kingdom, [6]Nutricia Ltd, Trowbridge, United Kingdom

Background: In Germany, Peter Burgard and Udo Wendel developed modular teaching slide sets to assist communication with new families with an IMD. These modules contain core information explaining the condition, principles of management and genetics. They enable information to be given in a structured way.
Methods: The modules have now been adapted for UK families, by a working group of metabolic dietitans from the BIMDG Dietitians Group. Initially dietitans prioritised the development of modules to support expanded newborn screening. So far seven teaching modules have been developed: glutaric aciduria type 1, homocystinuria, isovaleric acidaemia, MSUD, phenylketonuria, tyrosinaemia and galactosaemia.
Results & Conclusions: Similarly to Burgard and Wendel modules, a common structure has been followed: including basic information about the condition, management principles, diet, emergency management and genetics of the condition. A design team was involved with the final production to improve readability and presentation. Preliminary evaluation by other health professionals is encouraging. The modules are likely to

provide a foundation to ensure that information is delivered in a consistent way to new families by health professionals involved in the care of IMD children. Further modules, covering all dietary treated IMD conditions will be developed.
Conflict of Interest declared.

P-097

A new dietary treatment approach to Fanconi-Bickel Syndrome

Kiykim E[1], Gunaydin D[1], Zubarioglu T[1], Altay S[1], Aktuglu Zeybek A C[1]

[1]Div Nutr Metab Dis, Ist Univ Cer Med Fac, Istanbul, Turkey

Background: Fanconi-Bickel syndrome (FBS) is a rare metabolic disorder characterized by hepatorenal glycogen accumulation. Typical clinical findings include hepatomegaly, glucose and galactose intolerance, fasting hypoglycemia, characteristic tubular nephropathy, rickets, and marked growth retardation. Here we report the possible positive effects of high protein diet supported with high caloric intake with complex carbohydrates in FBS patients.
Methods: Three FBS patients, $1^{2/12}$, $9^{6/12}$ and 17 years of age, followed by Pediatric Metabolism unit were enrolled to the study. All patients were given a galactose restricted diet with frequent feedings supported by raw starch that consist a higher caloric and protein content that was previously described. Carbohydrates were selected from complex carbohydrates.
Results: Full dietary compliance was achieved only in 17 years old male patient. At the end of 24 months follow up, pre and postprandial blood glucose swings were taken under control; 19 cm and 25.5 kg increase were achieved in length and weight, respectively. Hepatomegaly regressed with decrease in serum liver transaminases. No difference was seen in lipid profile.
Conclusion: The medical nutrition therapy with high protein and caloric content along with complex carbohydrates and raw starch consumption may help achieving good metabolic control and catch-up growth in FBS patients.

P-098

The value of dietetic school/nursery visits in children with IMD on diet therapy

Kitchen S M[1], Daly A[1], Chahal S[1], Ashmore C[1], MacDonald A[1]

[1]Birmingham Childrens Hospital, Birmingham, United Kingdom

Introduction: Children with IMD spend significant time attending nursery or school. Teachers may receive limited professional instruction either by telephone or letter and there is little information online.
Objectives: We have evaluated the purpose and benefit of dietetic nursery/school visiting in IMD.
Methods: Between May 2014 /April 2015, IMD dietitians visited nurseries/schools of 70 children with dietary treated IMD (UCD: n=9, OAA: n=13; FAOD; n=6; CHO disorders: n=5, amino acidopathies [MSUD, PKU, HT1, HCU] n= 37). The purpose, information given and outcome of each visit were documented.
Results: At least 60% of parents were unable to explain their children's health needs effectively. Dietitians gave teachers individual/practical instruction on: administration of protein substitute or dietary supplements (n=55), condition specific education, including impact on behaviour and learning (n=68); school meal planning (n=59); illness management (n=33), tube feeding (n=10), and exercise management (n=8). This close school liaison led to improvement in dietary adherence (n=20); safer feeding practices (particularly when fasting times were limited), and reduced parental anxiety. It also led to increased allocation of carer support time for educational/health needs (n=5).
Conclusions: Direct dietetic nursery/school visiting enabled safe child care by improving teacher IMD knowledge and practical skills.

P-099

Metabolic group study for consensus development on nutritional treatment for phenylketonuria

Cakir Bicer N[7], Gokcay G[2], Altay S[1], Ozer I[3], Saglam T[2], Gunden F[4], Aktuglu Zeybek A C[1], Saglam H[4], Cesur Y[5], Onal H[6], Demirkol M[2]

[1]Div Nutr Metab Dis, Ist Univ Cer Med Fac, Istanbul, Turkey, [2]Ped Nutr Metab, Ist Med Faculty Ist Univ, Istanbul, Turkey, [3]Ped Metab Dis, Goztepe Edu Res Hosp, Istanbul, Turkey, [4]Uludag Univ, Div Metab Dis, Bursa, Turkey, [5]Ped Metab Dis, Bezmialem Vakif Uni, Istanbul, Turkey, [6]Div Metab Dis, KSS Edu Res Hosp, Istanbul, Turkey, [7]Arel Univ, Dep Nutrition and Dietetic, Istanbul, Turkey

Background: Nutritional treatment for phenylketonuria (PKU) needs life-long compliance but patients sharing information on social media search different treatment modalities and consult other centers.

Objectives: The aim of this study was to discuss nutritional treatment for PKU from nearby centers aiming to develop a regional consensus.
Methods: Six pediatric metabolic centers in Marmara region surrounding Istanbul, establishing the Marmara Metabolic Group organized eight meetings on PKU management during 2014-March 2015. Protein, energy and nutrient intake, elimination diet, breastfeeding, complementary feeding, medical foods, phenylalanine (phe) content of exchange lists, target blood phe levels in PKU treatment were evaluated.
Results: Nutritional treatment strategies of six centers responsible for 1734 PKU patients were evaluated. Differences in the dietary management were in phe levels at treatment initiation and follow-up. Breastfeeding was practiced in all clinics but the duration of elimination diet, method of complementary feeding and intervals for monitoring phe levels differed among centers. Different exchange list containing 30 mg, 15 mg or 50 mg phe were used in centers.
Conclusion: Discussing problems and sharing experiences on nutritional management of PKU were useful for regional guidelines development which may be the initial step to the development of national guidelines.

P-100

Managing feeding problems in a case of fructose 1,6-bisphosphatase deficiency (F16BPD)

McSweeney M[1], Fitzachary C[2]

[1]Dept Metab Med, Great Ormond St Hosp, London, United Kingdom, [2]Dietetic Dept, Great Ormond St Hosp, London, United Kingdom

Background: F16BPD is a disorder of gluconeogenesis that frequently presents with a severe life- threatening episode of marked hypoglycaemia and lactic acidosis. Treatment is aimed at avoidance of prolonged periods of fasting and adequate supply of glucose during illnesses such as infections, diarrhoea and vomiting. Well children require no dietary modification although uncooked cornstarch (UCCS) is used to prevent hypoglycaemia in some.
Case Report: A three year old girl who had a nasogastric tube inserted to deliver oral glucose solution during an episode of tonsillitis. She subsequently developed feeding difficulties and enteral feed dependency. Recurrent episodes of hypoglycaemia (< 3.5mmol/L) occurred when attempting to withdraw nasogastric tube support leading to parental anxiety and further dietary intervention.
Methods: Enteral feeds were titrated with food. An UCCS load test was undertaken to establish safe fasting period and continuous glucose monitoring (CGM) was used to measure glucose control.
Results: CGM was 99% and 95% within range (3.5-7.8mmol/L). UCCS load prior to removal of tube showed 8hours of normoglycaemia.
Discussion: CGM and UCCS enabled safe transition from full enteral feed to oral diet and provided parental reassurance. This case study highlights our management of feeding difficulties that can be encountered in a case of F16BPD.

P-101

Accuracy of reported ingestion of special low protein foods in patients with phenylketonuria

Ramos P C[1], Almeida M F[1 3], Pinto A[1], Rocha J C[1 2 4]

[1]Centro de Genética Médica JM, CHP EPE, Porto, Portugal, [2]Faculdade de Ciências da Saúde, UFP, Porto, Portugal, [3]UMIB/ICBAS/UP, Porto, Portugal, [4]Cent Health Technol Serv Res (CINTESIS), Porto, Portugal

Background: Special low protein foods (SLPF) used in phenylketonuria treatment are fully reimbursed in Portugal and national acquisition and delivery is coordinated by Center of Medical Genetics.
Objective: To compare reported SLPF ingestion with billing data in diet-treated patients.
Patients and Methods: A sample of 73 patients (17.0±8.0 y; 1-33 y; 43% females) was studied. Daily reported SLPF ingestion (kcal/day) was collected at the annual routine nutritional status evaluation (ARNSE). Billing data from 2014 was collected to estimate daily SLPF ingestion (kcal/day). Good metabolic control was defined as median blood [phenylalanine] < 6 mg/dL or < 8 mg/dL in patients < or >12 y, respectively.
Results: Reported and estimated daily SPLF ingestion were similar (421.3 [205.2; 599.5] vs. 425.4 [162.0; 682.6]; p=0.533), even considering age (0-10y; 10-19y and >19y) (p=0.158), gender (p=0.569), disease severity (p=0.479) and metabolic control (p=0.621). Food history underestimated the ingestion of ice-creams, cakes, cakes/pancake mix, chocolates, energy bars, jelly, savory foods, baby cereals and bread (p< 0.001; p< 0.001; p=0.003; p< 0.001; p=0.001 and p< 0.001, respectively), while overestimated cookies ingestion (p< 0.001).
Conclusions: Although global reported and estimated SLPF ingestions were similar, patients manifested a tendency to underestimate the consumption of some SLPF subgroups with high energy content.

P-102

Abnormal feeding behavior in children with inborn errors of metabolism treated with strict dietary regimens

Manganozzi L[1], Gallo G[1], Deodato F[1], Maiorana A[1], Caviglia S[2], Bernabei S[3], Dionisi-Vici C[1]

[1]Div Met Dis, Dep Ped,Bamb Gesù Child hos, Rome, Italy, [2]Un Clin Psy, Dep Neur, Bamb Gesù child h, Rome, Italy, [3]Un Art Nut, Dep Chir, Bamb gesù child ho, Rome, Italy

Background & objectives: Patients with inborn errors of metabolism (IEM) often require special and restrictive dietary regimens to treat and prevent life-threatening metabolic decompensation. Abnormal feeding behaviour has been reported in PKU, UCDs and FAO defects. In 2013, the Diagnostic and Statistical Manual of Mental Disorders, re-organize in a single category the main feeding and eating disorders (FED).
Patients & methods: To report the frequency of FED, we began a systematic evaluation of feeding modalities in patients with IEM on strict dietary regimens.
Results: Among 204 patients, 15 present FED (53% restrictive food intake, 40% restriction of food choice, 7% both of them). We analysed in detail two patients: pt1 with methylmalonic acidaemia (22 y) who presents since adolescence food aversion and markedly selective behaviour till to require parenteral nutrition; pt2 with ornithine transcarbamylase deficiency (15y) from childhood is still able to eat only semiliquid food.
Discussion & Conclusion: Abnormal feeding behaviour is present in a relevant proportion of patients with IEM requiring strict dietary regimens. This observation highlights the need of a multidisciplinary approach. The long-term follow-up should not be limited to biochemical and clinical monitoring, but requires an individualized approach, to limit the potential impact of dietary restriction in the development of FED.

P-103

Ketogenic diet in nonketotic hyperglycinemia: case report

Gomez-Castro J F[1]

[1]Pediatrics Div, Fundacion Valle del Lili, Cali, Colombia

Ketogenic Diet (KD) in nonketotic hyperglycinemia (NKH): case report
NKH is an inborn error of metabolism characterized by refractory epilepsy and severe neurological impairment. Treatments intended to prevent glycine deposit in brain and stop NMDAr hyperactivation, have not shown clinical improvement, even when started early after onset of symptoms. KD is an effective treatment for some specific metabolic disorders, and is an alternative for refractory epilepsy in certain settings. Recent reports documents control of seizures in NKH patients treated with KD, and although mechanisms by KD works remains unknown, it suggests a new and promising therapeutic choice for NKH. We present a case series in which a group of patients with NKH and conventional treatment were selected for classic modality of KD, resulting in substantial seizure control. First: female, 2 years with KD and second, male, 6 months with KD. Seizures reduction 100% (20 to 0 per day) and 78% (9 to 2) respectively; hospitalizations for epilepsy were reduced 100% both. We applied the ECAVIPEP: score 73 and 72 points, classified as good quality of life. We recommend further studies exploring KD in NKH, and explore impact for preclinical settings and other end points like prevention of development delay.
Conflict of Interest declared.

05. New metabolic disease groups

P-104

Muscular dystrophy and mitochondrial Ca^{+2} up take disease in two brothers

Eyaid W[1 2], Shehhi W[1], Dress K[1 2], Balwi M[1 2], Twaijri W[1 2], Alassiri A[1 2], Mootha V[3]

[1]king abdulaziz medical city., riyadh, Saudi Arabia, [2]King Saud bin Abdulaziz Univ for Health, Riyadh, Saudi Arabia, [3]Cent.for Hum. Genet.Research, Mass.Gen H, Boston, Massachusetts, United States

Background: Mitochondrial calcium uptake has a central role in cell physiology by stimulating ATP production, shaping cytosolic calcium transients and regulating cell death. Mitochondrial Ca+2 uptake is mediated by the Ca+2 uniporter complex in the inner mitochondrial membrane, MCU. The MICU1 is a subunit of the MCU. It regulates channel opening in response to intracellular calcium content and calcium transients.
Case report: Here we report a mutation in the *MICU1* gene in two brothers who presented with incidental finding of elevated creatinine kinase, CK level. Both of them are having mild proximal weakness, and have no abnormal movement or learning difficulty. Muscle biopsy showed minimal myopathic changes. The whole exome sequencing, WES showed that they have a homozygous mutation c.547C>T (p. Q183X) in the *MICU1* gene, and both parents were heterozygous.
Conclusion: This is the second report in the literature that shows that mutation in the *MICU1* gene is directly linked to a form of neuromuscular disease in children.

P-105

Hereditary spastic paraplegia with predominant cerebellar signs due to *KIF1C* mutation in two brothers

Ozgul R K[1 2], Serdaroğlu E[3], Yalnizoglu D[3], Yucel-Yilmaz D[1 2], Topcu M[3], Gormez Z[4], Sagiroglu M[4], Dursun A[1]

[1]Div Metab Dis, Hacettepe Univ Child Hosp, Ankara, Turkey, [2]Inst of Child Health, Hacettepe Univ, Ankara, Turkey, [3]Div Pediatric Neurol, Hacettepe Univ, Ankara, Turkey, [4]TÜBİTAK, BİLGEM, İGBAM, Kocaeli, Turkey

Background and objectives: Hereditary spastic paraplegias (HSPs) are a group of genetic disorders resulting in pyramidal tract impairment especially of lower limbs. Distal neuropathy generally results from dysfunctional axonal transport. KIF1C from kinesin family is recently identified as one of the genetic causes of HSP.
Case report: Two brothers born to consanguinous Turkish parents were analysed. They presented with ataxia at 3-4 years of age, and later developed pyramidal findings. Metabolic screening and electrophysiological studies did not lead to a specific diagnosis. Cranial magnetic resonance imaging showed cerebral and upper cervical spinal atrophy, bilateral symmetrical pyramidal tract involvement, and focal subcortical changes.
Methods and Results: Whole exome sequencing revealed a homozygous mutation (c.463C>T;p.R155*) in *KIF1C* gene, coding a member of the transporter kinesin family.
Discussion/Conclusion: Hereditary spastic paraplegias (HSPs) are characterized by weakness and spasticity of bilateral lower limbs. Patients with additional findings other than pyramidal tract involvement are classified as complicated HSP. Here we report two brothers who presented with cerebellar findings in early childhood. Pyramidal findings developed later while ataxia, dysarthria and tremor worsened. We suggest consideration of complicated HSP in the differential diagnosis of early childhood ataxia.
This study was supported by TÜBİTAK, TURKEY (Project no:111S217)

P-106

***SNX14* (Sorting Nexin 14) gene mutation causes a new syndromic form of cerebellar atrophy in a Turkish family**

Ozgul R K[1 2], Yucel-Yilmaz D[1 2], Gerdan O F[4], Yalnizoglu D[3], Sagiroglu M[4], Dursun A[1]

[1]Div Metab Dis, Hacettepe Univ Child Hosp, Ankara, Turkey, [2]Inst of Child Health, Hacettepe Univ, Ankara, Turkey, [3]Div Pediatric Neurol, Hacettepe Univ, Ankara, Turkey, [4]TÜBİTAK, BİLGEM, İGBAM, Kocaeli, Turkey

Background: The hereditary cerebellar ataxias are a group of clinical conditions presenting with imbalance, poor coordination and atrophy and/or hypoplasia of the cerebellum. A common hallmark of the cerebellar ataxias is a progressive cerebellar neurodegeneration due to Purkinje cell loss.
Case reports: In this study, two children with cerebellar atrophy, coarsening of facial features, and mental retardation were investigated by whole exome sequencing to identify the disease causing gene. Exome sequencing analysis obtained from affected siblings were analysed and rare variants were filtered by computer programs with different databases. All known diseases were excluded by clinical findings, biochemical tests, enzyme analyses, and radiological findings and known genetic diseases were ruled out by exome sequencing data analysis. *SNX14* (Sorting Nexin 14) gene was selected as the most probable candidate gene. In this family survey, we have identified two more affected cousins with same phenotype, and *SNX14* gene mutation (c.2670delT; p.C890*) was also detected in these patients. Segregation analysis strongly supports that *SNX14* gene was the disease causing gene in this family.
Conclusion: This family has a new syndromic cerebellar atrophy caused by mutation in *SNX14* gene, mediating the fusion of lysosomes with autophagosomes.
This study was supported by TÜBITAK, TURKEY (Project no: 111S217)

P-107

The p.R85W mutation in *HNF4A* gene causes Fanconi-Bickel syndrome type II

Diodato D[1 2], Niceta M[6 7], Diomedi-Camassei F[4], Lepri F[5], Emma F[3], Novelli A[6], Tartaglia M[6 7], Dionisi-Vici C[1]

[1]Metab Div, Child Hosp Bambino Gesù, Rome, Italy, [2]Neuromusc Div, Child Hosp Bambino Gesù, Rome, Italy, [3]Nephrol Div, Child Hosp Bambino Gesù, Rome, Italy, [4]Pathol Unit, Child Hosp Bambino Gesù, Rome, Italy, [5]Mol Gen Unit, Child Hosp Bambino Gesù, Rome, Italy, [6]Med Gen, Child Hosp Bambino Gesù, Rome, Italy, [7]Istituto Superiore Sanità, Rome, Italy

Background: Fanconi-Bickel syndrome (FBS, #227810) is a rare autosomal recessive disorder of carbohydrate metabolism due to *SLC2A2* gene mutations. FBS is characterized by hepatorenal glycogen accumulation, proximal renal tubular dysfunction and defective utilization of glucose and galactose. The R85W change

(NP_000448.3) in *HFN4A* (previously associated to maturity-onset diabetes of the youth or MODY1, #125850) has been recently linked to an atypical dominant renal Fanconi syndrome with neonatal hyperinsulinism and macrosomia, in one case associated to transient hepatic dysfunction. We report a further patient harboring the p.R85W mutation who presents a hepatorenal phenotype.
Case report: A 1-year boy was admitted with a clinical picture characterized by growth delay, hepatopathy and renal tubulopathy compatible with FBS. Laboratory tests showed glycosuria, proteinuria, phosphaturia, aminoaciduria, hyperuricosuria and hypouricemia. Bone x-ray showed delayed calcification. Liver and renal biopsy displayed respectively glycogen accumulation and lipid storage. *SLC2A2* sequencing revealed no mutations. WES analysis identified a missense change in *HNF4A* predicting the p.R85W substitution, absent in the parents.
Conclusion: Our patien's clinical picture combined with literature data, clearly indicates that the heterozygous *HFN4A* p.R85W is responsible for a FBS phenotype. We then propose the name of Fanconi-Bickel type II for this clinical entity.

06. Phenylketonuria: general

P-108

Incidence of renal impairment in adult patients with phenylketonuria: a sigle center study

Chan H[1], Micciche A[1], Lateef A[1], Rahman Y[1], Ramachandran R[1]

[1]Centre for IMD, St Thomas' (GSTT), London, United Kingdom

Background: Hennermann et al reported increased incidence of impaired renal function (CKD) in adult Phenylketonuria (PKU) patients on treatment. We conducted a retrospective audit to look at the incidence of CKD in our patients.
Methods: All adult PKU patients attending clinics at our centre were included. Renal function was estimated using serum creatinine and eGFR.
Results: 109 [46 male (age: median 26.1 yrs, range 18 – 64 yrs; 63 female (age: median 28.5 yrs, range 18 – 66 yrs)] were included. 32/46 males and 56/63 females were on a phenylalanine restricted diet. 105/109 (96%) patients had renal functions checked at least once in the last three years. Serum creatinine: male [median 72 umol/L; range 52 – 120 umol/L (reference range: 59 - 104)]; female [median 60 umol/L; range 43 – 79 umol/L (reference range: 45 - 84)]. Only one male patient had raised creatinine of 120 umol/L. eGFR was available on 57/63 females and 35/46 males; all > 60 mL/min.
Conclusion: Only one patient (0.9%) had mild renal impairment unrelated to PKU. Estimated incidence of renal impairment in adults over 18 years of age in England is 9%. We found no evidence of increased incidence of renal impairment in our cohort of PKU patients.

P-109

The influence of serum phenylalanine and tyrosine levels on prolactin concentration in adult PKU patients

Reismann P[1], Kiss E[2], Simon E[2], Szatmári I[2], Rácz K[1]

[1]2nd Depart of Med, Semmelweis Univ, Budapest, Hungary, [2]1st Depart of Pediat, Semmelweis Univ, Budapest, Hungary

Objective: It has been previously postulated that high phenylalanine (Phe) might disturb intracerebral dopamine production, the main regulator of pituitary prolactin secretion. A study with PKU children showed a positive association between Phe and hyperprolactinemia. We investigated whether this association is also probable for adults patients.
Patients and Methods: In a monocentric study 158 adult PKU patients were enrolled. Serum Phe, Tyrosine (Tyr), Prolactin (PRL), TSH levels were measured, Phe/Tyr ratio was calculated. Male and female data were divided since prolactin normal level is sex dependent.
Results: Male group (n = 68); age: 31± 7.3 years, Phe: 689 ±247 micromol/L, Tyr: 54±22 micromol/L; PRL: 10±7.5 ng/ml, Female group (n = 90): age: 30± 7.0 years, Phe: 582±286 micromol/L, Tyr: 50±22 micromol/L; PRL: 16 ±9.6 ng/ml. Correlation has not been found with Phe, Tyr or Phe/Tyr ratio and serum prolactin level either in the male or in the female group. (Male group: p-value: PRL-Phe: 0,014; PRL-Tyr: 0,538; PRL-Phe/Tyr: 0,119) (Female group: p-value: PRL-Phe: 0,478; PRL-Tyr: 0,580; PRL-Phe/Tyr: 0,251)
Conclusion: In adult PKU patients neither Phe, nor Tyr serum level might not have a substantive impact on intracerebral dopamine production, since prolactin level is not influenced by the Phe-Tyr amino acid serum concentrations.

P-110

Prevalence of PKU in Pediatrics in Algiers

Sadaoui B[1], Arrada Z[1]

[1]Consult. Metab. Dis., Dep. of Ped. H.Dey, Algiers, Algeria

Background and objectives: Algeria is a country in the Maghreb region of North Africa on the Mediterranean coast, with a total area of 2.381.741 Km², the population is estimated at 38.700.000 in 2014 with more than 978000 births per year, 95% in health facilities.To date, more than 120 cases of phenylketonuria have been diagnosed, and only targetted screening exists but we are developing plans to begin a pilot program.
Methods: We want to share our experience about PKU in our department by a retrospective study of all children followed in our consultation of metabolic diseases, recently inaugurated.
Results: - Our consultation currently monitors 63 PKU patients. -Sex ratio: male predominance. - Age of patients followed: Newborn to 33 years- Our patients come from throughout the country, guided by colleagues or by hearsay. - Average age at diagnosis: over 2 years old- The majority of our patients were recruited in an advanced state. A specific diet and drug treatment significantly improved the condition of our patients.We have done 10 BH4 tests with 50% positive.
Conclusion: The recurrent challenge in our country is to establish newborn screening for PKU and other metabolic diseases.

P-111

White matter abnormalities in early treated phenylketonuria: a retrospective longitudinal long term study

Mastrangelo M[1], Berillo L[1], Caputi C[1], Di Biasi C[2], Carducci C[3], Chiarotti F[4]

[1]Pediatr. Neurol.- Univ.-Sapienza, Rome, Italy, [2]Emerg Radiol- Univ. Sapienza –, Rome, Italy, [3]Dep. Exp. Med.- Univ. Sapienza, Rome, Italy, [4]National Institute of Health, Rome, Italy

Background: Although several transversal studies had been performed in the past, no data are available on the longitudinal progression, if any, of white matter lesions in phenylketonuria (PKU).
Objective: to study white matter alterations progression and their relationships with phenylalanine blood levels and intellectual quotient by analyzing serial MRI exams in a cohort of early treated PKU patients.
Methods: Fourty seven early treated PKU patients (mean age 25.10 years, standard deviation 5.58, range 12-37 years) were enrolled according to the following criteria: a) two or more consecutive brain MRIs; b) median blood phenylalanine assessed at moment of MRI examination. White matter abnormalities were evaluated through a previously published severity score. (Leuzzi et al, Neuropediatrics 1993) Serial intellectual quotient tests were performed during the follow up in 35/47 patients. The relationships and the variations between the white matter abnormalities severity scores, blood phenylalanine and intellectual quotient according to the age and the sex of the patients were analyzed.
Results & Conclusions: Multiple regression analysis evidenced that white matter abnormalities severity score was higher in PKU patients with higher blood phenylalanine and in older patients while no evident relationships were found between these parameters and intellectual quotient. No differences were detected between males and females.

P-112

Neuropsychiatric comorbidities in adults with phenylketonuria: A retrospective cohort study

Cohen-Pfeffer J L[1], Bilder D A[2], Kobori J A[3], Johnson E M[1], Jurecki E R[1], Grant M L[4]

[1]BioMarin Pharmaceutical Inc., Novato, CA, United States, [2]University of Utah School of Medicine, Salt Lake City, UT, United States, [3]Kaiser Permanente, San Jose, CA, United States, [4]Drexel University College of Medicine, Philadelphia, PA, United States

Background: Adults with phenylketonuria (PKU) have been reported to experience neuropsychiatric symptoms, including anxiety, depression and cognitive functioning problems. Identifying medical and psychiatric comorbidities associated with PKU may assist in the primary and specialty care management of these adults.
Methods: This study used ICD-9 codes from the MarketScan insurance claims databases (US-based) from 2006-2012. Estimated prevalences of the neurocognitive and psychiatric comorbidity diagnoses for adults with PKU were compared with two matched comparison groups (diabetics and general population.)
Results: The estimated prevalence of neurological, cognitive, and psychiatric co-morbidities were determined. Compared to diabetic and general controls (matched for age, gender, insurance-type), respectively, adults with PKU had significantly increased relative risks (RR) for anxiety (RR=1.15, 1.70), intellectual disability (RR=5.46, 7.03), dementia (RR=1.38, 1.57), movement disorders (RR=1.17, 2.34), eating disorders (RR=1.72, 3.93), ADD/ADHD (RR=1.20, 1.79), behavioral conduct disorders (RR=1.77, 3.03), developmental disorders (RR=3.47, 6.56), OCD (RR=2.21, 3.83), autism spectrum disorders (RR=3.74, 5.06) and Tourette/tic disorders (RR=4.40, 3.67).
Conclusions: This study estimates the prevalence of neuropsychiatric comorbidities in adults with PKU. This analysis suggests that adults with PKU experience increased rates for these

comorbid disorders. These results support continued monitoring of behavioral, psychiatric and neurocognitive functioning across the life-span in individuals with PKU.
Conflict of Interest declared.

P-113

Investigating the metabolome for monitoring PKU patients under treatment using high resolution nuclear magnetic resonance spectroscopy (NMRS) in urine

Cannet C[1], Trefz F K[2], Okun J G[2], Bayat A[3], Schäfer H[1], Spraul M[1], Hoffmann G F[2]

[1]Bruker BioSpin GmbH, Rheinstetten, Germany, [2]Dep Ped, Div Neuroped Metab Med,Univ Heid, Heidelberg, Germany, [3]Kennedy Centre,Center for PKU, Glostrup, Denmark

Introduction: Monitoring of treatment in PKU patients is based on blood phenylalanine. Fluctuation of phenylalanine is often not well understood. Progress in NMRS technology may offer a new horizon to monitor metabolic diseases. We used urinary analyses of treated patients with PKU as a model to demonstrate the advantage of treatment monitoring.
Methods: 60 urines of patients (age 1-35 years) with dietary (47) or sapropterin (13) treatment were measured using a Bruker Avance IVDr 600 MHz System. Multivariate statistical analysis against a reference of 311 healthy children using Hotelling´s T-squared statistic and principal component analyses (PCA) were performed.
Results: The urine profiles of PKU patients did not show differences to the healthy reference group (95% CI). However, PCA analyses in patients under dietary treatment revealed 5 outliers. All Sapropterin treated patients had an excellent metabolic profile under a higher natural protein supply compared to the other patients.
Discussion: Investigation of the metabolome in urine using NMRS and statistical analysis is a promising approach to further monitor the quality of and patient compliance to treatment. Other than PKU, the monitoring of treatment of e.g. organic acidurias, urea cycle disorders and mitochondrial defects may profit from this approach.

P-114

Gut microbia in patients with phenylketonuria: a study using next-generation sequencing

Oliveira F P[1 2], Mendes R H[1 2], De Souza C F[1], Vairo F P[1], Dobbler P[4], Sperb-Ludwig F[1 3], Roesch L F W[4], Schwartz I V D[1 2 3]

[1]Med Gen Serv/HCPA, Porto Alegre, Brazil, [2]PPGBM/Fed Univ Rio Grande do Sul, Porto Alegre, Brazil, [3]PPGCM/Fed Univ Rio Grande do Sul, Porto Alegre, Brazil, [4]PPGCB/Fed Univ Pampa, São Gabriel, Brazil

Background: The human gut is composed by 10^{13}-10^{14} microbial cells, whose compositional and functional features have shown significant differences in states of health and disease.
Aim: To analyze the gut microbia of patients with phenylketonuria (PKU), an inborn error of metabolism characterized by high blood Phe concentration.
Methodology: Stool samples were collected from 9 PKU patients on treatment (median age= 2.6 years; mean blood Phe concentration= 5.6 mg/dL), and 12 healthy controls (median age= 4.8 years). Bacterial DNA was extracted and the 16S rRNA gene was sequenced using the PGM Ion Torrent™. Bioinformatic analyses were performed following the recommendations of Brazilian Microbiome Project.
Results: Significant differences in microbial abundance were observed between microbial communities from PKU and controls (pseudo F= 39.5; p= 0.001). *Prevotella* and *Peptostreptococcaceae*, which are involved in the metabolism of complex glycans, including plant polysaccharides, were the main microbial groups abundant in PKU. The control group presented abundance of Clostridiales, *Coprococcus*, *Ruminococcus*, *Lachnospira*,*Dorea*, and *Odoribacter*, most of which are associated with fat metabolism.
Conclusions: Our results suggest there are differences of gut microbia between the PKU and control groups, which may reflect the effects of dietary treatment.

P-115

Emergence of neuropsychiatric symptoms in phenylketonuria

Symeon C[1], Barrington S[1], Ramachandran R[1], Rahman Y[1], Pitkanen M[1]

[1]King's Health Partners, London, United Kingdom

Background: Phenylketonuria (PKU) is a medical success story with a positive shift in diagnosis and management resulting in a decrease of the severe phenotype. However more subtle neuropsychiatric symptoms can occur which are commonly under-diagnosed and untreated.
Aim: To investigate neuropsychiatric and neurocognitive presentations in PKU.
Method: Retrospective review of 12 patients who were referred with memory complaints to a joint Adult Inherited Metabolic Disorders-Neuropsychiatry clinic. Patients

underwent neuropsychiatric assessment, neurocognitive testing, FDG-PET and blood tests.
Results: 9/12 FDG-PET imaging scans confirmed hypometabolism in frontotemporal regions. This correlated with high Phe-levels and reduced performance in frontal executive, memory and manual dexterity tests. 11/12 patients also had significant psychiatric co-morbidity, mainly depression and anxiety, and high Phe-levels (mean 856 μmol/L). Most of these patients had stopped their Phe restricted diet and were lost to metabolic follow-up between the ages of 13 and 20 years.
Conclusions: The study suggests that the incidence of subtle neurocognitive and neuropsychiatric problems with associated metabolic brain pathology and high Phe-levels is common in PKU patients who complain of memory problems. This highlights the need to have mechanisms in place to identify this cohort in the current PKU population.

P-116

Clinical and molecular findings of patients with hyperphenylalaninemia in Korea

Lee D H[1]

[1]Dep. Pediatr, Soonchunhyang University, Seoul, Korea, Republic of

Background: The aim of this study is finding out clinical and molecular findings of patients with HPA in Korea.
Methods: Clinical and biochemical, genetic analysis were done retrospectively from January 1999 to July 2014 in Soonchunhyang University Hospital.
Results: 207 patients were diagnosed with hyperphenylalaninemia. Among them, 194 patients were confirmed to classic PKU (n=160), BH4 responsive PKU (n=27) and benign hyperphenylalaninemia (n=7). All patients with classic PKU were treated with low phenylalanine diet therapy. After BH4 loading, blood phenylalanine level decreased over 30% in BH4 responsive PKU. Among BH4 responsive PKU, R241C allele mutation was identified most commonly (41%) in genetic analysis. Thirteen patients were diagnosed with BH4 deficiency. Among them, eleven patients were 6-pyruvoyl-tetrahydropterin (PTPS) deficiency, one patient was dihydropteridine reductase (DHPR) deficiency and one patient was not confirmed. C.259C>T mutation was identified most commonly in PTPS gene analysis. All patients with BH4 deficiency were treated well with L-Dopa, BH4 and 5-hydroxytryptophan. Most of the early treated patients have a good tolerance for drugs.
Conclusion: In our study of 207 patients with HPA, the breakdown was: classic PKU (n=160), BH4 responsive PKU (n=27), benign hyperphenylalaninemia (n=7), BH4 deficiency was found in 13 patients (6.2%) in Korea.

P-117

Not healthy, not ill: the social stigma of adult phenylketonuria

Wiig I[1], Diesen P S[1]

[1]Centre for Rare Dis, Oslo Univ Hosp, Oslo, Norway

Background: A medical sociological study investigating social implications from living with PKU, as experienced by young adults.
Patients & Methods: Qualitative, in-depth, interviews with eleven early treated Norwegian adults with PKU, 20-30 years old.
Results: The study revealed experience of stigma in food-related situations. When eating together with semi-intimate relations, participants had to deal with ignorance and questions regarding PKU. Dietary compliance could result in disbelief and difficulties in giving socially valid answers to the question on risk from non-compliance. Additionally, they struggled with stigma and shame as PKU could be associated with mental illness.
Conclusion: Early adulthood is characterized by increased autonomous living, new social structures and new acquaintances. The study showed that participants had to deal with complex decision-making when eating with others. To avoid stigma, shame or unwanted attention, participants could react with temporary or prolonged non-compliance, placing their health at risk. A paradox of living with PKU seems to lie in the opposites between fitting in socially by eating normal food or remaining free from clinical risks by being dietary compliant. Compliant participants found it difficult to explain their condition and reason with others. Non-compliant participants experienced difficulties in reasoning and justifying their decision for themselves.

P-118

Parenting children with phenylketonuria (PKU): emotional and psychosocial outcomes and their association with blood phenylalanine levels

Borghi L[2], Vegni E[2], Salvatici E[1], Paci S[1], Rovelli V[1], Giovannini M[1]

[1]Cl Dept of Ped, San Paolo Univ Hosp, Milan, Italy, [2]Clin Psych, San Paolo Univ Hosp, Milan, Italy

Background and objectives. Newborn screening and early treatment turned phenylketonuria into a treatable condition with good health outcomes. The parents' role is crucial for disease management and child wellbeing, nevertheless, PKU parents may suffer from several emotional and psychosocial maladjustments. We conducted a cross-sectional study aimed to: 1) evaluate the emotional and psychosocial characteristics of parents of PKU children; 2) assess if these variables are related to blood phenylalanine (Phe) levels of their children.
Methods. 150 parents' socio-demographic data, psychological (SCL-90R, STAI-Y, BDI-2 and STAXI-2) and quality of life (SF-36) outcomes, and patients' clinical data were collected.
Results. Parents have emotional and psychosocial outcomes comparable to the general population. Gender (female), lower educational level, single/divorced marital status and children's critical age (e.g., neonatal) are associated with poor emotional outcomes in parents. Children blood Phe levels are directly related to state-anxiety (r=0.353, p=0.002), trait-anxiety (r=0.362, p=0.002) and anger expression-out (r=0.249, p=0.041).
Conclusions. Parenting children with PKU does not have implications for emotional outcomes or quality of life. Maladjustments arise in specific socio-demographic conditions or with children's high Phe levels. Results provide useful insights to set up prevention programs and help clinicians to identify specific situations at risk for non adherence to dietary therapy.

P-119

How to secure care and treatment: e-learning for professionals caring for patients with a late treatment start for PKU

Wiig I[1], Iversen K[1], Heivang S[1]

[1]Centre for Rare Dis, Oslo Univ Hosp, Oslo, Norway

Why: The Centre for Rare Disorders is an interdisciplinary competence centre for information and counselling on rare disorders like PKU.
Most late treated PKU patients in Norway are adults; some are adolescents and children from immigrant families. Cognitively impaired patients need care and assistance and many live in care homes. Staff turn-over in institutions requires ongoing education of personnel.
E-learning is a tool to increase care givers' knowledge and optimize treatment.
How: The course is module based:
- General knowledge of PKU and treatment history
- The PKU-diet – including calculation of phenylalanine intake, cooking and use of low-protein foods
- Ethical considerations and problems in follow-up
Teaching instruments include texts, short films and oral communications. To ensure security, students receive a password and username after enrollment. We use online tests and mail correspondence to monitor progress.
Conclusion: Our experience is that e-learning is effective in providing knowledge and information, students show involvement and dedication. E-learning makes practical everyday information on PKU readily available. Studying is possible at any time and place; learning and reviewing is done at individual pace. This flexibility is appreciated. Revisions and change of topics are easily done at the Centre.

P-120

The personal burden of phenylketonuria for caregivers of paediatric patients in the UK: a cross-sectional study investigating time burden and costs

MacDonald A[1], Smith T A[2], Eijgelshoven I[3], Van Loon J[3], De Silva S U[2]

[1]Dietetic Dept, The Child Hosp, Birmingham, United Kingdom, [2]Mapi Ltd, London, United Kingdom, [3]Mapi Ltd, Houten, Netherlands

Objectives: To identify the time and financial burden for caregivers of children with PKU managed by dietary control.
Methods: A cross-sectional questionnaire-based survey of caregivers of paediatric PKU patients attending one of four specialist UK metabolic centres.
Results: Of 195 invited participants, 106 caregivers of children (aged 1–17 years; median age, 7 years) with PKU (n=45 [mild or moderate PKU]; n=60 [severe PKU]; severity data missing for one respondent) completed the survey. PKU dietary management (baking, cooking, and supervision) took a median of 19.3 hours/weekly but there were no differences between children adherent and non-adherent with diet. PKU severity and control of Phe levels showed little association with the time spent on dietary management. 21% of caregivers reduced their working hours, with a further 24% discontinuing employment. Out-of-pocket costs (OOPC) (not including Phe-free L-amino acid supplements/low-protein specialist foods available on UK NHS free prescription for under-16s) were incurred for attendance at PKU events, PKU-related equipment, and additional expenditure for holidays.
Conclusion: PKU dietary management is associated with a considerable time burden for caregivers of PKU patients; a personal financial burden also arises due to OOPC and lost earnings.
Conflict of Interest declared.

P-121

Untargeted metabolomics in human disorders affecting phenylalanine metabolism

Jans J J[1], Sain-van der velden M[1], Pras-Raves M[1], Van der Ham M[1], Gerrits J[1], Prinsen H C M[1], Verhoeven-Duif N M[1]

[1]Div Med Gen, Univ Med Center, Utrecht, Netherlands

Background & Objectives: Elevated phenylalanine levels require further examination (BH4 loading test, pterins, enzyme, mutation analysis). Here, we aim to pinpoint the cause of phenylalanine elevation using a single mass spectrometric approach (MS) in dried blood spots (DBS).
Patients & Methods: High resolution-MS equipped with nanoelectrospray ionization technology was applied to DBS from patients with increased phenylalanine. DBS of patients with (treated/untreated) PKU (n=200/4), liver disease (n=7), DHPR (n=1) and controls (n=34) were extracted with methanol and measured (positive and negative modes). A bioinformatics pipeline was developed including peak detection, grouping and identification.
Results: Phenylalanine was increased in all patients (proof of concept). In addition, phenyllactate, phenylpyruvate and acetylphenylalanine are useful markers for PKU while glutamylphenylalanine, phenylethylamine and phenylacetate were not. To further discriminate PKU from DHPR secondary hyperphenylalaninemia, metabolites were ranked based on t-test or oneway ANOVA. Interestingly, multiple metabolites were differentially present in DHPR vs PKU.
Conclusion: This approach contributes to a better understanding of the metabolic pathways. A complete metabolic profile may render additional investigation superfluous. Independent validation of the identified masses in additional patients is essential. For newborn screening, the original DBS may be used as second tier, reducing the time to establish a final diagnosis.

P-122

Social and social cognitive functioning in patients with early and continuously treated PKU: The PKU-COBESO study

Jahja R[1], Van Spronsen F J[1], De Sonneville L M J[2], Van der Meere J J[3], Bosch A M[4], Hollak C E M[4], Rubio-Gazalbo M E[5], Brouwers M C G[5], Hofstede F C[6], De Vries M C[7], Janssen M C H[7], Van der Ploeg A T[8], Langendonk J G[8], Huijbregts S C J[2]

[1]Div Metab Dis, Beatrix Child Hosp, UMCG, Groningen, Netherlands, [2]Leiden Univ, Dep Child Adol Studies, Leiden, Netherlands, [3]Univ Groningen, Dep Dev Clin Neuropsy, Groningen, Netherlands, [4]Acad Med Cen, Amsterdam, Netherlands, [5]Acad Hosp Maastricht, Maastricht, Netherlands, [6]Wilhemina Child Hosp, Univ Med Cen, Utrecht, Netherlands, [7]Univ Med Cen St Radboud, Nijmegen, Netherlands, [8]Erasmus Med Cen, Rotterdam, Netherlands

Background and objectives: PKU is associated with shortages in dopamine and serotonin, even after (dietary) treatment from birth onwards. As dopamine and serotonin are related to social and social cognitive functioning, PKU patients were expected to have problems in these domains, as they do with respect to executive functioning.
Patients and methods: 95 PKU (mean age 21.6 years ±10.2) and 95 healthy individuals (19.6 years ±8.7) completed tasks measuring social cognitive skills and questionnaires assessing social functioning. PKU patients and controls were compared using multivariate analyses of variance.
Results: Generally, PKU patients showed more difficulties in social cognitive tasks and social functioning ($p< 0.001$), with some indications for greater differences compared to controls in adolescents ($p< 0.01$) and adults ($p< 0.001$). There were no associations between phenylalanine levels and social outcome for PKU children and adolescents. However, poorer social functioning in PKU adults was related to high lifetime phenylalanine levels (r=-0.26, $p< 0.05$), rather than concurrent phenylalanine (r=0.03, $p>0.05$).
Conclusion: PKU patients exhibit difficulties in social cognitive tasks and experience problems with social interactions in daily life compared to controls, particularly observed in adults, although social demands also increase with age. Social cognition and functioning are important outcome measures in PKU treatment and monitoring.
Conflict of Interest declared.

P-123

Psychiatric disturbances in adolescent and adult phenylketonuric patients

Manti F[1], Nardecchia F[1], Carducci C[3], Carducci C[2], Leuzzi V[1]

[1]Dep Ped Child Neur Psyc, Univ Sapienza, Rome, Italy, [2]Dep Exper Med, Univ Sapienza, Rome, Italy, [3]Dep Molec Med, Univ Sapienza, Rome, Italy

Background and objectives. Psychiatric symptoms are a challenging aspect in adolescent and adult early treated phenylketonuric (ETPKU) patients. To assess the risk for these disturbs,

we explored: a) psychiatric vulnerability and the occurrence of symptoms requiring psychiatric intervention in ETPKU patients; b) their correlation with the quality of biochemical control.
Patients and methods. Forty-six ETPKU patients (aged 12 to 44) and 30 healthy controls were subjected to cognitive and psychiatric assessment by means of self-report questionnaires and psychiatric interview. Psychiatric diagnoses, if detected, were made according to DSM-5 criteria. Concomitant neuropsychological functioning, and historical and concurrent biochemical metabolic control were included in the statistical analysis.
Results. Twenty five out of 46 ETPKUs showed clinical scores on at least one scale of the psychiatric assessment (in controls 7/30); anxiety and withdrawn were the most frequent self-reported symptoms. 17 patients (0 controls), met criteria for a psychiatric diagnosis, most of them belonging to the Anxiety Disorders category. Psychiatric disorders were not associated with the quality of metabolic control.
Discussion/Conclusion. PKU patients are at high risk for psychiatric disturbances, whose pathogenesis deserves further investigation. In the management of these patients metabolic clinicians should be aware of the occurrence of these disturbances.

P-124

Optic neuropathy in an adult PKU patient during long-term follow-up

Nardecchia F[1 2], Danti F R[1], Berillo L[1], Manti F[1], Mittica A[1], Nebbioso M[4], Carducci C[3]

[1]Dept of Ped and Child Neurol and Psych, Rome, Italy, [2]Dept of Physiol and Pharmacol, Rome, Italy, [3]Dept of Experim Med, Rome, Italy, [4]Dept of Sense Organs, Rome, Italy

Background: Visual loss in PKU patients has been recently described in two adults several years after diet termination. Dietary Phe restriction led to recovery of the symptom.
Case report: We report a new case of progressive loss of visual acuity in a 30 year-old patient wich ameliorated after 6 months of diet resumption. The patient was diagnosed with classical PKU at 4 years of age because of psychomotor delay and dietary treatment was started. He maintained serum levels of Phenylalanine (Phe) < 600 μM until adolescence when he went off diet (levels range 800-1800 μM). Serial MRI showed bilateral and symmetric white matter involvement. At 30 years he reported gradual visual impairment. At that time the ophthalmologic examination found bilateral visual acuity loss (2/10); visual evoked potentials (VEP) were altered; and MRI scan showed involvement of optic radiation. Phe-restricted diet was restarted obtaining blood Phe levels < 360 μM. Visual acuity was partially restored after 6 months, with partial attenuation of the MRI hyperintensities, and improvement of VEP.
Conclusions: Pathogenesis of optic neuropathy in PKU patients is unknown. Rising reports of neurological complications in adult PKU patients support a long-term follow-up.

P-125

Investigation of L-carnitine levels in phenylketonuric patients

Kumru B[2], Bağcı C[1], Hişmi B[2], Kaplan D S[1]

[1]Dep Physiol, Health Sci, Gaziantep Univ, Gaziantep, Turkey, [2]Div Metab Dis, Gaziantep Child Hosp, Gaziantep, Turkey

Background and objectives: In phenylketonuric (PKU) patients, L-carnitine levels may vary due to metabolic or dietary reasons. Studies have shown that PKU patients on supplementation with phe-free amino acid mixtures that do not contain L-carnitine have lower plasma L-carnitine levels when compared with the controls.
Patients and Methods: The 42 classical PKU patients have been classified into two groups according to the diagnosis times: Early diagnosed (Group-A; n=20) and late diagnosed (Group-B; n=22). The early diagnosed patients have been classified into two sub-groups: The good dietary adherence (Group-A_1) and the bad dietary adherence (Group-A_2). The late diagnosed patients have been classified into two sub-groups as the ones who supplemented phe-free amino acid mixture (Group-B_1) and the ones who supplemented with LNAA mixture (Group-B_2). The L-carnitine levels of the patients were compared with age-matched controls.
Results: The mean plasma L-carnitine levels of patients and controls: Group-A_1;36.8 μmol/L, Group-A_2;32.0 μmol/L, Control-A;29.8 μmol/L and Group-B_1;28.7 μmol/L, Group-B_2;26.7 μmol/L, Control-B;31.7 μmol/L.There is no significant difference between patients and controls in terms of plasma L-carnitine levels.
Conclusion: Although in patients who receive LNAA mixture for about one year, L-carnitine does not exist in LNAA mixture, there is no significant difference in L-carnitine levels.

P-126

Neurotransmitter metabolites, melatonin and dopamine, as biomarkers to optimize treatment in phenylketonuria: effects of sapropterin

Yano S[1], Moseley K[1], Fu X[2], Azen C[3]

[1]Genet, Pediatr, Univ Southern California, Los Angeles, United States, [2]Path, CHLA, Univ Southern California, Los Angeles, United States, [3]Clinical Translational Sci Inst, USC, Los Angeles, United States

Background: PKU individuals have shown a higher prevalence of neuropsychological deficits. Abnormal neurotransmitter metabolism, particularly low serotonin and dopamine levels in the brain, is believed to be involved. Trp and Tyr are precursors for serotonin and dopamine, respectively. Both Trp hydroxylase and Tyr hydroxylase require tetrahydrobiopterin (BH4) as a cofactor.
Objective: (1) Evaluate the effects of sapropterin, a synthetic form of BH4, in serotonin and dopamine metabolism in PKU. (2) Evaluate the effects of concurrent use of large neutral amino acids (LNAA) with sapropterin.
Study Design: Nine adults with PKU completed the study consisting of four 4-week phases: (1) LNAA supplementation, (2) washout, (3) sapropterin therapy, and (4) LNAA with sapropterin therapy. An overnight protocol measured serum melatonin, a serotonin metabolite, and urine6-sulfatoxymelatonin and dopamine in first void urine specimens after each phase.
Results: Three subjects responded to sapropterin with increased serum melatonin levels (Phase 3 vs. Phase 2), two of whom showed synergistic effects in Phase 4 vs. Phase 1. Eight subjects showed negative association between serum melatonin and plasma Phe levels as well as urine dopamine and plasma Phe levels.
Conclusion: Melatonin levels increased in PKU individuals with sapropterin in association with Phe reduction.
Conflict of Interest declared.

P-127

The effect of elevated blood phenylalanine level on the immune status of PKU patients

Bushueva T V[1], Kurbatova O V[1], Samohina I V[1], Miroshkina L V[1], Freidlin E F[1], Kuzenkova L M[1], Borovik T E[1], Petrichuk S V[1]

[1]Scient Cent of Child Health, Moscow, Russian Federation

Background: Phenylketonuria (PKU) is characterized by disturbance of phenylalanine metabolism.
Purpose: To determine the effect of elevated blood phenylalanine level on the immune status of PKU patients younger and older than 12 years.
Patients and Methods: 45 PKU patients at age from 6 months to 36 years were examined. The lymphocytes subsets of peripheral blood was evaluated by flow cytometry (FC 500, BC). The Phe blood level was determined by fluorimetric method.
Results: The absolute number of T lymphocytes in PKU patients with elevated Phe blood level was decreased (p=0.023). The degree of reduction of T-cells corresponded to normal Me=48.8% (31,2-63,3) in children under 12 years and was lower than normal in patients over 12 years Me=-20.3% (-50.0 -18.7),p=0.048. Number of cytotoxic T-cells had been reduced in children over 12 years with elevated Phe blood level compared with PKU children with normal levels and younger than 12 years (?=0,0003). Most patients with PKU showed a decrease in the number of B-lymphocytes. The number of NK-cells in patients with PKU corresponded to the age norm.
Conclusion: These data indicate varying sensitivity of immune system cells to the action of elevated blood Phe level. Age dynamics of peripheral blood lymphocytes in patients with PKU corresponded to the same age dynamics of healthy individuals.

P-128

Evaluation of lean body mass in patients with phenylketonuria diagnosed and treated early

Kanufre V C[1 2], Soares R D L[1 2], Alves M R A[1], Starling A L P[1 2], Norton R C[1 2], Camatta G C[1], Mata Y A[1], Almeida L M[1], Lopes J C C[1], Aguiar M J B[1 2]

[1]Nupad, Fed Univ Min Ger, Belo Horizonte, Brazil, [2]HC, Fed Univ Min Ger, Belo Horizonte, Brazil

Objective: To evaluate the percentage of lean mass in patients with phenylketonuria.
Methods: A cross-sectional study was performed with 77 patients between 5 and 25 years old, with early diagnosis and treatment who attended the consultation from 12.03.2014 to 02.25.2015. All of them underwent anthropometric measurements: weight, height, arm circumference and triceps skinfold thickness. Subsequently, participants were classified according to BMI-for-age, height-for-age and the percentage of lean mass.
Results: Among the patients, 32 (41.5%) had muscle mass deficit of which 28 (87.5%) were normal weight-for-age and 25 (78%) adolescents. The rating of height-for-age was used among 66 patients aged between 5 and 19 years; 65 (98.5%) of them presented appropriate parameters.
Discussion: The high percentage of lean body mass deficiency in normal weight patients, as found in this study, could either be a result of poor adherence to the intake of the amino acid mixture - which is observed mainly in adolescents - or excessive intake of lipids and carbohydrates, or by a possible sedentary lifestyle.

Conclusion: It is evidence of the need for new strategies to improve the acceptance of the amino acid mixture and reduce the excessive consumption of carbohydrates and lipids in these patients.

P-129

Cognitive performance of adult patients with PKU compared to a healthy control group using the Cambridge Neuropsychological Test Automated Battery (CANTAB)

Bartus A[1], Palasti F[1], Kiss E[2], Simon E[2], Szatmari I[2], Bokay J[2], Reismann P[1]

[1]2nd Depart of Med, Semmelweis Univ, Budapest, Hungary, [2]1st Depart of Pediat, Semmelweis Univ, Budapest, Hungary

Objectives: In 2014 we conducted a pilot study for scanning cognitive dysfunctions in adult PKU patients regarded to be on strict diet (Phe < 600 micromol/L) or loose diet (Phe > 600 micromol/L). Now, this PKU patient population was compared to a healthy control group.

Patients & Methods: Patients with PKU (n = 45; mean age 29.9 years, mean Phe level 631±255 micromol/L) and healthy controls (n = 31; mean age 26.5 years, mean Phe level 44±9 micromol/L) performed CANT AB tests measuring neurocognitive functions: sensorimotor (MOT), executive function (SOC) and spatial working memory (SWM).

Results: PKU patients had significantly worse test results in memory, problem solving skills and strategy independent of whether on strict diet or loose diet. However, there was no significant difference in response speed and initial thinking time between PKU patients and controls. Furthermore, tyrosine levels did not correlate with the test results.

Conclusion: Results of this study demonstrate that cognitive abilities, measured by a selection of CANTAB tests, are influenced by high phenylalanine levels except the speed of response.

P-130

Bone mineral density and vitamin D levels in Chilean phenylketonuria and hyperphenylalaninemia patients

Leiva C A[1], Cornejo V[1], Bravo P[1], Castro G[1]

[1]LabGEM-INTA-Univ de Chile, Santiago, Chile

Background: It is well known that the phenylketonuria population has decreased bone mineral density (BMD) compared with non-PKU.

Aim: To measure the BMD in three groups: PKU (Phe levels:160-750 umol/l), mild hyperphenylalaninemias (HPHE) (Phe levels:60-240 umol/l) and controls.

Methods: BMD of spine (L2-L4), femur and total body, and levels of 25-OH-VIT D and intact Parathormone (PTH) were measured and Vitamin D intake was calculated.

Results: 16 patients by group (age range 6-23 years old) were included. There were not significant differences ($p< 0,05$) between groups in BMD and intact PTH. Significant difference with 25-OH-Vitamin D level between PKU and HPHE (PKU:38,9 ng/ml; HPHE: 28 ng/ml) (NV: >32 ng/ml) were observed. Moreover, PKU patients consumed 440 UI/day of Vit D, HPHE: 150 UI/day and controls: 145 UI/day, showing a difference between PKU compared with HPHE and control groups ($p< 0,05$) (RDI: 400-600 UI/day).

Conclusion: We concluded that the PKU group does not have significant risks of bone mineral disease and it does not show deficiency of Vit D. The HPHE group showed levels and vitamin D intake below the normal range, which could affect their long-term bone architecture. This suggests more studies are necessary in the HPHE group to prevent future abnormalities in the BMD.

P-131

Epilepsy debut in late diagnosed phenylketonuric patients after introduction of phenylalanine restricted diet

Gonzalez M J[1 4 5], Itzep D[4], Lambruschini N[5 6], Artuch R[2 3 5], Campistol J[1 4 5]

[1]Neurometab, Sant Joan de Deu Hospital, Barcelona, Spain, [2]Biochemical Dep, Sant Joan de Deu Hosp, Barcelona, Spain, [3]C Biomed Netw Research Rare Dis, CIBERER, BCN, Spain, [4]Neuroped Dep, Sant Joan de Deu Hosp, Barcelona, Spain, [5]PKU follow-up Unit Sant Joan de Deu Hosp, Barcelona, Spain, [6]Gast -Nutrit Dep, Sant Joan de Deu Hosp, Barcelona, Spain

Objective: To describe the clinical features of seizures and biochemical findings in delayed diagnosis PKU patients, after introduction of restricted diet in phe.

Method and patients: 34 PKU patients with late diagnosis were analyzed after introduction phe-restricted diet, 6 had previous seizures at the beginning of the diet and were excluded. Of the rest just 3 debuted with seizures after starting the diet.

Results: Three patients aged 3-33 years started seizures after diet, all had severe intellectual disabilities. The period between onset of diet and seizures appearance was between 15 and 90 days. The phe levels at the begining of seizures (2.8—11.31mg/dl) showed a large decrease compared to the values at the diagnosis (17.85-28.26 mg/dl). At debut of epilepsy 3 patients with diet presented secondarily generalized focal seizures. Awake

critic EEG-record presented secondarily generalized focal pattern with normal inter-critic record. CBZ and CZP association controlled seizures.
Conclusions: A prevalence of 25- 50% of epilepsy in PKU late diagnosis has been described. In this review prevalence was 11% and paradoxically occured in only 3 patients while introducing the diet. This has not been reported in the literature. The mechanisms of seizure activity and its relationship to the sharp decline in the levels of Phe are unknown.

P-132

Micronutrient and fatty acid status in phenylketonuria, and effects on bone health

Bosch A M[1], Demirdas S[1], Hollak C E M[2], Van der Lee J H[3], Van Spronsen F J[4], Rubio-Gozalbo M E[5], Bisschop P H[2], Vaz F M[6], Ter Horst N[7], Wijburg F A[1]

[1]Dep of Paed, Emma Child Hosp, AMC, Amsterdam, Netherlands, [2]Dep Int Med, Div Endo & Metab, AMC, Amsterdam, Netherlands, [3]Clin Research Unit, Acad Med Centre, Amsterdam, Netherlands, [4]Div Metab Dis, Univ Med Centre, Groningen, Netherlands, [5]Dep of Ped & Lab GMD, Univ Med Centre, Maastricht, Netherlands, [6]Lab GMD, Acad Med Centre, Amsterdam, Netherlands, [7]Dep of Diet, Acad Med Centre, Amsterdam, Netherlands

Introduction: In phenylketonuria the natural protein restricted diet, supplemented with micronutrient fortified amino acid mixtures prevents severe cognitive impairment due to high phenylalanine levels. Nutrient deficiencies may occur due to dietary restrictions. We evaluated intake and blood levels of micronutrients and essential fatty acids (FA), bone mineral density (BMD), bone turnover markers (BTM) and fracture history.
Methods: Sixty early and continuously treated patients with phenylketonuria (aged 1- 39 years), were included. Dietary intake, blood micronutrients, amino acids, erythrocyte FA, BMD, fracture history and BTM were investigated.
Results: Selenium and 25-OH vitamin D2+3 dietary intake and serum levels were low. Zinc serum levels were low despite adequate intake. Folic acid serum and intake levels were high. Despite safe total protein and fat intakes, arginine plasma levels and erythrocyte eicosapentaenoic acid were low. In patients low BMD was slightly more prevalent but lifetime fracture prevalence was comparable to the general population. BTM were elevated.
Conclusions: Nutrient status in phenylketonuria is overall non-remarkable, but we did find low intake and blood levels of zinc, selenium, 25-OH vitamin D2+3, arginine, eicosapentaenoic acid. Furthermore, high levels of folic acid were observed. A slightly more prevalent low BMD and elevated BTM warrant further investigation.
Conflict of Interest declared.

P-133

Life satisfaction and prevalence of anxiety and depression in adult phenylketonuria (PKU) patients: experience from Leipzig

Thiele A G[1], Mütze U[1], Kiess W[1], Baerwald C[2], Beblo S[1]

[1]Div Metab Dis, Univ Child Hosp, Leipzig, Germany, [2]Div Metab Dis, Depart Int Med, Leipzig, Germany

Background and objectives Adult care of PKU-patients represents a challenge and is of increasing importance with respect to the recommended lifelong diet. Insufficient metabolic control influences negatively patients' psychological and physical wellbeing. We report on life satisfaction and prevalence of anxiety and depression in adult PKU-patients.
Patients and Methods Cross-sectional study of early and continuously treated, adult PKU-patients (n=30, 17f, median age 30.7y). Using "Questions on Life Satisfaction-module", Beck-Depression-inventory and Beck-anxiety-inventory to evaluate life satisfaction, anxiety and depression. Comparison to controls (n=41, 22f, median age 26.0y).
Results Mean metabolic control was satisfactory during the last year prior to enrolment (mean phenylalanine-concentration: 725±239μmol/l). Life satisfaction was comparable to healthy controls, with better scores in PKU-patients regarding single items e.g. health or income. Living situation was significantly better in PKU compared to controls (9.8±5.7 vs.6.3 ±5.9; P=0.022). 24% of the PKU-patients reached scores indicating mild depression (controls: 26.8%) and 24% mild to moderate anxiety (controls: 39.0%). One patient and one control attained scores indicating a clinical relevant anxiety disturbance.
Conclusion PKU patients' risk for depression and anxiety disorders appears lower than in the healthy population despite their chronic disease. Specialized care in adulthood should be continued to consolidate this positive result.

P-134

Novel p.Gln226Lys mutation in phenylalanine hydroxylase gene resulting in classical PKU

Klaassen K[1], Stojiljkovic M[1], Brasil S[2], Djordjevic M[3], Desviat L R[2], Pavlovic S[1], Perez B[2]

[1]IMGGE, University of Belgrade, Belgrade, Serbia, [2]CEDEM, CBMSO, CIBERER, UAM, Madrid, Spain, [3]Mother and Child Healthcare Institute, Belgrade, Serbia

Phenylketonuria (PKU) is the most frequent inborn disorder of amino acid metabolism and it is caused by mutations in human phenylalanine hydroxylase gene (*PAH*), leading to PAH enzyme de?ciency.
We detected a previously undescribed nonsynonymous substitution p.Gln226Lys in *PAH* (c.676C>A) in two unrelated Serbian patients with classical PKU. This substitution was predicted to be damaging by PolyPhen2, SIFT and MutPred prediction algorithms, and the protein sequence alignment using Clustal Omega pointed out to evolutionary conservation of Gln226 residue. Therefore, we performed functional analysis of p.Gln226Lys PAH. Using an *in vitro* expression hepatoma system, we demonstrated that the amount of mutant p.Gln226Lys PAH-FLAG detected by Western blot was only 1.2% compared to wt PAH-FLAG. This result indicated that Lys226 residue probably impairs protein folding and/or leads to accelerated degradation of PAH protein. Furthermore, the addition of sepiapterin, intracellular precursor of BH4, did not significantly increase the amount of mutant protein. These ?ndings were in accordance with severe patients' phenotypes.
This is the first previously unreported *PAH* mutation in Serbian population. It was concluded that p.Gln226Lys is a disease causing mutation, and it does not respond to BH4. Therefore, our study contributes to functional landscape of *PAH* mutations and personalized medicine in PKU.

P-135

The perception of the multiprofessional team about the care and the treatment of the Phenylketonuria

Soares R D L[1 2], Goulart L M H[2], Kanufre V C[1 2], Alves M R A[1], Starling A L P[1 2], Norton R C[1 2], Melo V R[1 2], Camatta G C[1 2], Aguiar M J B[1 2]

[1]Nupad, Fed Univ Min Ger, Belo Horizonte, Brazil, [2]HC, Fed Univ Min Ger, Belo Horizonte, Brazil

Introduction: In the State of Minas Gerais (Brazil), the treatment of phenylketonuria (PKU) has been a major challenge for the multidisciplinary team of doctors, nutritionists, nurses, psychologists, pedagogues and social workers.
Objective: To understand the perception of the multidisciplinary team about the care and treatment of PKU.
Methods: This is a qualitative study that has used Focus Group with the participation of 8 professionals. Data were analyzed by Content Analysis Method.
Results: The team recognized their role as a mainstay for those families and reported feelings of powerlessness and frustration, especially when there is not the expected adherence. Participants revealed that there are difficulties to maintain strict diets throughout life, since the affected individual in regular treatment presents no clinical signs of disease.
Conclusions: The treatment of PKU is still a challenge that often brings up the feelings of powerlessness and professional frustration, since the theoretical and biological approaches are not sufficient to encompass the complexity of care and treatment. Nevertheless, the integration of professional led to the comprehension of the factors that can influence the treatment and then actions were suggested as strategies for dealing with the problems and face challenges.

P-136

Breastfeeding infants with phenylketonuria: a single centre experience in Turkey

Aksoy B[1], Kuyum P[1], Tuncer N[3], Arslan N[2], Ozturk Y[2]

[1]Dept Pediatrics, Dokuz Eylul Univ S Med, Izmir, Turkey, [2]Div Ped Metab Dis,Dokuz Eylul Univ S Med, Izmir, Turkey, [3]Dokuz Eylul Univ Hosp Dietetics, Izmir, Turkey

Background: This study aimed to detect the prevalence and duration of breastfeeding and serum phenylalanine (Phe) levels in infants with phenylketonuria (PKU) in our unit.
Methods: Charts were reviewed for infants diagnosed with PKU between 2008 and 2015. One hundred and thirty two charts were evaluated and 41 of them had complete records and were enrolled in the study.
Results: Fourty-one infants with PKU were diagnosed by the national neonatal screening programme at postpartum 25±18 days. Of the 41 infants with PKU, 40 (97.6%) were breastfed following delivery whereas only one (2.4%) was bottle fed. After the diagnosis 25 (61%) infants continued breastfeeding together with *phenylalanine-free amino acid* based protein *substitute*. The mean duration of breastfeeding was 7.4±4.0 (1-15) months. There was not any difference in serum Phe concentrations (456±180μmol/L) between breastfed and non-breastfed infants with PKU. Frequency of malnutrition (7.3%) did not differ between the two groups. Mean weight gain for a month was higher in breastfed infants with PKU ($p < 0.05$). Mean follow-up duration was 51±25.6 (3-90) months.
Conclusion: Rate of breastfeeding in PKU infants after the diagnosis was 61%. The duration of breastfeeding was only 7.4 months. The weight gain was better in breastfed infants with PKU. Breastfeeding should be supported especially in specific disorders as PKU in Turkey.

P-137

Neuro-psychiatric involvement in adult patients with phenylketonuria (PKU): the wide spectrum of brain vulnerability

Pilotto A[1 2], Trefz F K[3], Zipser C[6], Charyasz E[4], Freisinger P[5], Ethofer T[4], Gramer G[3], Hoffmann G F[3], Berg D[1]

[1]Neurodegeneration, Tuebingen University, Tuebingen, Germany, [2]Neurology, University of Brescia, Brescia, Italy, [3]Pediatrics, University of Heidelberg, Heidelberg, Germany, [4]Biomedical MRI, Tuebingen University, Tuebingen, Germany, [5]Pediatrics, Reutlingen Hospital, Reutlingen, Germany, [6]Neurology, Tuebingen University, Tuebingen, Germany

Background: PKU patients identified by newborn screening are entering middle age. There is no comprehensive evaluation of neuro-psychiatric involvement in late adulthood so far. Brain aging may increase the risk of neurological decline given the co-presence of neurotransmitter deficits, oxidative damage and metabolic abnormalities.
Methods: Inclusion criteria: classical PKU patients older than 30 years and age-matched controls (Ctrl, n=15). Investigations: Extensive neurological evaluation, neuropsychological testing, 3T-MRI, transcranial ultrasound, sensory and motor evoked potentials, blood and urine analyses. Voxel-based morphometry (VBM) analyses on MRI adjusted for age, gender and educational levels in order to detect gray (GM) or white matter (WM) lesions/atrophy in patients compared to Ctrl.
Results: 11 PKU patients (mean age 40.5y, range 30-54y) with (n=9) or without (n=2) current dietary treatment were included. Adult PKU subjects presented heterogeneous cognitive impairment, ranging from very mild single-domain to severe multi-domain deficits, variably associated with neurological, electrophysiological and MRI abnormalities. VBM analyses showed greater GM and WM damage, especially in the left putamen, bilateral frontal and temporal structures in adult PKU compared to Ctrl.
Conclusion: Our preliminary results show that neurological impairment in adult PKU patients is significant with a heterogeneous spectrum, involving both cortical and subcortical functions and structures.

P-138

Blood phenylalanine monitoring in phenylketonuria: a single centre experience in the UK

Stonestreet K[1], Gribben J[1], Winstone R[1], Van Wyk K[1], Campbell T[2], Vara R[2]

[1]N&D Dept, St. Thomas' Hosp, London, United Kingdom, [2]Ctr Inherited Met Dis, St Thomas' Hosp, London, United Kingdom

Background: Optimal control of phenylalanine concentrations in phenylketonuria (PKU) is essential for maximising neurocognitive outcomes. Recent reports suggest some benefit from aiming for lower phenylalanine levels than previously recommended.
Methods: A database of home phenylalanine monitoring results was established (August 2013-August 2014). Classical PKU patients solely on dietary treatment were included and compared to existing national recommendations.
Results: Eighty-one patients were identified, 47 male. Median age 6 years (range 0-15y). Patients were grouped by age (0-4, 5-9, 10-14, 15-16y). The proportion of phenylalanine concentrations within or below national guidelines for each patient was calculated (median for each age group = 68%, 86%, 88%, 100% respectively), along with mean blood phenylalanine (median 318umol/L, 336umol/L, 467umol/L, 415umol/L respectively), and proportion of expected samples received (median 83%, 114%, 100%, 62% respectively).
Conclusion: Our data compared favourably to previous published papers for all groups except 0-4years. Our centre uses lower target ranges than current national recommendations for ages 5 and above which may account for the comparatively positive results in these groups. This suggests that there may be a benefit to lowering the target range for all groups in order to achieve the national recommendations and perhaps improve neurocognitive outcomes in adulthood.

P-139 - Withdrawn

P-140

Teaching parents to take a quality blood spot in the treatment of phenylketonuria

Mumford N[1], Skeath R[1], Cleary M A[1]

[1]GOSH, London, United Kingdom

Background: Infants diagnosed with PKU have ongoing blood phenylalanine levels monitored through dried blood spot analysis. Parents are taught how to take a heel prick blood spot by the clinical nurse specialist through practical demonstration and visual teaching aids. A new local policy based on a recent national screening policy has specified an increased sample size to give better accuracy of results came in to practice on 14th april 2015.

Aim: To audit the quality of blood spots sent in by parents according to policy recommendations.
Method: Review blood spots sent to our laboratory over a three months period pre and post the implementation of the policy (n=750-900) Number of blood spots of acceptable quality for anaylsis was compared.
Results: Around 5% of samples were rejected on the grounds of either being too small, multi-spotted, contaminated or had no identification, before the new policy implementation. Rejection rates have increased with the stricter guidelines of the new policy.
Conclusion: This audit has highlighted the need for frequent teaching of families and regular audits to ensure good quality blood spots to meet the new standards and reduce rejection rates.

P-141

Monitoring body composition of adult patients with classical phenylketonuria

Boros F[1], Gyure E[1], Racz G Z[1], Papp F[1]

[1]Dept Ped, Univ Szeged, Szeged, Hungary

Background: Dietary treatment may be associated with an increased risk of obesity and other metabolic complications in phenylketonuria (PKU).
Patients and methods: We examined the nutritional status and body composition by InBody 720 device of 27 adult patients with classical PKU aged between 16 and 42 years.
Results: Overweight status, defined as body mass index (BMI) greater than 25 kg/m^{2,} was found in 13 patients (48%) and 3 of them (11%) were obese (BMI >30 kg/m^2). Overweight patients had elevated body fat percentage (BFP) compared to age and sex matched reference values. In 5 further cases (18%), we observed abnormally increased BFP despite normal BMI. Decreased protein and mineral levels together with decreased skeletal muscle mass were found in 2 patients (7%), which indicates insufficient nutrition intake. In contrast, in 26% of patients (n=7) the skeletal muscle mass was higher than matching normal reference values despite normal protein levels.
Conclusion: Determination of body composition of PKU patients might be a valuable tool for the evaluation of nutritional status and dietary treatment. Compared to BMI, the BFP is a more accurate parameter for determining obesity: 20% of our patients had high BFP contrary to normal BMI.

P-142

Plasma phenylalanine concentration in adult PKU patients is correlated with global decrease in antioxidant genes expression in blood leukocytes

Veyrat-Durebex C[2 4], Debeissat C[1], Blasco H[2 4], Patin F[2 4], Hénique H[3 4], Emond P[2 4], Antar C[2], Hérault O[1 4], Maillot F[3 4]

[1]Univ Hosp, Hematol Biol, CNRS 7292 Lnox, Tours, France, [2]Univ Hosp, Biochem Lab, INSERM U930, Tours, France, [3]Univ Hosp, Dept Int Med, INSERM U 1069, Tours, France, [4]Univ François Rabelais, Tours, France

Background & Objectives:Oxidative stress has been implicated in the pathophysiology of PKU. The objective of this study was to investigate the relationship between the metabolic disturbances of PKU and oxidative stress in blood leukocytes.
Patients & Methods: Blood samples were collected from 9 untreated adult PKU patients and 9 age and sex matched healthy controls. The level of reactive oxygen species (ROS) in leukocytes was evaluated by flow cytometry (DCFDA staining). The expression of major antioxidant genes was also quantified using qRT-PCR (patent WO2012085188 A1-2012/06/28). Plasma amino acids were quantified by chromatography. Statistical analyses were performed using Wilcoxon and Pearson tests.
Results: PKU patients presented higher plasma concentrations of phenylalanine and lower tyrosine than controls ($p< 0,01$). The level of ROS was significantly higher in monocytes and neutrophils of PKU patients. Global antioxidant gene expression was lower in PKU patients with a lower expression of superoxide dismutase 1 and 2, glutathione reductase, peroxiredoxins, glutathione peroxidases and glutaredoxins ($p< 0,05$), which was correlated with higher phenylalanine concentrations ($p< 0,05$).
Discussion & Conclusion: This is the first study analyzing the expression profile of major antioxidants genes in blood leukocytes of adult PKU patients. These data highlight a lower antioxidant arsenal associated with hyperphenylalaninemia.
Conflict of Interest declared.

P-143

Maternal phenylketonuria: Impact of nutritional treatment on the offspring outcome

Saglam T[1], Yesil A[1], Cakir Bicer N[2], Balci M C[1], Gokcay G[1], Demirkol M[1]

[1]Ped Nutr Metab, Ist Med Faculty Ist Univ, Istanbul, Turkey, [2]Arel Univ Dep Nutr Dietetic, Istanbul, Turkey

Background: In maternal phenylketonuria (MPKU) patients, high blood phenylalanine (phe) levels during pregnancy may lead to teratogenic effects on fetus.
Objectives: The aim was to examine the relationship between nutritional therapy onset, compliance and outcome of offspring in MPKU patients.
Methods: MPKU patients diagnosed during 2008-2014 have been evaluated. Gestational diet initiation time, dietary records, blood phe/tyrosine(tyr) levels and teratogenic findings in the offspring were recorded.
Results: Eight MPKU patients with a mean age of 26.6±4.8 years (range=18-34), 14 pregnancies (nine untreated and diagnosed after pregnancy, three started treatment after and two before pregnancy) were evaluated. In late-/early-treated patients, mean daily intakes were 792±475/1125±151 mg phe; 59(23/36)/61(31/28) g protein (natural/PKU formula); 1510 ±561/2147±401 kcal energy and mean blood phe/tyr levels were 489±151/39±8 and 172±94/49±15 mcmol/L during pregnancy. Clinical findings in the offspring of untreated patients were neonatal deaths (2/9), microcephaly (6/8), facial dysmorphism (7/8), congenital cardiac anomalies (4/8), developmental delay (3/7), intrauterine growth retardation (3/9). In the late-treated group microcephaly (1/3) and dysmorphism (1/3) were recorded, while early treated offspring were normal.
Conclusion: MPKU patients benefit from early nutritional therapy. Compliance to dietary treatment was poor in late-starters. It is estimated that the higher phe/tyr ratio of late-treated group may be explained by the catabolic state caused by deficient energy intake.

P-144

Phenylketonuria as a protein misfolding disease: probing aggregation pathways as a tool to identify new therapeutic targets

Lino P R[1 2 3], Leandro J[1 3], Amaro M P[1 3], Almeida A J[2 3], Leandro P[1 3]

[1]Metabolism & Genetics Group, Lisbon, Portugal, [2]Nanostruct Syst Overcom Biol Barr Group, Lisbon, Portugal, [3]Res Inst Medicines, Fac Pharm, Univ Lisb, Lisbon, Portugal

Background: Phenylketonuria (PKU; #OMIM 261600) is considered a loss-of-function conformational disorder with a large fraction of PKU mutations associated with misfolded phenylalanine hydroxylase (hPAH) variants presenting elevated propensity to self-associate and form higher-order oligomers which will be targeted for degradation.
Objectives: Our aim was to characterize the aggregation pathways of wild-type (WT) hPAH in order to identify key points for future therapeutic targeting.
Methods: The hPAHWT was heterologous expressed and purified via affinity and size exclusion chromatographies. Differential scanning fluorimetry (DSF) and dynamic light scattering (DLS) was used to characterize the stability of the functional tetramers.
Results: DSF revealed two thermal transitions ($T_{m,1}$ ~47°C and $T_{m,2}$ ~54°C). The T_{agg} as determined by DLS was 39.50±0.08°C.The aggregation kinetics at 37 and 42°C monitored by DLS revealed a t_{lag} of 7.3±0.7 and 1.5 ±0.2 min and a rate constant K_I of 0.067±0.006 and 0.374 ±0.038 min^{-1}, respectively. Interestingly the aggregation process involves the dissociation of tetramers into dimers before the formation of the higher order oligomers.
Conclusion: By using DLS we were able to identify essential processes in the aggregation of hPAHWT that could be crucial in the development of new strategies to stabilize the WT protein and to rescue aggregation-prone hPAH variants.

P-145

Multicenter study on long-term growth in patients with PKU

Belanger A[1], Stanescu S[1], Dokoupil K[2], Ahring K[3], Rocha J C[5], Gokmen Ozel H[4], Robert M[9], Van Dam E[6], Lammardo A M[7], MacDonald A[8]

[1]U. Enf. Metabolicas, Hosp Ramon y Cajal, Madrid, Spain, [2]Dp Metab Nutr, DrHauner Child Hosp, Munich, Germany, [3]Center for PKU, Kennedy Institute, Glostrup, Denmark, [4]Dp Nutrition Dietetics, Hacettepe Univ, Ankara, Turkey, [5]Centro Genética Médica JM, Porto, Portugal, [6]S. Metab Dis, Univ Med Centre Groningen, Groningen, Netherlands, [7]Dp Pediatric, San Paolo Hosp, Milan, Italy, [8]Inh Metab Disorders, Children's Hosp, Birmingham, United Kingdom, [9]Dp Nutr Metab, Hosp Uv Enf Reine Fabiola, Brussels, Belgium

Background. The majority of the studies on PKU have focused on the the neurological development of patients. Studies regarding the physical development usually focus on a short period of time, do not include dietary information, and results are contradictory.
Objectives. The aim of this study is to determine whether the patients with PKU reach their final genetic height and if there is a relation between the dietery restrictions and the growth parameters.
Material and methods. This is a retrospective multicentre multinational study including PKU patients from 8 centers from

different countries. Data of growth parameters and dietary regimes were collected from birth until the age of 18.
Results. The study included 176 PKU patients. The analyzed growth parameters (weight, height and body mass index) are in the normal range and the patients reach their final genetic height. Body mass index z-score show a tendency to obesity. Results are put in relation to the different dietary trends.
Conclusions. PKU patients should have an adequate final physical development. It is important to collect longitudinal growth data throughout childhood and adolescence in PKU that considers any change in growth in relationship to dietary patterns.

P-146

Increased acetylcholinesterase activity in the brain of rats submitted to an animal model of hyperphenylalaninemia

Ferreira G C[1], Macan T P[2], Oliveira M B[3], Citadin S[3], Kist L W[4], Pereira E G[2], Marques S O[5], Souza C T[5], Streck E L[2], Bogo M R[4], Budni J[3], Zugno A I[3], Schuck P F[2]

[1]Instit Bioq Med, Univ Fed RJ, Rio de Janeiro, Brazil, [2]Lab Inb Err Metab, Univ Extr Sul Catarin, Criciúma, Brazil, [3]Lab Neurosci, Univ Extr Sul Catarin, Criciúma, Brazil, [4]Pontif Univ Cat RS, Porto Alegre, Brazil, [5]Lab Fisiol Bioquim Exerc, Univ Extr Sul, Criciúma, Brazil

Background and Objective: Phenylalanine accumulation in tissues and body fluids is the hallmark of phenylketonuria (PKU). Brain injury is a clinical characteristic of PKU patients, although the pathophysiology of this damage is poorly understood. We here investigated the *in vivo* and *in silico* effects of Phe on acetylcholinesterase (AChE) activity.
Materials and methods: For the *in vivo* experiments, animals received a single subcutaneous administration of saline (control group) or 5.2 μmol/g phenylalanine plus 0.9 μmol/g p-chlorophenylalanine (HPA group). One hour after the administration, the animals were euthanized by decapitation; brain structures were isolated and homogenized. AChE activity and its relative RNA expression, PKA and PKC content were determined and the interaction between Phe and AchE by molecular dynamics was analyzed.
Results: HPA animals showed increased AChE activity in striatum, without altering its expression. Furthermore, PKA and PKC content were not altered. *In silico*, the affinity of ACh to AChE was found decreased in the presence of Phe due to steric hindrance.
Conclusions: Our results suggest that Phe induces cholinergic alterations. Since cholinergic imbalance is associated to failure and progressive neurologic decline in learning/memory functions, it is possible that AChE alterations might contribute to the intellectual deficiency observed in PKU patients.

P-147

Phenylalanine variability as a determinant factor during neurodevelopment. Outcomes in higher cognitive functions

Santander D[2], De la Parra A[3], Cornejo V[3], Ossandón T[1]

[1]Dep Psych, Med Sch, Cent Int Neur, PUC, Santiago, Chile, [2]PhD Neuro Prog, PUC, Santiago, Chile, [3]LabGEM, INTA, Universidad de Chile, Santiago, Chile

Background: Phenylketonuria (PKU) patients who have been correctly treated display mild deficits in cognitive functions like attention and working memory, which emulate the symptoms observed in attention deficit hyperactivity disorder (ADHD). In both PKU and ADHD the underlying cause of cognitive symptoms may relate to a deficit in catecholamines, which are products of tyrosine metabolism. We established a quantitative relationship between high-level cognitive functions in PKU and ADHD patients.
Methods: Clinical data from 129 early-treated PKU patients with no concomitant ilnesses were analyzed (age range: 6 months - 15 years), sixty ADHD patients and sixty controls.
Results: Indicators of psychomotor and mental development, and IQ data are negatively correlated with both the mean phenylalaninemia levels and variability, especially starting from the 18th month since birth. Although age and IQ are uncorrelated, the IQ drops with age. Both groups show worse verbal IQ results than performance IQ, and a low score in a digit retention subtest.
Conclusion: Though phenylalaninemia levels are universally accepted as the most relevant indicator to assess the success of PKU treatment, our results highlight the importance to maintain stable levels throughout as much at the neurodevelopment stage as at the rest of a patient's life.

P-739

Coexistence of phenylketonuria and primary adrenal insufficiency

Coskun T[1], Pektas E[1], Buyukyilmaz G[2], Alikasifoglu A[2], Dursun A[1], Sivri H S[1], Tokatli A[1]

[1]Div Metab Dis, Hacettepe Univ Child Hosp, Ankara, Turkey, [2]Ped Endocrinol,Hacettepe Univ Child Hosp, Ankara, Turkey

Phenylketonuria (PKU) is an autosomal recessive disease. Some other autosomal recessive diseases accompanying PKU have previously been reported. We present, co-existence of Primary Adrenal Insufficiency (PAI) in a newborn with PKU detected on newborn screening.A 16-day-old girl was referred to our hospital after having been found to be hyperphenylalaninemic. She was the first child born to parents who are second-degree cousins. Blood phenylalanine (Phe) level was 57.7 mg/dl. She was put on phenylalanine-free formula for 5 days (first washout). Since her blood Phe level did not drop to desired levels despite two more washout trials and she lost weight, we suspected the presence of another disease accompanying PKU. Detection of hyponatremia, hyperkalemia, high blood ACTH and renin and low cortisol and aldosterone levels led us to the diagnosis of PAI in our patient. After treatment for PAI she started gaining weight quickly and blood Phe levels dropped to normal ranges dramatically.We conclude that PAI could be a rare coincidental event in children with PKU and the high rate of consanguineous marriages in Turkey might increase the possibility of co-existence of these two in the same patient.

07. Phenylketonuria: treatment, BH4

P-148

Tetrahydrobiopterin (BH4) was safe and effective in patients less than 4 years old with BH4-responsive PKU in Japan

Shintaku H[1], Ohura T[2]

[1]Dept Pediat Osaka City Univ Grad Sch Med, Osaka, Japan, [2]Div Pediatr Sendai City Hospital, Sendai, Japan

Background and objectives: We reported the efficacy and safety of BH4 therapy initiated in patients under 4 years old among 43 patients with BH4 responsive PKU (BPKU) at the end of 2011. In this study we evaluated the efficacy and safety of BH4 therapy among BPKU patients with or without diet therapy.
Patients and Methods: At the end of 2013, 49 patients were receiving BH4 therapy, of whom 25 were under 4 years of age when treatment began. We conducted a longitudinal retrospective study which examined plasma phenylalanine levels and BH4 dosages of BPKU on normal diets (23 patients) and compared them with on protein-restricted diets (26 patients).
Results: The plasma phenylalanine values were slightly lower (308.1μmol/L) in restricted diet group than (403.5 μmol/L) in normal diet group. The dosages of BH4 were almost the same (10.0 and 10.6 mg/kg, respectively). In all 49 patients plasma Phe values were maintained within a favorable range and no considerable side effect was reported.
Conclusions: BH4 therapy initiated before 4 years of age was very effective to maintain favorable plasma phenylalanine levels in Japanese BPKU patients and was safe. Therefore, it should reduce the protein-restricted diet by increasing the dose of BH4.
Conflict of Interest declared.

P-149

Evaluation of multiple dosing regimens in phase 2 studies of 'rAvPAL-PEG' for control of blood phenylalanine levels in adults with phenylketonuria

Adams D[1], Thomas J A[2], Longo N[3], Zori R[4], Burton B K[5], Wasserstein M[6], Grange D K[7], Vockley J[8], Hillman R[9], Harding C[10], Shur N[11], Rice G M[12], Rizzo W B[13], Whitley C[18], Goodin K M[14], McBride K L[15], Decker C[16], Merilainen M[16], Li M[16], Schweighardt B[16], Dimmock D[17]

[1]Goryeb Childrens Hospital, Morristown, United States, [2]University of Colorado, Aurora, United States, [3]University of Utah, Salt Lake City, United States, [4]University of Florida, Gainesville, United States, [5]Lurie Childrens Hospital, Chicago, United States, [6]Mount Sinai Hospital, New York, United States, [7]St Louis Childrens Hospital, St Louis, United States, [8]Childrens Hospital Pittsburgh, Pittsburgh, United States, [9]University of Missouri, Columbia, United States, [10]Oregon Health Sciences, Portland, United States, [11]Albany Medical College, Albany, United States, [12]University of Wisconsin, Madison, United States, [13]Nebraska Medical Center, Omaha, United States, [14]Weisskopf Child Evaluation Center, Louisville, United States, [15]Nationwide Childrens Hospital, Columbus, United States, [16]BioMarin Pharmaceutical Inc, Novato, United States, [17]Medical College of Wisconsin, Milwaukee, United States, [18]University of Minnesota, Minneapolis, United States

Background: Phenylketonuria (PKU) is caused by a deficiency of phenylalanine hydroxylase. PEGylated Recombinant Anabaena variabilis phenylalanine ammonia lyase (rAvPAL-PEG) is a potential enzyme substitution therapy to treat PKU.
Methods: In study PAL-002, various fixed, weight-based doses of rAvPAL-PEG were administered weekly to 40 subjects during an 8-week induction period, followed by 8 weeks of dose titration. Study PAL-004 explored various daily dose regimens of rAvPAL-PEG in 24 subjects with higher starting doses in an attempt to achieve an earlier blood phenylalanine (Phe)-lowering effect.
Results: Overall, weekly doses in the PAL-002 study did not lead to substantial Phe reductions. Treatment was generally well tolerated, although all subjects experienced at least one adverse event (AE) and most (78%) experienced a mild-to-

moderate hypersensitivity AE (HAE). The most common AEs were injection site reactions. In Study PAL-004, a substantial mean blood Phe reduction (-929 ± 691 μmol/L) was observed at Week 2; however, this reduction was not sustained in most subjects due to more frequent HAEs requiring dose modification.
Conclusions: Dosing in early Phase 2 studies did not achieve optimal blood Phe reduction. Dosing was generally well tolerated in PAL-002, but higher starting doses in PAL-004 resulted in more frequent HAEs requiring dose reductions.
Conflict of Interest declared.

P-150

Evaluation of an induction, titration, and maintenance dosing regimen in a phase 2 study of 'rAvPAL-PEG' for control of blood phenylalanine levels in adults with phenylketonuria (PKU)

Zori R[3], Thomas J A[1], Shur N[4], Rizzo W B[6], Decker C[5], Merilainen M[5], Li M[5], Schweighardt B[5], Longo N[2]

[1]University of Colorado, Aurora, United States, [2]University of Utah, Salt Lake City, United States, [3]University of Florida, Gainesville, United States, [4]Albany Medical College, Albany, United States, [5]BioMarin Pharmaceutical Inc, Novato, United States, [6]Childrens Hospital and Medical Center, Omaha, United States

Background: Phenylketonuria (PKU) is caused by a deficiency of phenylalanine hydroxylase. PEGylated Recombinant Anabaena variabilis phenylalanine ammonia lyase (rAvPAL-PEG) is a potential enzyme substitution therapy to treat PKU.
Methods: Study 165-205 was a multi-center, open-label, fixed dose-finding study of rAvPAL-PEG in 24 subjects with weekly low-dose 4-8 week induction followed by at least 4 weeks of titration to a target dose maintaining blood phenylalanine (Phe) levels ≤ 600 μmol/L for 4 consecutive weeks.
Results: Mean baseline Phe (1169 ± 291 μmol/L) was reduced to 618 ± 529 μmol/L by the end of the 24-week study. Eleven of 24 subjects achieved target dose; the remaining 13 did not sustain Phe ≤ 600 μmol/L during the study. All AEs were mild or moderate. One subject experienced a serious AE unrelated to treatment. Hypersensitivity AEs and injection site reactions each occurred in 92% of subjects. All subjects developed antibody against PAL and most developed antibody against PEG.
Conclusions: This study demonstrated that weekly, low-dose introduction of rAvPAL-PEG, followed by gradual dosage and frequency increases was well tolerated, with HAEs limited to mild or moderate severity. Blood Phe reduction appears dependent on individual immune response against rAvPAL-PEG, dose and duration of the treatment.
Conflict of Interest declared.

P-151

Phase 2 studies contribute to rAvPAL-PEG Phase 3 trial design

Burton B K[4], Harding C[5], Longo N[2], Thomas J A[1], Zori R[3], Bilder D A[2], Posner J[7], Lieberman P[8], Merilainen M[6], Gu Z[6], Schweighardt B[6], Weng H[6], Levy H[9]

[1]University of Colorado, Aurora, United States, [2]University of Utah, Salt Lake City, United States, [3]University of Florida, Gainesville, United States, [4]Lurie Childrens Hospital, Chicago, United States, [5]Oregon Health Sciences, Portland, United States, [6]BioMarin Pharmaceutical Inc, Novato, United States, [7]Kings College, London, United Kingdom, [8]Univ of Tennessee College of Medicine, Germantown, United States, [9]Bostons Childrens Hospital, Boston, United States

Background: Phenylketonuria (PKU) is caused by a deficiency of phenylalanine hydroxylase. PEGylated Recombinant *Anabaena variabilis* phenylalanine ammonia lyase (rAvPAL-PEG) is a potential enzyme substitution therapy for the treatment of PKU.
Methods: Four Phase 2 studies informed the ongoing Phase 3 study on the safety and efficacy of the introduction and maintenance of treatment with rAvPAL-PEG subcutaneous injections using different dose levels, methods and timelines in dose titration.
Design: Phase 2 data indicate that the majority of patients can be treated to meaningful blood Phe reductions with daily doses of 20 and 40 mg. Weekly, low-dose introduction of treatment, followed by gradual dosage and frequency increases, result in fewer hypersensitivity events. The data demonstrate that rAvPAL-PEG blood Phe reduction is dependent on the individual immune response against the rAvPAL-PEG compound, dose, and duration of the treatment. Most Phase 2 subjects entered the long-term extension study, where the majority of subjects achieved at least 2 consecutive blood Phe values ≤ 600 μmol/L in 25-40 weeks.
Conclusions: Data from multiple early-phase clinical studies informed the design of the Phase 3 trial. In order to safely achieve blood Phe target concentrations, dosage, duration of treatment, and individual immunological responses must be considered.
Conflict of Interest declared.

P-152

Molecular characterization of *QDPR* gene in Iranian families with BH4 deficiency; reporting novel and recurrent mutations

Abiri M[1 3], Foroozania H[2], Salehpour S[4], Bagherianf H[5], Alaei M R[4], Khatami S[3], Azadmehr S[5], Setoodeh A[1], Rejali L[5], Rohani F[1], Zeinali S[3 5]

[1]TUMS, Tehran, Iran, Islamic Republic of, [2]IAU, tehran, Iran, Islamic Republic of, [3]Pasteur Institute, tehran, Iran, Islamic Republic of, [4]SBUMS, tehran, Iran, Islamic Republic of, [5]Kawsar Human Genetics Research Center, tehran, Iran, Islamic Republic of

Newborn screening for PKU has been in practice in Iran since 2007. Some hyperphenylalaninemia cases have tetrahydrobiopterin (BH4) biosynthesis deficiency/disorder. DHPR (dihydropteridine reductase deficiency) is one form of the BH4 deficiency. This deficiency is presented by psychomotor retardation, myoclonic epilepsy, microcephaly, febrile attacks, hypertonia of the trunk with limb hypertonia and fatal course due to neurotransmitters. Diagnosis is confirmed by measuring pterins and neurotransmitter metabolites in urine and cerebrospinal fluid. Measurement of the DHPR enzyme activity in dried blood spot or fibroblast is also possible. Five genes including *QDPR* (encoding DHPR enzyme, the necessary cofactor for PAH activity) have been identified in the synthesis or recycling of BH4. We identified a total number of 93 BH4-deficient families. A multiplex set of STR markers linked to 4 genes responsible for the BH4 deficiency (i.e. *GCH1*, *PCBD1*, *PTS* and *QDPR* genes) was used to quickly determine which gene may be responsible to cause the disease. Mutation analysis of *QDPR* gene revealed some known and novel mutations. We found 16 types of mutations that most of the mutations (10 out of 16) were novel. Our findings show that no common mutation predominates and they are scattered throughout the causative gene in our population.

P-153

A randomized, placebo-controlled, double-blind study of sapropterin to treat symptoms of ADHD and executive dysfunction in children and adolescents with phenylketonuria

Cohen-Pfeffer J L[2], Grant M[4], McCandless S M[5], Stahl S M[6], Bilder D A[1], Jurecki E R[2], Yu S[2], Sanchez-Valle A[7], Dimmock D[3]

[1]University of Utah, Salt Lake City, United States, [2]BioMarin Pharmaceutical Inc, Novato, United States, [3]Medical College of Wisconsin, Milwaukee, United States, [4]Drexel University College of Medicine, Philadelphia, United States, [5]Case Western University, Cleveland, United States, [6]University of California, San Diego, La Jolla, United States, [7]University S. Florida,College Medicine, Tampa, United States

Introduction: Children with phenylketonuria (PKU) may exhibit symptoms of neurocognitive impairment.
Methods: A sub-analysis was performed on pediatric subjects (< 18 years) with PKU from a larger cohort to determine the impact of sapropterin therapy on neurocognitive deficits. Subjects were randomized to blinded treatment with sapropterin (N = 43) or placebo (N = 43) for 13 weeks. Behavior Rating Inventory of Executive Function (BRIEF) Global Executive Composite (GEC), Behavioral Regulation Index (BRI), and Metacognition Index (MI) T-scores were assessed at weeks 13 and 26. Attention Deficit Hyperactivity Disorder Rating Scale (ADHD-RS) was obtained at screening and weeks 4, 8, 13, and 26.
Results: For subjects on sapropterin after 13 weeks, average blood phenylalanine decreased from baseline of 501 ± 305 mmol/L to 394 ± 344 mmol/L. The least squares (LS) mean change differences from baseline between the sapropterin-treatment and placebo groups were significant for BRIEF-GEC (p=0.04) and BRIEF-MI (p=0.04). The LS mean change differences in ADHD-RS from baseline between the sapropterin-treatment and placebo groups were significant for ADHD-RS Total Score (p=0.01), Hyperactivity/Impulsivity (p=0.02), and Inattention (p=0.04).
Conclusions: In a double-blind, placebo controlled study, sapropterin use was associated with neurocognitive improvements in children and adolescents compared to placebo.
Conflict of Interest declared.

P-154

Tetrahydrobiopterin (BH4) responsiveness in hyperphenylalaninemic (HPA) patients, a longitudinal study: experience of the paediatric department - San Paolo Hospital - University of Milan

Selmi R[1 2], Selmi R[1 2]

[1]Div Metab Dis, Paed Depar, San Paolo Hos, Milan, Italy, [2]Arab Organization for Rare diseases, Gaza, Palestine

Objectives: This study aims to evaluate potential BH4 nutritional effects as additional therapy beside dietary treatment in HPA patients.
Patients and Methods: Ten patients (6 males and 4 females), aged 7-24 years, underwent two years follow up including periodical assessments of anthropometric measurements (z-score was evaluated), dietary intakes and blood nutritional indices (plasma phenylalanine (Phe) levels, iron status,

proteins, albumin and lipid profile), in order to evaluate potential BH4 effect upon these parameters.
Results: Plasma phenylalanine levels were found to be considerably decreased in the first (T1) and second (T2) year after the beginning of BH4 treatment (- 40.2% and - 42.28%, respectively), always remaining within the safe range (< 360 mcmol/l). Significant dietary Phe and dietary natural protein intakes increases could also be achieved (Phe intakes: + 142.3%, p=.008, at T1 and +149.7%, p=0.043, at T2, respectively; natural protein intakes: +145.5%, p =.008 at T1 and + 198.3%, p=0.043 at T2, respectively).
Conclusions: This study suggests that HPA patients receiving a diet restricted in Phe can benefit of a BH4 additional therapy in order not only to increase natural protein and Phe intakes but also to obtain better nutritional status and subsequent better ponderal and stature anthropometric outcome

P-155

Genotype–phenotype associations in French patients with phenylketonuria and importance of genotype for full assessment of tetrahydrobiopterin responsiveness

Jeannesson-Thivisol E[1], Feillet F[1], Chéry C[1], Perrin P[1], Battaglia-Hsu S F[1], Herbeth B[1], Cano A[2], Barth M[3], Fouilhoux A[4], Mention K[5], Labarthe F[6], Arnoux J B[7], Maillot F[6], Lenaerts C[8], Dumesnil C[9], Wagner K[10], Terral D[11], Broué P[12], De Parscau L[13], Gay C[14], Kuster A[15], Bédu A[16], Besson G[17], Lamireau D[18], Odent S[19], Masurel A[20], Guéant J L[1], Namour F[1]

[1]Nancy Univ Hosp and INSERM U954, Vandoeuvre-lès-Nancy, France, [2]Timone Hosp, Marseille, France, [3]Angers Univ Hosp, Angers, France, [4]Hospices Civils de Lyon, Bron, France, [5]Jeanne de Flandres Hosp, Lille, France, [6]Tours Univ Hosp, Tours, France, [7]Necker Enfants Malades Hosp, Paris, France, [8]Amiens Univ Hosp, Amiens, France, [9]Rouen Univ Hosp, Rouen, France, [10]Lenval Hosp, Nice, France, [11]Hotel-Dieu Hosp, Clermont-Ferrand, France, [12]Children Hosp, Toulouse, France, [13]Brest Univ Hosp, Brest, France, [14]Saint-Etienne Univ Hosp, Saint-Etienne, France, [15]Nantes Univ Hosp, Nantes, France, [16]Mere-Enfant Hosp, Limoges, France, [17]Grenoble Univ Hosp, Grenoble, France, [18]Pellegrin Enfants Hosp, Bordeaux, France, [19]Rennes Univ Hosp, Rennes, France, [20]Dijon Univ Hosp, Dijon, France

Background: Sapropterin (BH4) is an important therapeutical strategy in phenylketonuria. Phenylalanine hydroxylase (*PAH*) is a highly polymorphic gene and it is difficult to identify BH4-responsive genotypes. Our aim is to improve prediction of BH4-responsiveness through determination of genotypes, BH4-loading test, predictions of responsiveness according to the literature and types and locations of mutations.
Methods: 364 French patients with phenylketonuria benefited from a 24-hour BH4-loading test and *PAH* gene sequencing.
Results: 31.6% of patients were BH4-responsive. 127 different mutations were found, including 26 new mutations. The mutations c.434A>T, c.500A>T, c.529G>C, c.1045T>G and c.1196T>C were newly classified as being BH4-responsive. We identified 261 genotypes, among which 26 were newly recognized as being BH4-responsive. Even though patients carry 2 responsive alleles, BH4-responsiveness cannot be predicted with certainty unless they present mild hyperphenylalaninemia. BH4-responsiveness cannot be predicted in patients carrying one responsive mutation only. In general, the milder the phenotype is, the stronger the BH4-response is. Only missense mutations, particularly in exons 12, 11 and 8, are associated with BH4-responsiveness. Any other type of mutation predicts a negative response.
Conclusion: Patients should benefit from a combination of BH4-loading test and molecular analysis for optimal prediction of both phenylketonuria severity and efficacy of BH4 pharmacotherapy.

P-156

The effect of L-carnitine on phenylalanine-induced DNA damage

Deon M[1 3], Landgraf S S[4], Mescka C P[1 5], Lamberty J F[1], Moura D J[2], Saffi J[2], Wajner M[1 5], Vargas C R[1 3 5]

[1]Serviço de Genética Médica, HCPA, Porto Alegre, Brazil, [2]Lab Genética Toxicológica, UFCSPA, Porto Alegre, Brazil, [3]PPGCF,UFRGS, Porto Alegre, Brazil, [4]IFRJ, Rio de Janeiro, Brazil, [5]PPGBioq, UFRGS, Porto Alegre, Brazil

Background. L-Carnitine (LC) has an antioxidant property. Phenylketonuria (PKU) has been associated with oxidative stress.
Objectives. The aim of this study was to verify the effect of LC on phenylalanine-induced DNA damage.
Methods. The *in vitro* effect of different concentrations of LC (30, 120 and 150 μM) on DNA damage-induced by phenylalanine (Phe)(1000 μM) was examined in whole blood cells from normal individuals using the comet assay. Urinary 8-hydroxydeoguanosine (8-OHdG) levels, a biomarker of oxidative DNA damage, were measured in 8 patients with classical PKU (Phe blood level: 641 ± 36.37 μmol/L), under dietary therapy and supplemented with a special formula containing LC, and in controls individuals.

Results. The *in vitro* co-treatment with Phe and LC reduced significantly DNA damage index when compared to Phe group. The urinary excretion of 8-OHdG presented similar levels in both groups analyzed. In treated PKU patients, urinary 8-OHdG levels were positively correlated with blood Phe levels and negatively correlated with blood LC concentration.
Conclusions. The present study yields experimental evidence that LC can reduce the *in vitro* DNA injury induced by Phe, as well as, allows to hypothesize that LC protect against DNA damage in patients with PKU. Financial support: CAPES, CNPq, FIPE/HCPA.

P-157

Fifth interim analysis of the Kuvan® adult maternal paediatric european registry (KAMPER): pregnancies

Feillet F[7], Lagler F B[1], Rogoff D[2], Stucker F[2], Alm J[3], Muntau A C[4], Burlina A[5], Trefz F K[6]

[1]Paracelsus Med Univ, Salzburg, Austria, [2]Merck Serono S.A., Geneva, Switzerland, [3]Karolinska Univ Hosp, Stockhom, Sweden, [4]Univ Child Hosp, Univ Med Cent, Hamburg Eppendorf, Germany, [5]Univ Hosp, Padova, Italy, [6]Sch of Med Univ, Tuebingen, Germany, [7]Hôpital d'enfants Brabois, Vandoeuvre lès Nancy, France

Objectives: To describe the efficacy and safety of the use of sapropterin dihydrochloride in a group of eight BH_4-responsive pregnant women with phenylketonuria.
Methods: Data were collected from the maternal sub-registry of KAMPER, which records data on the management, efficacy and safety of sapropterin in pregnant patients.
Results: Eight patients (mean age 28.1; range 22.4–35.0, total of 9 pregnancies). From those, only 4 were under a Phe-restricted diet before the onset of pregnancy. Mean dose of sapropterin during pregnancy was 11.8±5.9 mg/kg/day (range 3.3–21.0 mg) and the median duration of sapropterin use was 203 days. Six patients delivered normal babies (5 male, 1 female). One patient was expected to give birth after the database lock for this fifth interim analysis and the outcome of the remaining patient is unknown. Of those patients for whom data are available, two breastfed and two did not. Three patients each experienced a mild adverse event, one of which was considered related to study medication (sapropterin overdose, prior to the pregnancy).
Conclusion: In a small population of pregnant women with hyperphenylalaninaemia, therapy with sapropterin during gestation was shown to be safe and well tolerated and resulted in normal healthy births.
Conflict of Interest declared.

P-158

Population pharmacokinetics analysis of sapropterin dihydrochloride in 0–4 year-old children with phenylketonuria: results from the SPARK study

Muntau A C[2], Uhl W[1], Mould D R[3], Munafo A[4]

[1]Merck KGaA, Darmstadt, Germany, [2]Univ Child Hosp, Univ Med Cen, Hamburg Eppendorf, Germany, [3]Projections Res Inc., Phoenixville, United States, [4]Merck Inst Pharm, Lausanne, Switzerland

Objectives: To develop a population pharmacokinetic model for sapropterin dihydrochloride in 0–4 year-old children with phenylketonuria.
Methods: Plasma samples were collected at baseline for endogenous BH_4 and sparsely between weeks 5–12 after oral administration of sapropterin 10 mg/kg/day. Non-linear mixed-effect modelling was applied to estimate the pharmacokinetic parameters and their variability.
Results: Data from 52 subjects aged 1–48 months (of whom 26 received the drug, 26 diet alone). A 1-compartment model with first-order input following a lag time and first-order elimination, including an endogenous baseline component, best described the data. The only covariate retained was body weight, parameterised using an allometric function, affecting both clearance and volume. The final parameters estimates were 2780 L/hr for CL/F, 3870 L for V/F, and K_a of 0.234 hr^{-1}. The elimination was absorption-rate limited, the effective terminal half-life was 3 hr. The exposure across all age groups was regarded as comparable, slightly lower than in older patients, supporting a conservative and safe approach.
Conclusion: The model adequately described the data in these 0–4 year-old children with phenylketonuria. The effect of weight was substantial and dose adjustments based on weight are appropriate. From a pharmacokinetic perspective, the once-daily regimen is justified.
Conflict of Interest declared.

P-159

Long-term treatment of pediatric hyperphenylalaninemia patients with sapropterin

Zerjav Tansek M[1], Groselj U[1], Kelvisar M[2], Kobe H[1], Repic Lampret B[1], Battelino T[1 2]

[1]UMC Ljubljana, Univ Child Hosp Ljubljana, Ljubljana, Slovenia, [2]Faculty of Medicine, Univ of Ljubljana, Ljubljana, Slovenia

Background: Sapropterin (BH4) is shown to reduce phenylalanine (Phe) levels in responders with hyperphenylalaninemia, enabling relaxation of dietary restrictions. The study has assessed long term clinical effects of treatment with BH4.
Material and methods: Nine pre-pubertal patients with a proven BH4 responsiveness were treated with BH4. The median dietary tolerance to Phe, blood Phe, tyrosine (Tyr), zinc, selenium and vitamin B12 levels in the two year periods before and after the introduction of BH4 treatment were analyzed.
Results: The daily Phe tolerance had tripled, from pretreatment median value of 620 mg (IQR 400–700 mg) to 2000 (IQR 1000–2000 mg) ($p< 0.001$). The median blood Phe levels before BH4 therapy were in recommended range (200 μmol/l, IQR 191-302) and did not change significantly on therapy (190 μmol/l; IQR 135–285 μmol/l) (p=Ns). The median blood Phe/Tyr before introducing the BH4 therapy was 4.7 and had lowered significantly on therapy to 2.4 ($p=0.01$). Median zinc, selenium and vitamin B12 levels had not changed (p=Ns).
Conclusions: BH4 therapy had enabled patients much higher dietary Phe intakes. Median blood Phe and Tyr levels each did not change significantly on therapy, but median Phe/Tyr ratios had lowered despite discontinuing the Tyr supplemented medical food.

P-160

Fifth interim analysis of the Kuvan® adult maternal pediatric european registry (KAMPER): insights into management/practice in patients with PKU

Lagler F B[1], Muntau A C[2], Feillet F[6], Rogoff D[3], Moreau-Stucker F[3], Alm J[4], Burlina A[5], Trefz F K[7]

[1]Paracelsus Med Univ, Salzburg, Austria, [2]Univ Child Hosp, Univ Med Cent, Hamburg Eppendorf, Germany, [3]EMD Serono Inc., Billerica, United States, [4]Karolinska Univ Hosp, Stockholm, Sweden, [5]Univ Hosp, Padova, Italy, [6]CHU Brabois Hôpital d'Enfants, Vandoeuvr les Nanacy, France, [7]Sch of Med Univ, Tuebingen, Germany

Objectives: To present a long-term safety follow-up in phenylketonuria (PKU) patients receiving sapropterin dihydrochloride. Additionally, to present data on real-life management of PKU and patient population.
Methods: The Kuvan® Adult Maternal Paediatric European Registry (KAMPER), an observational, multicentre drug registry in eight European countries, provides long-term safety and efficacy outcomes of sapropterin treatment in hyperphenylalaninaemic patients.
Results: Data from 452 PKU patients (n=223 females, 49.3%); median age 10.1 (range 3.2-39.7) years is included. The minimum and maximum follow-up period was 1 day and 4.4 years. A total of 127 patients experienced 261 adverse events (AEs); eight patients reported 11 serious AEs (tachycardia, Niemann-Pick disease, upper limb fracture, testicular seminoma, drop attacks, headache, stimuli unresponsiveness, abortion, abnormal behaviour, suicidal ideation and nephrolithiasis; only headache was considered related to study medication). Data on baseline and follow-up sapropterin dose; Phe and Tyr levels; dietary intake; ingested and prescribed Phe intake; growth, psychiatric, behavioural and neurological assessment; and school/work performance will be presented.
Conclusion: Interim results from the KAMPER registry continue to show that sapropterin has a favourable safety profile. The large number of subjects included in this registry makes it a valuable source of information on real-life management and challenges of PKU patients.
Conflict of Interest declared.

P-161

Effect of divided daily doses of sapropterin in phenylketonuria

Kör D[1], Şeker Yılmaz B[1], Bulut D[1], Yolburun G[1], Gönkek S[1], Özakçaoğlu T[1], Ceylaner S[2], Onenli Mungan N[1]

[1]Div Metab Dis, Univ Cukurova, Adana, Turkey, [2]Intergen Genetics Laboratory, Ankara, Turkey

Background: Treatment of PKU includes a lifelong phenylalanine-restricted diet. BH_4 is a co-factor for the PAH and can improve PAH activity and Phe tolerance with long-term studies.
Methods: After 72-h BH4-loading test sapropterin was initiated to 50 responsive patients. Patients were divided into two groups; sapropterin using divided 2-daily doses (group-1) and using a single dose (group-2). We evaluated the efficacy of divided daily doses of sapropterin.
Results: No significant differences were found in the age of diagnosis, age of treatment, mean Phe-levels at diagnosis and mean daily Phe-consumption at the beginning and in the sixth months of treatment between two groups. But, mean daily consumption of Phe increased significantly after the first year of treatment in the divided daily doses group ($p< 0,05$).Also, during long-term treatment with sapropterin, 7 patients in group-1 and one patient in group-2 quit Phe-free diet. No clear genotype and sapropterin-responsiveness correlation could be determined.
Conclusion: To the best of our knowledge this is the first study evaluating the efficacy of divided daily doses of sapropterin. Sapropterin given 2-daily doses was found to be more efficacious then once daily dose at the first year of treatment, but further studies are needed to predict the long-term effects.

P-162

Large neutral amino acid (LNAA) supplementation exerts its effect through three synergistic mechanisms: proof of principle in PKU mice

Van Vliet D[1 2], Bruinenberg V M[2], Mazzola P N[1 2], Van Faassen H J R[3], De Blaauw P[3], Kema I P[3], Heiner-Fokkema M R[3], Van Anholt R D[4], Van der Zee E A[2], Van Spronsen F J[1]

[1]UMCG, Beatrix Child Hosp, Div Metab Dis, Groningen, Netherlands, [2]Univ of Groningen, Dept Mol & Neurobiol, Groningen, Netherlands, [3]UMCG, Dept Lab Med, Groningen, Netherlands, [4]Deventer, Deventer, Netherlands

Background and objectives: Insufficient understanding of the effects of LNAA supplementation on neurocognitive dysfunction in PKU hampers its clinical application. As a proof of principle, this study investigated all hypothesized biochemical treatment objectives of LNAA supplementation without dietary phenylalanine restriction in PKU mice.
Methods: From postnatal day 37, C57Bl/6 *Pah-enu2* (PKU) and wild-type mice received either a LNAA supplemented diet, an isonitrogenic/isocaloric high-protein control diet, or normal chow. After 6 weeks of treatment, blood and brain were collected to determine the effect of LNAA treatment on 1) blood phenylalanine, 2) brain phenylalanine, 3) brain LNAA, and 4) brain neurotransmitters.
Results: In PKU mice, LNAA supplementation reduced blood and brain phenylalanine concentrations by 33% and 26%, respectively, compared to normal chow ($p< 0.01$), while alleviating the brain deficiencies of some but not all supplemented LNAA. Moreover, LNAA supplementation in PKU mice significantly increased brain serotonin and norepinephrine concentrations from 35% to 71% and from 57% to 86% of wild-type levels ($p< 0.01$), but not brain dopamine ($p=0.307$).
Conclusion: LNAA supplementation without dietary phenylalanine restriction in PKU mice improves brain biochemistry through three synergistic mechanisms. By this, LNAA supplementation becomes an interesting treatment strategy for PKU, necessitating additional research to define the optimal regimen.
Conflict of Interest declared.

P-163

Targeted large neutral amino acid supplements in PKU: from therapeutic brain modulation to revealing underlying mechanisms of brain dysfunction

Van Vliet D[1 2], Bruinenberg V M[2], Mazzola P N[1 2], Van Faassen H J R[3], De Blaauw P[3], Kema I P[3], Heiner-Fokkema M R[3], Van der Zee E A[2], Van Spronsen F J[1]

[1]UMCG, Beatrix Child Hosp, Div Metab Dis, Groningen, Netherlands, [2]Univ of Groningen, Dept Mol & Neurobiol, Groningen, Netherlands, [3]UMCG, Dept Lab Med, Groningen, Netherlands

Background and objective: Despite many scientific efforts, insufficient knowledge about the role of phenylalanine toxicity and neurotransmitter depletion in neurocognitive dysfunction in PKU hampers the development of pathophysiology-based treatment strategies. Using various large neutral amino acid (LNAA) supplements aimed to reduce brain phenylalanine, increase brain LNAA, or enhance brain neurotransmitters, we studied the pathophysiological cascade by which high blood phenylalanine impairs brain biochemistry and investigated therapeutic opportunities to restore this.
Methods: Different LNAA supplements including 1) *leucine+isoleucine*, 2) *all LNAA* (with and without threonine), 3) *tyrosine+tryptophan*, and 4) *threonine* alone were investigated in *Pah-enu2* (PKU) mice.
Results: Brain phenylalanine decreased with 24-29% on *leucine+isoleucine* and *all LNAA* ($p< 0.01$). Regarding brain non-phenylalanine LNAA, all regimens increased the LNAA that were supplemented, while *leucine+isoleucine* further reduced most other LNAA. Brain serotonin and norepinephrine were increased by 80% and 39%, respectively, on *all LNAA* ($p< 0.01$), and increased by 40% and 28% on *tyrosine+tryptophan* ($p< 0.01$).
Conclusions: Different LNAA regimens serve different biochemical treatment objectives in PKU. Interestingly, increasing neurotransmitter precursor amino acids rather than decreasing brain phenylalanine improved brain neurotransmitters, while the combination of both strategies was most effective. Supplementation of all LNAA was most effective, achieving all three brain biochemical treatment objectives.
Conflict of Interest declared.

P-164

Fluctuations of blood phenylalanine levels in children and adolescents with phenylketonuria

Feldmann R[1], Nguyen T[1], Schallert M[1], Och U[1], Marquardt T[1], Weglage J[1], Rutsch F[1]

[1]Div Metab Dis, Univ Child Hosp, Muenster, Germany

Objectives: We examined the impact of fluctuations in metabolic control on the cognitive development in children and adolescents with early-treated phenylketonuria (PKU).
Methods: We investigated 50 patients with early-treated PKU aged 6 to 18 years, 27 being on a Phe restricted continous diet, and 23 treated with sapropterin. For 26 weeks, patients were

assessed weekly for their blood phenylalanine (Phe) levels. Data were analyzed for fluctuations indicated by the standard deviation of the individual blood Phe levels. Fluctuations were compared to the standard deviation of 26 individual blood Phe level measurements before study interval (sapropterin treatment). We assessed the concurrent Full Scale IQ (FSIQ) of the patients.
Results: Blood Phe level fluctuations in patients on diet were higher than in patients treated with sapropterin. FSIQ showed a negative correlation with the blood Phe level fluctuations before study interval in patients later treated with sapropterin. This negative correlation was not found anymore after treatment with sapropterin had begun.
Conclusions: Fluctuations were reduced in patients treated with sapropterin. FSIQ was correlated negatively with fluctuations before study interval in these patients. Thus, fluctuations may have a detrimental impact on the FSIQ in patients with relatively low blood Phe levels due to their PAH activity.

P-165

First steps towards living quality improvement of PKU children in Romania by administration of sapropterine

Moldovanu F[1], Nanu M[1], Muresan M[2], Iorgulescu D[1]

[1]Inst Mother&Child Care, Dep of Pediatric, Bucharest, Romania, [2]Clinical Children Hospital, Cluj, Romania

Background: During the first four years of dietary treatment of PKU children in Romania, our experience revealed difficulties in maintaining a good metabolic control of the desease because of a variable compliance to the diet.
Objectives: Identification of PKU children responsive to sapropterin loading test in Romania, long term treatment with sapropterin of the responsive patients, follow up of Phe tolerance increase under treatment.
Pacients and method: A number of 24 PKU children, aged 4-18y, under protein restricted diet and with a plasmatic Phe level of 134-1870 μmol/L have been tested with 20mg/kg/d of sapropterin for seven days. The cut-off > 30% of Phe level decrease was considered. The responsive patients received 20mg/kg/d of sapropterin and tolerance to Phe has been followed under treatment.
Results: A number of 9 patients (38%) were responsive to sapropterin loading test, of which 7 patients received 20mg/kg/d of sapropterin on the long term.The tolerance to dietary Phe was monitored. After three months of treatment, dietary Phe mean tolerance of these patients increased significantly from 475mg Phe/d to 794mg Phe/d (paired t-test: p=0.0002).
Conclusion: The administration of sapropterin to responsive PKU children contributes to the improvement of metabolic control and life quality of patients.

P-166

Effects of Casein Glycomacropeptide (CGMP) versus free synthetic amino acid on growth and body composition in early treated mice with Phenylketonuria (PKU)

Ahring K[1], Dagnaes-Hansen F[2], Christensen M[5], Jensen E[3], Jensen T G[2], Johansen K S[2], Lund A M[4], Madsen J G[2], Nielsen K B[4], Pedersen M[2], Pedersen S G[2], Poulsen K[6], Fuglsang M K[2], Moller L B[1]

[1]PKU dept, Kennedy Centre, CPH Uni Hosp, Copenhagen, Denmark, [2]Dept of Biomedicine, Aarhus Uni, Aarhus, Denmark, [3]AFI, Aarhus, Denmark, [4]Genetic Clinic, CPH Uni Hosp,, Copenhagen, Denmark, [5]Metab Lab, CPH Uni Hosp, Copenhagen, Denmark, [6]Statens Serum Institut, Copenhagen, Denmark

Introduction: CGMP is a natural protein low in phenylalanine with a neutral taste. CGMP is a potential dietary alternative to AAs and may improve PKU nutrition on several aspects
Objective: To determine the effects of CGMP versus free AA on growth and body composition in early treated PKU mice.
Materials and Methods: 28 mice (classical PKU) were divided into five groups and each group followed a special diet for 12 weeks:1: Semi-free diet (SFD) + CGMP 2: SFD + CGMP+AA 3: Control 4: CGMP +protein free diet (PFD) 5: AAM + PFD
Results: Growth and body composition were measured after 12 weeks. Growth ranged from an average of 4.9 g to 9.56 g: 1: 4.9 g (70% compared to start weight) 2: 6.5 g (78%) 3: 5.84 g (82%)
4: 9.56 g (173%) 5: 9.14 g (151%)
Body scan (average fat & lean body mass): 1: 1.50 g/11.70 g 2: 1.73 g/12.79 g 3: 1.78 g/11.18 g 4: 2.06 g/ 13.15 g 5: 2.26 g/13.40 g
Conclusions: Mice fed on CGMP or AA + PFD demonstrated the best average growth. Body scan supported this result.
Conflict of Interest declared.

P-167

48-hour BH4 loading test versus algorithmic phenylalanine challenge: analysis of an open-label multicentre study

Lagler F B[1], Möslinger D[3], Brunner-Krainz M[4], Fekete A[5], Konstantopoulou V[3], Moder A[1], Plecko-Startinig B[2]

[1]Inst Inborn Errors of Metab, PMU, Salzburg, Austria, [2]Children´s hospital, Zürich, Switzerland, [3]Kinderklinik, Med Uni Wien, Vienna, Austria, [4]UK f Kinder- und Jugendheilkunde, MUG, Graz, Austria, [5]Metabolic Center, Vienna, Austria

Background: The BH4 loading test (BLT) recommended by the European Phenylketonuria Group should be performed at ≥400 μmol/l blood phenylalanine. However many patients, who qualify for Kuvan® treatment target lower levels. Furthermore BLT doesn't capture an increase of phenylalanine tolerance (PheTol). Thus we developed a novel algorithm with a BLT followed by a 13 days standardised algorithmic Phe challenge (APC).
Primary Objective: Feasibility and validity of the APC vs. BLT.
Patients/Methods: 22 PKU patients, aged 4-18 years with potentially BH4 responsive genotype were eligible. Responsiveness was defined as a ≥30% decrease in blood Phe levels and/or an increase of PheTol by ≥10 mg/kg/d (or ≥350 mg/d absolute).
Results: Both tests showed a response rate of 17/20 and a failure rate of 3/20 (2 patients lost-to-follow-up). Mean PheTol at baseline increased significantly by 8 mg/kg/d (CI95 5 -11) after the BLT (PheTol1). APC allowed for a further significant increase of PheTol1 by 5 (CI95 2-8) mg/kg/d in the long term assessment. No participant showed harmful hyperphenylalaninemia.
Conclusion/Discussion: Our data revealed the APC protocol as an appropriate diagnostic tool with the add-on benefit of challenging the patient´s PheTol to the maximum level.
Conflict of Interest declared.

P-168

The effects of tetrahydrobiopterin (BH4) treatment on cerebral metabolism in phenylketonuric mice

Pascucci T[1 2], Fiori E[1], Carducci C[1], Leuzzi V[1], Puglisi-Allegra S[1 2]

[1]Sapienza, University of Rome, Rome, Italy, [2]IRCCS Santa Lucia Foundation, Rome, Italy

Background and objectives: Phenylketonuria (PKU) is a genetic metabolic disease caused by deficiency of the enzyme phenylalanine hydroxylase (PAH), using tetrahydrobiopterin (BH4) as a cofactor. As BH4 is not only a cofactor for PAH, but also for tyrosine- and tryptophan hydroxylase enzymes, effects of BH4 treatment on brain neurotransmitter synthesis has been evaluated in ENU2 mice, the genetic murine model of PKU.
Materials and Methods: WT and ENU2 BTBR adult mice are treated with BH4 (100 mg/kg i.p.) or vehicle. Cerebral aminergic metabolism has been evaluated by analysis of biogenic amines and their metabolites, using a HPLC system coupled with a coulometric detector, in punches of several brain areas collected postmortem.
Results: Acute treatment with BH4 tendentially increased biogenic amine metabolism in all investigated brain areas, although the effect was only statistically significant in prefrontal cortex.
Discussion and Conclusion: Our data support the hypothesis that BH4, besides being a cofactor for PAH, passes the blood-brain barrier and acts as a cofactor for tyrosine hydroxylase and tryptophan hydroxylase that are also present in the brain. Additional experiments are currently in progress to evaluate effect of repeated treatment with lower doses of BH4 on brain metabolism.

P-169

A German multi-centre study of women with phenylketonuria (PKU) before, during and after pregnancy

Burgard P[1], Dokoupil K[2], Heddrich-Ellerbrok M[7], Jung A[3], Kühnemund N[4], Lier D[11], Meyer U[5], Newger M[6], Och U[8], Schick P[1], Schwarz M[9], Strittmatter U[10]

[1]Div Metab Dis, Univ Child Hosp, Heidelberg, Germany, [2]Univ Child Hosp, Munich, Germany, [3]Metabolic Centre, Charité Univ Hosp, Berlin, Germany, [4]Dpt Internal Medicine, Univ Hosp, Leipzig, Germany, [5]Pediatr Adolesc Med, Med School, Hannover, Germany, [6]Dpt Gastroenterology, Univ Hosp, Düsseldorf, Germany, [7]Univ Child Hosp Eppendorf, Hamburg, Germany, [8]Univ Child Hosp, Münster, Germany, [9]Practice Int Med Metab Dis, Karst, Germany, [10]Univ Child Adolesc Hosp, Ulm, Germany, [11]Hosp Pediatr Adolesc Med, Reutlingen, Germany

Background: Pregnancies in women with PKU bear a risk for severe phenylalanine embryofetopathy. Recommendations for treatment are based on evidence from planned studies suggesting pre-conceptional start of control of phenylalanine intake and blood phenylalanine as well as close monitoring the complete pregnancy. Much less is known how recommendations are followed in practice.
Methods: Using filed historical data and retrospective interviews of 110 women from 11 German metabolic centres (for all research sites http://www.mpku.org), we investigated first pregnancies ending with childbirth, from the pre-conceptional phase until post-conception. Childrens' outcome was measured by standardized IQ tests and parental rating of behavioural/emotional problems.

Results: Before planning pregnancy most participants were on relaxed diets including amino acids mixtures in about 50%. Nearly all women underwent pre-conceptional dietary training, and started treatment pre-conceptionally. Periods between start of treatment and conception can be long. Compliance with recommendations was perfect in nearly all cases. Developmental outcome of children was normal. Analysis of filed data revealed that women declining participation in the study had poorer compliance with recommendations.
Conclusion: International data bases and registries for maternal conditions are necessary to get more systematic knowledge of pregnancies of women with PKU, but also with other metabolic conditions.
Conflict of Interest declared.

P-170

Are we giving our phenylketonuric patients the adequate amount of micronutrients?

Sánchez-Pintos P[1], De Castro M J[1], Crujeiras V[2], Iglesias J A[1], Aldamiz-Echevarria L[3], Dalmau J[4], Vitoria I[4], Couce M L[1]

[1]Metab Dis Unit, Clinic Univ Hosp.CIBERER, Santiago de Compostela, Spain, [2]Gastroenterol Nutrit Unit, Clin Univ H, Santiago de Compostela, Spain, [3]Metab Unit. Hosp de Cruces, Barakaldo, Spain, [4]Metab Unit. Univ Hosp La Fe, Valencia, Spain

Background: The mainstay of therapy in PKU is dietary restriction of natural proteins. This requires the use of medical foods including phenylalanine (Phe)-free protein substitutes. However it is not yet clearly established what is the optimal supplementation of vitamins and minerals.
Objectives: To describe the micronutrient status in patients with PKU.
Patients and Methods: We conducted a cross-sectional observational study in patients with PKU. Phenylalanine (Phe) levels, phe tolerance, anthropometric measurements and biochemical parameters (calcium, phosphorus, selenium, zinc, folic acid) were assessed according to the severity of their disorder, age, diet, BH4 supplementation and adherence to treatment.
Results**:** A total of 156 patients were included. Calcium, phosphorus and zinc levels were normal in the majority of cases. Selenium levels were under the lower limit in 25% of patients (94.87% with PKU phenotype). Surprisingly folic acid levels were above the upper limit in 39% of patients.
Conclusion: A high prevalence of selenium deficiency and folic acid overload was noted in our study. The composition of Phe-free protein substitutes for PKU patients regarding micronutrients should be reconsidered.

P-171

One year follow up of 11 sapropterin treated PKU patients with positive 24 hour sapropterin tests

Senden M[1], Forsell E[1], Heidenborg C[1], Nordenström A[1], Lajic S[1], Alm J[1], Zetterström R H[1], Nergårdh R[1]

[1]Div Metab Dis, Karolinska Univ Hosp, Stockholm, Sweden

Background and objectives: PKU patients with possible tetrahydrobiopterin responding mutations were tested in a 24h sapropterin loading test. To validate the significance of positive test, data from the year before and after one year on saproterin treatment were compared.
Patients and Methods: 14 patients with a positive 24 h loading test, (>30% reduction in phenylalanine levels), were given sapropterin. For 11 patients data on phenylalanine levels and data on daily protein intake the year before and the first year after start of sapropterin treatment were available. Phenylalanine intake was calculated from self-reported food registration. Plasma phenylalanine levels were measured from filterpaper samples.
Results: Mean phenylalanine levels were significantly lower (579 μmol/L ± 245 vs 471 μmol/L ± 205) and phenylalanine intake was significantly higher (1083 mg/day ± 915,9 vs 2188 mg/day ± 894) the first year on tetrabiopterin treatment compared to pretreatment levels.
Conclusion: PKU patients with a positive sapropterin loading test will have a positive response on phenylalnine levels and an increase in protein intake with saprotein treatment. The question how many of the patients with negative loading that would respond to sapropterin treatment still remains unanserwed.

08. Sulphur amino acid disorders

P-172

Chronic hyperhomocysteinemia increases inflammatory markers in skeletal muscle of rats

Kolling J[1], Scherer E B S[1], Siebert C[1], Longoni A[1], Dos Santos T M[1], Zanotto B S[1], Wyse A T S[1]

[1]UFRGS, Porto Alegre, RS, Brazil

In the present study, we investigated the effect of chronic homocysteine administration on some parameters of inflammation, such as cytokines (TNF-α, IL-1β and IL-6) and chemokine monocyte chemotactic protein-1 (MCP-1) with

commercial kits, in skeletal muscle and serum of rats. Since cholinergic system has been associated with inflammation, we also evaluated the effect of homocysteine on acetylcholinesterase and butyrylcholinesterase activities in skeletal muscle and serum of rats, respectively. Wistar rats received daily subcutaneous injections of homocysteine (0.3–0.6 μmol/g body weight) or saline (control) from the 6th to the 28th days-of-age. Results showed that chronic hyperhomocysteinemia significantly increased pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) and chemokine monocyte chemotactic protein-1 (MCP-1) in skeletal muscle and serum of rats. Acetylcholinesterase activity was increased in skeletal muscle and butyrylcholinesterase activity was not changed in serum of rats. Creatine prevented the increase in acetylcholinesterase, therefore acetylcholine levels could remain high, contributing to the anti-inflammatory action of the cholinergic pathway. Thus, indirectly, creatine can reduce the levels of proinflammatory mediators. According to our results, severe hyperhomocysteinemia increases inflammatory parameters and alters acetylcholinesterase activity, suggesting that this process might be associated, at least in part, with the muscular dysfunctions characteristic of some patients with severe hyperhomocysteinemia. Supported by CNPq

P-173

Gestational hypermethioninemia alters oxidative/nitrative status in skeletal muscle and biomarkers of muscular injury and inflammation in serum of rat offspring

Schweinberger B M[1], Turcatel E[1], Rodrigues A F[1], Wyse A T S[1]

[1]UFRGS, Porto Alegre, RS, Brazil

Background and objectives: In the present study we investigate oxidative/nitrative stress parameters (reactive oxygen species production, lipid peroxidation, sulfhydryl content, superoxide dismutase, catalase and nitrite levels) in gastrocnemius skeletal muscle of the offspring from rats with hypermethioninemia. Muscular injury and inflammation were evaluated by creatine kinase activity, creatinine levels, urea and C-reactive protein and the increase of cardiac troponin I in serum.
Materials and Methods: Wistar female rats received injections of methionine (2.68 μmol/g body weight) or saline during gestational period. After the rats give birth, pups were sacrificed at the twenty-first day of life for removal of muscle and serum.
Results: Methionine treatment increased reactive oxygen species production and lipid peroxidation, and decreased sulfhydryl content, antioxidant enzymes activities and nitrite levels in skeletal muscle of the offspring. Creatine kinase activity was reduced and urea and C-reactive protein levels were increased in serum of pups.
Conclusion: Our findings showed that maternal hypermethioninemia altered oxidative/nitrative status in gastrocnemius skeletal muscle of the offspring, which represent a mechanism able to contribute to myopathies. In addition, we believe that these results may be relevant since gestational hypermethioninemia could cause damage to the skeletal muscle during intrauterine life.

P-174

Lipid profile and alterations in the enzymes paraoxonase and butyrylcholinesterase in plasma of patients with homocystinuria due CBS deficiency: the vitamin B_{12} and folic acid importance

Mescka C P[1 2], Vanzin C S[1 2], Hammerschimidt T G[2], Vilarinho L[3], Nogueira C[3], Wajner M[1 2], Wyse A T S[1], Vargas C R[1 2]

[1]Universidad Fed. do Rio Grande do Sul, Porto Alegre, Brazil, [2]Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil, [3]Inst. Nac. de Saúde Doutor Ricardo Jorge, Porto, Portugal

Homocystinuria is an inborn error of metabolism mainly caused by cystathionine-β-synthase (CBS) deficiency. Homocysteine (Hcy) and methionine (Met) accumulate in the body of patients. Despite to the fact that thromboembolism represent the major cause of morbidity in CBS-deficient patients, the mechanisms of cardiovascular alterations found in homocystinuria remain unclear. In this work, we evaluated the lipid profile and the activities of the enzymes paraoxonase (PON1) and butyrylcholinesterase (BuChE) in plasma of CBS-deficient patients at diagnosis and during the treatment (protein-restricted diet supplemented by pyridoxine, folic acid, betaine and vitamin B_{12}). We also investigate the effect of folic acid and vitamin B_{12}. We found a significant decrease in HDL-cholesterol and apolipoprotein A1 (ApoA1) levels, as well as in PON1 activity in both untreated and treated CBS-deficient patients when compared to controls. BuChE activity was significantly increased in not treated patients. Furthermore, vitamin B_{12} was positively correlated with PON1 and ApoA1 levels, while folic acid was inversely correlated with tHcy (total homocysteine) concentration, demonstrating the importance of this treatment. Our results demonstrated that CBS-deficient patients presented important alterations in biochemical parameters, possibly caused by the metabolites of Hcy, and that the adequate adherence to the treatment is essential to revert or prevent these alterations.

P-175

Cyclic pyranopterin monophosphate treatment trial in a newborn with molybdenum cofactor type A deficiency

Hişmi B[1], Sarıkabadayı Ü[3], Veldman A[4], Özçelik A[6], Santamaria-Araujo J A[5], Coskun T[2], Sivri S[2], Tokatli A[2], Karlı-Oğuz K[7], Schwarz G[5]

[1]Div Metab Dis, Gaziantep Child Hosp, Gaziantep, Turkey, [2]Div Metab Dis, Hacettepe Univ Child Hosp, Ankara, Turkey, [3]Div Neonatol, Sanko Univ Hosp, Gaziantep, Turkey, [4]Div Neonatol, Monash Univ Hosp, Melbourne, Australia, [5]Intsitute of Biochemistry, Cologne Univ, Cologne, Germany, [6]Div Ped Neurol, Gaziantep Univ Hosp, Gaziantep, Turkey, [7]Depart Radiol, Hacettepe Univ, Ankara, Turkey

Background: Molybdenum cofactor deficiency (MoCD) is a devastating disease and an experimental treatment with cyclic pyranopterin monophosphate (cPMP) is available for type A deficiency. Here we report a prenatally diagnosed newborn with MoCD type A, in whom experimental treatment with cPMP was started.
Case report: Baby was born by elective caesarean section with a gestational age of 35+3 weeks. The timing for delivery was chosen to balance risk of prematurity with the risk of in-utero sulphite toxicity. cPMP treatment (240 mcg/kg) was started at postnatal 4th hour. Labile blood pressures with periods of severe hypertension alternating with significant hypotension dominated the first 3 days of life. Clinical seizures were observed on day 2 and 3 of life, it was questioned if the hypertension was associated with sub-clinical seizures. Treatment with esmolol, propranolol and phenobarbital were started. An MRI performed on day 2 of life revealed discrete atrophy and some edema with reduced diffusion compatible with acute cytotoxicity. The blood pressures were controlled on day 3 of life. She developed stage-III necrotising enterocolitis on day 6 of life and died despite aggressive supportive treatment. According to the biomarkers, she was efficiently treated with cPMP.
Discussion: Despite premature delivery, the baby was severely affected from MoCD.

P-176

Betaine depletion in hyperhomocysteinemia

Imbard A[1 2], Benoist J F[1 2], Esse R[3], Gupta S[4], Lebon S[5], De Vriese A S[6], Ogier de Baulny H[7], Krugger W[4], Schiff M[7], Blom H J[8]

[1]Biochem Lab, RDB Hosp, APHP, Paris, France, [2]Paris Sud Univ, Chatenay Malabry, France, [3]Ins Med Pharm Sc, Lisbon, Portugal, [4]Cancer Biol Prog Foc Chase Center, Philadelphia, United States, [5]INSERM UMR1141, RDB Hosp, Paris, France, [6]Renal Unit , Dep Int Med, AZ Sint Jan, Bruges, Belgium, [7]Ref Cent for Metab Dis, RDB Hosp, Paris, France, [8]Lab for Clin Biochem and Metab Univ Hosp, Freiburg, France

Background & objectives: Betaine-homocysteine methyltransferase (BHMT) is an alternate methyl-donor for homocysteine removal. We assumed that BHMT may be predominant in situations of hyperhomocysteinemia (HHcy). As a consequence betaine may become deficient in plasma and tissues from patients and animal models with HHcy of genetic or acquired causes.
Materials, Patients & Methods: S-adenosyl-methionine, S-adenosyl-homocysteine and betaine were quantified by ESI-LC-MS/MS in plasma from 17 patients with primary or secondary HHcy before any treatment, and in plasma and tissues from rats with dietary induced HHcy and CBS deficiency mice. *CHDH*, *BADH*, *BHMT*, *DMGDH*, *SARDH* and *SLC6A12* expression was quantified by RTqPCR.
Results: All patients and animal models showed decreased betaine concentrations in plasma, liver, heart and brain but it was unaffected in kidney. In the CBS deficient model, *BHMT* expression and activity was decreased in liver while in kidney *BHMT* and *SLC6A12* expression (coding for BGT1- the cellular betaine transporter) was increased.
Discussion & Conclusion: We showed that HHcy induces betaine depletion in plasma and tissues -whatever its etiology- highlighting the key role of the BHMT pathway for the removal of homocysteine. In kidney betaine also plays a major role as an osmolyte; its concentration is conserved, possibly due to overexpression of *SLC6A12*.

P-177

Hepatic phospholipids modification in cystathionine beta-synthase deficiency

Imbard A[1 2], Schlemmer D[1], Krugger W[4], Esse R[3], Gupta S[4], Lebon S[5], Blom H J[6], Benoist J F[1 2]

[1]Biochem Lab, RDB Hosp, APHP, Paris, France, [2]Paris Sud Univ, Chatenay Malabry, France, [3]Ins Med Pharm Sc, Lisbon, Portugal, [4]Cancer Biol Prog Foc Chase Center, Philadelphia, United States, [5]INSERM UMR1141, RDB Hosp, Paris, France, [6]Lab for Clin Biochem and Metab Univ Hosp, Freiburg, France

Background and objectives: Moderate hepatic steatosis is a complication of homocystinuria due to cystathionine beta-synthase (CBS) deficiency. CBS deficiency induces

homocysteine and S-adenosylhomocysteine (AdoHcy) accumulation that could be responsible for abnormal phosphatidylcholine (PC) metabolism. Therefore we investigated hepatic phospholipid metabolism in a hypomorphic CBS -/- mouse model compared to wild type.
Materials and methods: S-adenosylmethionine (AdoMet), AdoHcy, methionine, homocysteine, and a phospholipids profile were assessed by ESI-LC-MS/MS in CBS -/- liver. mRNA expression of genes involved in PC synthesis pathway were analyzed by RT-qPCR.
Results: Methionine,homocysteine, AdoMet and AdoHcy were increased while AdoMet/AdoHcy was decreased. We found a qualitative change in the phospholipids profile including i) an increase of some PC containing mono- or di-unsaturated fatty acids, ii) a decrease in PC containing various polyunsaturated fatty acids, and iii) an increase of phospatidylethanolamine (PE) containing poylunsaturated fatty acids. Only *CHPT1* gene expression was increased.
Discussion/conclusion: Accumulation of unsaturated PE together with decrease in unsaturated PC suggests an inhibition of PC synthesis through the phosphatidylethanolamine methyltransferase (PEMT) by AdoHcy. The PEMT pathway uses preferentially unsaturated PE for PC synthesis. The increase of *CHPT1* expression and of saturated PC might suggest an induction of the PC synthesis through Kennedy pathway.

P-178

Update on the European network and registry for homocystinurias and methylation defects (E-HOD)

Kozich V[1], Huemer M[2 6], Groenendijk M[3], Dionisi-Vici C[4], Morris A[5], Brachet E[7], Baumgartner M R[6], Koelker S[8], Blom H J[3]

[1]Inst IEM, Charles Univ-1st Faculty Med, Prague, Czech Republic, [2]Landeskkrankenhaus Bregenz, Bregenz, Austria, [3]Dept Gen Pediatr Adol Neonat, Univ Med C, Freiburg, Germany, [4]Div Meta Child Hosp Bambino Gesu, Roma, Italy, [5]Centre Genomic Med, St Mary's Hosp, Manchester, United Kingdom, [6]Div Metab Dis, Univ Child Hosp, Zurich, Switzerland, [7]Orphan Europe, Paris, France, [8]University Childrens´ Hospital, Heidelberg, Germany

Background: The aim of E-HOD is to improve medical awareness, optimize diagnosis and treatment, and to enable networking between healthcare professionals and patients with homocystinurias (HCU), methylation defects (MD) and folate defects (FD).
Methods: E-HOD is funded by the EC-CHAFEA and started on February 15, 2013. The project has three major activities: 1/ collecting longitudinal data into a registry; 2/developing evidence-based consensus diagnostic and clinical care protocols; 3/ evaluating different newborn screening programs for HCU and producing a position paper.
Results: As of April 2015 E-HOD consists of almost 100 partners from 28 countries linking health care professionals, patient's representatives and industry. A website was developed (http://www.e-hod.org), patient information brochures are being prepared and the E-HOD registry (based on the same platform as E-IMD) contains data on more than 300 patients. Recommendations on newborn screening were published (Huemer et al, JIMD 2015, PMID: 25762406) and three manuscripts on guidelines for CBS deficiency, remethylation disorders and methylation defects are in preparation.
Conclusion: E-HOD is an active consortium with an impact beyond Europe and strives to ensure that all patients with HCU, MD and FD in Europe have an equal access to the best up-to-date care.
Acknowledgement: Supported by CHAFEA/EAHC-2012-12-02.
Conflict of Interest declared.

P-179

Diagnostic method development for homocystinemia on human plasma using GC-MS-SIM following two step derivatisation

Yoon H R[1], Jeong H J[1]

[1]Coll Pharmacy Duksung Women's Univ., Seoul, Korea, Republic of

Background: If early diagnosis is not made, patients with metabolic disorders such as homocystinemia rapidly progress to develop physical defects or mental retardation resulting from storage of the toxic material in the brain.
Objective: A de novo analytical method was developed to quantify sulfur amino acids (methionine, homocysteine, methoinine sulfoxide) and methyl malonic acid, after two-step derivatisation with good sensitivity and specificity on human plasma.
Method: The formation of the trimethylsilyl derivative of the carboxylic functional group was performed by adding MSTF A. Then 10 μL of methyl orange was added to the residue until the color was changed to yellow. Consecutively, the trifluoroacyl (TFA) derivative of the amino (-NH2) functional group was produced by adding MBTFA. GC-MS was setup with specific ions of the TMS-TFA derivative of methionine (m/z 243, m/z 317) for selected ion monitoring, methyl malonic acid (m/z 299, m/z 147), homocysteine (m/z 212, m/z 384), and methionine sulfoxide(m/z 254, m/z 334).
Results: A calibration curve showed a linear relationship in pooled plasma showing 0.9936 ~ 0.9992 in the range of 0.1 ~ 300ng investigated.

Conclusion: The described method could be useful for routine analysis, monitoring, and clinical diagnostic application of homocystinemia on dietary therapy.

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High homocysteine concentrations lower the production of H_2S by cystathionine beta-synthase. Is there a link with cardiovascular disease?

Mendes M I[1], Leandro P[2], Çoker M[3], Kluijtmans L A J[4], Salomons G S[1], Smith D E[1]

[1]Metabolic Unit, VU Univ Med Center, Amsterdam, Netherlands, [2]Met & Gen gr, iMed.UL, Univ Lisbon, Lisbon, Portugal, [3]Dep Ped, Ege Univ Med Fac, Izmir, Turkey, [4]Transl Met Lab, Radboud Univ Med Cent, Nijmegen, Netherlands

Background: Hyperhomocysteinemia is a risk factor for cardiovascular disease (CVD). H_2S is a signaling molecule in cardiovascular and nervous systems. The main enzyme forming H_2S is cystathionine beta-synthase (CBS). CBS deficient patients present high plasma and urine homocysteine and early cardiovascular events. CBS catalyzes the condensation of serine with homocysteine, generating cystathionine. Alternatively, CBS can use cysteine instead of serine, forming cystathionine and H_2S.
Methods & Results: Human embryonic kidney (HEK293) and fibroblast cell lines (four controls and four CBS deficient patients) were incubated with [^{13}C]serine, [D_2]cysteine and increasing concentrations of homocysteine. After 2h incubation, cell pellets were used to measure intracellular [^{13}C]cystathionine and [D_2]cystathionine.In control cell lines, in the presence of normal concentrations of homocysteine (0.5 – 2 μM) 80 % of cystathionine is generated from serine and 20 % from cysteine, with the formation of H_2S. Higher amounts of homocysteine (25 μM) disturbed the ratio between the two reactions. The reaction using cysteine (and producing H_2S) was decreased.
Conclusions: Our study suggests that increasing homocysteine disturbs the balance between the use of serine/cysteine by CBS in controls, affecting the production of H_2S. The decrease in production of H_2S warrants further research into a possible involvement of H_2S in CVD in mild hyperhomocysteinemia.

P-181

Muscle disease in S-adenosylhomocysteine hydrolase deficiency: dystrophy as a consequence of dysmethylation?

Petkovic Ramadza D[1], Majic Z[3], Cuk M[1 2], Prutki M[4], Brcic L[5], Zekusic M[3], Lochmüller H[7], Pazanin L[6], Zarkovic K[5], Bilic K[3], Sarnavka V[1], Barisic N[1 2], Fumic K[3], Baric I[1 2]

[1]Dep of Pediatr, Univ Hosp Centr Zagreb, Zagreb, Croatia, [2]School of Medicine, Univ of Zagreb, Zagreb, Croatia, [3]Dep of Lab Diagn, Univ Hosp Centr Zagreb, Zagreb, Croatia, [4]Dep of Radiol, Univ Hosp Centr Zagreb, Zagreb, Croatia, [5]Dep of Pathol, Univ Hosp Centr Zagreb, Zagreb, Croatia, [6]Univ Hosp Centr Sisters of Charity, Zagreb, Croatia, [7]Inst of Hum Genet, Newcastle Univ, Newcastle upon Tyne, United Kingdom

Background: S-adenosylhomocysteine hydrolase (SAHH) deficiency is an autosomal recessive methionine cycle disorder. Pathogenesis seems to be complex and inhibition of methyltransferases due to decreased S-adenosylmethionine/S-adenosylhomocysteine (AdoMet/AdoHcy) ratio probably contributes to it. Biochemical hallmarks of the disease are elevated AdoHcy, AdoMet and hypermethioninemia. Clinical presentation is variable, but muscle disease with high CK is a constant feature.
Methods: We performed pathological studies and muscle MRI to get insight into muscular pathology, and established myoblast cell cultures to test the effect of altered methylation index on muscle in SAHH deficient patients.
Results: Muscle biopsy demonstrated destructive myopathy with myelin figures and immunohistochemistry revealed normal expression of muscle structural proteins. Muscle MRI showed degeneration predominantly affecting lower leg muscles and progression correlated with age. Muscle spectroscopy showed increased lipid and water peaks consistent with fatty degeneration and edema. Results on myoblast culture showed different methylation index and structural differences between SAHH deficient and control cell lines under various concentrations of AdoMet and AdoHcy.
Conclusion: The pattern of affected muscles and progression with age indicate a contraction-induced injury as seen in dystrophinopathies. Altered methylation seems to be a contributing factor to the muscle pathology. Better understanding of pathogenesis could lead to a more tailored treatment.

P-182

Investigating the relationship between sulfur amino acids and lipid metabolism: classical homocystinuria as a model

Poloni S[1], Spritzer P M[3], Mendes R H[1], Castro K[3], Sperb-Ludwig F[1], Tucci S[2], Blom H J[2], Schwartz I V D[1]

[1]Lab BRAIN, HCPA/UFRGS, Porto Alegre, Brazil, [2]Lab Clin Bioch,University Medical Center, Freiburg, Germany, [3]HCPA/UFRGS, Porto Alegre, Brazil

Classical homocystinuria (CH; CβS deficiency) is characterized by high levels of homocysteine and methionine, and by deficiency of cysteine. Growing evidence suggests that sulfur amino acids (SAA) affect body composition and lipid metabolism. CβS deficient mice present with a 50% reduction in fat mass and a marked suppression of the lipogenic enzyme SCD-1 in liver.
Objective: To investigate body composition, lipid metabolism, and SCD-1 indices in treated patients with CH.
Design: In this cross-sectional study, 10 treated patients with CH and 16 healthy controls were included. Body composition was assessed by DXA. Lipoproteins, free fatty acids, acylcarnitines, AdoHcy, AdoMet, leptin, glucose and insulin were measured in plasma. HOMA-IR index was used to determine insulin resistance. SCD-16 and SCD-18 indices were estimated as product/precursor ratios of fatty acids.
Results: No significant changes in body composition, acylcarnitines and HOMA-IR were found. AdoMet and AdoHcy levels were higher among patients, while LDL was reduced ($p< 0.01$). C16:1 level and the SCD-16 index were reduced among patients ($p< 0.05$), as well leptin (median: 2.6 ng/mL *vs* 6.2 ng/mL, p=0.02). BMI showed a positive correlation with C16:1/C16 ratio and with C16:1 levels ($p< 0.05$).
Conclusions: These results suggest that SAA, via leptin and SCD-1, modulate lipid metabolism also in humans.

P-183

Milder clinical phenotype in molybdenum cofactor deficiency type B associated with a novel mutation – case report

Sykut-Cegielska J[1], Taybert J[2], Schwarz G[3]

[1]Screening Dept, Institute Mother & Child, Warsaw, Poland, [2]Metab Outpat Clinic, Inst Mother&Child, Warsaw, Poland, [3]Dept Chem, Institute Bioch, Cologne Univ, Koeln, Germany

Background: Molybdenum cofactor deficiency (MoCD) is an ultrarare fatal disease with severe neurological damage and death usually in early childhood. One-third of patients carrying mutations in the *MOCS2* gene are classified as having MoCD type B.
Case report: We report a 3-year-old girl with uncomplicated pre- and perinatal history, but focal seizures of limbs and eyelids since Day 2, which resolved spontaneously, but reappeared (as clonic jerks) at Day 14. In the neonatal period she presented with apathy, variable muscle tone and feeding difficulties. Microcephaly, dysmorphic features and lens dislocation (at age of 3 months) were noticed. Excessive weight gain, axial hypotonia, decreased motor activity and psychomotor retardation were observed during infantile period. Seizures were controlled initially by phenobarbital and then by valproate. Brain MRIs revealed progressive cerebral atrophy, abnormal basal ganglia, thin brainstem and corpus callosum, hypoplastic cerebellum. Since 16 months the patient requires respiratory assistance during night-time, but she is in a stable clinical condition. Very low serum uric acid level and positive urine sulphite reaction were detected. A novel mutation - 2bp-deletion in exon 7 of the *MOCS2* gene (c.539_540delAA) was found.
Conclusion: This result confirms MoCD type B diagnosis associated with a novel mutation in the patient with a milder phenotype.

P-184

Poor adherence to treatment is the commonest cause of a persistently elevated homocysteine in patients with classical homocystinuria

Stepien K M[1], Hendriksz C J[1], Hiwot T[2], Dawson C[2]

[1]Mark Holland Metabolic Unit, SRFT, Salford, United Kingdom, [2]Dept Endo Metab,UHB, Birmingham, United Kingdom

Background: Classical homocystinuria (HCU) is an inherited disorder of homocysteine (HCy) metabolism caused by cystathionine beta-synthase deficiency. Untreated, it predisposes to thromboembolism. Reducing homocysteine level to < 100 umol/L with vitamin cofactors of enzymes required for HCy metabolism reduces the risk of thromboembolism to that of the non-HCU population. In this study we explore why many patients on treatment fail to achieve target HCy levels.
Methods: Patients with classical HCU and HCy level >100 umol/L attending two UK adult metabolic centres were identified from patient databases held within the two centres. Their clinical records were examined for data that may explain their failure to achieve a target HCy.
Results: 23 patients with classical HCU had HCy level >100 umol/L. The commonest reason for failing to achieve target was poor adherence to treatment (17/23; 74%). All poorly adherent patients were on at least three medications +/- dietary protein restriction and 4/17 (25%) had intellectual disability.
Conclusion: Poor adherence is common in the HCU population. Risk factors include multiple daily dosing with drugs taken in high doses and intellectual disability associated with the disorder. We suggest that specialist pharmacists could work with this patient cohort to address this issue.

P-185

Observations from spectral domain ocular coherence tomography (SDOCT) in two infants with early-onset cobalamin C defect (cblC)

Weisfeld-Adams J D[1 2], McCourt E A[1 2]

[1]University of Colorado-Denver, Denver, United States, [2]Children's Hospital Colorado, Aurora, United States

Background: Ocular involvement and visual impairment in the cobalamin C defect (cblC) is a common early disease sequela, and is of unique character among organic acidemias and inherited hyperhomocysteinemias.
Methods: Detailed SDOCT imaging was performed on two young cblC patients.
Results: SDOCT showed outer plexiform layer and outer nuclear layer thinning with exaggerated foveal depressions. Retinal pigment epithelium changes and lack of inner/outer segment definition were seen in both patients. Inner plexiform layer thickening could represent a remodeling response to retinal degeneration.
Discussion: Our observations further delineate the course of early macular injury in patients with cblC, with progression coinciding with a possible critical period of postnatal foveal development. Relentlessly progressive visual loss may occur despite near-normalization of biochemical abnormalities that characterize the condition and that have been historically used to assess treatment efficacy (hyperhomocysteinemia, methylmalonic acidemia, hypomethioninemia). SDOCT appears destined to become a key component of our understanding of pathomechanisms of retinal disease in cblC. Systematic, multi-center natural history studies of cblC are required to confirm these observations, expand the existing knowledge base of pathomechanisms in cblC, and inform future therapeutic development.

P-186

Biomarkers of the one-carbon metabolism and B-vitamins in two healthy pediatric populations

Caldeira Araujo H[1 2], Rivera I[3 4], Castro R[3 4], Tavares de Almeida I[3]

[1]Unit Medical Sciences, Univ Madeira, Funchal, Portugal, [2]Centro Química Madeira, Univ Madeira, Funchal, Portugal, [3]Met&Gen, iMed.UL, Fac Pharmacy, Lisbon, Portugal, [4]Dep Bioch Human Biol, Fac Pharmacy, Lisbon, Portugal

Derangements of one-carbon metabolism may underlie several pathologies. Evaluation of nutritional status in pediatric ages is important to uncover deficiencies and to set age-specific reference ranges.
Two healthy pediatric groups, 9-year-old (n=195, 107M: 88F) and 17-year-old (n=128, 57M: 71F) were studied. The one-carbon metabolic pathway was explored by assessing plasma levels of folate, total cobalamin (tCbl), holo-transcobalamin (holo-TC, active form of Cbl), methylmalonic acid (MMA) and total homocysteine (tHcy). SNPs of key enzymes (*MTHFR-677C>T* and *1298A>C*, *MTR-2756A>G*, *TCNII*-776C>G and *MTR*-66A>G) were identified and correlated with the levels of metabolites.
Plasma concentrations (mean±stds; 9 versus 17-year-old) were: folates (22.87±9.25; 11.65±4.17nmol/L); total Cbl (514.74±165.53; 302.51±118.32pmol/L); holo-TC (100.60 ±31.63; 66.20±25.10pmol/L); MMA (0.239±0.095; 0.180 ±0.105μmol/L) and tHcy (3.75 ± 1.78;8.72±3.41μmol/L). Significant ($p < 0.05$) gene-nutrients interactions were only observed in the 17Y-old group, for the *TCNII*776 genotypes with folates, *MTR*2756 genotypes with tCbl and *MTHFR*677 genotypes with tHcy. Significantly decreased levels of folates, total Cbl, holo-TC and MMA and increased tHcy levels were observed in the 17Y population when compared to the 9Y one. Moreover, in the 17Y-old group, an inverse correlation was observed between MMA and holo-TC plasma levels, which is in agreement with the putative role of plasma MMA as an early marker of Cbl deficiency.

09. Other amino acid disorders

P-003

Three cases of hereditary tyrosinaemia type 1 (HT-1); neuropsychiatric outcomes and functional brain imaging following treatment with NTBC

Walker H L[1], Barrington S[2], Rahman Y[3], Pitkanen M[4]

[1]Sou Lon Maud Men Heal Tru, London, United Kingdom, [2]Div PET Imag, St Thom Hosp, London, United Kingdom, [3]Dept Metab Dis, St Thom Hosp, London, United Kingdom, [4]Dept Neuropsy, St Thom Hosp, London, United Kingdom

Objective: To examine neuropsychiatric outcomes and results of brain imaging in adults with hereditary tyrosinaemia type I (HT-1), treated with 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC).
Method: A retrospective review of records, FDG-PET brain scans and standardised neuropsychiatric testing was conducted on three adults with tyrosinaemia type 1. The patients on NTBC treatment had been referred to a joint neuropsychiatry/inherited metabolic disorders clinic. 2

patients were on NTBC from 6 weeks of age and the third from 9 years of age.
Results: All patients performed below expectations on formal tests of wide range of cognitive functions. FDG-PET brain scans were normal in two patients however one early NTBC treated patient had confirmed bilateral reduced metabolism in temporal and mediofrontal areas. This imaging change correlated with the neurocognitive testing results.
Conclusions: The study confirms HT-1 patients treated with NTBC all underperformed in cognitive testing regardless of the point when the NTBC was first started. However, the imaging correlated in only one of the patient's neurocognitive performance. It should be noted that this study reports only a small case series of a rare disorder and further systematic, longitudinal, multi-centre studies are necessary in order to understand the relationship between HT-1, NTBC treatment and neurocognitive outcome.

P-010

Taste and palatability acceptance of a nitisinone suspension in children with hereditary tyrosinemia type 1 (HT-1)

McKiernan P[2], Lindner M[3], Valayannopoulos V[4], Breen C[5], Lotz-Havla A S[6], Vara R[7], Schlune A[8], Rudebeck M[1], Olsson B[1]

[1]Swedish Orphan Biovitrum (Sobi), Stockholm, Sweden, [2]Child hosp, Birmingham, United Kingdom, [3]Univklin Zentr für Kind Jugendmed, Heidelberg, Germany, [4]Necker hosp, Paris, France, [5]St Mary's Hosp, Manchester, United Kingdom, [6]Klin der Univ, München, Germany, [7]Evelina Child hosp, London, United Kingdom, [8]Heinrich-Heine-Univ, Univ Child Hosp, Düsseldorf, Germany

Background: A nitisinone suspension was developed to facilitate administration in children with HT-1. Patients who cannot swallow capsules currently mix the content with food/fluids.
Methods: An open-label, non-controlled, 3-day taste/palatability study in 18 HT-1 patients (n=6/age group: 1 month-under 2 years, 2 - under 12 years and 12 - under 18 years) to determine the acceptance of the suspension (see A-020).
On a 5-graded verbal/numerical scale with incorporated faces with higher values representing better taste/palatability, subjects 5 - under18 years rated taste/palatability, while parents of subjects under 5 years rated their child's acceptance. Overall acceptability was assessed after last dose (Yes/No).
Data analysis was performed for 2 groups: under 5 and 5-under 18 years.
Results: Median acceptability score was 5.0 (5=very well) for patients under 5 years, and median taste score and palatability score for patients 5 - under 18 years was 4.0 for both (4=good).
80% of patients swallowing capsules whole and 75% of those mixing capsule content with food/fluids would accept taking the suspension again. All non-acceptance responses were from patients over 12 years, for reasons that were not obviously correlated to their taste/palatability/acceptability scores.
Conclusion: The nitisinone suspension was well accepted in paediatric HT-1 patients. A majority would accept taking the suspension again, both those currently mixing capsule content with food/fluids and those swallowing capsules whole.
Conflict of Interest declared.

P-013

The role of the metabolic biochemistry laboratory in monitoring maple syrup urine disease (MSUD) patients undergoing liver transplant

Agalou S[1], Ramachandran R[2], Rahman Y[2], Connolly G[3], Cardy D[3], Loughrey C[4], Carling R[1]

[1]Viapath, Biochem SciDep, St Thomas' Hosp, London, United Kingdom, [2]Inh Met Dis Dep Evelina Chil Hosp, London, United Kingdom, [3]Clin Biochem Royal Vic Hosp, Belfast, Ireland, [4]Dept of Clinical Biochemistry, Belfast, Ireland

Case report: A 23-year-old male with classical MSUD underwent liver transplant following an 18-month deterioration in metabolic control with recurring encephalopathy associated with fluctuating plasma leucine.
Methods: Real-time monitoring of the branched-chain amino acids (BCAA) in the immediate pre-/peri-/ post-operative period is imperative for monitoring graft function and resultant metabolic control.
Currently no UK laboratory provides a metabolic biochemistry service outside of normal working hours (OOH, 9am-5pm, Monday - Friday). An ad-hoc OOH service was arranged. This was particularly challenging because the timing of surgery could not be predicted since it relied on a suitable liver becoming available from a cadaveric donor.
Results: Bloodspot BCAA analysis (the preferred method in our laboratory OOH) is logistically easier to perform than plasma analysis. However, bloodspot analysis is not available in other laboratories OOH. Hence, plasma and blood spot samples were collected to enable analysis in another laboratory if needed. Retrospective comparison of results for BCAA in plasma and bloodspot were significantly different ($p < 0.005$) and therefore cannot be used interchangeably.

Conclusion: This case also highlights the need for a network of laboratories with shared OOH protocols for both analysis and on-call cover to meet the likely increasing demand from similar clinical scenarios in the future.

P-187

Type I hyperprolinemia: Atypical presentations in a family

Ersoy M[1], Tatli B[4], Cakir Bicer N[2], Bursal Duramaz B[3]

[1]Child Metab Dis,Sadi Konuk Res&Tra Hosp, Istanbul, Turkey, [2]Depart Nutr&Diet., Arel Univ, Istanbul, Turkey, [3]Depart Pedia. Sadi Konuk Res&Tra Hosp, Istanbul, Turkey, [4]Div Pediat Neur, Istanbul Medical Fac, Istanbul, Turkey

Background: Type I hyperprolinemia (HPI) is a rare metabolic disease characterized by mental retardation, epilepsy, autism, schizophrenia and behavior problems. However, various cases remain asymptomatic even in the same family. So, there are still unknown areas of HPI. We report the members of the same family that have high proline levels with different presentations.
Cases: A four-year-old girl evaluated with mild mental retardation and atypical autistic features. The use of nonverbal behaviors, such as eye-to-eye gaze, facial expression were impaired. Plasma proline level was 984μmol/L (normal: 97-329). Because the level of urinary pyrroline-5-carboxylate was within normal range, she was diagnosed with HPI. The patient was started to protein restricted diet together with antioxidant agents. The level of proline decresed to 50% of initial level after 6 months treatment. Eye to eye gaze period and learning ability improved. Her mother who was investigated for mutible sclerosis with dysarthria, sensory-motor gating and poor memory, showed high plasma proline level (731μmol/L). We screened the other 9-year-old sibling; although she had not any symptoms, her proline level was 610μmol/L.
Discussion: While the clinical manifestations in HPI are not well characterized, patients with neuropsyciatric symptoms should be investigated for HPI.

P-188

Hyperprolinemia induces DNA, protein and lipid damage in blood of rats: antioxidant protection

Ferreira A G K[1], Scherer E B S[1], Da Cunha A A[1], Manfredini V[1], Biancini G B[1], Vanzin C S[1], Vargas C R[1], Wyse A T S[1]

[1]UFRGS, Porto Alegre, RS, Brazil

Hyperprolinemias are metabolic diseases caused by enzyme deficiencies in proline catabolism. This study investigated the effects of hyperprolinemia on oxidative damage to protein, lipids and DNA, antioxidant status and the influence of antioxidants on the effects proline elicited in blood of rats. Rats received two daily injections of proline and/or vitamin E plus C (6^{th}-28^{th} day of life). Hyperprolinemia significantly increased carbonyl content, malondialdehyde levels and a greater damage index in comet assay, while the non-enzymatic antioxidant potential (TRAP) was decreased. The antioxidants treatment completely prevented the oxidative damage to proteins, but partially prevented lipids, DNA damage and TRAP. The plasma levels of vitamins E and C significantly increased in rats treated exogenously with these vitamins, but when proline was co-administered, the vitamins levels were similar to those found in plasma of control and proline rats. Our findings suggest that hyperprolinemia promotes oxidative damage to the three major classes of macromolecules in blood of rats. Such effects were accomplished by TRAP and exogenous vitamins decrease. Since vitamins significantly prevented oxidative damage to biomolecules studied, our data points antioxidants as an effective adjuvant therapeutic to limit oxidative damage in hyperprolinemia. Supported by CNPq.

P-189

D-glyceric aciduria does not cause hyperglycinemia: a historic case correction

Sass J O[1], Spector E[2 5], Kronquist K[2 5], Creadon-Swindell G[5], Walter M[3], Christensen E[4], Van Hove J[2 5]

[1]Bonn-Rhein-Sieg Univ Appl Sciences, Rheinbach, Germany, [2]University of Colorado, Aurora, CO, United States, [3]Univ Child Hosp, Lab Clin Biochem Metab, Freiburg, Germany, [4]Rigshospitalet, Dept Clin Genet, Copenhagen, Denmark, [5]Children´s Hospital Colorado, Aurora, CO, United States

Nonketotic hyperglycinemia (NKH) is a neurometabolic disease characterized by deficient activity of the glycine cleavage system (GCS) and elevated glycine concentrations. D-glyceric aciduria (DGA) has been described with overlapping symptoms. It is caused by deficient D-glycerate kinase activity due to mutations in the *GLYCTK* gene. The first DGA patient reported by Brandt et al. in 1974 presented in addition to glyceric aciduria with elevated glycine levels and decreased GCS activity in autopsy liver. Based on this case, it was assumed that glyceric aciduria can cause NKH, and should be considered in the differential diagnosis. We reexamined fibroblasts from this original patient, who had an apparent

homozygous single base deletion c. 1448delT in the *GLYCTK* gene causing a frameshift p.Pro483Serfs*2 resulting in undetectable D-glycerate kinase (Sass et al. 2010). Now, an additional homozygous missense mutation c.350C>T changing a conserved amino acid (p.Ser117Leu) was identified in the *AMT* gene, causing NKH. Both mutations are homozygous and located in proximity on chromosome 3. However, a custom-made microarray found no evidence for an exonic deletion. This historic patient indeed had a co-occurrence of two separate rare conditions. We conclude that glyceric aciduria does not induce NKH and the diagnostic algorithms should be updated.

P-190

Determination of autosomal dominant or recessive methionine adenosylmethionine I/III deficiencies based on clinical and molecular studies

Kim Y M[2], Lee B H[1], Kim J H[1], Kim G H[1], Kim J M[1], Kang M[1], Choi I H[1], Cheon C K[2], Sohn Y B[3], Yoo H W[1]

[1]Med Genet Center, Asan Medical Center, Seoul, Korea, Republic of, [2]Dep Pediatr, Pusan Nat Univ Child Hosp, Yangsan, Korea, Republic of, [3]Dep Pediatr, Ajou Medical Center, Suwon, Korea, Republic of

Background: MAT I/III deficiency can be inherited either in autosomal dominant (AD) or recessive (AR) traits. In this study, nineteen Korean patients with MAT I/IIIdeficiency from fifteen unrelated families were described.
Methods: Thirteen patients had AD type of MATI/III deficiency, whereas the other six patients had AR type.
Results: Plasma methionine (137 vs. 733μmol/L, $P< 0.05$) and homocysteine levels (12.3 vs. 18.6μmol/L, $P< 0.05$) were lower in AD type than in AR type. In the AD type, in addition to the only AD mutation reported to date (p.Arg264His), two novel *MAT1A* mutations, p.Arg249Gln and p.Gly280Arg, were found. In AR type, six mutations including two novel mutations, p.Arg163Trp and p.Tyr335*, were identified. Macromolecular analyses predicted that all the mutations in AD type are located on the dimer interface and or substrate-binding site, hindering MATI/III dimerization or substrate binding. In contrast, those in AR type are distant from the interface or substrate-binding site.
Conclusion: The results of our study indicates that molecular remodeling as well as evaluation of clinical and genetic findings help to determine MATI/III deficiency either in AD or AR type, which is important for the management of a patient regarding neurological outcome as well as genetic counseling.

P-191

Functional characterization of novel missense variants identified in the *GLDC* gene of Spanish nonketotic hyperglycinemia patients

Bravo-Alonso I[1], Navarrete R[1], Ugarte M[1], Perez-Cerdá C[1], Rodríguez-Pombo P[1]

[1]CEDEM, CBM-SO, UAM-CSIC, CIBERER, IDIPAZ, Madrid, Spain

Background & Aim: Nonketotic Hyperglycinemia (NKH) is an autosomal recessive disorder of the glycine metabolism caused by a defective activity of the glycine cleavage system (GCS) and biochemically characterized by a massive accumulation of glycine in body fluids. We present the functional analysis of 10 *GLDC* missense-variants identified in a cohort of 27 Spanish NKH-patients displaying a wide range of clinical phenotypes.
Methods & Results: To analyse the impact of mutants on protein stability and enzyme activity, we over-expressed wild-type or mutant pCMV-*GLDC* constructs in COS7 cells. According to our results, for cells over-expressing p.T146L, p.L173P, p.P581R, p.P267A, p.R373W, p.D866H and p.V905G mutants, GCS activities were less than 5% of the wild-type. When cells were transfected with either of the variants p. R461W, p.K376E and p.M1L, the activities detected were between 20-50%. Except for p.V905G, p.R373W and p.D866H constructs that expressed a normal amount of GLDC protein, the decreased amount of protein detected on the Western blot correlated with the retained activity.
Conclusion: Highlighting the impact of the GCS residual activity on the clinical outcome in our series of NKH patients, the less severe clinical phenotypes, established in terms of CSF-glycine levels, clinical symptoms and survival, corresponded to those patients carrying mutations retaining the highest residual activities.

P-192

Lysinuric protein intolerance: An overlooked diagnosis

Tumer L[1], Olgac A[1], Ozgul R K[3], Yenicesu I[2], Ezgu F S[1], Biberoglu G[1], Hasanoglu A[1]

[1]Div Pediatr Metabolism, Gazi Univ Hosp, Ankara, Turkey, [2]Div Pediatr Hematology, Gazi University, Ankara, Turkey, [3]Div Pediatr Metab, Hacettepe Univ Hosp, Ankara, Turkey

Introduction: Lysinuric protein intolerance (LPI) is an autosomal recessively inherited inborn error of metabolism (IEM) caused by mutations in *SLC7A7 gene*, which encodes a

component of the dibasic amino acid transporter found in the intestinal and renal tubular cells. Long term complications are pulmonary alveolar proteinosis, dyslipidemia, hematological abnormalities, renal disease and osteoporosis.
Case report: 15 year old girl, who had been followed-up in multiple centers for short stature and hepatosplenomegaly for 8 years, was admitted to our clinic. She had a history of rejecting high protein foods since infancy. Physical examination revealed short stature, hepatosplenomegaly and retarted puberty. Transaminases were elevated. Decresed levels of lysine, arginine and ornithine in plasma, and high amounts of lysine excretion in urine were detected. Postprandial ammonium levels were increased. Hemophagocytosis and megaloblastic changes were detected in bone marrow examination. SLC7A7 gene analysis showed a previously known c.344_347delTTGC, p.Leu115sX53 homozygous mutation. The patient was started on low protein diet and L-citrulline, and is stil being followed-up in our department.
Conclusion: LPI is a rare IEM with significant long term complications that should be kept in mind while evaluating children with short stature, retarded puberty, hepatosplenomegaly and osteoporosis. Early diagnosis is of great importance for the initiation of appropriate treatment.

P-193

Tyrosinemia type III in an asymptomatic Polish girl

Szymanska E[1], Sredzinska M[1], Ciara E[2], Halat P[2], Rokicki D[1], Tylki-Szymanska A[1]

[1]Dept Ped Nutr Met Dis,Ch Mem Health Ins, Warsaw, Poland, [2]Dept Genet, Ch Mem Health Ins, Warsaw, Poland

Background: Hereditary tyrosinemia type III (HT3) is a rarely detected inborn error of tyrosine metabolism caused by mutations in the *HPD* gene encoding 4-hydroxyphenylpyruvate dioxygenase (4HPD), which are transmitted in an autosomal recessive trait. This metabolic error is characterized by elevated levels of tyrosine in body fluids and massive excretion of tyrosine derivatives into urine. Only few cases with variable clinical features ranging from asymptomatic individuals to patients with neurologic manifestations including mental retardation and ataxia have been described in the literature so far.
Case report: We report an 11 years old girl presenting with normal mental development without neurological symptoms, who has been diagnosed with HT3 on the basis of elevated serum level of tyrosine ranging from 425 to 535 umol/L (normal values: 29-86 umol/L), and elevated urinary excretion of p-hydroxyphenyl derivatives; the defect was confirmed genetically. The girl has been presenting only with recurrent proteinuria, of unknown mechanism. A phenylalanine- and tyrosine-restricted diet has never been administered.
Conclusion: The spectrum of hepatic and neurologic symptoms in HT is most likely associated with tyrosine derivatives which do not accumulate in HT3. It is not clear, whether nephrologic complications in our patient are associated with HT3. Moreover, since HT3 may be asymptomatic, its prevalence may be underestimated.

P-194

Serine deficiency - serial monitoring and response to treatment

Croft J M[1], Leong H Y[3], Desurkar A[2], Watkinson J[1], Smith L[1], YAP S[3]

[1]Clin Chem, Sheffield Children's Hosp, Sheffield, United Kingdom, [2]Paed Neurology, Sheffield Childrens Hosp, Sheffield, United Kingdom, [3]Metab Medicine, Sheffield Childrens Hosp, Sheffield, United Kingdom

Background: Disorders of serine deficiency have been diagnosed in only a few children. Clinical features include congenital microcephaly and development of psychomotor retardation and intractable seizures.
Case Report: A 13 month old boy of consanguineous parents was referred with severe developmental delay, microcephaly, dystonia, visual impairment and West syndrome with uncontrolled seizures. Initial EEG showed hypsarrthymia. Relevant investigations showed low plasma serine (27μmol/L ref. 40-280) and glycine (74μmol/L ref.100-425) and low CSF serine (5μmol/L ref. 35-80) and glycine (< 5μmol/L ref. 0-10). The patient was started on L-serine 417mg/kg/day initially, adjusted to 600mg/kg/day following titration by plasma serine concentration with Vigabatrin continued. Following 4 doses of 500mg serine, plasma serine was 280μmol/L and glycine was 322μmol/L. The patient became seizure free with resolution of hypsarrhythmia by 6 weeks of treatment. He also showed improved visual alertness and good control of dystonia. A CSF sample at 1 month post treatment showed serine 38μmol/L and glycine 3μmol/L. A blood sample has been sent for molecular analysis.
Conclusion: This case demonstrates the treatment and response to serine with serial biochemical monitoring resulting in clinical and EEG ablation of seizures.

P-195

Unusual isoleucine concentration kinetic in the follow-up of 2 patients affected by maple syrup urine disease

Imbard A[1 2], Pichard S[3], Paquay S[3], Paviolo M[3], Rigal O[1], Acquaviva C[4], Janvier P[3], Greneche M O[3], François L[3], Benoist J F[1 2], Schiff M[3]

[1]Biochem Lab, RDB Hosp, APHP, Paris, France, [2]Paris Sud Univ, Chatenay Malabry, France, [3]Ref Cent for Metab Dis, RDB Hosp, Paris, France, [4]Lab of Metab Biochem,, Bron, France

Background and objectives: Maple Syrup urine disease (MSUD) is due to branched chain keto-acid dehydrogenase deficiency that leads to accumulation of branched chain amino-acids. Treatment relies on a protein-restricted diet supplemented with branched chain amino acid-free medical foods. Plasma leucine concentrations are regularly checked and kept between 150 and 250 μM, since leucine is viewed as the most toxic compound.
Cases report: We report on 2 neonatal MSUD patients diagnosed at 28 and 6 days of life with neonatal coma. Both patients were genetically confirmed (homozygous for *BCKDHA* p.Gln177Ter and *BCKDHB* p.Gln267Ter respectively). Surprisingly, during the follow-up, they presented episodes of dramatically increased isoleucine (up-to 1700 μM) and alloisoleucine (up-to 1350 μM) uncorrelated with leucine or valine concentrations. These episodes were not associated with any clinical deterioration and occurred during periods without any isoleucine supplementation and usually following relative leucine depletion.
Discussion: Isoleucine likely comes from endogenous source as patients had adapted isoleucine intake, but dissociation with valine and leucine remains unexplained. Molecular mechanisms underlying our findings remain to be elucidated. Moreover, long-term exposure to high isoleucine levels could impact the outcome whereas low-protein diet does not seem to influence isoleucine levels in these particular patients.

P-196

Secondary brain creatine deficiency may lead to diagnosis of ornithine aminotransferase (OAT) deficiency

Paviolo M[2 3], Germanaud D[2 3], Paquay S[2 3], Pichard S[2 3], Imbard A[2 3], Bui Quoc E[1 2], Benoist J F[2 3], Schiff M[2 3]

[1]Ophthalmology Unit, Hosp R Debré, Paris, France, [2]University Paris-Diderot, Sorbonne, Paris, France, [3]Ref Cent for Metab Dis, R Debré Hosp, Paris, France

Background. OAT deficiency is a rare autosomal recessive disorder associated with gyrate atrophy of choroid and retina that leads to the diagnosis. Patients develop hyperornithinaemia, which inhibits arginine-glycine amidinotransferase, a rate-limiting enzyme in creatine production. Thus, high ornithine concentrations cause secondary creatine deficiency. We report the case of an OAT deficient patient diagnosed through secondary brain creatine deficiency.
Case report. A 3-year-old boy presented with partial complex non febrile seizures, delayed psychomotor development, and a gradually slowing of head growth. Brain MRI spectroscopy showed increased NAA/creatine and choline/creatine ratios which suggested a brain creatine deficiency. Very-low urinary excretion of creatine and guanidinoacetate and low blood creatine suggested AGAT deficiency. Blood amino acids disclosed high ornitihine levels (1107μmol/l, normal:26–56μmol/l) thus pointing towards OAT deficiency. Urinary excretion of ornithine, was highly increased. Ophthalmological exam showed normal visual acuity but funduscopy revealed areas of retinal thinning confirmed by optical coherence tomography. Electroretinogram revealed abnormal electrogenesis. Genetic testing revealed a known homozygous nonsense mutation in the OAT gene. A 4-week trial of pyridoxine showed no change in ornithine plasma level. Significant reduction of plasma ornithine was achieved by dietary restriction of arginine.
Conclusion. Secondary creatine deficiency may be a diagnostic clue for OAT deficiency.

P-197

Clinical and laboratory findings of four patients with glutathion synthetase deficiency and effect of natrium hydrogen carbonate

Gunduz M[1], Unal O[1], Mungan N O[3], Kavurt S[2], Turk E[1]

[1]Ankara Hematology Oncology Children's, Ankara, Turkey, [2]Etlik Women's Hospital Res and Train, Ankara, Turkey, [3]Cukurova Univ Div Metab Dis, Adana, Turkey

Background: Glutathione is one of the most important antioxidants. It is synthesised and metabolised in gamma-glutamyl cycle. Deficiency of glutathione synthetase (GS) is the most common inborn error of glutathione metabolism. It is manifested by severe metabolic acidosis, hemolytic anemia, and massive excretion of pyroglutamic acid in the urine.
Material Method: Clinical and laboratory findings of patients with GS deficiency, and effect of sodium hydrogencarbonate treatment in the follow up period were evaluated. Molecular genetic analysis or enzyme analysis was used to confirm the diagnosis.

Results: Four patients were diagnosed with GS. All patients presented in the neonatal period. At first presentation, three patients had metabolic/lactic acidosis and hemolytic anemia, in one patient, main clinical finding was hemolytic anemia. One patient died at 4 months old. Current age range of three patients is between 3-9 years old. One patient's growth and development is normal, and two patients have developed intellectual disability and seizures in the long term. Three surviving patients benefited very well from sodium hydrogencarbonate treatment and did not develop acidosis attack.
Conclusion: Prognosis of GS deficiency is difficult to predict, and, vary significantly between different patients. Sodium hydrogencarbonate treatment may be useful as an anti-acidosis treatment agent.

P-198

CSF/serum glycine ratios in non-ketotic hyperglycinemia patients

Kilic M[1], Genç-Sel Ç[2], Kasapkara Ç S[1], Ozkan M[2], Aksoy A[2], Kayilioglu H[2], Yuksel D[2], Karaer K[4], Ceylaner S[4], Aydin H I[3]

[1]Div Metab Dis, Dr Sami Ulus Child Hosp, Ankara, Turkey, [2]Ped Neurol Dis, Dr Sami Ulus Child Hosp, Ankara, Turkey, [3]Div Met Dis, Turgut Ozal Univ Hosp, Ankara, Turkey, [4]Intergen, Ankara, Turkey

Background and objectives: Nonketotic hyperglycinemia (NKH) is an autosomal recessive disorder of glycine metabolism caused by a defect in the glycine cleavage enzyme complex (GCS). Early hypotonia, hiccups, seizures, apnea, lethargy, coma can be seen in the newborn and infant period. Severe mental-motor retardation and intractable seizures are seen in surviving patients. A glycine CSF/serum glycine ratio of 0.06 (some sources 0.08) or higher is considered indicative of a diagnosis.
Patients and Methods: Five patients with NKH diagnosis were enrolled in this study.
Results: Female/Male ratio was 4/1. All patients had clinical symptoms (poor sucking, hypotonia, seizures and most of them needed mechanical ventilation due to respiratory failure) in the newborn period. CSF glycine/serum glycine ratio ranged from 0:16 to 0:25. However, in one patient, this ratio was 0.047, but found to be 0:17 in a repeated test. Burst suppression pattern and hypsarrythmia were observed on electroencephalography. One patient had died. Others are being followed with severe developmental delay and intractable seizures.
Conclusion: In suspected NKH patients with CSF glycine/serum glycine ratio from 0:04 to 0:06, cranial MR and MRS should be performed and in the presence of glycine peak, molecular genetic analysis should be made.

P-199

Maple syrup urine disease (MSUD) in Ireland: phenotypes, genotypes, and determinants of outcome

Knerr I[1], Crushell E[1], Hughes J[1], Monavari A A[1], Deverell D[2], Mayne P D[2 3], Naughten E[1], O'Shea A[2], Wala A[4], Häberle J[4], Treacy E P[1 5]

[1]NCIMD, TSCUH, Dublin, Ireland, [2]Metabolic Laboratory, TSCUH, Dublin, Ireland, [3]Nat Newborn Bloodsp Screen Lab, TSCUH, Dublin, Ireland, [4]Div Metab, Univ Child Hosp, Zurich, Switzerland, [5]Mater Hospital, Dublin, Ireland

Objective: To study phenotypes, genotypes and determinants of outcome in MSUD patients diagnosed on newborn screening in Ireland (1972-2011).
Methods: 17 patients (10 females, 7 males; 2 deaths); individual yearly medians of leucine (Leu), isoleucine, valine, maximum Leu levels and area under the curve (AUC) were determined. Leu results were grouped into 3 clusters according to metabolic control. Clinical results were grouped according to clinical outcome/IQ test results (n=14).
Results: Mean age 20.2 yrs. (±11.4, range 0.6-33.7); 17,834 data sets, mean 58/pt/year; 314.2 patient-years. Clinical clusters and biochemical (Leu) clusters were congruent. The mutational profile was heterogeneous (*BCKDHA*, *BCKDHB*, and *DBT* gene mutations). One *BCKDHB* mutation (*c.885delT*) occurred in 4 different families; affected individuals were found in each cluster. A heterozygous state for both *BCKDHA* and *DBT* mutations was found in 3 patients partially responsive to thiamine. Determinants of outcome were early diagnosis/treatment, peak Leu concentrations, long-term control/AUC Leu, year of treatment and, as a trend, therapeutic Leu:Ile:Val ratios.
Conclusions: Ireland has over 40 years of experience with MSUD. This study provides an opportunity to give an Irish perspective to the international experience with MSUD, to contribute to achieving optimal metabolic control in MSUD patients, and to maximizing long-term outcomes.
Conflict of Interest declared.

P-200

A novel glycine decarboxylase gene mutation in a neonate with nonketotic hyperglycinemia

Bülbül S[1], Aliefendioğlu,Didem D A[2], Oncul,Nur N O[1], Ceylaner,Serdar S C[3]

[1]Kirikkale Univ, Div Metab Dis,, Kırıkkale, Turkey, [2]Kirikkale Univ, Div Neonatology, Kırıkkale, Turkey, [3]Intergen Genetic Center, Ankara, Turkey

Background: Non-ketotic hyperglycinemia (NKH) is an autosomal recessive inherited disorder of metabolism. It is caused by a deficiency in the mitochondrial glycine cleavage system. Glycine cleavage system is made up of several protein subunits called the P-protein (glycine decarboxylase), T-protein (aminomethyl transferase) and H-protein (glycine cleavage system H protein).These proteins are encoded by *GDLC*, *AMT* and *GCSH* genes. In studies of non-keotic hyperglycemia cases, most of the mutations are seen *in GLDC* gene. Mutations in *AMT* and *GCSH* genes are rarely seen. Here we report a neonate who was referred with complaints of hypotony and cyanosis.
Case: It was the first living full-term baby of first degree relative parents. A distinct hypotony, microcephaly and atypical face movement was noticed. With high cerebrospinal fluid (CSF), plasma, urine glycine levels (131, 1579 and 20600 μmol/L, respectively), and elevated (CSF)/plasma glycine ratio, the baby was diagnosed with nonketotic hyperglycinemia. A novel mutation, p.L101*(c.302deIT), was detected in *GLDC* gene.
Conclusion: This mutation causes an early stop codon and truncating variation. Although it is a novel mutation, as the variation causes a severe defect on protein, it is most probably a disease causing mutation.

P-201

Neurocognitive outcome in hepatorenal tyrosinaemia – a pilot study

Stange M[1], Prüfe J[1], Hartmann H[1], Das A M[1]

[1]Ped Kid, Liv, Metab Dis, Hannover Med Sc, Hannover, Germany

Objective: Nitisinone has revolutionized the outcome in hepatorenal tyrosinaemia (HT1). The risk of developing liver failure, renal dysfunction and hepatocellular carcinoma is low if treatment is started early. However, with increased survival impairment of neurocognitive development becomes evident in some patients. This could result from elevated tyrosine, succinylacetone, nitisinone or low phenylalanine levels, or toxicity before treatment is initiated.
Methods: We investigated 5 HT1-patients. Patients < 3y were assessed using Bayley Scales of Infant Development III. In older patients Wechsler Preschool and Primary Scale of Intelligence (WPPSI-III) and Wechsler Intelligence Scale for Children (WISCIV) were used.
Results: 4 patients showed significant cognitive deficits regarding perceptional reasoning and processing speed. We found an inverse correlation between IQ and age, no correlation between age at start of treatment, nitisinone, tyrosine, succinylacetone, phenylalanine and IQ. Deficits were more pronounced in older patients.
Conclusions: Despite considerable diagnostic and therapeutic progress, neurocognitive deficits in HT1-patients increase with age. Only a correlation between patient age at testing and the neurocognitive deficit was found. We found no statistically relevant correlation between IQ and average nitisinone, tyrosine, succinylacetone or phenylalanine concentrations. The cause of progressive neurocognitive deficits in HT1-patients remains unknown. Studies in larger patient populations are needed.

P-202

Ketogenic diet in the management of nonketotic hyperglycinemia: There is still some hope.

Aktuglu Zeybek A C[1], Topuz H S[2], Kiykim E[1], Zubarioglu T[1], Gunaydin D[1], Altay S[3], Onal H[4], Cam H[1]

[1]Div Nutr Metab Dis, Ist Univ Cer Med Fac, Istanbul, Turkey, [2]Ist Public Health Inst, Istanbul, Turkey, [3]Dep Nutr Diet, Trakya Univ, Edirne, Turkey, [4]Div Metab Dis, KSS Edu Res Hosp, Istanbul, Turkey

Background: The neonatal nonketotic hyperglycinemia (NKH) is characterized by the appearance of lethargy, myoclonic jerks with rapid progression to intractable seizures and coma, in the first days of life. The outcome is usually poor despite the use of scavenges drugs and diet. Ketogenic diet (KD) has been successfully tried in childhood epilepsy with multiple drug resistance.
Methods: We report the clinical and laboratory results of five cases with neonatal NKH and early myoclonic encephalopathy treated with KD.
Results: The mean age at the start of treatment was 12.7±9 months while the mean period for the follow up was 8.4 ±3.8 months. KD was started on a ratio of 60% of the daily caloric amount and increased up to 90%. The mean plasma glycine level was 1278 ± 726,1 mcmol/L before treatment and 338 ± 138 mcmol/L after treatment. Reduction in seizure frequency was achieved in all patients but there was no significant difference in EEG recordings. KD side effects were observed in two patients, resolving after reduction of the ratio to 75%.
Conclusion: Our data suggest that KD along with standard therapy can help reduction of plasma glycine level and seizures that can result in improvement of the quality of life.

P-203

An autosomal dominant oculocutaneous albinism with a novel *OCA2* mutation in a Korean family.

Hwang S K[1], Kwon S H[1]

[1]Pediactrics, Kyungpook Nat Univ Hosp, Daegu, Korea, Republic of

Background and objectives: Oculocutaneous albinism is an inborn error of tyrosine metabolism which is caused by mutations in genes that regulate melanin synthesis. Here we performed whole exome sequencing on three-generations of a family with oculocutaneous albinism.
Case report: A 4 year old Korean male patient was referred to our hospital for evaluation of albinism. The albinism showed autosomal dominant inheritance through the family history of his father, one of his aunts, grandfather, and great-grand mother. It was unique and astonishing that hair color and skin tone of the affected family members started to change back to normal pigmentation at late twenties.
Materials and Methods: Targeted exonic regions were captured with Agilent SureSelect Human All Exon Kit v4 and exome sequencing was performed on Illumina. Candidate genes were screened and further validated by Sanger sequencing.
Results: A novel heterogeneous *OCA2* mutation (NM_000275:exon22:c.2338A>G) was identified in the chromosomes of individuals with albinism, but not in the family members with normal phenotype.
Conclusion: This is the first report describing an autosomal dominant oculocutaneous albinism family with a novel *OCA2* mutation. Uncovering pathomechanisms of *OCA2* mutation may provide new treatment and further more understanding of oculocutaneous albinism.

P-204

Case Report: The Hartnup-disease found by NMR spectroscopic investigations of urine

Aygen S[1], Hegele P[1], Kunig J[1], Spraul M[2], Schäfer H[2], Cannet C[2], Özates E[3]

[1]INFAI, Cologne, Germany, [2]Bruker BioSpin, Rheinstetten, Germany, [3]Medilife Capa Hastanesi, Istanbul, Turkey

Background: Hartnup disease is a rare, autosomal recessive metabolic disorder affecting the absorption of non-polar amino acids. The estimated prevalence is approximately 1:30.000. This disease is characterized by abnormal renal and gastrointestinal transport of neutral amino acids.
Methods: The urine sample was investigated by using a 600 MHz AVANCE III NMR system.
Patient and Results: Two urine samples were collected from a male child, premature with metabolic acidosis and acute renal failure. Sample 1 was collected on day 28 and sample 2 on day 31. Both samples were containing several neutral amino acids in concentrations above normal levels, as well as multiple organic acids. Furthermore, D-glucose and N-acetyltyrosine were found in high concentrations in both samples, compatible with Hartnup-disease.
Conclusion: We have found up to 11 of 13 pathologic metabolites for Hartnup disease in the urine samples obtained from that patient. All of these concentration levels are suspiciously high and are indicating a amino acid transport deficiency. Additionally, high concentrations of D-glucose, may also be a consequence arising from this. All of these observations are possibly indicating Hartnup disease. NMR spectroscopy of body fluids may be considered as alternative analytical approach for diagnosing known, but also as yet unknown, inborn errors of metabolism.

P-205

L-carnitine supplementation decreases DNA damage in treated MSUD patients

Mescka C P[1 3], Guerreiro G B G[2], Hammerschmidt T[3], Faverzani J L[3], Monalissa A[3], Coelho D M[3], Manfredini V[4], Wayhs C A Y[3], Wajner M[1 3], Dutra-Filho C S[1], Vargas C R[1 2 3]

[1]PPGBioq, UFRGS, Porto Alegre, Brazil, [2]PPGCF, UFRGS, Porto Alegre, Brazil, [3]Serv. Genét. Médica, HCPA, UFRGS, Porto Alegre, Brazil, [4]Univ. Fed. do Pampa, Uruguaiana, Brazil

Background and objectives: Maple syrup urine disease (MSUD) is a disorder of branched-chain amino acids (BCAA). Patients generally present psychomotor delay, mental retardation and brain abnormalites. Studies have shown that oxidative stress may be involved in neuropathology of MSUD. In this regard, it was recently reported that MSUD patients have deficiency of L-carnitine (l-car), a compound with antioxidant properties that is used as adjuvant therapy in various inborn errors of metabolism. In this work, we investigated DNA damage determined by the alkaline comet assay in peripheral whole blood leukocytes of MSUD patients submitted to a BCAA-restricted diet supplemented or not with l-car.

Results: We observed a significant increase of DNA damage index (DI) in leukocytes from MSUD patients under BCAA-restricted diet as compared to controls and that l-car supplementation significantly decreased DNA DI levels. It was also found a positive correlation between DI and MDA content, a marker of lipid peroxidation, and an inverse correlation between DI and l-car levels.
Conclusion: Taken together, our results suggest the involvement of oxidative stress in DNA damage in this disorder. Since l-car reduced DNA damage, it is presumed that dietary supplementation of this compound may serve as an adjuvant therapeutic strategy for MSUD patients.

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Marple Syrup Urine Disease - New insights from a French cohort

Abi Warde M T[1], Roda C[4], Arnoux J B[1], Habarou F[2], Brassier A[1], Servais A[3], Valayannopoulos V[1], Barbier V[1], Alili J M[5], Husson M C[5], Ottolenghi C[2], De Lonlay P[2]

[1]Div Met Dis, Necker Univ Child Hosp, Paris, France, [2]Div Metab Biochem, Necker Univ Hosp, Paris, France, [3]Div Nephr, Necker Univ Hosp, Paris, France, [4]Div Met Bioch, Bichat Univ Hosp, Paris, France, [5]Ass Pub Hop Par- AGEPS, Paris, France

Background: MSUD causes neonatal coma and recurrent decompensations.
Objectives & Methods: In order to evaluate our practice, we reviewed the files of 35 MSUD patients (born between 1964 and 2011) revealed by neonatal coma, their leucine levels, and metabolic events (leucine >5mg/dL requiring emergency diet and/or hospitalization). We defined scores for neurological outcome, autonomy, psychological profile, and family compliance to treatment and diet.
Results: At last follow-up date, patients were 16 year-old on average. The mean (SD) age at diagnosis was 10 (5) days. The IQ ranged from 53 to 126. Fourteen % of the patients needed adapted education, and 1/3 of adults were not autonomous. No statistical relation between neonatal coma and neurological outcome was found. We found statistical relations between family compliance, mean lifetime leucine levels and number of metabolic events. Compliance to treatment decreases with age with more frequent metabolic events after 15 years of age. Fifty six % of the patients needed psychological counseling. The eldest patients needed significantly more psychological care, independently of the frequency of metabolic events.
Conclusions: The importance of patients' education in avoiding metabolic events is evident. However, as chronic leucine intoxication may have psychiatric consequences, hepatic transplant is considered in the treatment of adult MSUD patients when compliance to treatment decreases.

P-207

Hereditary tyrosinemia type 1 in Algeria: National survey

Saadaoui B[1], Belbouab R[2], Dahlouk D[4], Baghdali N[2], Boubidi S[3], Arrada Z[1], Benalioua H[2], Benhafessa L[8], Benmati F[8], Chaou M[5], Cherfi S[6], Hamlaoui M T[1], Mekki A[3], Rahmoun H[7]

[1]Service de Pédiatrie B CHU N .Hamoud, algiers, Algeria, [2]1 Service de Pédiatrie CHU Mustapha, Algeries, Algeria, [3]Service de Pédiatrie A CHU N. Hamoud, Algeries, Algeria, [4]Service de Pédiatrie HCA, Algeries, Algeria, [5]Service de Pédiatrie B CHU Beni-Messous, Algeries, Algeria, [6]Service de Pédiatrie CHU Oran, Oran, Algeria, [7]Service de Pédiatrie CHU Setif, Setif, Algeria, [8]service de neonatologie HCA, Algeries, Algeria

Introduction: The incidence of tyrosinemia type 1 in Algeria is unknown. Better information on the disease and the development of urinary succinylacetone assay have helped to increase the number of diagnosed cases. Its prognosis has been improved after the introduction of Nitisinone drug (NBTC) ten years ago.
Objective: This is a descriptive and retrospective study making an inventory of the disease in order to harmonize its management.
Methods: A questionnaire including the socio-demographic data, forms of monitoring and outcome of treated patients was sent to all prescriber pediatricians of NTBC identified from the central pharmacy of hospitals.
Results: 51 children were identified with consanguinity in 66% of cases. The median age at diagnosis was 7 months (1- 54). Only 31% of children had specific diet. Twenty-one cases of cirrhosis (41%) and 3 hepatocellular carcinoma were identified. Two children have received liver transplants. Great heterogeneity in monitoring was found.
Conclusions: This study identified a delay in diagnosis, and dietary management of the disease. Standardization of follow-up adapted to local opportunities proves to be essential.

P-208

Hypertriglyceridemia in a 43-year-old female with lysinuric protein intolerance

Yamamoto S[1], Takayanagi M[2]

[1]Dept Ped, Shimoshizu National Hosp, Yotsukaido, Japan, [2]Div Metab, Chiba Children's Hosp, Chiba, Japan

Lysinuric protein intolerance (LPI) is an inherited defect of cationic amino acid transport at the basolateral membrane of intestinal and renal tubular cells caused by mutations in *SLC7A7* encoding the y+LAT1 protein. Combined hyperlipidemia has been reported in patients with lysinuric protein intolerance (1). We report a 43-year-old female with lysinuric protein intolerance presenting hypertriglyceridemia without hypercholesteremia.
Case Report: The diagnosis of LPI was made at the age of 14 year old, and low-protein diet and L-citrulline supplementation were started. Gene analysis of *SLC7A7* revealed homozygous mutation of p.S328F (2). Chylemia appeared at the age of 43 year old. She was slightly obese (body mass index: 25.0 kg/m^2). The highest level of serum triglyceride was 1102 mg/dl. Serum concentrations of LDL-C and HDL-C were decreased; the values were 34 mg/dl and 23 mg/dl, respectively. Plasma level of post-heparin lipoprotein lipase decreased to 42% of the mean of controls. Serum level of apolipoprotein C II was 7.3 mg/dl, and slightly elevated (controls: 1.5 – 3.8). These findings suggest that hypertriglyceridemia might be caused by shortage of lipoprotein lipase activity.
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P-209

Carnosine/homocarnosine synthesis involves two enzymes: a non-specific synthase and a metabolite-repair dipeptidase

Veiga-da-Cunha M[1]

[1]de Duve Inst, Univ catholique de Louvain, Brussels, Belgium

Background: Carnosine (β-alanyl-histidine) is an abundant (≈ 10 mM) dipeptide present in skeletal muscle, where it plays mainly the role of a pH buffer, due to the convenient pKa (6.3) of its imidazole moiety. Homocarnosine (γ-aminobutyryl-histidine) is a related dipeptide abundant in human brain with a still unknown function. Carnosine and homocarnosine synthesis is catalysed by the same ATP-dependent ligase (CARNS1) from L-histidine and β-alanine or GABA. CARNS1 is, however, a rather non-specific enzyme, which can also use basic amino acids (L-lysine, L-ornithine and L-arginine) at rates of 2-70 % of that at which it uses L-histidine, forming 'abnormal' dipeptides without significant buffering capacity at neutral pH.
Methods & Results: As these peptides are virtually absent from tissues, we searched for an enzyme that specifically destroys them, without hydrolyzing carnosine and homocarnosine, and identified the Zn^{2+} dipeptidase PM20D2, as the enzyme catalyzing this reaction.
Conclusions: This indicates that specific synthesis of carnosine and homocarnosine involves both a synthetic enzyme and a metabolite-repair enzyme. It is likely that a deficiency in either of the two enzymes will lead to a metabolic disease affecting muscle and brain. Due to the abundance of these dipeptides in tissues, NMR spectroscopy could help to diagnose such defect.

P-210

Neurogenic crises in two cases with tyrosinemia type 1

Canda E[1], Köse M[1], Akçay Arduç A[2], Kalkan Ucar S[1], Karapınar B[3], Çoker M[1]

[1]Div Metab Dis, Ege Univ Med Faculty, Izmir, Turkey, [2]Div Ped Neurol, Denizli State Hosp, Denizli, Turkey, [3]Div Ped İntensive Care, Ege Univ Med Fac, İzmir, Turkey

Increase of δ-aminolevulinic acid (ALA) in urine may cause porphyria-like symptoms especially in patients with Hereditary Tyrosinemia Type 1 that are non-adherent to treatment. We presented two cases with neurogenic crises.
Case 1: A 7-year 4-month old male patient, who presented with acute liver failure when he was 7 months old, received a diagnosis of Tyrosinemia Type 1. At the age of 7 years, the patient presented with brain fog, hypertension and convulsion following a period of approximately 1.5 months without treatment. Urinary ALA level was high.
Case 2: A 14-year 5-month old female patient, who presented to an external clinic with the complaint of failure to thrive when she was 11 months old, was taken under follow-up with the finding of renal tubular acidosis. She had the diagnosis of Tyrosinemia type 1 at the age of 2 years. The patient, who showed non-adherence to treatment, presented with brain fog and seizures. Hypertension, tachycardia, metabolic acidosis, and hyponatremia were found. Urinary ALA level was high.
Neurological crisis is one of the life-threatening complications seen in patients with Tyrosinemia Type 1. It has been reported in literature as a cause of death by 10% in untreated patients.

P-211

Hyperlysinemia in a child and his mother

Pektas E[1], Hismi B[3], Unal O[2], Dursun A[1], Sivri H S[1], Tokatli A[1], Coskun T[1]

[1]Div Metab Dis, Hacettepe Univ Child Hosp, Ankara, Turkey, [2]Div Metab Dis,Hematol Oncol Hosp, Ankara, Turkey, [3]Div Metab Dis, Child Hosp, Gaziantep, Turkey

Hyperlysinemia is a rare autosomal recessive disease caused by a defect in lysine-degradation pathway. Here, we present a child and his mother with familial hyperlysinemia with a novel mutation. A 6-year-old boy was referred to our hospital due to difficulty in identifying colors and numbers. He was the firstborn child of parents who are first-cousins. His initial physical examination, routine biochemical and hematologic tests and cranial MRI were normal. Wechsler Intelligence Scale for Children – Revised analysis showed high-normal intelligence, but learning difficulty which was restricted to social skills. Aminoacid analyses revealed normal argininosuccinic acid and high blood and urine lysine levels. The whole family was screened for hyperlysinemia. His mother, who had learning dificulties and an IQ of 66, also had high urine and serum levels of lysine. Sequence analysis of *AASS* (2-aminoadipic semialdehyde synthase) gene confirmed the diagnosis by identifying c.185C>T (p.Ser62Leu) homozygous mutation in the child and the mother, a mutation that has not been previously reported. Due to the high rate of consanguineous marriages in multiple generations in Turkey, if any hyperlysinemia patient is detected, all family members should be evaluated; not only the siblings, but the parents as well.

P-212

The use of ketogenic diet in classical neonatal nonketotic hyperglycinemia (NKH)

Balasubramaniam S[1 2], Robertson A[3], Kava M[4]

[1]Metab Unit, Princess Margaret Hosp, Perth, WA, Australia, [2]School Paeds, Child Health, UWA, Perth, WA, Australia, [3]Dietetics Dept, Princess Margaret Hosp, Perth, WA, Australia, [4]Dept of Neurol, Princess Margaret Hosp, Perth, WA, Australia

NKH is a devastating neurometabolic disorder affecting glycine metabolism characterised by profound intellectual disability, intractable seizures and poor quality of life. Therapies directed at decreasing glycine concentration and blocking its effect at the *N*-methyl-D-aspartate receptor have remained unsatisfactory. Ketogenic diet has been used to reduce seizures in a small cohort of patients with NKH. We present an infant with classical neonatal NKH and epileptic encephalopathy treated with ketogenic diet.

The male patient developed lethargy, poor feeding, hypotonia and respiratory insufficiency necessitating assisted ventilation on the third day of life. Seizures with burst suppression pattern on EEG developed the following day. Amino acid analysis showed highly elevated glycine concentrations in plasma (2358 umol/L, normal < 150-560), cerebrospinal fluid (CSF) (535.3 umol/L, normal 3.8-8) and urine (6370 umol/mmol creatinine, normal <1097). CSF/plasma glycine ratio was markedly elevated (0.23). Brain MRI showed a thin corpus callosum.

Refractory seizures persisted despite treatment with sodium benzoate, dextromethorphan, anti-convulsants and mild dietary protein restriction. Ketogenic diet commenced at 6 months of age, but temporarily disrupted for 5 weeks due to difficulties in maintaining glycaemic control and weight gain, lead to reduction in spasticity, seizure frequency, improvements in EEG, plasma glycine normalisation and enhanced quality of life.

P-213

Heterozygous donor liver transplantation in maple syrup urine disease – First procedure performed in the South of Brazil

Donis K C[2], Lorea C F[2], Silva A A[2], Souza C F M[2], Vairo F P[2], Tonon T[2], Refosco L F[2], Zanotelli M L[1], Vieira S M G[1], Benkert A[3], Strauss K A[3], Schwartz I V D[2]

[1]Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil, [2]Med Genet Serv, Hosp Clin Porto Alegre, Porto Alegre, Brazil, [3]Clinic for Special Children, Strasburg, United States

Background and objectives: Maple syrup urine disease (MSUD) is an autosomal recessive disorder caused by deficiency of the branched-chain ketoacid dehydrogenase complex (BCKDH). Liver transplantation is an effective therapy for classic MSUD. We report the first case of liver transplantation with a related living donor in a patient with MSUD performed at the Hospital de Clínicas de Porto Alegre, Brazil.

Case Report: A 2 year-old male was born to consanguineous parents. At 7 days of life, he was admitted with irritability, hypotonia, opisthotonus, sweet-smelling urine, and coma. Amino acids analysis showed: leucine 2130,6 μM/L (48-160), isoleucine 701,1 μM/L (31-86), and valine 863 μM/L (64-294). He had multiple metabolic decompensations and the family chose to pursue liver transplantation using a related donor (his father), who was heterozygous for BCKDH deficiency. The procedure was performed with no complications. Alloisoleucine decreased immediately after the transplantation. The pre-transplant values ranged from 103 to 490 and after one month, alloisoleucine was undetectable. Four months after the transplant, he is on an unrestricted diet and leucine, isoleucine, and valine levels are normal.

Discussion/Conclusion: Our successful experience confirms liver transplantation with related donors should be considered as a therapeutic option to MSUD patients.

P-214

Cerebral influx of amino acids in Brazilian patients with maple syrup urine disease

Cruz A F[1], Barbosa T M C[2], Mendes M O[3], Lima W P[4], Kanufre V C[1], Adelino T E R[5], Lopes J C C[1], Mata Y A[1], Valadares E R[6]

[1]HC, Fed Univ Min Ger, Belo Horizonte, Brazil, [2]Set pes des lab Herm Pard, Belo Horizonte, Brazil, [3]Dep tox lab Herms Pard, Belo Horizonte, Brazil, [4]assec cient lab herm par, Belo Horizonte, Brazil, [5]Lab Bio Mol Serv Viro e Riq Fund Eze Di, Belo Horizonte, Brazil, [6]Dep Prop Compl Fac Med Fed Univ Min Ger, Belo Horizonte, Brazil

Background/Objectives: Maple syrup urine disease is an inborn error of amino acids metabolism caused by deficiency of branched chain keto acid dehydrogenase. Symptoms are neurological, probably because of the competition of neutral amino acids for the influx through blood-brain barrier. The aim of this study was to evaluate the amino acids' cerebral influx in patients with the disease on dietary restriction of branched chain amino acids.
Materials and Methods: Amino acids in plasma of five patients aged 2 to 14 years were measured by high performance liquid chromatography, monthly during four months. The control group was of 60 healthy children, in the same age group. Cerebral influx was estimated by an equation described by Smith & Stoll in 1998, which considers blood concentration of ten amino acids and its affinity (Michaelis constant) for large amino acid transporter type 1.
Results: All patients presented increased levels of branched chain amino acids in blood; deficiencies of amino acids in central nervous system, specially tyrosine, tryptophan and valine were frequent.
Discussion and Conclusion: Serotonin and catecholamines are very important for the neurological outcome. Deficiencies of these neurotransmitter precursors should be cautiously investigated using cerebral influx equation and properly treated by nutritional management.

P-215

Molecular study of Brazilian patients with maple syrup urine disease

Cruz A F[1], Adelino T E R[2], Marcão A M L[4], Vilarinho L F T[4], Lopes J C C[1], Mata Y A[1], Valadares E R[3]

[1]HC, Fed Univ Min Ger, Belo Horizonte, Brazil, [2]Lab Bio Mol Serv Viro e Riq Fund Eze Di, Belo Horizonte, Brazil, [3]Dep Prop Compl Fac Med Fed Univ Min Ger, Belo Horizonte, Brazil, [4]Dp Gen Hum Inst Nac Sa D Ric Jor INSA IP, Porto, Portugal

Background/Objectives: Maple syrup urine disease is an inborn error of metabolism of branched-chain amino acids, with autosomal recessive inheritance. The aim of this study was to evaluate six patients with classic maple syrup urine disease from the state of Minas Gerais, Brazil, regarding molecular characteristics.
Materials and Methods: The molecular study was based on sequencing of exons and flanking intron regions of the three genes involved in the classic form of the disease, utilizing an automated DNA sequencer and DNA extracted from blood in filter paper.
Results: Patient 1 was homozygous for the c.595_596delAG mutation in *BCKDHB*. Patient 2 was homozygous for the c.1193T>C mutation in *DBT*. Patient 3 was heterozygous for c.359T>C and c.1159C>T mutations in *BCKDHB*. Patient 4 was homozygous for c.670G>T mutation in *DBT*. Patient 5 was heterozygous for c.261delA and c.670G>T mutations in *DBT*. Patient 6 was homozygous for c.108+6T>C mutation in *BCKDHA*.
Discussion and Conclusion: Locus heterogeneity and great allelic heterogeneity were observed in maple syrup urine disease Brazilian patients from Minas Gerais state. Novel mutations were described, all potentially pathogenic because they result in truncated proteins (c.359T>C, c.1159C>T, c.261delA) or with inclusion of intronic portions (c.108+6T>C).

P-216

Understanding the suboptimal neurocognitive outcome in NTBC treated tyrosinemia type 1 patients

Van Ginkel W G[1], Van Vliet D[1], Heiner-Fokkema M R[2], Van Spronsen F J[1]

[1]Beatrix Child Hosp, UMCG, Groningen, Netherlands, [2]Dep Lab Med, UMCG, Groningen, Netherlands

Background & Aim: Tyrosinemia type 1 (HT1) patients have neuropsychological deficits for executive functions and social cognition. It is unclear whether these deficits are due to high tyrosine or low phenylalanine concentrations. Both phenylalanine and tyrosine are large neutral amino acids (LNAA) that only enter the brain by competitive transport across the blood-brain barrier. Therefore, plasma LNAA profiles strongly determine -but do not directly reflect- brain LNAA concentrations. As theoretical experiment, we studied how plasma LNAA profiles of HT1 patients on NTBC and diet may influence brain influx of all LNAAs (except for tryptophan).

Methods: With blood LNAA concentrations of 8 HT1 patients (142 samples) and 341 controls (341 samples), z-scores of brain influx for individual LNAAs were estimated using Michaelis-Menton (K_m) and the equations of Strauss (2010).
Results: Mean plasma tyrosine and phenylalanine concentrations were 390 and 31 μmol/l. Z-scores of estimated brain tyrosine and phenylalanine influx were 17.8 and -3.6, respectively, others being within -1 and +1 range.
Discussion: Plasma tyrosine concentrations and estimated brain influx were strongly increased in HT1 patients. For phenylalanine, however, while mean plasma concentrations were only mildly reduced, estimated brain influx was markedly affected. This is important for improving treatment in HT1 patients

P-217

Newborn screening improves neurologic evolution and quality of life in maple syrup urine disease patients

Sánchez-Pintos P[1], De Castro M J[1], García Cazorla A[2], Ramos F J[2], Bueno M A[3], Camba M J[1], Bóveda M D[1], Couce M L[1]

[1]Metab Dis Unit, Clinic Univ Hosp.CIBERER, Santiago de Compostela, Spain, [2]Neurometab Unit. Hosp S. Joan de Déu., Barcelona, Spain, [3]Metab Unit. Univ Hosp Virgen del Rocío, Sevilla, Spain

Background-Objectives: Newborn screening (NBS) for maple syrup urine disease (MSUD) has not yet been implemented for most Spanish regions.
Objective: Assess clinical features and outcome in MSUD patients, diagnosed either by NBS or by clinical symptoms.
Patients & Methods: Retrospective comparative study of 14 MSUD patients during 13 years. Maximum leucine peak, interval with leucine concentration>1000μM, clinical findings, hospitalization admissions and cognitive assessment by Psychomotor-Development-Index (PDI) and gross-motor-function-measure (GMFM) was assessed.
Results: In NBS group (n=8) average age at detection was 4.6days with a leucine plasmatic concentration at diagnoses of 1807μM(263-2500), vs 11,5days and 2355μM(1600-3241) in late diagnoses patients (LD)(n=6). Follow-up period: 70 and 41 months respectively. All NBS patients remain asymptomatic, with no learning difficulties (5/8 on scholar age). The period of toxic leucine levels was 0.7days (0-4), mean hospitalization duration: 8.75days and the mean number of hospitalizations: 1.6 (0-5). All LD cases presented early acute encephalopathy, showed a longer period with leucine >1000μM [mean: 6.6 days (1-13); p: 0,001] and higher duration [mean: 53days; p: 0.005] and number of hospitalizations [mean:5.3 (4-7)]. PDI was ≥85 in 100% NBS vs 66% LD cases and GMFM normal in all.
Conclusions: MSUD prompt detection by NBS reduces the period with toxic leucine levels and hospitalization days and improves life quality and neurological outcomes.

P-218

NMR Spectroscopy as a tool in differential diagnosis, our experience in a patient suspected for tyrosinemia type I

Vulturar R[1 4], Nicolescu A[2 5], Pop T[3], Deleanu C[2 5]

[1]Univ. of Medicine and Pharmacy Cluj, Cluj-Napoca, Romania, [2]P. Poni Inst of Macromolecular Chemistry, Iasi, Romania, [3]Univ. of Medicine and Pharmacy Cluj, Cluj-Napoca, Romania, [4]Cogn Neuroscience Lab, Univ Babes Bolyai, Cluj-Napoca, Romania, [5]C. Nenitescu Inst Org Chemistry, Ro Acad, Bucharest, Romania

Case-study: A three months old boy was analyzed being suspected for tyrosinemia-type I or galactosemia (presented with failure to thrive-acute liver failure-coagulopathy). The blood analysis revealed very high levels of methionine and moderate increase of tyrosine; besides, the test for hepatitis B virus infection was positive, high viremia. The urinary-NMR-Spectroscopy (with Bruker-Avance-III-400-MHz) showed very high concentrations of lactate, 4-hydroxyphenyl acids [4-hydroxyphenyl-lactic acid (2014-2441 mmol/molCreatinine) and 4-hydroxyphenyl pyruvic acid (925-1024 mmol/molCreatinine)]. The galactose, succinylacetone-succinylacetoacetate were not detected; the diagnosis of galactosemia was excluded, and tyrosinemia type I become less likely. The 1H-NMR spectra were recorded with NOESY water-presaturation-pulse-sequence. The limit-of-detection-in-the-spectral-region of these metabolites (4-hydroxyphenyl derivatives and succinylacetone) for creatinine concentration of 1 mmol/l is 100 mmol/mol Crn.
Discussions, conclusions: The clinical manifestations of tyrosinemia type I are variable, and an affected individual can present at any time from the neonatal period-adulthood; symptoms usually appear in the first months of life (failure to thrive, jaundice, bleeding abnormalities, etc). The main advantage of NMR-Spectroscopy is that it produces a global biochemical profile of the-analyzed sample, without requiring predefined analytical conditions. Repeated investigations excluded the diagnosis of galactosemia or tyrosinemia type I; the high levels of methionine, tyrosine and the high excretion of 4-hydroxyphenyl derivatives were included into secondary changes due to the B virus hepatitis.

10. Urea cycle disorders

P-008

Selective regulation of Sirtuin 5 in human liver samples of patients with urea cycle disorders

Potthast A B[1], Bednarczyk J[1], Baumann U[1], Das A M[1]

[1]Ped Metab Dis, HMS, Hannover, Germany

Background: The NAD^+-dependent desuccinylase/demalonylase Sirtuin 5 (SIRT5) is known to regulate the activity of carbamylphosphate synthase 1 (CPS1), the rate-limiting enzyme of the urea cycle under physiological conditions. Patients with urea cycle defects (UCD) have a reduced urea cycle flux as the deficient enzyme becomes rate-limiting. In patients with CPS 1-deficiency, SIRT5 may be up-regulated as a last ditch attempt to recruit residual CPS- activity.
Methods: We analysed SIRT5 in liver biopsies of a patient with CPS-deficiency undergoing liver transplantation. Liver from a patient who was liver transplanted because of citrullinaemia served as a pathological control. Results were compared to liver biopsy material from patients without a metabolic disease. SIRT1, which is not involved in the regulation of the urea cycle, was measured too. Furthermore, sirtuins were measured in cultured skin fibroblasts.
Results: In liver, SIRT5 was up-regulated by 150% at protein and 150% at transcript level in CPS1-deficiency and by 150% and 40% in citrullinaemia, respectively, compared to controls. SIRT1 was not altered. In fibroblasts, SIRT5 was not up-regulated.
Conclusion: SIRT5 is up-regulated in the liver of UCD-patients at protein and transcript level which may be a compensatory mechanism. In the cells a fine tuning of SIRT5 can be performed.

P-219

Status of glutathione in Indian children with citrullinemia type I

Kudalkar K V[1], Jalan A B[1], Jalan R A[1], Shinde D H[1], Borugale M A[1], Joshi M M[1], Mahakal J M[1], Sonalkar N D[1]

[1]NIRMAN, Div of Biochemical Genetics, Navi Mumbai, India

Background: Citrullinemia is an autosomal recessive disorder caused by deficiency of argininosuccinate synthetase (ASS).
Objective: To analyze the levels of glutathione in patients with citrullinemia. To determine the relationship between levels of ammonia & citrulline in these patients with oxidative stress.
Materials and method: We analysed glutathione levels in 15 patients with citrullinemia. Among them, 4 patients survived the neonatal period and were followed up on a regular basis for their glutathione levels along with citrulline, ammonia, orotic acid, calcium, phosphorus, alkaline phosphatase, total proteins and albumin.
Result: We found considerably low levels of glutathione in patients with citrullinemia (24.42±14.98 nmol/mg Hb, n=15) compared to healthy individuals (46.64±17.51 nmol/mg Hb, n=54). The patients who succumbed in the neonatal period showed even lower levels than others in the same group; (16.24 ± 13.33 nmol/mg Hb, n=11).
Conclusion: Glutathione levels were found to vary inversely with levels of ammonia indicating increased oxidative stress with increasing levels of ammonia. Citrulline did not vary significantly with glutathione levels. The 4 patients who survived showed significant improvement of glutathione levels upon controlling the levels of ammonia. Better levels of glutathione within the group seemed to improve the chances of survival.

P-220

Evolution of the first patient of Mayan ancestry with argininemia found through the expanded newborn screening program of Yucatan, Mexico

Campos-Garcia F J[3], Contreras-Capetillo S[2], Maldonado-Solis F A[4], Moreno-Graciano C M[4], Martinez-Cruz P[4], Vela-Amieva M[1]

[1]Lab Err Inn Met, INP, D.F., Mexico, [2]Genet Div, O'Horan Gen Hosp, Merida, Mexico, [3]Med Super, TamizMas, Merida, Mexico, [4]Tamizaje Plus, Villahermosa, Mexico

Background: Homozygous mutations in the human *ARG1* gene provoke an autosomal recessive neurometabolic disorder known as argininemia (OMIM #207800). Worldwide frequency is 1:2,000,000 newborns, but in Mexico its prevalence is unknown.
Objective: Evaluate the evolution and neurological development of the first patient with argininemia from Yucatan.
Methods: The expanded newborn screening program of Yucatan has screened 112,500 neonates, from 2008 to 2015. We look for suspicious patients and the local general hospital implements treatment.
Results: No familial history for genetic or metabolic diseases, congenital malformations, nor intellectual disability was recorded; including his 4 siblings. He was born at his house after an uneventful pregnancy. Developmental milestones were normal. A newborn

screening sample was taken at 25 days of age, with an arginine blood level of 391.5 μmol/L (cut-off value: 74 μmol/L). Confirmatory test performed at 6 weeks of age showed an arginine of 690 μmol/L and mild hyperammonemia (102 μmol/L). A regime of 250 mg/Kg/day sodium benzoate and a non-essential aminoacid-free milk formula were started. Arginine and ammonium levels are within normal parameters. No neurologic manifestations have occurred in the patient to date.
Conclusion: Our patient remains asymptomatic to date with nutritional and pharmacological management.

P-221

Role of low plasma citrulline and arginine for the diagnosis of proximal urea cycle disorders

Borugale M A[1], Jalan A B[1], Joshi M M[1], Mahakal J M[1], Sonalkar N D[1], Kudalkar K V[1], Jalan R A[1], Shinde D H[1], Haeberle J[2]

[1]NIRMAN, Div of Biochemical Genetics, Navi Mumbai, India, [2]Div of Metabolism, Univ Children Hosp,, Zurich, Switzerland

Background: CPS/NAGS deficiencies are proximal urea cycle defects (UCDs) characterized by low plasma citrulline and arginine with low urinary orotic acid. We investigated in our patient cohort the role of citrulline and arginine levels for the diagnosis of proximal UCDs.
Material and Method: We studied 8 children (5 males, 3 females) with hyperammonemia, low citrulline, arginine and orotic acid, in which gene analyses were done. We present a retrospective analysis of these patients.
Result: Our patients presented with lethargy, poor feeding, vomiting and seizures. Biochemical profile of these patients showed hyperammonemia (381±328μmol/L), low orotic acid (1.08±2.82μmol/L), low citrulline (2 ±4.02μmol/L) and arginine (4±11.71μmol/L). Molecular studies were performed in 5 patients for CPS/NAGS deficiency, of which only one was *CPS1* deficient (c.2339G>A/p.Arg780His homozygous). Two patients were analysed for only *NAGS*, who were normal. These two children are now healthy and on normal feeds.
Conclusion: Low citrulline and arginine are not always indicative of CPS/NAGS deficiency. In our cohort of 8 patients, only 1 was confirmed to have *CPS1* deficiency. In the remainingpatients no mutation in *CPS*/*NAGS* genes could be ascertained. Therefore other causes of low citrulline and arginine need to be assessed in newborns with this biochemical profile.

P-222

Citrullinaemia type 2 (CTLN2): How should one manage a child with an incidental diagnosis?

Hulley S L[1], Kirk R[2], Harrison C[3], Sharrard M J[1]

[1]Paediatric med, Sheffield Child Hosp, Sheffield, United Kingdom, [2]Sheffield Diagnostic Genetics Service, Sheffield, United Kingdom, [3]Paediatric Med, Rotherham Hosp, Rotherham, United Kingdom

Background: CTLN2 may present as prolonged neonatal cholestasis, usually with recovery by one year, childhood failure to thrive and dyslipidaemia, or adult hyperammonaemia and hepatopathy with poor outcome.
Case report: A girl, born to consanguineous parents of Asian origin, underwent investigations after birth as a sibling was affected with isovaleric acidaemia. Plasma acylcarnitines and urine organic acids were unremarkable, but plasma citrulline was elevated (123 μmol/L, 0-40). Cholestasis was absent and a liver ultrasound normal. Citrulline (348 μmol/L) and threonine (687 μmol/L, 10-400) had both risen at 4 weeks but by 10 weeks had normalised.
Results: Sequencing of the *SLC25A13* gene showed homozygosity for a previously reported mutation in exon 17 (c.1763G>A, p.Arg588Gln). At 6 months she developed hepatosplenomegaly with elevated transaminases and triglycerides, and abnormal hepatic texture on ultrasound. A galactose free diet led to a clinical improvement.
Discussion: Children diagnosed neonatally with CTLN2 may show carbohydrate aversion in childhood and may develop symptoms in adulthood. There is little evidence on when to treat asymptomatic individuals. Here we demonstrate that early instigation of a galactose free diet may lead to clinical improvement in symptomatic infants and we recommend starting dietary treatment in the asymptomatic CTLN2 infant.

P-223

Mild citrullinemia in Turkish patients: Report of three patients

Aktuglu Zeybek A C[1], Kiykim E[1], Zubarioglu T[1], Cansever M S[2], Cam H[1], Aydin A[1]

[1]Div Nutr Metab Dis, Ist Univ Cer Med Fac, Istanbul, Turkey, [2]Cent Lab, Ist Univ Cer Med Fac, Istanbul, Turkey

Background: Classical Citrullinemia type I (CTI) is a rare metabolic disorder representing with hyperammonemia and neurologic manifestations in the neonatal period. A

considerable number of patients with a mild and asymptomatic disease course have been reported also resulting from mutations in the *ASS1* gene. We aimed to report the clinical and molecular characteristics of 3 Turkish patents with mild type CTI.
Methods: Biochemical, clinical findings and mutations in the *ASS1* gene were investigated in three mild type CTI patients.
Results: First patient was referred at 30 days of age with increase in citrulline level detected in expanded newborn screening (ENS). The second patient was 10 years old female sister of first patient, detected by family screening after the index patient was identified. The third patient was first identified by ENS, followed with low-protein diet initially and was referred for further evaluation at one year of age. Mutation analysis revealed a missense mutation on *ASS1* gene (c.1085G>T; p.Gly362Val) of all patients. None of the patients had to receive low-protein diet or ammonia scavenger during the follow-up and had normal developmental milestones.
Conclusion: We provided information about clinical and mutational spectrum of Turkish mild type CTI patients.

P-224

Clinical utility of urinary phenylacetylglutamine (U-PAGN) concentration as an adherence biomarker for patients with urea cycle disorders (UCDS) treated with glycerol phenylbutyrate (GPB)

Scharschmidt B F[1], Mokhtarani M[1], Diaz G A[2], Lichter-Konecki U[10], Berry S A[3], Bartley J[4], McCandless S E[5], Smith W[6], Harding C[7], Le Mons C[8], Coakley D F[1], Lee B[9]

[1]Hyperion Therapeutics, Brisbane, CA, United States, [2]Mt Sinai School of Medicine, New York, NY, United States, [3]University of MN, Minneapolis, MN, United States, [4]Long Beach Memorial Hospital, Long Beach, CA, United States, [5]Case Western Res Univ, Cleveland, OH, United States, [6]Maine Med Ctr, Portland, ME, United States, [7]Oregon Health Sci Univ, Portland, OR, United States, [8]National Urea Cycle Disorders Found, Pasendena, CA, United States, [9]Baylor College of Med, Houston, TX, United States, [10]Columbia Univ Med Ctr, New York, NY, United States

Plasma and urine metabolites are useful dosing biomarkers in UCD patients treated with sodium phenylbutyrate or GPB. Although U-PAGN is a direct measure of drug-mediated waste nitrogen excretion, timed (24h) collection is difficult in both adults and children. We therefore examined PAGN concentration in spot urines as a GPB dosing biomarker.
Methods: The relationship between U-PAGN concentration and GPB dose was analyzed in 280 spot urine samples from 134 healthy adults and UCD patients during steady state GPB dosing.
Results: Mean total GPB doses (mL/day) were higher for subjects with a body surface area (BSA) >1.3 m^2 vs. ≤1.3 m^2, which separated pediatric subjects ages < 11 years from older subjects; however, mean GPB doses adjusted for BSA (mL/m^2/day) were similar for the two groups. Linear regression showed a negative correlation between BSA and U-PAGN concentration, ($r = -0.347$, $p < 0.0001$). U-PAGN concentrations (9000, 7000, and 5000 mcg/ml) were identified, corresponding to the lower 25th percentile of U-PAGN concentrations in relation to age and BSA sub-groups (< 2 years old, >2 years with BSA < 1.3m^2 and BSA >1.3m^2, respectively).
Conclusions: Age-related U-PAGN concentration appears a promising marker for monitoring UCD patients regarding adherence to and/or effectiveness of GPB drug administration.
Conflict of Interest declared.

P-225

Glutamine and hyperammonemic crises (HAC) in patients with urea cycle disorders (UCDS)

Scharschmidt B F[1], Lee B[9], Diaz G A[2], Rhead W[10], Lichter-Konecki U[11], Feigenbaum A[12], Berry S A[3], Le Mons C[8], Bartley J[4], Longo N[13], Nagamani S C[9], Berquist W[14], Gallagher R[15], Harding C[7], McCandless S E[5], Smith W[6], Schulze A[16], Marino M[7], Coakley D F[1], Mokhtarani M[1]

[1]Hyperion Therapeutics, Brisbane, CA, United States, [2]Mt Sinai School of Medicine, New York, NY, United States, [3]University of MN, Minneapolis, MN, United States, [4]Long Beach Memorial Hospital, Long Beach, CA, United States, [5]Case Western Res Univ, Cleveland, OH, United States, [6]Maine Med Ctr, Portland, ME, United States, [7]Oregon Health Sci Univ, Portland, OR, United States, [8]National Urea Cycle Disorders Found, Pasendena, CA, United States, [9]Baylor College of Med, Houston, TX, United States, [10]The Med College of Wisc, Milwaukee, WI, United States, [11]Columbia Univ Med Ctr, New York, NY, United States, [12]UC San Diego, San Diego, CA, United States, [13]Univ of UT, Salt Lake City, UT, United States, [14]Stanford University, Palo Alto, CA, United States, [15]UCSF, San Francisco, CA, United States, [16]Hospital for Sick Children, Toronto, Canada

Plasma glutamine, which is less affected than ammonia (NH_3) by blood sampling procedures, is a widely used UCD biomarker. However, its diurnal variability and utility versus NH_3 have not been studied.
Methods: We analyzed a database of >1000 timed blood samples from 100 stable UCD patients (Lee, Genet Med 2014).

Results: Mean (SD) glutamine (μmol/L) at baseline was 740.1 (234.6) (ULN = 746); 18% of patients had values ≥ 900μmol/L. The correlation coefficient between glutamine and concurrent NH_3 measurements varied from 0.17 - 0.29. Glutamine >900μmol/L was associated with a ~2x risk of HAC [odds ratio (OR)=1.98; p=0.173]. When the analysis included baseline NH_3, the predictive value of glutamine was diminished (OR=1.47; p=0.439) as compared with that of abnormal baseline NH_3 (OR =4.96; p=0.013). Analysis of 100-200μmol/L increments in glutamine yielded similar results. Among 10 adults with serial 24-hour amino acid and NH_3 samples, glutamine exhibited a diurnal pattern different from NH_3 with less daily variability (coefficient of variation = 16% vs 66%).
Conclusions: The findings in this UCD population suggest that glutamine is a weaker predictor of HACs than NH_3 and that the utility of the predictive value of glutamine will need to take into account concurrent NH_3.
Conflict of Interest declared.

P-226

The role of hyperammonemia in acute liver failure in urea cycle disorders

Laemmle A[1], Stricker T[1], Baumgartner M R[1], Gallagher R C[2], Häberle J[1]

[1]Div Metab Dis, Univ Child Hosp, Zurich, Switzerland, [2]Dep Pediatrics, Div Medical Genet, UCSF, San Francisco, United States

Background: Acute liver failure (ALF) and coagulopathy are known complications of hyperammonemia and have been anecdotally reported in ornithine transcarbamylase deficiency (OTCD) and other urea cycle disorders.
Patients and Methods: To evaluate the prevalence of coagulopathy in OTCD, we analyzed the Swiss cohort of patients enrolled in the Urea Cycle Disorders Consortium (UCDC) and European registry and network for Intoxication type Metabolic Diseases (E-IMD). Data from 20 female and 5 male patients (total of 120 patient years) were collected from 09/2008 to 04/2015 and analyzed retrospectively. To investigate our hypothesis of ammonia-induced inhibition of hepatic protein synthesis, we performed *in vitro* experiments quantifying albumin synthesis in HepG2 cells treated with ammonium-chloride.
Results: Liver involvement with relevant coagulopathy was found in 44% of patients at least once in the course of disease. Hyperammonemia was closely correlated with (laboratory) coagulopathy as reflected by increased values for international normalized ratio (INR) and low levels of hepatic coagulation factors. In vitro, we found a dose-dependent reduction of albumin synthesis in HepG2-cells treated with ammonium-chloride.
Conclusion: In conclusion, coagulopathy is a common and likely underdiagnosed complication of OTCD because clinically often inapparent. Cell culture experiments suggest an ammonia-induced inhibition of hepatic protein synthesis as pathophysiological explanation for ALF.

P-227

Hyperammonemia in pediatric intensive care: differences between hepatic and metabolic causes

Blasco-Alonso J[1], Serrano-Nieto J[1], Gil-Gómez R[1], Sánchez P[1], Navas V M[1], Yahyaoui R[1], Milano G[1], Sierra C[1]

[1]Hospital Regional Universitario, Málaga, Spain

Backgrounds and objectives: Patients experiencing acute elevations of ammonia present to the pediatric intensive care unit (PICU) with encephalopathy. When hyperammonemia is not thought to be the result of liver failure, treatment for an occult disorder of metabolism must begin prior to confirmation of an etiology.
Methods: We describe cases of hyperammonemia admitted to our PICU between 2001 and 2014.
Results: Among over 6000 children admitted, 21 episodes of severe acute hyperammonemia appeared in 18 patients: 8 acute liver failure, 7 urea cycle disorders (UCD) (citrullinemia 2/7, OTC deficiency 4/7, CPS deficiency 1/7) and 3 propionic acidemia. Among the hepatic hyperammonemia, median age 1.5 years (5 days-8 years), ammonia levels 432+/-246 μmol/L; 4 stage 2 encephalopathies and 2 stage 3 ones. Among the metabolic patients: average age 7+/-4.1 days, ammonia 928.4+/-613.8 μmol/L and 3.7+/-3.1 days in hyperammonaemic coma. Two patients were referred in for liver transplantation, eleven subjects died (3 UCD) and three developed severe neurological sequelae (greater ammonia peak level (1062 vs 760 μmol/l) and longer coma (2 vs 4 days).
Conclusion: High morbidity and mortality in symptomatic hyperammonemia, requiring a wide number of intensive care techniques. UCD are serious diseases with hyperammonemia level and duration as key notes.

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Bleeding disorders in patients with argininemia

Kiykim E[1], Zubarioglu T[1], Aktuglu Zeybek A C[1], Cansever M S[2], Aydin A[1]

[1]Div Nutr Metab Dis, Ist Univ Cer Med Fac, Istanbul, Turkey, [2]Cent Lab, Ist Univ Cer Med Fac, Istanbul, Turkey

Background: Argininemia is an autosomal recessive disorder caused by the hepatic arginase deficiency. Hyperammonemia episodes can cause transient elevations of liver transaminases and coagulopathy in argininemia patients but a permanent coagulopathy has never been reported. Here we report five patients with argininemia and bleeding disorder.
Case reports: We report five patients diagnosed with argininemia and bleeding disorders. In our cases, 4 of patients had elevation on liver transaminases, one patient had mild hepatic fibrosis but no hepatic dysfunction was documented. Four patients had factor VII and factor IX deficiency while other K vitamin binding factors like II and X were in normal ranges. Patient 5, had impaired coagulation but no factor deficiency was documented. Three of the patients had minor bleeding problems like petechia and ecchymosis while none of our patients had severe life-threating hemorrhages. All of the patients had prolonged PT despite aPTT levels were normal.
Discussion: The classic onset of argininemia includes spastic paraparesis, mental retardation and episodic hyperammonemia mostly start in early childhood. Uncommon presentations of argininemia with neonatal cholestasis and cirrhosis have been reported. In our cases neither cirrhosis nor cholestasis was present. In conclusion patients affected with argininemia should be investigated for bleeding abnormalities.

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Brain-derived neurotrophic factor (BDNF) -dependent synaptic biomarkers in a group of patients with urea cycle disorders (UCD) and maple syrup urine disease (MSUD)

Tristán-Noguero A[1 2 3], Castells A A[7], Ramos F J[1 3], Piñeiros M[1 3], Cortés-Saladelafont E[1 2 3], Gonzalez J[1 3], Armstrong J[3 4 6], Castejón E[3 5], Meavilla S[3 5], Pérez-Dueñas B[1 3 6], Campistol J[1 3 6], Artuch R[3 4 6], Alcántara S[7], García-Cazorla A[1 2 3 6]

[1]Neurology Dept, Sant Joan de Déu Hosp, Barcelona, Spain, [2]Lab Synapt Metab, Sant Joan de Déu Hosp, Barcelona, Spain, [3]Metabolic Unit, Sant Joan de Déu Hosp, Barcelona, Spain, [4]Lab Molec Biochem, Sant Joan de Déu Hosp, Barcelona, Spain, [5]Gastro & Nutr Dept,Sant Joan de Déu Hosp, Barcelona, Spain, [6]CIBERER, Instituto de salud Carlos III, Barcelona, Spain, [7]Neural Development Research Group, UB, Barcelona, Spain

Introduction: Brain-derived neurotrophic factor (BDNF) has been involved in the regulation of amino acid (AA) metabolism. BDNF has important neurobiological functions including synaptic plasticity. Due to the scarce knowledge about synaptic dysfunction mechanisms in inborn errors (IEM) of AAs, we aimed to study the role of BDNF signaling in UCD and MSUD patients.
Methods: BDNF plasma levels were measured in 16 patients with UCD and 9 with MSUD (ELISA kit). Values were compared to a control group. mRNA from 24 genes involved in the synaptic machinery and BDNF signaling were measured in whole blood samples. cDNA was analyzed by RT PCR using Taqman Low Density Array Cards.
Results: UCD patients showed a tendency towards low BDNF plasma levels. Two genes related to BDNF and synaptic function were upregulated in UCD and MSUD: *ADORA2A* and *MECP2*. In MSUD patients, 11 more genes were upregulated and 2 were downregulated (*LIN28A* and *NCOR*).
Conclusions: Abnormal AAs levels induce changes in genes involved in BDNF signaling and synaptic remodeling. These changes seem to be more intense in MSUD than in UCD. Although further studies are needed to confirm these results, this approach could improve our knowledge about neuronal dysfunction in IEM.

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The challenging face of arginase 1 deficiency: early neonatal presentation with severe liver disease, secondary hemophagocytosis and bladder dysfunction suggesting paraparesis

Kuster A[1], Benoist J F[3], Caillaux G[2], Muller J B[2], Acquaviva-Bourdain C[4]

[1]Div Metab Dis, Univ Child Hosp, Nantes, France, [2]Neonatology, Univ Child Hosp, Nantes, France, [3]Biochemistry, Hôpital Robert Debré, Paris, France, [4]Biochemistry, Univ Child Hosp, Lyon-Bron, France

Background: Classical presentation of arginase 1 deficiency includes cognitive decline and progressive spasticity responsible of loss of ambulation and bowel and bladder control. Neonatal presentation is unusual, with only 6 cases reported and exceptional two patients described with neonatal cholestatic liver disease.
Case report: A preterm boy born at 28 WG presented at day 9 with a poorly understood megabladder with rupture and haemorrhagic shock. Severe liver dysfunction occurred at day 64 with elevated enzymes and cholestasis that were respectively attributed to sepsis and parenteral nutrition. Simultaneous bicytopenia led to bone marrow aspiration revealing hemophagocytosis. Metabolic work-up showed hyperammonemia (185 μM) with marked increase in plasma arginine (2170 μM) without orotic aciduria. Protein restriction

and nitrogen scavenging drugs were initiated, but the patient died from liver and secondary renal failure. Subsequent molecular analysis of *ARG1* gene found a novel homozygous c.316G>C (p.Gly106Arg) mutation. There were no abnormal CGH array findings and severe combined immunodeficiency syndromes were ruled out. Therefore, arginase deficiency was supposed to be responsible for neonatal liver failure with secondary hemophagocytosis.
Conclusion: Work-up of neonatal cholestasis should integrate arginase deficiency screening. Moreover, abnormalities of bladder innervation in unmyelinated infants missing signs of paraparesis may indicate an inborn error of metabolism.

11. Organic acidurias: branched-chain

P-016

Propiogenesis in the brain: implications for the management of disorders of propionate metabolism

Morton D H[1], Muenke N[1], Hendrickson C[1], Benkert A[1], Hill C[3], Mahoney C[3], Smolen G[3], Kelley R I[2], Clasquin M F[3]

[1]Clinic for Special Children, Strasburg Pennsylvania, United States, [2]Boston Children's Hospital, Boston MA, United States, [3]Agios Pharmaceuticals, Cambridge MA, United States

Background: Propionic acidemia (PA) treatment reduces propionyl-CoA in brain by restricted dietary protein, carnitine-C3 conjugation, and amino acid blends that modulate LAT1 transport across the blood-brain barrier. However, restriction of LAT1-dependent Met-Thr-Val can be detrimental to brain growth and function. Amino acids and citrate support mitochondrial repletion of succinyl-CoA, which is impaired in variants of PA, MMA and Cobalamin-C.
Methods: Amino acids were quantified by OPA-HPLC, methylcitrate and citrate by GC/MS, under IRB-approval. Comparisons included urine methylcitrate and citrate/methylcitrate ratios compared with plasma Ile-Val-Thr-Met levels and calculated LAT1-dependent flux. *In vitro* kinetic-flux-profiling used LC-Q-Exactive-MS-detection.
Results: Treatment of PA reduces methylcitrate excretion, increases citrate/methylcitrate ratio in urine, and controls calculated LAT1-influx for Ile-Val-Met-Thr.For plasma Met-Thr-Val-Ile, only the concentration of isoleucine correlated with the urine methylcitrate excretion. *MUT*(*0*) cells fed $^{13}C^{15}N$-Ile and Val synthesized propionylcarnitine almost exclusively from isoleucine.
Discussion: Our results suggest that isoleucine is strongly propiogenic. Excessive restriction of Met-Thr-Val, reflected in chronically low LAT1 transport values, probably cause CNS-depletion and poor neurological outcome without significantly reducing brain propionyl-CoA levels.
Conclusion: Future studies should assess the relative contributions of $^{13}C^{15}N$ Ile-Val-Thr-Met to propiogenesis in patients with the aim to prevent chronic, iatrogenic CNS Met-Thr-Val depletion in disorders of propionate metabolism.
Conflict of Interest declared.

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Methylmalonyl-CoA epimerase deficiency presenting as propionic aciduria

Abily-Donval L[4], Torre S[4], Tebani A[1], Samson A[1], Acquaviva C[3], Marret S[4], Benoist J F[2], Bekri S[1]

[1]Metab Biochem Rouen University Hospital, Rouen, France, [2]Biochemistry Robert Debré-APHP, Paris, France, [3]Metab Biochem Lyon University Hospital, Lyon, France, [4]Neonatal Medecine Rouen University Hospi, Rouen, France

Background: Methylmalonyl-CoA epimerase (MCE) converts D-methylmalonyl-CoA epimer to L-methylmalonyl-CoA epimer in the propionyl-CoA to succinyl-CoA pathway. Only 6 cases of isolated MCE deficiency have been described. The reported clinical pictures are variable, with two asymptomatic patients, three patients with chronic neurologic impairment and one patient with metabolic acidosis attacks. Two additional cases presented with a combined MCE and sepiapterin deficiency.
Case report: A male patient, born to non-consanguinous parents following an uneventful pregnancy presented, at 5 years of age, with feeding intolerance, metabolic acidosis, polypnea and hyperammonemia.
Results: Metabolic investigations were consistent with propionic acidemia with a high urinary excretion of 3-hydroxypropionate and methylcitrate and a large increase of plasmatic propionylcarnitine. Unexpectedly, priopionyl-CoA carboxylase activity was within the reference range. Afterward, an intermittent and mild excretion of methylmalonic acid was characterized. Plasmatic propionylcarnitine was constantly elevated, while methylmalonylcarnitine was never detected. Methylmalonic pathway gene set analysis using NGS approach allowed the identification of a homozygous nonsense mutation (p.R47*) in the methylmalonyl-CoA epimerase gene (*MCEE*).
Discussion: The finding of additional cases of MCE deficiency may help to have a better insight on the clinical impact of this rare condition and to consider it as a cause of increased methylmalonic acid.

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Two patients with atypical form and one with infantile form of HSD10 disease were identified in Japan

Fukao T[1], Sasai H[1], Aoyama Y[7], Akiba K[2], Goto M[2], Hasegawa Y[2], Kobayashi M[3], Ida H[3], Akagawa S[4], Hasegawa Y[5], Yamaguchi S[5], Shigematsu Y[6]

[1]Dep of Pediatr, Gifu Univ, Gifu, Japan, [2]Tokyo Metrop Child Med Centre, Tokyo, Japan, [3]Dept of Pediatr, JIkei Univ Med, Tokyo, Japan, [4]Nakano Child Hosp, Osaka, Japan, [5]Dept of Pediatr, Shimane Univ, Izumo, Japan, [6]Dept of Health Sci, Fukui Univ, Eiheiji, Japan, [7]Dept Biomed Sci, Chubu Univ, Kasugai, Japan

Background: 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency (HSD10 disease) is a rare X-linked recessive inborn error of metabolism, typically characterized by progressive neurodegenerative disease. Less than 30 cases have been reported worldwide.
Case reports: We report three patients from Japan. Case 1 is a 6-year-old boy, who presented with severe ketoacidosis following 5-day history of gastroenteritis. Case 2 is a 4-year-old boy, who presented with hypoglycaemia and metabolic acidosis, following one day episode of fever and appetite loss. Case 3 is a newborn who presented with hypoglycaemia, lactic acidosis at the age of 1 day. Their urinary organic acid analysis showed massive excretion of 2-methyl-3-hydroxybutyrate and tiglylglycine: mitochondrial acetoacetyl-CoA thiolase (MAT) deficiency was suspected. However, MAT enzyme activity was found to be normal. They were identified to be hemizygous for p.A154T, p.A157V, and p. Q226R mutations, respectively, in *HSD17B10* gene. The former two mutations were new mutations.
Discussion: Case 1 and Case 2 developed normally and classified to be atypical form of HSD10 disease. Case 3 showed regression from the age of 5 months, consistent with infantile form. There is wide clinical heterogeneity in this disorder and we added two patients to atypical form. We are now characterizing mutations identified in these patients.

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A case of methylmalonic acidemia (MMA) complicated by type IV renal tubular acidosis (RTA)

Lee B H[1], Yoo H W[1]

[1]Ped & Med Genet, Asan Med Ctr, Ulsan Uni, Seoul, Korea, Republic of

Background: The patients with MMA are highly likely to develop chronic renal insufficiency probably due to long-lasting tubulointerstial nephritis caused by toxic metabolites. Both proximal and distal renal tubular acidosis have been described in MMA patients.
Case: A 5 year old girl was diagnosed with MMA at 8 month of age, based on urine organic acid and mutation analysis of the *MUT* gene. Though the newborn screening result indicated elevation of propionylcarnitine, she was not further evaluated. At age of 8 months, she was brought to medical attention because of recurrent altered consciousness. The initial laboratory findings were as follows: Na- K- Cl: 131-6.5-111 mEq/L, HCO3- 11.8 mEq/L, Cr 0.2 mg/dL, urine organic acid: methylmalonic acid 1091.2 mmol/mol cr (N: 0-32.9 mmol/mol cr), methylcitric acid 14.4 mmol/mol cr, *MUT* gene p.[R108C]+[R727X], basal renin 6.1 ng/mL/hr (N: 2.3-37 ng/mL/hr), aldosterone 2060 pg/mL (N: 50-900 pg/mL). The serum electrolyte has been stably maintained till age of 2 with proper management. Since then, glomerular filtration function was progressively deteriorated. At present (5.8 yr old), her creatinine level is 2.6 mg/dL.
Conclusion: Type IV renal tubular acidosis, characterized by metabolic acidosis, chronic hyporeninemic pseudohypoaldosteronism with hyperkalemia & hyponatremia, is a rare complication of MMA.

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Hepatic impairment in methylmalonic and propionic acidurias

Garcia N[4], Imbard A[1 2], Tardieu M[5], Ogier de Baulny H[3], Labarthe F[5], Vogel H[8], Slama A[7], Broué P[6], Schiff M[3], Benoist J F[1 2]

[1]Biochem Lab, RDB Hosp, APHP, Paris, France, [2]Paris Sud Univ, Chatenay Malabry, France, [3]Ref Cent for Metab Dis, RDB Hosp, Paris, France, [4]Cent for Mol Dis, CHUV, Lausanne, Switzerland, [5]Ped Unit, CHRU, Tours, France, [6]Hepatolog and IEM Unit, Children hosp, Toulouse, France, [7]Biochem Lab, CHU Bicetre, Le Kremlin Bicetre, France, [8]Neuropathology, Stanford Univ Med Cent, Palo alto, United States

Background: Patients affected with methylmalonic (MMA) and propionic acidemias (PA) exhibit various long-term complications such as cardiomyopathy, renal failure, poor neurological outcome. Until now, hepatic impairment has never been described. The aim of this study was to characterize and extensively evaluate liver involvement in MMA and PA patients.

Patients and methods: We retrospectively collected clinical, biochemical and liver ultrasound data from patients with MMA (N=11) and PA (N=15) from 2003 to 2013.
Results: Alpha-fetoprotein (αFP) concentrations were elevated in 67% and 45% of PA and MMA patients respectively and tend to increase with age. Hepatomegaly (88 and 17 %) and liver hyperechogenicity was disclosed by liver ultrasound in 88% of PA and 50% of MMA patients. Frequency of liver impairment was higher in neonatal onset forms of PA.
Discussion: The mechanism of the liver damage expressed as a chronic liver fibrosis/cirrhosis observed in our patients is not fully understood. The increase of αFP with age suggests a mechanism of progressive toxicity which could be due the metabolites that accumulate in PA and MMA. These metabolites (e.g. methylmalonic acid, propionic acid, propionylcarnitine) have already been reported to have mitochondrial toxicity as confirmed by the results in two of our MMA patients.

P-235

Dietary treatment of 140 children with IVA - European practices

Daly A[1], Assoun M[20], Belanger-Quintana A[45], Bernabei S M[38], Bollhalder S[49], Cassiman D[29], Champion H[2], Chan H[9], Dalmau J[44], De Boer F[18], De Jong-Ter Horst N M[16], De Meyer A[33], Desloovere A[32], Dianin A[37], Dixon M[52], Dokoupil K[29], Dubois S[20], Evans S[1], Eyskens F[33], Faria A[35], Fasan I[39], Favre E[23], Feillet F[23], Fekete A[40], Ferreira Almeida M[34], Fitzachary C[52], Gallo G[38], Gingell C[3], Gribben J[10], Jankowski C[7], Janssen-Regelink R[14], Jones I[33], Jouault C[22], Kaalund Hansen K[12], Kahrs G E[42], Kok I L[19], Kowalik A[47], Laguerre C[21], Le Verge S[20], Lilje R[43], Maddalon C[50], Mayr D[41], Meyer U[27], Micciche A[9], Och U[26], Robert M[31], Rocha J C[34], Rogozinski H[11], Rohde C[25], Ross K[5], Saruggia I[24], Schlune A[28], Singleton K[51], Sjoqvist E[48], Stolen L H[43], Terry A[4], Timmer C[15], Tomlinson L[6], Tooke A[3], Van Dam E[18], Vande Kerckhove K[30], Van den Hurk T A M[19], Van der Ploeg E M C[17], Van Driessche M[32], Van Rijn M[18], Van Teeffelen-Heithoff A[26], Van Wegberg A[14], Vasconcelos C[36], Vestergaard H[46], Vitoria I[44], Webster D[7], White F J[13], White L[8], Zweers H[14], MacDonald A[1]

[1]Birmingham Children's Hospital, Birmingham, United Kingdom, [2]Addenbrookes Hospital, Cambridge, United Kingdom, [3]Nottingham University Hospitals, Nottingham, United Kingdom, [4]Alder Hey Children's NHS Trust, Liverpool, United Kingdom, [5]Royal Aberdeen Children's Hospital, Aberdeen, Scotland, United Kingdom, [6]University Hospitals Birmingham, Birmingham, United Kingdom, [7]Bristol Royal Hospital for Children, Bristol, United Kingdom, [8]Sheffield Children's Hospital, Sheffield, United Kingdom, [9]Centre for IMD, St Thomas' (GSTT), London, United Kingdom, [10]Evelina London Children's Hospital, London, United Kingdom, [11]Bradford Teaching Hospitals NHS Trust, Bradford, United Kingdom, [12]National Hosp Neurology & Neurosurgery, London, United Kingdom, [13]Central Manchester University Hospitals, Manchester, United Kingdom, [14]Radboud UMC, Nijmegen, Netherlands, [15]Academic Medical Centre, Amsterdam, Netherlands, [16]Emma Children's Hospital AMC, Amsterdam, Netherlands, [17]Maastricht University Medical Centre, Maastricht, Netherlands, [18]Uni Groningen, Uni Medical Centre, Groningen, Netherlands, [19]Wilhelmina Children's Hospital, UMC, Utrecht, Netherlands, [20]Hospital Necker Enfants Malades, Paris, France, [21]Hopital de Enfant Chu de Toulouse, Toulouse, France, [22]CHU, Angers, France, [23]CHU, Nancy, France, [24]CHU Timone, Marseille, France, [25]Hosp Child & Adol, Univ Hospitals, Leipzig, Germany, [26]University Hospital of Munster, Munster, Germany, [27]Hannover Med School, Clinic Paed, Hannover, Germany, [28]Dept Gen Pediatrics, Univ Child Hosp, Dusseldorf, Germany, [29]Dr von Hauner Children's Hospital, Munich, Germany, [30]University Hospitals Leuven, Leuven, Belgium, [31]Hosp Uni des enfants Reine Fabiola, Brussels, Belgium, [32]University Hospital Ghent, Ghent, Belgium, [33]Div Metab Dis, Univ Hosp Antwerp, Antwerp, Belgium, [34]Central Hospital of Porto EPE, Porto, Portugal, [35]Hosp Pediatr, Centro Hosp Uni Coimbra, Coimbra, Portugal, [36]Unit of Metab Dis, Centro Hospital Sao Joao, Porto, Portugal, [37]Inherit Metab Dis Unit, Dept of Ped, Verona, Italy, [38]Bambino Gesu Children's Hospital, Rome, Italy, [39]Dept Paediatrics, Uni Hospital Padova, Padova, Italy, [40]Metabolic Centre of Vienna, Vienna, Austria, [41]Dept Paed & Adol Medicine, Salzburg, Austria, [42]Haukeland University Hospital, Bergen, Norway, [43]Oslo University Hospital, Oslo, Norway, [44]Unit Metab, Hospital La Fe, Valencia, Spain, [45]Pediatric Dept, Hospital Ramon y Cajal, Madrid, Spain, [46]National University Hospital, Copenhagen, Denmark, [47]Children's Memorial Health Institute, Warsaw, Poland, [48]Children's Hospital, University Hospital, Lund, Sweden, [49]University Hospital Zurich, Zurich, Switzerland, [50]University Children's Hospital Zurich, Zurich, Switzerland, [51]University Hospital of Wales, Cardiff, Wales, United Kingdom, [52]Great Orman Street Hospital, London, United Kingdom

Introduction: Dietary treatment of IVA has received limited attention. There is little outcome data to guide optimal diet. We report results of a survey examining IVA dietary treatment within Europe.
Method: In 2014, a questionnaire was sent to dietitians working in IMD centres in Europe. Questions considered total and natural protein intake, use of leucine-free L-amino acids (LF-AA) and nutritional support in diet treated patients aged 0-6m, 6-12m, 1-10y, 11-16y, and >16y.

Results: One hundred and forty patients (neonatal onset, n=92) were identified from 38 centres representing 14 countries. Overall prescribed protein was low: mean total protein intake (natural and LF-AA) (g/kg/day): aged 0-6m, 1.8g; 7-12m, 1.8g; 1-10y, 1.6g; 11-16y, 1.2g; and >16y, 1.2g. Mean natural protein intake (g/kg/day) was 0-6m, 1.1g; 7-12m, 0.7g; 1-10y, 1.1g; 11-16y, 0.8g; and >16y, 0.8g. Twenty three centres (none UK) gave LF-AA in variable amounts: mean LF-AA (g/kg/day) 0-6m, 1.1g; 7-12m, 1.3g; 1-10y, 0.7g; 11-16y, 0.5g and >16y, 0.4g, providing a mean of 53% of total protein for all age groups. Seven patients required tube feeding.
Conclusions: In IVA, there is high use of LF-FAA with low natural protein intake. Overall, rigorous protein restriction is prescribed for age groups in IVA.

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European dietary practices in 179 propionic acidaemia patients

Daly A[1], Assoun M[20], Belanger-Quintana A[45], Bernabei S M[38], Bollhalder S[49], Cassiman D[29], Champion H[2], Chan H[9], Dalmau J[44], De Boer F[18], De Jong-Ter Horst N M[16], De Meyer A[33], Desloovere A[32], Dokoupil K[29], Dianin A[37], Dixon M[52], Dubois S[20], Evans S[1], Eyskens F[33], Faria A[35], Fasan I[39], Favre E[23], Feillet F[23], Fekete A[40], Ferreira Almeida M[34], Fitzachary C[52], Gallo G[38], Gingell C[3], Gribben J[10], Jankowski C[7], Janssen-Regelink R[14], Jones I[33], Jouault C[22], Kaalund Hansen K[12], Kahrs G E[42], Kok I L[19], Kowalik A[47], Laguerre C[21], Le Verge S[20], Lilje R[43], Maddalon C[50], Mayr D[41], Meyer U[27], Micciche A[9], Och U[26], Robert M[31], Rocha J C[34], Rogozinski H[11], Rohde C[25], Ross K[5], Sarrugia I[24], Schlune A[28], Singleton K[51], Sjoqvist E[48], Stolen L H[43], Terry A[4], Timmer C[15], Tomlinson L[6], Tooke A[3], Van Dam E[18], Vande Kerckhove K[30], Van Den Hurk T A M[19], Van Der Ploeg E M C[17], Van Driessche M[32], Van Rijn M[18], Van Teeffelen-Heithoff A[26], Van Wegberg A[14], Vasconcelos C[36], Vestergaard H[46], Vitoria I[44], Webster D[7], White F J[13], White L[8], Zweers H[14], MacDonald A[1]

[1]Birmingham Children's Hospital, Birmingham, United Kingdom, [2]Addenbrookes Hospital, Cambridge, United Kingdom, [3]Nottingham University Hospitals, Nottingham, United Kingdom, [4]Alder Hey Children's NHS Trust, Liverpool, United Kingdom, [5]Royal Aberdeen Children's Hospital, Aberdeen, Scotland, United Kingdom, [6]University Hospitals Birmingham, Birmingham, United Kingdom, [7]Bristol Royal Hospital for Children, Bristol, United Kingdom, [8]Sheffield Children's Hospital, Sheffield, United Kingdom, [9]Centre for IMD, St Thomas' (GSTT), London, United Kingdom, [10]Evelina London Children's Hospital, London, United Kingdom, [11]Bradford Teaching Hospitals NHS Trust, Bradford, United Kingdom, [12]National Hosp Neurology & Neurosurgery, London, United Kingdom, [13]Central Manchester University Hospitals, Manchester, United Kingdom, [14]Radboud UMC, Nijmegen, Netherlands, [15]Academic Medical Centre, Amsterdam, Netherlands, [16]Emma Children's Hospital AMC, Amsterdam, Netherlands, [17]Maastricht University Medical Centre, Maastricht, Netherlands, [18]Uni Groningen, Uni Medical Centre, Groningen, Netherlands, [19]Wilhelmina Children's Hospital, UMC, Utrecht, Netherlands, [20]Hospital Necker Enfants Malades, Paris, France, [21]Hopital de Enfant Chu de Toulouse, Toulouse, France, [22]CHU, Angers, France, [23]CHU, Nancy, France, [24]CHU Timone, Marseille, France, [25]Hosp Child & Adol, Univ Hospitals, Leipzig, Germany, [26]University Hospital of Munster, Munster, Germany, [27]Hannover Med School, Clinic Paed, Hannover, Germany, [28]Dept Gen Pediatrics, Univ Child Hosp, Dusseldorf, Germany, [29]Dr von Hauner Children's Hospital, Munich, Germany, [30]University Hospitals Leuven, Leuven, Belgium, [31]Hosp Uni des enfants Reine Fabiola, Brussels, Belgium, [32]University Hospital Ghent, Ghent, Belgium, [33]Div Metab Dis, Univ Hosp Antwerp, Antwerp, Belgium, [34]Central Hospital of Porto EPE, Porto, Portugal, [35]Hosp Pediatr, Centro Hosp Uni Coimbra, Coimbra, Portugal, [36]Unit of Metab Dis, Centro Hospital Sao Joao, Porto, Portugal, [37]Inherit Metab Dis Unit, Dept of Ped, Verona, Italy, [38]Bambino Gesu Children's Hospital, Rome, Italy, [39]Dept Paediatrics, Uni Hospital Padova, Padova, Italy, [40]Metabolic Centre of Vienna, Vienna, Austria, [41]Dept Paed & Adol Medicine, Salzburg, Austria, [42]Haukeland University Hospital, Bergen, Norway, [43]Oslo University Hospital, Oslo, Norway, [44]Unit Metab, Hospital La Fe, Valencia, Spain, [45]Pediatric Dept, Hospital Ramon y Cajal, Madrid, Spain, [46]National University Hospital, Copenhagen, Denmark, [47]Children's Memorial Health Institute, Warsaw, Poland, [48]Children's Hospital, University Hospital, Lund, Sweden, [49]University Hospital Zurich, Zurich, Switzerland, [50]University Children's Hospital Zurich, Zurich, Switzerland, [51]University Hospital of Wales, Cardiff, Wales, United Kingdom, [52]Great Orman Street Hospital, London, United Kingdom

Introduction: Nutritional support is the foundation of treatment in propionic acidaemia (PA) but protein requirements are controversial, even with guidelines. We aim to examine differences in dietary practices across Europe.
Methods: In 2014, a questionnaire was sent to dietitians working in IMD centres across Europe. Questions considered total and natural protein intake, use of methionine, threonine, valine and isoleucine-free L-amino acids (PFAA) and nutritional support in diet treated patients aged 0-6m, 6-12m, 1-10y, 11-16y, and >16y.
Results: 179 patients were identified from 46 centres representing 14 countries. 115 were diagnosed as neonates. Mean total protein prescription (g/kg/day) was: aged 0-6m, 2.3g; 6-12m, 1.4g; 1-10y, 1.4g; 11-16y, 1g; and >16y, 1g;

mean natural protein prescription (g/kg/day) was 0-6m, 0.8g; 6-12m, 0.6g; 1-10y, 0.6g; 11-16y, 0.6g; and >16y, 0.5g. 38 (83%) centres gave PFAA: mean PFAA g/kg/day was 0-6m, 1.5g; 6-12m, 0.4g; 1-10y, 0.5g; 11-16y, 0.4g, and >16y, 0.4g PFAA provided a mean of 38% of total protein across all age groups. Thirty seven (80%) centres used nasogastric/gastrostomy feeds to deliver >50% of nutritional requirements. Conclusions: Dietetic practices vary widely but mean natural protein intake is below WHO/FAO/UNU 2007 safe levels of intake. Protein requirements are unclear in PA.

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How do European dietary practices differ in B_{12} non responsive and B_{12} responsive methylmalonic acidaemia?

Daly A[1], Assoun M[20], Belanger-Quintana A[45], Bernabei S M[38], Bollhalder S[49], Cassiman D[29], Champion H[2], Chan H[9], Dalmau J[44], De Boer F[18], De Jong-Ter Horst N M[16], De Meyer A[33], Desloovere A[32], Dianin A[37], Dixon M[52], Dokoupil K[29], Dubois S[20], Evans S[1], Eyskens F[33], Faria A[35], Fasan I[39], Favre E[23], Feillet F[23], Fekete A[40], Ferreira Almeida M[34], Fitzachary C[52], Gallo G[38], Gingell C[3], Gribben J[10], Jankowski C[7], Janssen-Regelink R[14], Jones I[33], Jouault C[22], Kaalund Hansen K[12], Kahrs G E[42], Kok I L[19], Kowalik A[47], Laguerre C[21], Le Verge S[20], Lilje R[43], Maddalon C[50], Mayr D[41], Meyer U[27], Micciche A[9], Och U[26], Robert M[31], Rocha J C[34], Rogozinski H[11], Rohde C[25], Ross K[5], Sarrugia I[24], Schlune A[28], Singleton K[51], Sjoqvist E[48], Stolen L H[43], Terry A[4], Timmer C[15], Tomlinson L[6], Tooke A[3], Van Dam E[18], Vande Kerckhove K[30], Van Den Hurk T A M[19], Van Der Ploeg E M C[17], Van Driessche M[32], Van Rijn M[18], Van Teeffelen-Heithoff A[26], Van Wegberg A[14], Vasconcelos C[36], Vestergaard H[46], Vitoria I[44], Webster D[7], White F J[13], White L[8], Zweers H[14], MacDonald A[1]

[1]Birmingham Children's Hospital, Birmingham, United Kingdom, [2]Addenbrookes Hospital, Cambridge, United Kingdom, [3]Nottingham University Hospitals, Nottingham, United Kingdom, [4]Alder Hey Children's NHS Trust, Liverpool, United Kingdom, [5]Royal Aberdeen Children's Hospital, Aberdeen, Scotland, United Kingdom, [6]University Hospitals Birmingham, Birmingham, United Kingdom, [7]Bristol Royal Hospital for Children, Bristol, United Kingdom, [8]Sheffield Children's Hospital, Sheffield, United Kingdom, [9]Centre for IMD, St Thomas' (GSTT), London, United Kingdom, [10]Evelina London Children's Hospital, London, United Kingdom, [11]Bradford Teaching Hospitals NHS Trust, Bradford, United Kingdom, [12]National Hosp Neurology & Neurosurgery, London, United Kingdom, [13]Central Manchester University Hospitals, Manchester, United Kingdom, [14]Radboud UMC, Nijmegen, Netherlands, [15]Academic Medical Centre, Amsterdam, Netherlands, [16]Emma Children's Hospital AMC, Amsterdam, Netherlands, [17]Maastricht University Medical Centre, Maastricht, Netherlands, [18]Uni Groningen, Uni Medical Centre, Groningen, Netherlands, [19]Wilhelmina Children's Hospital, UMC, Utrecht, Netherlands, [20]Hospital Necker Enfants Malades, Paris, France, [21]Hopital de Enfant Chu de Toulouse, Toulouse, France, [22]CHU, Angers, France, [23]CHU, Nancy, France, [24]CHU Timone, Marseille, France, [25]Hosp Child & Adol, Univ Hospitals, Leipzig, Germany, [26]University Hospital of Munster, Munster, Germany, [27]Hannover Med School, Clinic Paed, Hannover, Germany, [28]Dept Gen Pediatrics, Univ Child Hosp, Dusseldorf, Germany, [29]Dr von Hauner Children's Hospital, Munich, Germany, [30]University Hospitals Leuven, Leuven, Belgium, [31]Hosp Uni des enfants Reine Fabiola, Brussels, Belgium, [32]University Hospital Ghent, Ghent, Belgium, [33]Div Metab Dis, Univ Hosp Antwerp, Antwerp, Belgium, [34]Central Hospital of Porto EPE, Porto, Portugal, [35]Hosp Pediatr, Centro Hosp Uni Coimbra, Coimbra, Portugal, [36]Unit of Metab Dis, Centro Hospital Sao Joao, Porto, Portugal, [37]Inherit Metab Dis Unit, Dept of Ped, Verona, Italy, [38]Bambino Gesu Children's Hospital, Rome, Italy, [39]Dept Paediatrics, Uni Hospital Padova, Padova, Italy, [40]Metabolic Centre of Vienna, Vienna, Austria, [41]Dept Paed & Adol Medicine, Salzburg, Austria, [42]Haukeland University Hospital, Bergen, Norway, [43]Oslo University Hospital, Oslo, Norway, [44]Unit Metab, Hospital La Fe, Valencia, Spain, [45]Pediatric Dept, Hospital Ramon y Cajal, Madrid, Spain, [46]National University Hospital, Copenhagen, Denmark, [47]Children's Memorial Health Institute, Warsaw, Poland, [48]Children's Hospital, University Hospital, Lund, Sweden, [49]University Hospital Zurich, Zurich, Switzerland, [50]University Children's Hospital Zurich, Zurich, Switzerland, [51]University Hospital of Wales, Cardiff, Wales, United Kingdom, [52]Great Orman Street Hospital, London, United Kingdom

Introduction: It is expected that in B_{12} responsive MMA (MMAr) dietary management should be less restrictive than in B_{12} non-responsive MMA (MMAnr). We have examined differences in dietary practices in MMA patients on diet therapy across Europe.

Methods: In 2014, a questionnaire was sent to dietitians working in IMD centres in Europe. Questions considered total and natural protein intake, use of methionine, threonine, valine and isoleucine-free L-amino acids (PF-AA) and nutritional support in diet treated patients aged 0-6m, 7-12m, 1-10y, 11-16y, and >16y.

Results: There were 219 MMAnr (43 centres), and 77 MMAr (23 centres) patients. There were few differences in total protein prescription (g/kg/day); in MMAnr: 0-6m, 2g; 7-12m, 1.3g; 1-10y, 1.5g; 11-16y, 1.1g; and >16y 1.2g; in MMAr: 0-6m, 1.8g;7-12m no data 1-10y, 1.4g; 11-16y, 1.3g; and >16y, 1.1g. However, natural protein prescription was significantly lower in the MMAnr patients aged 1-10y and 11-16y

than MMAr ($p< 0.05$) patients. Thirty three centres (77%) with MMAnr and 10 (42%) with MMAr patients prescribed PF-AA providing 36% and 19% respectively of total protein intake.
Conclusions: It is possible there is over restriction of total protein in MMAnr. Dietary protein prescription requires further study in both patient groups.

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Nutrition and child development in branched-chain aminoacidopathies

Köse M[1], Canda E[1], Atik Altınok Y[1], Kagnici M[1], Kalkan Ucar S[1], Habif S[2], Çoker M[1]

[1]Div Metab Dis, Ege Univ Med Faculty, İzmir, Turkey, [2]Div Clinical Biochemistry, Ege Univ Fac, İzmir, Turkey

In patients with branched-chain aminoacidopathies; prolonged mandatory protein limitation during frequent attacks, diets consisting from artificial proteins and not a strict adherence to the dietary limitations can cause malnutrition and retarded development in children. A low-protein diet and chronic acidosis might cause osteopenia and osteoporosis. Out of 43 patients, 20 were diagnosed with MMA, 16 with MSUD, 3 with PA and 4 with IVA. Mean follow-up period was 6.31±4.3 years. 21 patients had less than -2 SDS weight and 16 patients had less than -2 SDS height. A significant relationship between prealbumin, linear growth and number of attacks was observed. Patients with yearly mean attack number < 4 had significantly higher prealbumin levels compared to mean attack number >4 patients. Cut-off value for the analysis of linear growth, and number of attacks was 3.5. In bone density measurements of patients (33/44), 6 patients were diagnosed with osteopenia but no osteoporosis was detected. When the relationship between prealbumin and osteopenia was analyzed, all osteopenia patients had < 20 mg/dl prealbumin levels.In this study, we reviewed the effect of nutrition on growth and nutritional parameters and their results on bone. Simple measurements were developed to use during clinical follow-up period which might help with the treatment.

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A MSUD Case : Sodium phenylbutyrate treatment during attack period

Kalkan Ucar S[1], Köse M[1], Atik Altınok Y[1], Canda E[1], Kagnici M[1], Duyu M[2], Kabasakal C[3], Habif S[4], Çoker M[1]

[1]Div Metab Dis, Ege Univ Med Faculty, İzmir, Turkey, [2]Div Ped Intensive Care,Ege Univ Fac, İzmir, Turkey, [3]Div Ped Nephrol, İzmir, Turkey, [4]Div Clinical Biochemistry, Ege Univ Fac, İzmir, Turkey

In maple syrup urine disease, treatment for metabolic imbalance; in addition to basic dietary modifications; peritoneal dialysis, hemodiafiltration or using pharmacological therapy elimination of toxic metabolites can be employed.16-month old male patient was diagnosed with MSUD on his 45th day of life. Parents of the boy were 2nd degree cousins. After a issueless period; blood leucine level of the patient was 2904 mmol/l when the patient was hospitalized for upper respiratory tract infection. Leucine levels of the patient decreased sharply following hemodialysis (1900 mmol/l to 687 mmol/l) but increased dramatically to 2100 mmol/l after obligatory termination of hemodialysis due to technical difficulties. Sodium phenylacetate/sodium benzoate were added into the treatment and branched-chain amino acids were completely eliminated from the patient's diet. After 24 hours, blood leucine levels dropped to 897 mmol/l. Following that, sodium phenylbutyrate treatment was initiated. After sodium phenylbutyrate administration, leucine levels was dropped to 221 mmol/l without hemodialysis.Sodium phenylbutyrate treatments are known to decrease blood branched-chain amino acid levels Yet there are not enough studies on the application and efficacy of this treatment. Our case report sets an example of alternative treatments' efficacy when hemodialysis is not available due to technical difficulties during attack period.

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Branched-chain aminoacidopathy: Our experience in Ege University Faculty of Medicine

Çoker M[1], Köse M[1], Canda E[1], Kagnici M[1], Atik Altınok Y[1], Kalkan Ucar S[1], Habif S[2], Onay H[3]

[1]Div Metab Dis, Ege Univ Med Faculty, İzmir, Turkey, [2]Div Clinical Biochemistry, Ege Univ Fac, İzmir, Turkey, [3]Div Genetic Dis, Ege Univ Med Faculty, İzmir, Turkey

A group of metabolic diseases defined as "organic acidemia" is caused by enzyme dysfunctions in branched-chain amino acids, namely valine, leucine and isoleucine metabolism. The most commonly seen diseases in this group are maple syrup urine disease (MSUD), isovaleric acidemia (IVA) and methylmalonic acidemia (MMA). Mean follow-up period was 6.31±4.3 years. 19 patients were diagnosed during neonate period, 10 in the first six months of life, 6 in 6-12 month period and 12 after the patients' first birthday. 21 patients were

diagnosed with MMA, 17 with MSUD, 5 with PA and 4 with IVA. A significant relationship between number of attacks and neurological complications was observed. Patients with yearly mean attack number >4 had significantly lower IQ levels compared to mean attack number < 4 patients. Additionally, a striking relationship between initial leucine levels and final mental status in MSUD patients was detetcted. Patients with initial Leu > 1880 μmol/l are prone to mental deterioration and mental retardation. This study deals with the demographic and clinical results as well as disease-specific lab results of 47 patients diagnosed in our department and reviews their progress during follow-up period by comparing the results stated above.

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Challenge of two siblings with methylmalonic aciduria: Liver transplantation and beyond

Köse M[1], Canda E[1], Karakoyun M[3], Kagnici M[1], Yazıcı H[1], Atik Altınok Y[1], Kalkan Ucar S[1], Özgenç F[3], Aydoğdu S[3], Onay H[4], Habif S[2], Çoker M[1]

[1]Div Metab Dis, Ege Univ Med Faculty, İzmir, Turkey, [2]Div Clinical Biochemistry, Ege Univ Fac, İzmir, Turkey, [3]Div Ped Gastroenterology,Ege Univ Fac, İzmir, Turkey, [4]Div Genetic Dis, Ege Univ Med Faculty, İzmir, Turkey

Methylmalonic aciduria (MMA) is a branched-chain amino acid metabolism disease with serious morbidity and mortality rates, caused by the deficiency of methylmalonyl-CoA mutase (MCM) enzyme. A female patient with severe metabolic acidosis was presented to our department. Severe liver failure was developed in the patient during follow-up period. Liver transplantation (LT) was performed in 3 years old of age. The sister of the proband was also diagnosed with MMA in her first days of life. A homozygote c1372 G>A mutation was detected in *MUT* gene. Although a significant difference was observed between attack frequencies and MMA discharge following LT a striking increase in attack frequency was detected after the first 3 years' follow-up period. The prescribed immunosuppressive treatment regime was changed due to secondary damage potential of the toxicity to kidney and pancreas. During follow-up, both patients developed isoleucine replacement-resistant acrodermatitis dysmetabolica in both siblings during attack period. Even though LT can be a life saver in most severe cases, it is known that it does not eliminate all the complications the patient might experience during his lifetime. This study discusses the clinical results of 2 cases with normal neuromotor development but also with many complications of MMA.

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Secondary haemophagocytic lymphohistiocytosis (HLH) and posterior reversible encephalopathy syndrome (PRES) in a patient with B12-responsive late-onset methylmalonic acidemia

Paviolo M[1 2], Paquay S[1 2], Pichard S[1 2], Imbard A[1 2], Sachs P[1 3], Benoist J F[1 2], Schiff M[1 2]

[1]University Paris-Diderot, Sorbonne, Paris, France, [2]Ref Cent for Metab Dis, R Debré Hosp, Paris, France, [3]Ped Int Care Unit, Hosp R Debré, Paris, France

Case report & Results: A healthy 6-year-old girl, presented with a 3-day history of constipation, fever and vomiting progressively deteriorating into coma. Blood gas analysis revealed hypochloremic metabolic acidosis and an increased anion gap. Serum ammonia was 117 μmol/l and organic acid profile revealed urinary excretion of MMA (4200 μmol/mmol creatinine, normal < 5). Protein intake was stopped and intravenous (IV) glucose, sodium benzoate, L-carnitine and vitamin B12 therapy were started. On the 3rd day after admission, she exhibited microcytic anemia, thrombocytopenia, acute liver and renal failure and insulin resistant hyperglycaemia. Lactate dehydrogenase was 6500 IU/L (300-600) and serum ferritin was 1900 μg/L (15-80). Macrophage activation syndrome was confirmed by bone marrow analysis. We identified EBV reactivation at the origin of such secondary hemophagocytic lymphohistiocytosis (HLH). Dexamethasone and cyclosporine (2 μg/kg/d initially) were administered. On the 4th day of cyclosporine treatment, the patient presented with focal clonic seizures. MRI showed findings compatible with PRES. Levetiracetam treatment was initiated and cyclosporine withdrawn. Six months later, the patient is fully healthy and treated with mild protein restriction, 1 mg/day B12 and L-Carnitine.
Conclusions: This report emphasizes that mild B12-responsive MMA can present acutely with severe deterioration and coma in the setting of acute immune and inflammatory scenario such as post-EBV HLH.

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Two patients with cobalamin A deficiency and novel mutations

Gunduz M[1], Ozaydin E[1], Koc N[1], Unal O[1]

[1]Ankara Hematology Oncology Children's, Ankara, Turkey

Background: Adenosylcobalamin (AdoCbl) deficiency comprises CblA and CblB, two disorders characterised by

methylmalonic aciduria (MMA) which are often Cbl responsive. Mutations in the *MMAA* gene lead to Cbl A deficiency and, has been localised to chromosome 4q31.1-q31.2. Most CblA patients have good prognosis.
Case reports: Two patients presented with metabolic acidosis in the newborn period and methylmalonic acid were detected on urine organic acid analysis. Their current ages are 3 and 4 years old. Both patients were treated with protein restricted diet, carnitine and parenteral hydroxycobalamin treatment from the newborn period. Under treatment methylmalonic acid excretion was stopped in one patient. None of the patients develop metabolic crisis in the follow up period. However, global developmental delay and epilepsy occured in the patients. Novel mutations were deceted in both patients in the *MMAA* gene. Molecular genetic analysis showed p.T198fs*6 (c.593_596delCTGA) homozygous mutation and c.653_659delGAGGCGT frameshift homozygous mutation in the patients.
Conclusion: Although most of the patients with CblA deficiency respond to protein restriction and hydroxycobalamin treatment, some patients appear to become resistant, and late complications have been observed. Here we presented two patients with neurological complications and novel mutations in *MMMA* gene.

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A patient with isovaleric acidemia with novel mutations in two alleles accompanying with hypoparathyroidism

Gunduz M[1], Koc N[1], Unal O[1]

[1]Ankara Hematology Oncology Children's, Ankara, Turkey

Background: Isovaleric acidemia (IVA) is a rare metabolic disorder which disrupts metabolism of leucine. It is caused by mutations in the isovaleryl CoA dehydrogenase (*IVD*) gene located on chromosom 15q15.1. IVA has extreme clinical variability, reported mutations are highly heterogeneous and no genotype-phenotype correlation has been established. Primary hypothyroidism may be related to autoimmune disorders, some rare congenital disorders or iatrogenic. Coexistence of IVA and primary hypoprathyroidism has not been reported.
Case Report: The patient was presented with seizure and decreasing Ca levels up to 3 mg/dL at three years old, diagnosed with primary hypoparathroidism, and replacement treatments were started. When the patient was 10 years old meningeal irritation signs, and unconsciousness occured in an infectious disease. Urine organic analysis was checked and very high excretions of isovalerylglycine and 3-hydroxyisovalerate were detected on urine organic acid analysis. Protein restricted diet and carnitine treatmnets were commenced for isovaleric acidemia. She was evaluated for primary amenorrhea and autoimmune polyglandular syndrome was suggested. Molecular genetic analysis showed c.1583G>A (p.Arg53His)/c.535A>G (p.Met179Val) compund heterozygous mutation in *IVD* gene, and both of the alleles were novel mutation.
Conclusion: We presented a patient with rare two disorders, and two novel mutations in both alleles in *IVD* gene.

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High prevalence of movement disorders in four inborn errors of metabolism: considerable impact of involuntary movements on quality of life

Kuiper A[1], Coenen M A[2], Eggink H[1], Tijssen M A J[1], De Koning T J[3]

[1]Dep Neur, University Medical Center, Groningen, Netherlands, [2]Dep Clin Dev Neuropsychology, RUG, Groningen, Netherlands, [3]Dep Ped, University Medical Center, Groningen, Netherlands

Background: It is well-known that movement disorders (MD) can be present in many IEM. However, the prevalence of MD subtypes and how MD influence quality of life (QoL) remains unknown.
Patients and Methods: This is the first systematic study on MD in children and adults with IEM and the impact of MD on QoL. So far 40 patients (27 children) with galactosaemia (n=24), glutaric aciduria type I (n=9), propionic aciduria (n=4) and methylmalonic aciduria (n=3) were investigated by a standardized video protocol, scored by an expert panel. MD severity was scored using the Global Clinical Severity Index (GCI). QoL was measured with the PedsQL and SF-36.
Results: 22/40 patients (55%) had signs of a MD, 8 patients (20%) had a moderate-severe MD (GCI≥4/7). The predominant subtype was dystonia, followed by myoclonus, tremor and ataxia. In organic acidurias MD prevalence was considerably higher (81,3%) than in galactosaemia (37,5%). Pediatric patients with moderate-severe MD had significantly lower QoL scores than those without MD [mean PedsQL: 52, 1(SD: 10,6) vs 78,4(SD: 13,5) p=0,002]. There was a significant correlation between MD severity and PedsQL score (Pearson's correlation coefficient: -0,574, p=0,002).
Conclusion: The prevalence of MD is high in all four IEM, with considerable impact on QoL.

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Evaluation of the L-carnitine role on the urinary parameters of oxidative stress in maple syrup urine disease patients

Guerreiro G B G[1 3], Mescka C P[3], Coelho D M[3], Monalissa A[2], Dutra-Filho C S[4], Wajner M[3 4], Vargas C R[1 3 4]

[1]PPG Ciências Farmacêuticas UFRGS, Porto Alegre, Brazil, [2]Faculdade de Farmácia UFRGS, Porto Alegre, Brazil, [3]Serviço de Genética Médica HCPA, Porto Alegre, Brazil, [4]PPG Bioq UFRGS, Porto Alegre, Brazil

Maple syrup urine disease (MSUD) is a metabolic disease caused by a severe deficiency of the branched-chain α-keto acid dehydrogenase complex activity. The consequence is an accumulation in tissues and body fluids of branched-chain amino acids and their respective α-keto-acids. Studies have shown that oxidative stress may be involved in neuropathology of MSUD. L-carnitine (L-car) have an important role as antioxidant in some metabolic diseases. The main objective of this study was evaluated the role of L-car on the urinary parameters of oxidative stress. It was evaluated di-tyrosine, isoprostanes and antioxidant capacity in MSUD patients under protein-restricted diet supplemented or not with L-car capsules at a dose of 50 mg kg-1 day-1. It was also quantified the blood free L-car levels. It was found a deficiency of carnitine in patients before the L-car supplementation. Significant increases of di-tyrosine and isoprostanes, and decreases in the antioxidant capacity were observed in urine of MSUD patients before the treatment with L-car and the supplementation was able to reduce the di-tyrosine and isoprostanes levels and to increase the antioxidant capacity. Thus, it was possible to suggest that L-car could be helpful in the treatment of MSUD patients by preventing oxidative damage to the cells.

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Impaired mitochondrial oxidative phosphorylation and oxidative damage are detected in brain, heart and muscle of a mouse model of propionic acidemia

Gallego-Villar L[1], Rivera-Barahona A[1], Cuevas C[1], Sánchez-Aragó M[1], Guenzel A[3], Perez B[1], Barry M[3], Martin-Casanueva M A[2], Cuezva J M[1], Richard E[1], Desviat L R[1]

[1]Centro Biología Molecular Severo Ochoa, Madrid, Spain, [2]Hospital 12 de Octubre, Madrid, Spain, [3]Mayo Clinic, Rochester, United States

Propionic acidemia (PA) is caused by the deficiency of the mitochondrial enzyme propionyl-CoA carboxylase. PA may present neonatally or as a chronic late-onset form characterized by neurological deficits and cardiomyopathy. Propionyl-CoA accumulation has been described to inhibit several mitochondrial enzymes and to induce oxidative stress, pointing to a putative role of mitochondrial dysfunction in the development of the multiorgan complications observed. Using a hypomorph mouse model of PA, we have studied the expression of several proteins involved in mitochondrial function by Reverse Phase Protein Microarrays, OXPHOS activities, mtDNA copy number and parameters related to oxidative damage (lipid peroxidation, protein carbonylation). The results show decreased levels and activity of several OXPHOS complexes and mtDNA depletion in heart and muscle, while in brain an increase in mitochondrial mass is observed. Highest ROS production (DHE staining) and lipid peroxidation (TBARS assay) were observed in heart and brain. To characterise behavioural and locomotor defects, mice were subjected to the open-field test and rota-rod performance, revealing age-dependent alterations in locomotion, coordination and endurance. These data support the hypothesis that mitochondrial dysfunction and alterations in redox homeostasis may contribute to the pathophysiology of PA and pave the way for *in vivo* evaluation of antioxidant therapies.

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Treatment of extrapyramidal disorders in propionic aciduria (PA) and methylmalonic aciduria (MMA)

Celato A[1], Cazzorla C[1], Furlan F[1], Del Rizzo M[1], Giordano L[1], Antonini A[2], Sartori S[3], Burlina A B[1], Burlina A P[4]

[1]Div Metab Dis, Dep Ped, Padua Univ Hosp, Padua, Italy, [2]Div Neurol, Padua Univ Hosp, Padua, Italy, [3]Div Neurol, Dep Ped, Padua Univ Hosp, Padua, Italy, [4]Neurol Unit, St. Bassiano Hospital, Bassano del Grappa, Italy

Background: PA and MMA can present extrapyramidal movement disorders.
Case Reports: We report 1 PA and 2 MMA patients presenting dystonia-parkinsonism and status dystonicus. *PA*: Patient-1 is a 15-years-male who developed hypokinetic-rigid syndrome. Magnetic Resonance Imaging (MRI) displayed striatal hyperintensities. Symptoms improved with drugs (benserazide/L-DOPA, trihexyphenydyl hydrochloride) evolving into mild dystonia. *MMA*: Patient-2, a 12-years-female, presented dystonia. MRI showed putaminal infarctions. Briefly, she developed dystonic storming requiring benzodiazepines, propofol, fentanyl and mechanical ventilation together with levetiracetam, tetrabenazine, baclofen, phenobarbital, gabapentin. Due to poor drug effectiveness, globus pallidus internus-MRI-guided Deep Brain Stimulation (DBS) was performed with partial effectiveness, requiring additional drug treatments. After neurosurgical treatment she still presents limbs dystonia and muscle/tendon retractions. Patient-3 is a 6-years-female presenting acute metabolic acidosis. Despite extracorporeal detoxification, she developed status dystonicus with globi pallidi liquefaction. Drug treatment (propofol, benzodiazepines, morphine together with baclofen, tetrabenazine, gabapentin, levetiracetam) was poorly effective. Subthalamic

nucleus-MRI-guided DBS leaded to mild improvement, but dystonia persisted requiring drugs' add-on.
Conclusions: Our cases demonstrate basal ganglia degeneration in PA and MMA, even in the absence of metabolic decompensation. DBS may be effective in acute management, although it doesn't prevent secondary dystonia. Our experience underlines the need of a better understanding of the pathophysiology of dystonia in PA and MMA.

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Generation and characterization of iPS cells as a cellular model for propionic acidemia

Richard E[1], Alonso-Barroso E[1], Ugarte M[2], Pérez-Cerdá C[2], Perez B[2], Desviat L R[1]

[1]Cent Biol Mol, UAM, Madrid, Spain, [2]Cent Diag Enf Mol, UAM, Madrid, Spain

Background: The future hope of generated induced pluripotent stem cells (iPSC) from patients with inherited metabolic diseases is multifold. iPSC hold a great promise for advances in cell-based therapy and modeling of human disease since to date mostly patients-derived fibroblasts have been used with their limitations. 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. PA patients develop multiorgan complications, especially cardiomyopathy in the long-term.
Methods & Results: We have successfully achieved the generation of iPSC from PCCA patient-derived fibroblasts reprogrammed using CytoTune Sendai vectors which include the four Yamanaka factors (Oct4, Sox2, Klf4 and c-Myc). The iPSC generated have been characterized and presented the hallmarks for these cells: (1) typical iPSC-like morphology and growth characteristics, (2) conservation of the mutations identified in the PCCA patient, (3) positive staining for alkaline phosphatase activity, (4) expression of pluripotency-associated markers and (5) demethylation of the OCT4 and NANOG promoters. Our next step will be the iPSC differentiation into cardiomyocytes providing a basis for an experimental cellular model for PA disease.

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Chemokine production induced by 2-methylcitrate in 3D organotypic rat brain cell aggregates

Cung H P[1], Remacle N[1], Zavadakova P[1], Braissant O[2], Ballhausen D[1]

[1]Cent Mol Dis, Univ Hosp, Lausanne, Switzerland, [2]Biomed, Univ Hosp, Lausanne, Switzerland

Background: In a model of methylmalonic aciduria (MMAuria) using 3D rat brain cell aggregates, we recently showed that ammonium levels increased in culture medium within few hours after 2-methylcitrate (2-MCA) administration. Moreover, 2-MCA altered the morphology of developing brain cells and increased cleaved caspase-3 levels at day-in-vitro (DIV) 14.
Objectives & Methods: To better understand the mechanisms involved, we analyzed the time course of cytokine and chemokine production in culture medium of 3D rat brain cell aggregates exposed to 1 mM 2-MCA (DIV11 to 14), which were harvested from DIV12 to 19.
Results: Levels of the chemokines MIP-1α, MIP-2, MCP-1, MCP-3, GRO-α and IP-10 were increased by 2-MCA exposure. Compared to controls, the increase was most substantial on DIV15 for MIP-1α, MIP-2, MCP-1, MCP-3 and GRO-α and on DIV19 for IP-10. No significant change of cytokines was observed.
Conclusions: Chemokines, which are involved in neurodegenerative processes, were substantial increased after 2-MCA exposure. They are responsible for immune cell attraction, have anti-apoptotic properties and play an important role in CNS repair. The start of the chemokines increase on DIV15 suggests a link to the maximum of elevated cleaved caspase-3 on DIV14. Further studies are necessary to unravel the precise role of chemokines in MMAuria.

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Excellent outcome of patient with congenital lactic acidosis with riboflavin supplementation

Stanescu S[1], Alonso D[1], Bélanger-Quintana A[1], Cerdá C[2], Merinero B[2], Martínez-Pardo M[1]

[1]U. Enf. Metabolicas, Hosp Ramon y Cajal, Madrid, Spain, [2]CEDEM, Universidad Autonoma, Madrid, Spain

Background: Dihydrolipoamide dehydrogenase (DLD, E3) is a flavoprotein common to pyruvate, α- ketoglutarate and branched-chain α-keto acid dehydrogenases. E3 deficiency classically presents as severe and progressive neurological impairment with persistent lactic acidosis and high mortality.
Methods: We present a case of a male newborn which presents in the first 48 hours general deterioration and persistent hypoglycemia. Routine biochemical examinations displayed elevation of blood lactate (17 mmol/L) and ammonia (600 μmol/L), hyperCKemia, increase of LDH, elevation of blood trasaminases and severe coagulopathy.

Results: Metabolic investigations documented a characteristic profile of E3 deficiency: elevation of plasma branched-chain amino acids, detectable alloisoleucine and increased excretion of lactate, pyruvate, α-ketoglutarate, and branched-chain α-hydroxy and α-keto acids. The genetic tests identified a new mutation in the DLD gene. Dietetic treatment with branched-chain amino acid restriction, thiamine and riboflavin was initiated, resulting in a complete resolution of the metabolic derangement. After three years of unchanged treatment no further major crisis have occurred and he has had a normal psychomotor development.
Conclusions: The excellent outcome of our patient, in contrast with the usually described fatal outcome, suggests a potential therapeutic role for riboflavin in addition to protein restriction in E3-deficient patients.

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Molecular mechanisms and interactions in methylmalonic aciduria due to MMAA deficiency

Plessl T[1 3], Burda P[1], Baumgartner M R[1 2 3], Froese D S[1 2]

[1]Div Metab Dis, Univ Child Hosp, Zürich, Switzerland, [2]radiz - Rare Disease Initiative Zürich, Zürich, Switzerland, [3]Zur Cen Integr Hum Physiol, Univ Zurich, Zürich, Switzerland

Background: In humans, vitamin B12 (cobalamin, Cbl) is converted to adenosylcobalamin (AdoCbl) to function as cofactor for the mitochondrial enzyme methylmalonyl-CoA mutase (MUT). In this multi-step process, the protein MMAA is responsible for proper AdoCbl incorporation into MUT and its disturbed function causes the metabolic disorder methylmalonic aciduria. Although the structure of MMAA is known, knowledge of the mechanism of dysfunction caused by patient missense mutations in MMAA is still lacking.
Methods & Results: Here, we examined all 22 known *MMAA* missense mutations - 9 of which are novel and discovered in fibroblasts of 13 patients referred for diagnostic work-up at the Kinderspital Zurich - to determine their effect on protein stability, enzymatic function, and MUT interaction. Our data stratify these mutations into categories of defects, including those that cause mis-folding, those that result in reduced GTP binding or catalysis, and those that affect interaction. We further present clinical data of those MMAA patients referred to Zurich, including initial presentation, cellular biochemistry and response to treatment with cobalamin and dietary restrictions.
Conclusions: Our detailed analysis improves knowledge of the effect of disease-related mutations in methylmalonic aciduria and provides insight into the inter-relationship between MMAA and MUT.

P-253

Functional analysis of missense variants in D-2-hydroxyglutarate dehydrogenase gene

Pop A[1], Struys E A[1], Jansen E E W[1], Smith D E[1], Van Schaftingen E[2], Van der Knaap M S[3], Salomons G S[1]

[1]Metabolic Unit, VU Univ Med Center, Amsterdam, Netherlands, [2]de Duve Inst, Louvain Univ, Brussels, Belgium, [3]Dept of Child Neurol, VU Univ Med Center, Amsterdam, Netherlands

Background: D-2-hydroxyglutaric aciduria type I (D2HGA-type I), a neurometabolic disorder with a broad clinical spectrum, is caused by autosomal recessive mutations in the *D2HGDH* gene encoding D-2-hydroxyglutarate dehydrogenase (D-2-HGDH). We detected 45 potential pathogenic variants (52 patients) in *D2HGDH* of which 29 missense. We developed functional studies to investigate the effect of missense variants on the enzyme function.
Methods: Site directed mutagenesis was used to introduce the 29 missense variants in the pCMV5-*D2HGDH* expression vector. The wild type and missense variants were overexpressed in HEK293 cells. D-2-HGDH enzyme activity was evaluated based on the conversion of [$^2H_4$]D-2-HG to [$^2H_4$]2-ketoglutarate, subsequently converted into [$^2H_4$]L-glutamate as detected by LC-MS/MS.
Results: Overexpression studies showed that 17 variants resulted in the almost complete impairment of the D-2-HGDH enzyme, and thus indicationg their pathogenicity. Two variants showed comparable activities to wild type transfected cells, indicating that these variants have no negative influence on enzyme activity. For the remaining 10 variants with residual activities ranging between 17% and 68% of the wild type, further studies are warranted. Immunodetection confirmed that transfections were successful.
Conclusion: We developed a functional assay to evaluate the pathogenicity of novel *D2HGDH* variants, which is essential for classification of missense variants.

P-254

Genetic diagnosis of cobalamin defects by next generation sequencing

Vega A[1], Leal F[1], Desviat L R[1], Couce M L[2], De la Cruz X[4], Yahyaoui R[3], Gaspar A[5], Tavares de Almeida I[6], Ugarte M[1], Merinero B[1], Perez B[1]

[1]CEDEM, CBMSO, Uni. Autonoma de Madrid, Madrid, Spain, [2]Hosp Clínico Universitario de Santiago, Santiago de

Compostela, Spain, [3]Hospital Univ Carlos Haya, Malaga, Spain, [4]Vall d'Hebron Institute of Research (VHI, Barcelona, Spain, [5]Metab Dis Unit,, Paed Ser, Sta Maria Hos, Lisbon, Portugal, [6]Met&Gen Lab., Fac. Farm, Univ. Lisboa, Lisbon, Portugal

Background: Cobalamin (Cbl) is a complex molecule needed for just two metabolic reactions in human: methylation of homocysteine to methionine catalyzed by methionine synthase and conversion of methylmalonyl-CoA to succinyl-CoA catalyzed by methylmalonyl-CoA mutase. Deficiency of Cbl, inability to absorb Cbl or inability to process Cbl to its active cofactors can results in elevated levels of MMA and/or homocysteine (MMA/MMAHC).
Patients & Methods: Here we report genetic diagnosis in a validation cohort (5 patients) and a discovery cohort (24 patients,) in a retrospective and prospective study, as a second-tier test from neonatal screening program (NBS). We have used a custom panel including 105 IMD genes and the Illumina clinical-exome TruSightOne® combined with a genes virtual capture.
Results: The diagnosis yield was 100% in MMA patients while was of 85% in MMAHC patients. We have identified disease-causing mutations in *CD320*, *GIF* and *MMACHC* genes and some variants with uncertain clinical significance in *CUBN* and *SUCLA2* genes and the functional reported SNP p.Asp301Tyr in *TCN1* gene. Additionally pathogenic mutations in *ACSF3* gene in a patient from NBS were detected.
Conclusion: In summary the massive parallel sequencing will allow the genetic accurate diagnosis of Cbl defects improving the familiar genetic counselling and a more rational therapeutical intervention.

P-255

Biochemical and molecular analysis of Russian patients with methylmalonic aciduria

Kurkina M V[1], Baydakova G V[1], Mikhaylova S V[2], Zakharova E Y[1]

[1]Research Centre for Medical Genetics, Moscow, Russian Federation, [2]Russian Pediatric Regional Hospital, Moscow, Russian Federation

Methylmalonic aciduria (MMA) is a genetically heterogeneous disorder of methylmalonate and cobalamin (cbl; vitamin B12) metabolism. The most common form is isolated MMA, which iscaused by a mutation in the gene encoding methylmalonyl-CoA mutase (*MUT*). This leads to a partial, mut (-), or full, mut (0), loss of enzyme activity. The MMA diagnosis was confirmed in 26 patients on the basis of specific biochemical changes. Concentration of methylmalonic acid ranged from 1359 to 20182 mmol/mol creatinine (normal: 0 - 2 mM\M creatinine). Isolated MMA was confirmed in 25 patients and combined MMA with homocystinuria in 1 patient. Clinical severity and age of manifestation varied from 2 mo to 1,5 years. DNA diagnosis has been performed in 17 patients with isolated MMA. Mutations in the MUT genewere found in 13 patient: 7 of them were novel (?.Leu358Pro (2 allels), ?.Gl496Term, ?.Leu674Phe (2 alleles), c.2197_2200insTGCC, ?.Gly284Arg, IVS 8-1G>C, p.Arg616Pro) and 5 of them had been previously described (?.Arg727Term, ?.Asn219Tyr, ?.Arg369His, ?.Arg467Term, p. Lys223Arg). Two patients had mutations in the ???? gene (c.592_595delACTG, ?.Arg145Term, novel mutation p.Lys223Arg (2 alleles)). In 2 cases an analysis of four genes (MUT, MMAA, MMACHC, MMADHC) were performed and no mutations were found. Mutations in MUT gene are common cause of isolated MMA in Russia. Different presentation and severity of MMA may be due to mutations diversity in *MUT* gene.

P-256

Late-onset cardiac failure after liver transplantation in one patient with propionic acidemia

Nassogne M C[1], Vincent M F[1], Reding R[1], Sokal E M[1], Barrea C[1]

[1]UCL, Cliniques universitaires Saint-Luc, Brussels, Belgium

Background: Propionic acidemia (PA) is a metabolic disease with a severe prognosis including cardiac and neurologic complications. Since 20 years, liver transplantation (LT) has been proposed and previous studies have shown that the PA-associated cardiomyopathy is reversible after LT.
Case report: We report the unusual evolution of a child with PA with late-onset cardiac failure 4 years after LT. This boy was followed for a neonatal-onset PA. The child was hospitalized several times for metabolic decompensations treated without hemofiltration. Mild cardiac dysfunction was diagnosed at the age of 4 treated first with digoxine and diuretics, then with enalapril with a complete resolution at the age of 11. LT from related living donor was performed at the age of 12.5 without any postoperative complications. Immunosuppression was assured with tacrolimus without rejection. Mild protein restriction was maintained. During systematic follow-up, moderate systolic dysfunction (ejection fraction 42%) was detected 4.5 years after LT. Other causes of cardiac failure were excluded and enalapril was introduced without further deterioration after 6 months. Metabolic investigations were stable. Cardiac biopsy is still pending.

Conclusions: This single case raises the question of the mechanisms of cardiac involvement in PA, such as mitochondrial respiratory chain dysfunction and the relevance to perform LT in PA.

P-257

Expression of disease causing isovaleryl-CoA dehydrogenase in *Escherichia coli* for characterization and stability studies

Mohsen A W[1], Sharma V[1], Amieva M V[3], Karunanidhi A[1], Naylor E[2], Vockley J[1]

[1]Div Med Genet, Dept Pediatr, Univ Pitt, Pittsburgh, PA, United States, [2]Dept Ped, Medical Univ South Carolina, Charleston, South Carolina, United States, [3]Inst Nacio Ped, Univ Nacin Auton Mexico, Mexico City, Mexico

Background: Isovaleryl-CoA dehydrogenase (IVD) catalyzes conversion of isovaleryl-CoA to 3-methylcrotonyl-CoA in leucine catabolism. Deficiency of IVD leads to isovaleric acidemia (IVA). We have previously characterized in detail multiple missense mutations in patients with IVA identified through newborn screening. Since then, other *IVD* mutations have been published from East Asian, European, Middle Eastern, and North American cohorts.
Methods & Results: In this study, we report additional mutations identified through newborn screening (NBS) and in clinically diagnosed patients. Among them, p.T64I and p.M135T mutants (mature numbering), identified from DNA screening of NBS blood spots and clinical patients diagnosis at the Mexican Instituto Nacional de Pediatria, and p.K117E, p.I167M, p.T207I, and p.F350S, identified from patients of other ethnicities, were expressed in a prokaryotic system, and enzyme stability and function were assessed by western blotting and enzyme assay. *IVD* p.T64I, p.K117E, and p.M135T, were expressed in amounts comparable to wild type. Mutant enzyme activity was 9%, 21%, and 21% of the wild type enzyme, respectively. While *IVD* p.I167M antigen was present, no activity was detected. *IVD* p.T207I and p.F350S antigens were undetectable in cell free extract.
Conclusions: Characterization of pathogenicity of mutations identified through NBS provides the opportunity for better clinical care and counseling early in life for patients.

P-258

Molecular genetic characterization of 149 *mut* patients: the Basel/Zurich cohort

Forny P[1 2], Schnellmann A S[1], Froese D S[1 2], Baumgartner M R[1 2]

[1]Div Metab Dis, Univ Child Hosp, Zürich, Switzerland, [2]radiz – Rare Disease Initiative Zürich, Zürich, Switzerland

Isolated methylmalonic acidemia (MMAemia) is an autosomal recessive disorder of propionate metabolism which is most commonly caused by mutations in the methylmalonyl-CoA mutase (*MUT*) gene (*mut*-type MMAemia). We previously characterized a subset of *MUT* mutations biochemically. Here, we report a complete list of all mutations identified in patients referred for diagnostic testing to the UKBB/Kinderspital Zurich from 1977 to 2015, dissecting one of the largest *mut* cohorts in Europe. From 149 referred patients, 28 had the mut^- and 99 the mut^0 subtype, while 22 were unclear due to a lack of biochemical information. We identified a deleterious variant in 293 (total 298) patient alleles, in which 96 different mutations (35 novel) were detected. Forty-four mutations were identified only once, suggesting many patients carry private mutations. Investigation of the missense mutations revealed five hotspot positions (219, 328, 369, 615, 694), each of which were mutated at least 10 times. By contrast, only one mutation was found in the inter-domain linker region (aa 463-579), implying it is tolerant to substitution. Western blot analysis of patient fibroblasts revealed reduced MUT protein for all 35 cell lines tested. This large scale analysis helps characterize the landscape of *MUT* mutations and dysfunction.

P-259

Molecular genetics of maple syrup urine disease in Brazilian patients

Camelo Jr. J S[1], Margutti A V B[1], Schwartz I V D[2], Silva Jr. W A[1]

[1]Ribeirão Preto School of Medicine - USP, Ribeirão Preto, Brazil, [2]Federal University of Rio Grande do Sul, Porto Alegre, Brazil

Introduction: Maple syrup urine disease (MSUD) is an autosomal recessive disorder, caused by the defective function of the branched-chain α-ketoacid dehydrogenase (BCKD) complex, involved in the metabolism of branched-chain amino acids (BCAAs). Early diagnosis is crucial in preventing neurological deterioration. The objectives are to characterize the genetic basis through the identification of point mutations of *BCKDHA*, *BCKDHB* and *DBT* genes in Brazilian MSUD patients and to assess possible correlation genotype-phenotype.
Methods: Cross-sectional study with 25 MSUD patients belonging to the MSUD Network: Brazilian Network of Care and Research about MSUD. *BCKDHA*, *BCKDHB* and *DBT* genes were analyzed by direct sequencing of

DNA and *in silico* analysis. Clinical characteristics of patients were obtained.
Results: Twenty novel mutations were identified, four in *BCKDHA* gene, thirteen in *BCKDHB* gene and three in *DBT* gene. Nine previously described mutations were detected. A consistent genotype-phenotype association was not found.
Conclusion: Due to late diagnosis and lack of immediate treatment of most Brazilian MSUD patients, genotyping wasn't predictive of clinical severity of variant MSUD phenotypes; but might be of prognostic value in subjects identified in newborn screening, in whom early treatment probably shall alter the natural course of the disease.

P-260

The first reported case of pregnancy in 3-methylglutaconic aciduria type I (3-MGA type 1)

Davoren E[1], Mienie L J[1], Vorster B C[1], Dercksen M[1]

[1]Cent Hum Metabolomics, Univ of NW, Potchefstroom, South Africa

Background & Objectives: 3-Methylglutaconyl-CoA hydratase deficiency is phenotypically characterized by mild to severe neurological symptoms. We report on the natural course and management of a female patient, diagnosed at the age of 2, who conceived and gave birth to an apparently healthy baby boy.
Patient & Methods: The patient presented with neurological and gastric abnormalities accompanied by metabolic decompensation at time of diagnosis. Mild cognitive impairment with limited episodes of metabolic crisis remained after therapeutic intervention. She became pregnant at 27 years. Clinical and biochemical monitoring (including routine blood work, organic acid- and acylcarnitine assays) were performed during the antenatal and post-natal period.
Results: The patient's metabolic status deteriorated from the second trimester and proved difficult to manage. Lactic ketoacidosis and secondary carnitine depletion was noted, with limited improvement through therapeutic adjustment. Metabolic decompensation remained 3-4 days after delivery and subsequently improved thereafter. The metabolic workup of the baby was within normal limits.
Conclusion: Pregnancy induced an undesirable near-constant catabolic state in our patient. Although the metabolic workup in the neonatal period was normal, the long term sequelae of intra-uterine exposure to abnormal metabolites cannot be assessed at this stage and limited knowledge is available at present.

P-261

Low-leucine encephalopathy in maple syrup urine disease: could a shift in brain leucine be to blame?

Hamilton P K[1], Ryan K[1], Loughrey C[1], McStravick N[1], Cardy D[1], Connolly G M[1]

[1]Belfast Health and Social Care Trust, Belfast, United Kingdom

Background
Encephalopathy in maple syrup urine disease (MSUD) is typically associated with high leucine concentrations.
Case report
A 24 year old man with MSUD was admitted electively for stabilisation of amino acid levels. Two days post-discharge, he was re-admitted in an encephalopathic state. Neurological examination revealed brainstem abnormalities and imaging showed brainstem and cerebellar oedema. Plasma leucine, isoleucine and valine concentrations were 26 (reference range 133-187), 1040 (66-102) and 324 (215-289) micromol/L respectively, having been 861, 1125 and 293 micromol/L six days previously. No other cause for his encephalopathy was found descpite a detailed series of investigations. Magnetic resonance single voxel spectroscopy revealed a small peak in keeping with branched-chain alpha keto acid, but this was only minimally deviated from the baseline, supporting the notion of low intra-cerebral leucine concentrations. He was admitted to intensive care and required intubation and ventilation. He was treated with an intravenous carbohydrate and fat regimen, with parenteral nutrition to raise his blood leucine levels. He made a good neurological recovery.
Discussion
We postulate that this unusual presentation related to a marked shift in brain leucine levels, and that this represents a previously undescribed complication of MSUD.

P-262

Identification of a novel mutation in start codon of *MMAB* gene in an Indian family with methylmalonic acidemia using targeted next generation sequencing and prenatal diagnosis

Gupta D[1 4], Bijarnia-Mahay S[1], Ramprasad V L[3], Kohli S[1], Saxena R[1], Yamaguchi S[2], Deb R[4], Verma I C[1]

[1]Cen of Med Genet, Sir Ganga Ram Hosp, New Delhi, India, [2]Dept of Paed, Shimane Univ Schl Med, Izumo, Shimane, Japan, [3]Medgenome, Scigenome Labs, Cochin, Kerala, India, [4]Amity Inst Biotech, Amity Univ, Noida, UP, India

Background: Methylmalonic aciduria (MMA) is a common organic aciduria in Indian population. Although diagnosis of MMA is achieved on metabolite testing using MS/MS and GC/MS, molecular diagnosis is essential for accurate prenatal diagnosis.
Case Report: We report a prenatal diagnosis enabled by molecular analysis using next generation sequencing (NGS). The family had lost 2 children at 1.5 years and 3 months, with episodes of respiratory distress, lethargy and coma with no clear diagnosis. MS/MS analysis in second baby showed increased C3 metabolite, suggesting possibility of organic acidemia. In absence of proband's DNA, the couple was tested for multigene panel of organic acidemia using NGS.
Result: A heterozygous novel mutation, c.2T>G, p.M1?, in initiation codon was detected in female partner in *MMAB* gene. In-silico analysis predicted pathogenicity. After validating mutation by Sanger sequencing, husband was sequenced and noted to carry the same mutation. Prenatal diagnosis was performed on amniotic fluid by GC-MS as well as mutation analysis. The biochemical analysis on AF showed affected fetus, and the same was confirmed by mutation analysis (homozygous c.2T>G). The pregnancy was terminated after counselling.
Conclusion: Targeted panel sequencing proved useful as it allowed prenatal diagnosis and prevention of this burdensome disorder in the family.

P-263

Profound biotinidase deficiency: natural course of the disease and impact of treatment in adult patients

Demirkol M[1], Çakar E[1], Güneş D[1], Karaca M[1], Balcı M C[1], Türkoğlu Ü[1], Ozer I[2], Gokcay G[1]

[1]Ped Nutr Metab, Ist Med Faculty Ist Univ, Istanbul, Turkey, [2]Ped Metab Dis, Goztepe Edu Res Hosp, Istanbul, Turkey

Background: Profound biotinidase deficiency (PBD) (OMIM253260), disorder of biotin metabolism, can be detected by newborn screening and treated with biotin supplementation.
Objectives: The aim was to evaluate natural course and impact of treatment in adult patients detected by newborn screening (NS), family-screening (FS) or diagnosed and treated after the onset of initial clinical symptoms, symptomatic patients (SP).
Methods: Patients with PBD
Results: There were 44 patients (21 males, 23 females; mean age: 23.0±6.8years,range:18-45), 22 newborn-screened, 11 family-screened and 11 symptomatic patients. Consanguinity rate was 64%. Age at diagnosis in NS, FS and SP groups were 30.8±25.8days,range:5-97, 24.1±11.2years,range:4.9-40.6, 5.1±9.2years,range:0.3-30 and follow-up period after diagnosis 11.2±7.3years,range:0.1-19, 1.7±3.6years,range:0.1-12, 9.6±7.0days,range:0.5-21, respectively. In NS-group ten patients were symptomatic with dermatitis (n:7), hypotonia (n:1), hair loss(n:3) at minimum age of 23 days. In FS-group two siblings had developmental delay and dermatologic findings and two parents had hearing (n:1) and vision impairment (n:1). Symptomatic-patients had cutaneous findings(n:6), developmental delay(n:9), convulsions (n:7), hair-loss (n:6), bilateral optic atrophy(n:2), sensorineural hearing-loss(n:1), spinal involvement (n:2), ataxia (n:2) at minimum age of 35 days.
Conclusion: Prognosis improved significantly after diagnosis in late diagnosed patients but hearing loss, visual impairment and spinal findings showed resistance to high dose biotin treatment.

P-264

Organic acid disorders: Burden of disease in North India

Kumari C[1], Ramji S[1], Kapoor S[1]

[1]MAMC, New Delhi, India

Background: Organic acidemias are heterogeneous group of disorders resulting from the abnormality or absence of an enzyme or its cofactor, resulting into a wide spectrum of clinical manifestation and often poor outcome or even death early in life. The aim of this study is to screen the patients suspected with inborn errors of metabolism to know the incidence of disease in North India.
Materials and Methods: Patients having suspicion index of IEM were referred to our lab for urinary organic acids screening by GCMS and dried blood spots analysis by TMS.The results of both analysis are considered for the diagnosis of organic acid disorders.
Results: From 2010 to 2014, 2105 patients with red flag signs of IEM were screened. Out of these 90 were diagnosed as having organic disorders and classified as follows:- MMA 27, Glutaric aidemia 21/90,Lactic acidemia 10/90, Respiratory chain disorders 7/90, 4-hydroxybutyric aciduria 05/90, Propionic acidemia 04/90, 3MCC 3/90, MSUD 03/90, PKU 03/90,, fumaric aciduria 03/90, Dicarboxylic aciduria02/90 Isovaleric acidemia 01/90, and β-Ketothiolase deficiency 01/90.The most common disease group was MMA.
Conclusion: The point incidence of organic acid disorders is high in North Indian patients and needs further study to discover the disease.

P-265

Isovaleric acidemia and nephronophthisis: A case report

Donis K C[1], Poswar F[1], Lorea C F[1], Silva A A[1], Souza C F M[1], Vairo F P[1], Refosco L F[1], Schwartz I V D[1]

[1]Med Genet Serv, Hosp Clin Porto Alegre, Porto Alegre, Brazil

Background and objectives: Isovaleric acidemia (IVA) is an organic acidemia, caused by the deficiency of isovaleryl-CoA dehydrogenase (IVD). Nephronophthisis (NPHP) is an autosomal recessive ciliopathy, which may be caused by mutations in eleven different genes. We report a patient who presents both IVA and NPHP.
Case report: A 10-year-old female born to consanguineous parents. She had recurrent hospitalizations since 3 years of age with acute encephalopathy and metabolic acidosis. The diagnosis of IVA was established when the patient was 6 years. The *IVD* gene analysis showed the p.R53H mutation of in homozygosity. Hypoproteic diet and supplementation with L-carnitine 100mg/kg/day and glycine 250 mg/kg/day were initiated. At 10 years of age, she presented chronic renal failure. Renal ultrasound was compatible with NPHP. The DNA analysis showed a homozygous mutation in the NPHP4 gene (p.Asp1311Serfs*92). The patient is under evaluation for kidney transplantation.
Discussion/Conclusion: In this patient, the association of IVA and NPHP can be attributed to consanguinity. The optimal management is with hypoproteic diet and all the adjustments in the treatment should be done considering the renal disease effects

12. Organic acidurias: others

P-266

A novel frameshift mutation of malonyl-CoA decarboxylase deficiency

Ersoy M[1], Akyol M B[2], Ceylaner S[3], Bicer Cakır N[4], Bursal Duramaz B[5]

[1]Child Metab Dis,Sadi Konuk Res&Tra Hosp, Istanbul, Turkey, [2]Div Pedi Cardio, Sadi Konuk Res&Tra Hosp, Istanbul, Turkey, [3]Intergen Genetics Center, Ankara, Turkey, [4]Depart Nutr&Diet., Arel Univ, Istanbul, Turkey, [5]Depart Pedia. Sadi Konuk Res&Tra Hosp, Istanbul, Turkey

Background: Malonyl-CoA decarboxylase deficiency (MLYCD) is a rare inborn error of metabolism characterized by developmental delay, cardiomyopathy, seizures, episodes of hypoglycemia and acidosis. Despite the lack of consensus, most of the cases reveal clinical and biochemical response to diet therapy.
Aim: Determine the phenotype of a noval mutation and the benefits of treatment in late-diagnosed case.
Case report: A one-year-old boy was referred to the hospital with failure to thrive and neuromotor development delay. He had mild delay in motor area with normal social development. Free carnitine was 12.3 μmol/L (normal: 8.6-90); C3DC was 3.78μmol/L (normal: 0-0.6). Urine organic acids showed marked elevation of malonic and methylmalonic acid together withdicarboxylic aciduria. Echocardiogram revealed left ventricular noncompaction-cardiomyopathy with normal ejection fraction. Brain magnetic resonance imaging showed hyperintensity at dentate nucleuses and global cerebral atrophy with delayed myelination. MLYCD gene sequencing showed a novel homozygous insertion *c.13_14ins*G result in a frameshift mutation.The patient was started on levocarnitine 50mg/kg/day and a high carbohydrate/low fat diet.After 6-months treatment, significient biochemical andclinicalimprovementwas observed.
Conclusion: Although malonic aciduria is a rare inherited metabolic disease, diet therapy can facilitate improvement in late-diagnosed cases.

P-267

Functional characterization of novel variants found in *ASPA* gene associated with Canavan disease

Mendes M I[1], Smith D E[1], Pop A[1], Holwerda U[1], Salomons G S[1]

[1]Metabolic Unit, VU Univ Med Center, Amsterdam, Netherlands

Background: Canavan disease is a devastating degenerative brain disorder caused by mutations in *ASPA* gene. Over 80 mutations have been identified. In our laboratory 84 patients have been diagnosed with Canavan disease by DNA sequencing. Thirty nine different mutations were identified, of which 25 missense variants (13 novel). We present the development of a method for the characterization of *ASPA* variants.
Methods: Wild-type (WT) *ASPA* cDNA was cloned into the pCMV6 expression vector. The variants of interest were introduced by site-directed mutagenesis. HEK293 cells were used to express ASPA variants. Cell homogenates (48h after transfection) were used to measure ASPA activity using [$^{13}C_4$] N-acetyl aspartic acid as substrate. The product [$^{13}C_4$] aspartic acid was quantified by LC-MS/MS.
Results: The method was validated using the WT and the empty vector as positive and negative controls, respectively. The activity for the WT transfectants was 800-fold higher than the empty vector. Most variants presented with enzyme activities lower than 5% of the WT. Western-Blot detected ASPA protein for all variants.

Conclusion: We developed a fast and reliable method to characterize *ASPA* variants which is important not only for proper diagnosis but also for interpretation of next generation sequence data.

P-268

Living with intoxication-type inborn errors of metabolism - a qualitative analysis of focus group interviews with paediatric patients and their caregivers

Zeltner N A[1 2 7 8], Landolt M A[2 7], Baumgartner M R[1 8], Lageder S[7], Quitmann J[6], Sommer R[6], Mühlhausen C[5], Schlune A[4], Scholl-Bürgi S[3], Karall D[3], Huemer M[1 8]

[1]CRPP radiz - Rare Dis Initiative Zürich, Univ of Zürich, Switzerland, [2]Dept Psychosom Psychiat, Univ Child Hosp, Zürich, Switzerland, [3]Clinic for Pediatrics, Med Univ, Innsbruck, Austria, [4]Dept Gen Pediatrics, Univ Child Hosp, Düsseldorf, Germany, [5]Dept of Pediatrics, Univ Med Center, Hamburg-Eppendorf, Germany, [6]Dept Med Psychol, Univ Med Center, Hamburg-Eppendorf, Germany, [7]Dept Child and Adolescent Health Psychol, Univ of Zürich, Switzerland, [8]Div Metab, Univ Child Hosp, Zürich, Switzerland

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. Adherence to treatment requires intense efforts and fear of metabolic crises is always present. Therefore, health-related quality of life (HrQoL) of patients is a meaningful outcome parameter. With the aim of developing the first validated, disease-specific HrQoL assessment tool for IT-IEM, we involved patients and their caregivers as content experts.
Aim: To identify major psychosocial constraints and resources of IT-IEM patients to develop a disease-specific HrQoL assessment tool for children and adolescents.
Methods: Focus group interviews with 19 patients and 26 parents were conducted in four metabolic centres in Austria, Germany and Switzerland. Based on qualitative content analysis disease-specific HrQoL categories were established.
Results: Fourteen disease-specific HrQoL categories reflected the concerns of patients facing IT-IEM. Dietary restrictions and social stigmatization are major burdens. Not only physical limitations or cognitive deficits but also treatment issues such as tube feeding impair social life significantly.
Conclusion: Insight into patients' and caregivers' perceptions of stressors and resources allows precise assessment of HrQoL and facilitates definition of meaningful outcome parameters and personalized interventions.
Conflict of Interest declared.

P-269

Expanding clinical phenotype in CMAMMA and new tools for fast metabolic diagnostics

De Sain-van der Velden M G M[1], Van der Ham M[1], Jans J J[1], Visser G[2], Prinsen H C M[1], Verhoeven-Duif N M[1], Van Gassen K L I[1], Van Hasselt P M[2]

[1]Dept Med Genet, Univ Med Center (UMC), Utrecht, Netherlands, [2]Dept Metab Dis, Univ Med Center (UMC), Utrecht, Netherlands

Background: Mutations in the *ACSF3* gene are associated with combined malonic and methylmalonic aciduria (CMAMMA) (OMIM # 614265). The metabolic work-up for this disorder in urine is challenging, since excretion of malonic acid (MA) can be easily overlooked.
Patients and Methods: We describe a novel clinical presentation of CMAMMA in two sisters with CMAMMA. Both patients had a severe psychiatric presentation (including mutism) after a viral infection. Furthermore, one patient had corpus callosum lesions. A UPLC–MS/MS method for quantification of plasma MA was developed and applied to samples of these patients.
Results: MA excretion (13-226 μmol/mmol creatinine) was sometimes just above the highest control value. Plasma MA concentrations in both patients (range 0.85-3.43 μmol/L; n=6) were above the highest value observed in control subjects (0.31 μmol/L; range 0.08-0.79 μmol/L; n=87). To our surprise, plasma MA concentrations was also elevated in patients with classic methylmalonic acidemia (range 0.17-2.12 μmol/L; n=20). The MA/MMA ratio in plasma allowed full differentiation between classic methylmalonic acidemia and CMAMMA.
Conclusion: Calculating the MA/MMA ratio allows differentiation between CMAMMA and other causes of methylmalonic acidemia. The full clinical spectrum of CMAMMA remains to be delineated.

P-270 - Withdrawn

P-271

Ethylmalonic encephalopathy: diagnosis of a turkish case after unknown death of two children in the family

Onenli Mungan N[1], Kor D[1], Seker Yilmaz B[1], Bulut D[1], Mert G[3], Incecik F[3], Ceylaner S[2]

[1]Div Metab Dis, Univ Cukurova, Adana, Turkey, [2]Intergen Genetic Lab, Ankara, Turkey, [3]Div Ped Neurol, Univ Cukurova, Adana, Turkey

Background and Objectives: Ethylmalonic encephalopathy (EE) is a rare autosomal recessive disorder caused by mutations in the *ETHE1* gene and characterized by chronic diarrhea, encephalopathy, petechiae and acrocyanosis. Approximately 50 reports have been published describing this disease, most involving patients of Mediterranean or Arab origin.
Case Report: Two months old male infant brought to clinic with the only complain of restlessness, and history of death of two brothers at the ages of 5 and 6 without a specific diagnosis. His parents are first degree cousins. First physical examination, biochemical-metabolic tests and radiological investigations revealed no abnormality. The clinical status and laboratory values were normal until the age of 6 months. After a mild upper way infection his convulsions and persistent crying started and heavy ethylmalonic aciduria and lactic acidosis was detected. EE was suspected in the light of laboratory findings and mutation analysis confirmed the diagnosis with a mutation of p.R163W (c.487C>T) in *ETHE1* gene.
Conclusion: We want to emphasize the clinical heterogeneity of EE with this case. Although recurrent petechiae, acrocyanosis, and chronic diarrhea are the most striking features of EE, we neither detected these findings in index case nor revealed from the history of two brothers.

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In vivo magnetic resonance spectrocopy in an adult case of L-2-hydroxyglutaric aciduria

Ete M[1], Audoin B[1], Viout P[2], Guye M[2], Pelletier J[1], Kaphan E[1]

[1]Pole Neuroscience, AP-HM Timone, Marseille, France, [2]CRMBM, Fac Med Univ Med Aix-Marseille II, Marseille, France

Background and objectives: Neurometabolic disorders are a heterogeneous group of diseases. Diagnosis are often difficult. In vivo proton magnetic resonance spectsroscopy (MRS) is a new imaging technique and could probably become a precious diagnostic tool for this group of diseases. L-2-hydroxyglutaric aciduria is a rare neurometabolic disorder. Diagnosis is usually suggested by urinary organic acid chromatography and confirmed by molecular analysis. Brain descriptions by magnetic resonance imaging are extensive, but not specific of the disease. Data of MRS are nonexistent in term of diagnosis.
Case report: We report here an adult case of L-2-hydroxyglutaric aciduria confirmed by biochemical and molecular analysis.
Materials and Methods: Magnetic resonance examination was performed by using a 3.0-T MR system with multinuclear options (Verio; Siemens, Erlangen, Germany).
Results: MRS showed two peaks, corresponding to the L-2-hydroxyglutarate peaks, according to the human metabolome database.
Conclusion: We describe for the first time the L-2-hydroxyglutarate peak with brain MRS in a patient with L-2-hydroxyglutaric aciduria. MRS could become a diagnostic tool for neurometabolic disorders in the future.

P-273

Inborn errors of cerebral L-lysine metabolism: different degradation pathways and the role of brain peroxisomes

Posset R[1], Opp S[1], Struys E A[2], Völkl A[3], Mohr H[3], Hoffmann G F[1], Kölker S[1], Sauer S W[1], Okun J G[1]

[1]Div Neuroped Metab Med, Univ Child Hosp, Heidelberg, Germany, [2]Metabolic Unit, Dept Clin Chem, VUMC, Amsterdam, Netherlands, [3]Anatomy Cell Biol II, Univ of Heidelberg, Heidelberg, Germany

Background: Inherited deficiencies of cerebral L-lysine metabolism, e.g. glutaric aciduria type I, affect the CNS, however information about brain-specific L-lysine breakdown is lacking. While mitochondrial saccharopine pathway is considered as major hepatic L-lysine breakdown route, cerebral L-lysine degradation is supposed to be channeled via peroxisomal pipecolate pathway only.
Methods: Labeled stable L-lysine isotopes (L-[U-^{13}C; U-^{15}N]lysine; L-[α-^{15}N]lysine, L-[ε-^{15}N]lysine) were used to study in vivo kinetics and L-lysine degradation route(s) in brains and livers of wildtype and *Gcdh*$^{-/-}$ mice by measuring isotope-labeled intermediates with liquid chromatography tandem mass spectrometry. To better understand the role of brain peroxisomes, organelles were isolated and purified according to a newly established protocol.
Results: L-lysine degradation is not strictly tissue-specific, as hitherto assumed and cerebral L-pipecolate, an intermediate of L-lysine degradation, is produced along both pathways – to a minor proportion retrograde along the saccharopine pathway and to a major proportion anterograde along the pipecolate pathway. Brain peroxisomes are the most likely subcellular compartments of L-pipecolate degradation with low L-pipecolate oxidase activity (7 ± 2 μU/mg protein).
Conclusion: In the murine brain, L-lysine degradation is best described by a three-compartment model (cytosol, mitochondria and peroxisomes), with mainly pipecolate but also saccharopine pathway contributing to cerebral L-pipecolate production.

P-274

Anaplerotic therapy in propionic acidemia

Longo N[1 2], Price L B[1], Gappmaier E[3], Cantor N L[4], Ernst S L[1], Bailey C[1], Pasquali M[1 2]

[1]Dept Pediatrics, Univ Utah, Salt Lake City, United States, [2]Dept Pathology, Univ Utah, Salt Lake City, United States, [3]ARUP Laboratories, Salt Lake City, United States, [4]Primary Child Hosp, Salt Lake City, United States

Background: Propionic acidemia is caused by a deficiency of propionyl-CoA carboxylase, the enzyme that converts propionyl-CoA to methylmalonyl-CoA. Propionic acid sequesters oxaloacetate from the Krebs cycle to form methylcitric acid, leading to a deficiency in Krebs cycle intermediates and decreasing plasma glutamine levels. Our objective was to determine safety and efficacy of the anaplerotic agents glutamine (400 mg/kg/day), citrate (7.5 mEq/kg/day), or ornithine ketoglutarate (400 mg/kg) in patients with propionic acidemia.
Methods: Supplements were administered for four weeks with a two week washout period between supplements. Citrate was then supplemented for 30 weeks. Primary outcome was increases in plasma glutamine and/or urine Krebs cycle intermediates.
Results: Supplements were safe and not associated with significant side effects. They did not increase plasma glutamine levels, but citrate increased the urinary excretion of 2-ketoglutarate, succinate, and fumarate. Hospitalizations did not change during the trial period, but decreased significantly in the 2 years following the study (when citrate was continued) compared to the 2 years before and during the study.
Conclusions: Citrate enters the Krebs cycle and increases levels of 2-ketoglutarate, succinate, and fumarate. Citrate supplements were safe and might have contributed to reduce hospitalizations in patients with propionic acidemia.

P-275

A treatable cause for childhood bulbar pulsy, Fazio-Londe disease

Eyaid W[1 2], Rifai M[1 2], Alem H[1 2], Hazwani T[1 2]

[1]king abdulaziz medical city., riyadh, Saudi Arabia, [2]King Saud bin Abdulaziz Univ for Health, Riyadh, Saudi Arabia

Fazio-Londe disease is a progressive bulbar palsy with onset in childhood that presents with hypotonia and respiratory insufficiency. The profile of plasma acylcarnitines and urine organic acids are usually suggestive of a mild form of the multiple acyl-CoA dehydrogenation defect (MADD, ethylmalonic/adipic acid syndrome). It is due to mutations in the *SLC52A3* gene which encodes the intestinal (hRFT2) riboflavin transporter. We report on an infant who presented with progressive muscle weakness, ptosis and respiratory failure that led to hypoxic ischemic encephalopathy, HIE. The whole exome sequencing showed that he has a mutation in the *SLC52A3* gene (c.71G>A). He was started on 10mg/kg of riboflavin. Because of the HIE incident it was difficult to evaluate his response for riboflavin trial.

P-276

Malonic aciduria: marked clinical variability in a consanguineous family

Sudrié-Arnaud B[1], Torre S[3], Goldenberg A[2], Kundul F[1], Brasse-Lagnel C[1], Duhamel O[3], Salomons G S[4], Tourancheau E[3], Marret S[3]

[1]Metab Biochem Rouen University Hospital, Rouen, France, [2]Genetics Rouen University Hospital, Rouen, France, [3]Neonatal Medecine Rouen University Hospi, Rouen, France, [4]Dpt Clin Chem, VU Univ Med Center, Amsterdam, Netherlands

Background: Malonic aciduria is a rare inherited metabolic disease linked to malonyl-CoA decarboxylase deficiency. Only few cases have been described with systemic clinical involvement including neurologic and digestive symptoms, metabolic acidosis, cardiomyopathy and neonatal death. Indeed, malonic acid acts as an inhibitor of fatty acids mitochondrial β-oxidation which may constitute the pathophysiological bases of this disorder.
Case Report: We report different clinical presentations in two children who were found, after the establishment of the family tree, as belonging to the same family. The first case presented at the age of two days with convulsions and dilated cardiomyopathy. The second case was born preterm and was referred at the age of 5 years for developmental delay and behavioural troubles; cardiac function was normal.
Results: High excretion of malonic acid was characterized in both cases. Acylcarnitine profile showed the presence of high levels of malonylcarnitine. Malonyl-CoA decarboxylase activity in cultured fibroblasts was significantly decreased. Molecular analysis of gene *MLYCD* allowed the identification of a homozygous nonsense mutation (p.Gln116X).
Discussion: Carnitine supplementation and a high-carbohydrate, low-fat diet with medium-chain triglyceride (MCT)-supplementation were applied. Despite harboring the same gene mutation, these patients have marked clinical variability, ranging from no cardiac symptoms to severe cardiomyopathy.

P-277

Expanding the phenotype in aminoacylase 1 (ACY1) deficiency: characterization of the molecular defect in a 63-year-old woman with generalized dystonia

Sass J O[1], Vaithilingam J[2], Britschgi C[2], Delnooz C C S[5], Van den Warrenburg B P[3], Wevers R A[4]

[1]Bonn-Rhein-Sieg Univ Appl Sciences, Rheinbach, Germany, [2]Univ Child Hosp, Clin Chem Biochem, Zürich, Switzerland, [3]Radboud Univ Med Ctr, Dept Neurology, Nijmegen, Netherlands, [4]Radboud Univ Med Ctr, Dept Lab Med, Nijmegen, Netherlands, [5]Univ Med Ctr Groningen, Dept Neurology, Groningen, Netherlands

Aminoacylase 1 (ACY1) deficiency (ACY1D) is an organic aciduria due to mutations in the *ACY1* gene. It is considered much underdiagnosed. Most individuals known to be affected by ACY1D have presented with neurologic symptoms. We present here a cognitively normal 63-year-old woman who around the age of 12 years had developed dystonic symptoms that gradually evolved into generalized dystonia. Extensive investigations, including diagnostic exome sequencing, were performed to elucidate the cause of dystonia. Findings were only compatible with a diagnosis of ACY1D. The urinary metabolite pattern was characteristic and there was decreased ACY1 activity in EBV-immortalized lymphocytes. Compound-heterozygosity for two *ACY1* mutations was detected, one well-characterized c.1057C>T (p.Arg353Cys) and the other novel c.325A>G (p.Arg109Gly). Expression analysis in HEK293 cells revealed high residual activity of the enzyme with the latter mutation. However, following transfection of cells with stable expression of the c.1057C>T variant with either wild-type *ACY1* or the c.325A>G mutant, only the wild-type essentially normalized ACY1 activity, suggesting an inhibiting interference between the two variants. Although a causal relation to the clinical symptoms is not proven, our report seems to extend the clinical spectrum of ACY1D and underlines that screening for organic acidurias deserves consideration in patients with unexplained general dystonia.

P-278

Oxidative stress in patients with 3-hydroxy-3-methylglutaric aciduria and the role of L-carnitine therapy

Jacques C E D[1 2], Ribas G S[2], Dos Santos M M[1], Hammerschmidt T[2], Guerreiro G B G[1 2], Faverzani J L[2], Sitta A[2], De Moura D M[2], De Souza H M[2], Wajner M[2], Giugliani R[2], Vargas C R[1 2]

[1]PPG Ciên Farm, Univ Fed Rio Grande Sul, Porto Alegre, Brazil, [2]Serv Gen Méd, Hosp Clín Porto Alegre, Porto Alegre, Brazil

Background: 3-hydroxy-3-methylglutaric aciduria (HMGA) is a genetic disorder characterized by the accumulation of organic acids which can interfere in cellular redox homeostasis and in the energy metabolism. The aim of this work was to investigate oxidative stress in HMGA patients and the effect of the therapy with low-protein diet and L-carnitine on this process.
Patients and methods: We analyzed markers of protein (urinary di-tyrosine and plasma carbonyl and sulfhydryl groups) and lipid oxidation (determination of thiobarbituric acid-reactive substances (TBA-RS) in plasma) and the urinary antioxidant capacity in untreated HMGA patients and in patients treated with protein-restricted diet and L-carnitine (100 mg/kg/day).
Results: Untreated patients presented high di-tyrosine and TBA-RS, reduced urinary antioxidant capacity and low concentrations of free-carnitine in plasma. Treated patients also presented an increase in protein oxidation markers, however, they showed normal levels of TBA-RS, free-carnitine and urinary antioxidant capacity.
Conclusion: Considering that L-carnitine has demonstrated antioxidant properties in several pathologies, it is possible that the correction of lipid peroxidation in HMGA patients of this study can be related with the supplementation of this substance. Our results are the first reporting that a redox imbalance occurs in HMGA patients, reinforcing the importance of the antioxidant therapy in this disorder.

P-279 Withdrawn

P-280

A long term follow-up study of 8 individuals with asymptomatic propionic acidemia

Watanabe Y[1 2], Tashiro K[1], Aoki K[1], Inaba M[1], Yanagiuchi C[1], Suzutani Y[1], Ishii H[1], Kinoshita Y[1], Tajima G[3], Yorifuji T[5], Shigematsu Y[4], Maeda Y[7], Nakajima Y[6], Inokuchi T[1], Matsuishi T[1 2]

[1]Research Institute of GC/MS, Kurume Univ, Kurume, Japan, [2]Dept Ped and Child Health, Kurume Univ, Kurume, Japan, [3]Dept. Pediatrics, Hiroshima Univ., Hiroshima, Japan, [4]Dept. Health Science, Fukui Univ, Fukui, Japan, [5]Ped Endo Metab Osaka City Gen Hosp, Osaka, Japan, [6]Dept.Pediatrics, Fujita Health Univ, Nagoya, Japan, [7]Lab Hosp Pharm, Nagoya City Univ, Nagoya, Japan

Background: A longitudinal study of biochemical analytes in 8 individuals with asymptomatic propionic acidemia (PA) was conducted. Higher residual activities in propionyl-CoA carboxylase and the common mutation (Y435C) in *PCCB* in Japanese are known in individuals with asymptomatic PA. All of them remained asymptomatic on regular diet during the observation period (mean: 67 months). Our objectives were to study and compare the biochemical characteristics in individuals with asymptomatic PA to classic PA.
Methods: Acylcarnitines in blood spots on filter paper and in plasma, and urine organic acids were analyzed in 8 individuals (age: 4 months to 14y) at multiple points during the observation period. The specimens from each individual obtained as newborn were also analyzed. These values were compared to the ones in patients with classic PA.
Results: (1) C3 carnitine levels and C3/C2 ratios in dried blood spots in newborn screening as well as methylcitrate and 3-hydroxypropionate levels in the urine from the neonatal period showed lower values in the asymptomatic PA individuals. (2) Plasma C3 carnitine and urine methylcitrate levels in asymptomatic PA were lower than the lowest values of these analytes in classic PA.
Conclusion: Distinguishing asymptomatic PA from classic PA may be possible based on the biochemical analytes.

P-281

Ethylmalonic encephalopathy in an Indian boy: a report of mutations in *ETHE1* gene

Bijarnia-Mahay S[1], Gupta D[1], Shigematsu Y[2], Yamaguchi S[3], Saxena R[1], Verma I C[1]

[1]Center Med Genet, Sir Ganga Ram Hosp, New Delhi, India, [2]Dept Health Science & Pediatr,Univ Fukui, Fukui, Japan, [3]Dept of Ped, Shimane School Med, Izumo, Shimane, Japan

Ethylmalonic encephalopathy (EE) is a rare inborn error of metabolism affecting brain, peripheral blood vessels and gastrointestinal tract, with devastating consequences. Caused by recessive mutations in *ETHE1* gene, EE is characterized by neurodevelopmental delay and regression, recurrent petechiae, orthostatic acrocyanosis, and chronic diarrhea. Because of overlapping biochemical features of ethylmalonic acidurias, the disorder is often misdiagnosed as SCAD deficiency and thus gene studies are required for confirmation. We present a case of a 4-year-old boy who presented with typical features: developmental delay followed by regression after 2 years of age, hypotonia in infancy giving way to spasticity, chronic diarrhea, petechial spots and acrocyanosis. MRI Brain showed altered signal intensities: T2W/FLAIR hyperintensities in bilateral caudate and putamen, subtle hyperintensities in bilateral peritrigonal regions. The child succumbed to an acute illness with metabolic decompensation. Biochemical derangements included raised C4, C5 analytes on MS/MS and elevation of ethlymalonate, methlysuccinate, slight elevation of isobutyryl glycine and isovalerylglycine, mild increase in lactate on urinary GC-MS analysis. Sequencing of *ETHE1* gene revealed compound heterozygous mutations, c.488G>A (p.Arg163Gul) in exon 4, and c.375+5G>T in intron 3.To our knowledge, this is the first case report from India of EE with mutations in *ETHE1* gene.

P-282

Mutation screening study in Turkish patients with L-2-hydroxyglutaric aciduria

Yucel-Yilmaz D[1 2], Ozgul R K[1 2], Unal O[1], Coskun T[1], Sivri S[1], Tokatlı A[1], Dursun A[1]

[1]Div Metab Dis, Hacettepe Univ Child Hosp, Ankara, Turkey, [2]Inst of Child Health, Hacettepe Univ, Ankara, Turkey

L-2-hydroxyglutaric aciduria (L2HGA) is a rare autosomal recessive neurometabolic disease characterized essentially by the presence of elevated levels of L-2-hydroxyglutaric acid (LGA) in urine, plasma and cerebrospinal fluid. L2HGA is caused by a deficiency in the L2-Hydroxyglutaric dehydrogenase (L2HGDH) enzyme that is encoded by the *L2HGDH* gene. Clinically, the disease is characterized by neurological manifestations, including psychomotor retardation, cerebellar ataxia, and more variably macrocephaly, or epilepsy. In this study, mutation screening of ten exons of *L2HGDH* gene was performed in 11 unrelated Turkish patients with L2HGA by using direct sequence analysis. Three different types of pathogenic mutations (3 missense, 1 nonsense and 1 deletion) were detected in patients suspected with L2HGA. Four of them (p.Gly55Asp, p.Arg251*, p.Pro302Leu, c.1115delT) were reported previously. One mutation p.Pro280Leu was detected as a novel mutation in two different patients with L2HGA in this study.
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Mutation identification and prenatal diagnosis of biotinidase deficiency in Indian patients

Gupta D[1], Bijarnia-Mahay S[1], Kohli S[1], Verma J[1], Saxena R[1], Puri R D[1], Vyas B[1], Shigematsu Y[2], Yamaguchi S[3], Verma I C[1]

[1]Cen of Med Genet, Sir Ganga Ram Hosp, New Delhi, India, [2]Dept of Hlth Sci, Dept of Pead, Univ Fuk, Fukui, Japan,

[3]Dept of Paed, Shimane Univ Schl Med, Izumo, Shimane, Japan

Background: Biotinidase Deficiency (*BTD*) is a unique treatable inborn error of metabolism with excellent outcome on timely treatment. If untreated, disease can lead to intractable seizures, developmental delay, hearing loss, ataxia and death. We present our data with mutation analysis and prenatal diagnosis from India.
Patients and Methods: Twelve probands with deficient biotinidase enzyme level were taken up for mutation analysis. Sanger sequencing followed by computational analysis was performed to identify mutations in *BTD* gene.
Results: Among the 12 families, consanguinity was noted in 8, and seizures were presenting features in all. Mutations were identified in 10/12 probands. Mutation, c.98_104del7ins3 was the most common, present in 10/24 alleles (homozygous in 4 patients). Other previously reported mutations were: c.654G>C, c.1330G>C and c.476G>A. Two patients had novel mutations, c.1612C>A and deletion of exon 1. Five prenatal diagnoses (PNDs) were performed in 3 families. Three PNDs showed carrier fetuses, one normal and one affected fetus. Pregnancy with affected fetus was continued after counseling, and newborn baby was started on biotin therapy. He is doing well at 4 years of age.
Conclusion: Molecular testing is important to confirm Biotinidase deficiency, to chart future prognosis, institute appropriate therapy and provide prenatal diagnosis wherever requested.

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Organic acidurias diagnosis. A critical review of our experience

Echeverri O Y[1], Guevara J M[1], Pulido N F[3], Ardila Y A[3], Espinosa E[4], Cabarcas L[3], Correa L N[2], Barrera L A[1]

[1]I.E.I.M. Pont. Univ. Javeriana, Bogota, Colombia, [2]Clin. Univ. Colombia, Bogotá, Colombia, [3]Hosp. Univ. San Ignacio, Bogota, Colombia, [4]Univ. Mil. Nueva Granada - IOIR, Bogota, Colombia

Background and objectives: Organic-Acidurias (OAs) comprise more than 40 monogenic disorders, characterized by organic acids accumulation in body fluids. Clinically, OAs present with broad spectrum of onset ages and clinical manifestations. Biochemical diagnosis is done demonstrating abnormal urinary organic acids excretion. Although in many countries expanded newborn screening is performed, in Colombia, it is unavailable and OAs are diagnosed after the onset of clinical manifestations. The aim of this work is to show our experience in diagnosis and treatment of OAs in Colombia.
Methods: Retrospective analysis of GC-MS analysis, performed between 2009-2014 in the IEIM and in treatment with nutritional formulas registered recently in Colombia.
Results: Among 2300 samples analyzed we identified more than 40 OAs-patients. GAI was the most common diagnosis (>25%). More than 10% of the cases corresponded to very rare OAs. Nutritional treatment availability has improved the situation; more than 25 patients are currently under treatment. However, some difficulties need to be overcome including: high non-conclusive urinary profiles observed (30%); high occurrence of cases where metabolites derived from medication/diet interferes with profile interpretation, among others. In addition, around 20% of patients died before starting treatment.
Conclusion: This report shows our advances in diagnosis and treatment and some difference in the incidence of organic aciduria compared to other populations.
Conflict of Interest declared.

P-285

Behavioural and emotional problems, intellectual impairment and health related quality of life in patients with organic acidurias and urea cycle disorders

Jamiolkowsky D[1], Kölker S[1], Glahn E M[1], Barić I[3], Zeman J[4], Baumgartner M R[5], Mühlhausen C[2], Garcia Cazorla A[6], Gleich F[1], Haege G[1], Burgard - on behalf of the E-IMD consortium P[1]

[1]Div Metab Dis, Univ Child Hosp, Heidelberg, Germany, [2]Univ Child Hosp Eppendorf, Hamburg, Germany, [3]Dpt Pediatr, Univ Hosp Center, Zagreb, Croatia, [4]Dpt Pediatr, Charles University, Prague, Czech Republic, [5]Div Metab Dis, Univ Child Hosp, Zurich, Switzerland, [6]Div Neurology, Hosp San Joan de Deu, Barcelona, Spain

Background: Organic acidurias (OADs) and urea cycle disorders (UCDs) bear risks for acute/chronic metabolic decompensation and impairments of the CNS. Systematic studies of intellectual functioning (IF), behavioural/emotional problems (BEP), health related quality of life (HRQoL), and their interrelations are lacking.
Methods: BEPs, IF, and HRQoL of patients with OADs (n=100) and UCDs (n=44) from the European Registry and Network for Intoxication type Metabolic Diseases (E-IMD) were compared with normative data.
Results: BEPs are increased in OADs (UCDs) by a factor of 2.5 (3.0), in female asymptomatic carriers of x-linked inherited ornithine transcarbamylase deficiency (fasOTCD)

by a factor of 1.5. Patient groups show similar patterns of problems, comparable with an epidemiological sample. Mental retardation (IQ ≤70) was found in 31% of OAD, 43% of UCD, and in none of the fasOTCD subjects. HRQoL was in the normal band. BEPs were significantly associated with IF (OR=6.24,95%CI:1.39-27.99), but HRQoL was independent from both variables.
Conclusions: OADs and UCDs have a threefold risk for developing impairment of IF and BEPs. Disorders showed similar BEPs profiles compared to epidemiological data. Intellectual retardation and BEPs were strongly associated with mental impairment. HRQoL of the patients was in the normal band, possibly due to coping strategies of their families.

13. Carbohydrate disorders

P-004

Correction of glycogen storage disease type IV by AAV-mediated gene therapy

Sun B[1], Yi H[1], Zhang Q[1], Yang C[1], Kishnani P S[1]

[1]Div Med Genet, Duke Univ Med Ctr, Durham, NC, United States

Background and Objectives: Deficiency of glycogen branching enzyme (GBE) causes glycogen storage disease type IV (GSD IV), which is characterized by accumulation of less branched, poorly soluble polysaccharides (polyglucosan bodies) in multiple tissues. There is no treatment for this disease. We aimed to evaluate the efficacy of gene therapy with AAV vector in GSD IV mice.
Methods: An AAV9 vector containing a human GBE expression cassette driven by a ubiquitous promoter (AAV-GBE) was intravenously injected into two-week-old GSD IV mice at a dose of $5x10^{11}$ vector genome per mouse. Mice were euthanized at 3 months of age following overnight fasting.
Results: In the AAV-treated mice, GBE enzyme activity was highly elevated in heart (48 folds of wild-type) and skeletal muscles (2-3 folds of wild-type), which is consistent with the high copy numbers of viral vector genome. Complete glycogen clearance was achieved in these tissues as determined by glycogen content assay and PAS staining. Glycogen level was significantly reduced in brain but not in liver by the AAV treatment.
Conclusion: Systemic injection of an AAV9-GBE vector at young age eliminated glycogen deposition in cardiac and skeletal muscles, and significantly reduced glycogen level in the brain of GSD IV mice.

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Glycogen storage disease type III presents with a markedly reduced serum apolipoprotein C-III sialylation

Ondruskova N[1], Honzik T[1], Kolarova H[1], Poupetova H[2], Zeman J[1], Hansikova H[1]

[1]Dep Pediatr, Charles Univ, Gen Univ Hosp, Prague, Czech Republic, [2]IIMD, Charles Univ, Gen Univ Hosp, Prague, Czech Republic

Background and objectives: Recent studies have shown that patients with various disorders associated with altered lipid profiles, including metabolic syndrome, present with an increased level and abnormal glycosylation of apolipoprotein C-III (ApoC-III). Glycogen storage diseases (GSD) represent a group of metabolic disorders characterized by inherited disturbances in glycogen synthesis or degradation. Biochemically, hyperlipidemia is common in patients with GSD. It prompted us to examine the spectrum of sialylated ApoC-III isoforms in patients with GSD.
Materials and methods: Serum from 28 patients with various types of GSD (2x type 0, 6x type Ia, 3x type Ib, 6x type II, 6x type III, 1x type VI, 4x type IX) were analyzed by isoelectric focusing followed by a Western blot of ApoC-III.
Results: Normal to aberrant ApoC-III glycosylation was observed in patients with various types of GSD. The most profound hypoglycosylation was found in patients with GSD III (mean values: asialoApoC-III: 12.5%, monosialoApoC-III: 66.9%, disialoApoC-III: 20.7% vs. ref. range: 3.3%, 53.3% and 43.4%, respectively).
Discussion/Conclusion: ApoC-III hypoglycosylation detected in GSD patients is possibly associated with hypertriglyceridemia and it may reflect the disease course, at least in patients with GSD III.
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P-287

D-galactose alters behavioral and biochemical parameters in hippocampus and cerebellum of rats

Rodrigues A F[1], Biasibetti H[1], Sanches E F[1], Pierozan P[1], Zanotto B S[1], Sebotaio M[1], Wyse A T S[1]

[1]UFRGS, Porto Alegre, RS, Brazil

Background: Tissue accumulation of galactose (GAL) is a hallmark in classical galactosemia. The aim of this study was

to investigate the effects of intracerebroventricular administration of GAL on memory and motor coordination in Wistar rats.
Methods: Acetylcholinesterase activity (Ache) and nitric oxide (NO) levels in hippocampus and cerebellum were also investigated. Adult Wistar rats received intracerebroventricular injection of GAL (4mM) or saline (control). Beam walk was performed 1h and 24h after injecting GAL to evaluate the motor coordination. Training session of inhibitory avoidance task was performed 1h after the GAL injection and the test session was performed 24h after the training. Animals were decapitated; hipocampus and cerebellum were dissected for biochemical analyses.
Results: Results showed that GAL impaired the motor coordination after 24h, but not 1h after the treatment. Memory deficit was verified in the test session. In the hippocampus, GAL decreased NO levels at 1h, and increased Ache activity at 1h after injection. In the cerebellum GAL decreased NO levels at 1h, and increased Ache at 24h after injection.
Conclusion: Our findings showed alterations in motor and neurochemical parameters by GAL, which could contribute, at least in part, to understand the GAL neurotoxicity.
Supported by CNPq.

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Alternative night-time nutrition regimens in glycogen storage disease type I: a controlled crossover study

Hochuli M[2], Christ E[3], Meienberg F[4], Lehmann R[2], Krützfeldt J[2], Baumgartner M R[1]

[1]Div Metabolism, Univ Child Hospital, Zurich, Switzerland, [2]Div Endo and Diabetes, Univ Hosp, Zurich, Switzerland, [3]Div Endo and Diabetes, Univ Hosp, Bern, Switzerland, [4]Div Endo and Diabetes, Univ Hosp, Basel, Switzerland

Background and Objectives: To evaluate whether in adult patients with glycogen storage disease type I (GSDI), stable nighttime glucose control can be achieved with other types of slowly digested carbohydrates than uncooked corn starch (CS) or modified corn starch (MCS).
Methods: Nocturnal glucose control and fasting times were assessed with three different nutrition regimens in five patients, using continuous glucose monitoring in an outpatient everyday life setting. For each patient, continuous glucose profiles were measured after ingestion of (1) CS, (2) MCS or (3) a pasta meal at bedtime, during 5 to 6 consecutive nights for each regimen.
Results: Stable nocturnal glucose control was achieved for all patients with a pasta meal, with mean duration of glycemia >3.5 mmol/l of 7.6h (range 5.7-10.8), and >4 mmol/l of 7h (5.2-9.2), similar to CS and MCS. Fasting glucose before breakfast on workdays (after 7.1±0.8h) was not significantly different between the three interventions (CS 4.1±0.5mmol/l, MCS 4.6±0.7mmol/l, pasta 4.3±0.9mmol/l). During prolonged fasting on weekends, longer duration of normoglycemia was achieved with CS or MCS than with pasta.
Conclusion: Consumption of cooked pasta is a suitable and more palatable alternative to uncooked corn starch to achieve nighttime glucose control in adult patients with GSDI.

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Features of functioning of humoral immunity in children with hepatic forms of glycogen storage disease (GSD)

Kurbatova O V[1], Bushueva T V[1], Surkov A N[1], Zakirov R S[1], Polyakova S I[1], Miroshkina L V[1], Samokhina I V[1], Izmailova T D[1], Semenova G F[1], Nikitin A V[1], Freidlin E V[1], Melnichuk O S[1], Semikina E L[1], Petrichuk S V[1]

[1]FSBI Scientific Centre of Children Healt, Moscow, Russian Federation

The aim: To determine characteristics of humoral immunity in children with hepatic types of GSD.
Patients and methods: We examined 57 children with hepatic types of GSD (types I, III, IV and IX), a control group consisted of 34 healthy children. Immunocytochemical analysis of peripheral blood, which allows to determine the number of B-cells (B-lf) and the activity of succinatedehydrogenase (SDH), was performed on a flow cytometer FC500 (BC,USA). The immunoglobulin (Ig) concentration was determined on a UniCel DxC800 (BC,USA).
Results. In patients with GSD the absolute number of B-lf was reduced in 35% of cases, 40% were consistent with the age-related reference interval, and in 26% of cases B-If was increased. Most patients with GSD showed a reduction in the concentration of IgG (70% of cases) and normal levels of IgA and IgM relative to age-matched controls. We observed an inverse relationship between the content of B-lf and the of SDH-activity: in patients with low number of B-lf, SDH activity was increased and amounted to 136,7 (83,8-176,6); in patients with a normal number of B-lf and in patients with a high content of B-lf, SDH-activity activity was reduced (79,1 (59,3-93,2) and 67,3 (50,2-91,5), respectively, compared to a control group (107,8 (101,8-129,2)).
Conclusion.The results indicate a decreased function of humoral immunity in children with GSD, mainly determined by the presence of mitochondrial dysfunction in B-lymphocytes populations, but not a change in absolute lymphocyte number.

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Grey matter density abnormalities in patients with classic galactosemia: a voxel based morphometry study

Timmers I[1 2], Van der Korput L[2], Jansma B M[1], Rubio-Gazalbo M E[2 3]

[1]Dep of Cogn Neurosc, Maastricht Univ, Maastricht, Netherlands, [2]Dep of Ped, Maastricht Univ Med Ctr, Maastricht, Netherlands, [3]Lab Gen Met Dis, Maastricht Univ Med Ctr, Maastricht, Netherlands

Background: Grey matter (GM) abnormalities have been observed in patients with classic galactosemia, including cerebral and cerebellar atrophy. Our objective was to obtain a quantitative overview, and to find correlations with outcome.
Methods: We acquired T1-weighted MR images in 8 patients (aged 16-21y) and 8 matched controls (aged 15-20y). Voxel-based morphometry (VBM) was used to examine potential group differences in GM density.
Results: The patients showed clusters of GM density abnormalities, comprising *both decreases and increases*, compared to controls. We observed decreased GM density (bilaterally) in the pallidum (basal ganglia; *p-corr*< .01) and occipital cortex [*p-corr*< .01], and increased GM density in the medial [*p-corr*< .05] and inferior frontal cortex [*p-corr*< .05] (bilaterally, but to higher extent in the right hemisphere). No abnormalities were found in the cerebellum. Furthermore, correlations [|*r*|>.7] were found with age, age at initiation of dietary treatment, and with visual working memory.
Conclusion: Our study reveals decreases as well as increases in GM density in patients with classic galactosemia. The increases might reflect delayed maturation of the frontal regions, or compensatory mechanisms. The regional pattern of the abnormalities is in agreement with the observed cognitive profile including motor, language, visuo-spatial, and higher-order cognitive abnormalities.

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Polish patient diagnosed with glycogen synthase deficiency homozygous for newly identified mutation in *GYS2* gene

Szymanska E[1], Watrobinska U[3], Rokicki D[1], Ciara E[2], Halat P[2], Ploski R[4], Tylki-Szymanska A[1]

[1]Dept Ped Nutr Met Dis,Ch Mem Health Ins, Warsaw, Poland, [2]Dept Genet, Ch Mem Health Ins, Warsaw, Poland, [3]Dept Endocryn, Ch Mem Health ins, Warsaw, Poland, [4]Dept Med Genet, Warsaw Medic Univers, Warsaw, Poland

Background: Glycogen synthetase deficiency (glycogenosis 0) caused by mutations in the *GYS2* gene is characterized by lack of glycogen synthesis in the liver. It is a rare condition of disturbed glycogen homeostasis in the liver with less than 30 cases reported in the literature.
Case report: We report a 9 year old boy diagnosed with glycogenosis 0 due to the newly identified, highly pathogenic homozygous mutation: NM_021957.3:p.Phe574Leu/c.1720T>C in ex. 14. A random, asymptomatic hypoglycemia with ketonuria was found in this patient at the age of 7 years. His development was proper with normal weight and height parameters. On the oral glucose tolerance test, his blood levels of glucose, insulin and lactate were within normal values at baseline, and they increased after glucose intake. On fasting plasma glucose test, after 8 hours of fasting, glucose blood level of 34 mg/dl with no clinical symptoms. The results of these tests suggested glycogenosis type 0 which was genetically confirmed with WES analysis.Dietary management with frequent meals and addition of uncooked cornstarch was introduced.
Conclusion: Glycogenosis type 0 should be suspected in children with normal weight and growth, who usually present with fasting hypoglycemia and urinary ketones.

P-292

Resting energy expenditure in children with glycogen storage diseases

Szymanska E[1], Ehmke vel Emczynska E[1], Rokicki D[1], Tylki-Szymanska A[1], Ksiazyk J[1]

[1]Dept Ped Nutr Met Dis,Ch Mem Health Ins, Warsaw, Poland

Background: There are few studies in the variability of resting energy expenditure (REE) in GSD. We hypothesise that treatment should be individualized.
Materials and methods. Twenty four patients with hepatic GSDs, aged 7,5 years (range: 4-15,7 y) were studied. Total oral energy intake (TEI) was estimated. REESch was calculated with the use of Schofield's formula and indirect calorimetry was measured (REEIC) after approximately 4 hours fasting. Anthropometry was described by Z-score. Body fat content was assessed by BIA (body impedance analysis). Wilcoxon test was used for statistical analysis with $p < 0.05$ regarded as significant. Median REESch and REEIC were 42 kcal/kg b.m. (range: 19-59 kcal/kg body mass – b.m..) and 50 kcal/kg b.m. (32-88 kcal/kg b.m.) respectively; $p < 0.001$. Median TEI was 57 kcal/kg b.m. (18-104 kcal/kg b.m.): $p < 0{,}001$ for REESch and $p = 0.14$ (N.S.) for REEIC. Median Z-score for b.m. was -0.433, for height -0.393 and for BMI -0.277. Median BMI was 85 %. Median body fat content was 37,4 % (28,3-50,5).

Conclusion: REEIC in patients with hepatic GSDs was higher than REESch which can be related to short fasting period, however it was not different from TEI. High adiposity was found in the studied group.

P-293

Classic galactosemia: a *GALT* knockout zebrafish

Van Erven B[1 2], Vanoevelen J M[3], Bierau J[2], Smeets H J[3 4 5], Coelho A I[1 2], Rubio-Gozalbo M E[1 2]

[1]Dept Pediatr, MUMC, Maastricht, Netherlands, [2]Lab Genet Metab Dis, MUMC, Maastricht, Netherlands, [3]Dept Clin Genet, Clin Genomics, MUMC, Maastricht, Netherlands, [4]GROW Res School, MUMC, Maastricht, Netherlands, [5]CARIM Res School, MUMC, Maastricht, Netherlands

Background: Classic galactosemia is a genetic disorder characterized by a severe deficiency of galactose-1-phosphate uridylyltransferase (GALT), a key enzyme in galactose metabolism. At present, classic galactosemia's pathogenesis and timing of toxicity are still not fully elucidated, and current standard of therapy fails to prevent the development of burdensome long-term complications. This study aimed to develop a disease model amenable to high-throughput screening of pharmacological chaperones and that allows to gain new insights on brain and gonads damage throughout development.
Results: After the successful development of a *GALT* knockdown zebrafish [1], we have now generated a *GALT* knockout zebrafish using a TALEN-strategy. Two genotypes were identified, both resulting in a frameshift mutation with a premature stop and consequent loss-of-function of GALT. After transgenesis confirmation, expression profiles of the Leloir pathway enzymes (in situ hybridization), as well as biochemical and morphological phenotype, were assessed and compared to wild-type zebrafish. Further phenotype characterization is presently being performed. Additionally, a knock-in of the most common mutation (p.Q188R) is currently underway.
Conclusion: Modeling the human phenotype in a zebrafish model shall be a valuable tool for further pathophysiologic and therapeutic studies.
[1] Vanoevelen JM, van Erven B et al. A zebrafish model for classic galactosemia. JIMD 2013; 36Suppl2:S226.

P-294

Hepatic glycogen synthase deficiency: an infrequently recognized cause of ketotic hypoglycemia

Eminoglu F T[1], Korkmaz V[2], Dur O[2], Ekim M[3]

[1]Ankara Univ Hosp, Dept Ped Metab Dis, Ankara, Turkey, [2]Ankara Univ Hosp, Pediatrics, Ankara, Turkey, [3]Ankara Univ Hosp, Dept Ped Romatology, Ankara, Turkey

Background: Glycogen storage disease type 0 (GSD-0) is a rare form of fasting hypoglycemia presenting in infancy or early childhood and accompanied by high blood ketones and low alanine and lactate concentrations. Although feeding relieves symptoms, it often results in postprandial hyperglycemia and hyperlactatemia. Until recently, the definitive diagnosis of GSD0 depended on the demonstration of decreased hepatic glycogen on a liver biopsy. The need for an invasive procedure may be one reason that this condition has been infrequently diagnosed. Mutation analysis of the GYS2 gene is a non-invasive method for making this.
Case Report: A 10 year old girl was referred to our department due to non-symptomatic fasting hypoglycemia. Parents were the first degree cousins. Physical examination was unremarkable except for mild mental retardation. Biochemical evaluation as well as direct sequencing of exons and exon-intron boundary regions of the GYS2 gene was performed in this patient presenting fasting hypoglycemia, postprandial hyperglycemia and hyperlactatemia. We identified a previously reported homozygous nonsense mutation, c.13C>T in the *GYS 2* gene.
Conclusion: GSD-0 is more common than previously assumed. Recognition of the variable phenotype spectrum of GSD-0 and routine analysis of GYS2 is essential for the correct diagnosis.

P-295

Two successful pregnancies in a woman with glycogen storage disease (GSD) type IIIa

Paci S[1], Montanari C[1], Tagi V M[1], Re Dionigi A[1], Salvatici E[1], Giovannini M[1]

[1]Cl Dept of Ped, San Paolo Univ Hosp, Milan, Italy

Background: Because of the increased metabolic demand during pregnancy, females with GSD III are predisposed to hypoglycemia, ketosis and lactic acidosis, with higher risk of intrauterine growth retardation and low birth weight, and to cardiac function deterioration in subtype IIIa, particularly if a cardiomyopathy pre-exists.
Case report: We report on two successful pregnancies after intracytoplasmic sperm injection in a 39-year-old woman, diagnosed with GSD IIIa at 8 months of age by liver biopsy and routinely followed up. Her metabolic profile was good with frequent meals; she required regular uncooked cornstarch early in both pregnancies (1 g/Kg at bedtime). Transthoracic

echocardiograms showed mild left-ventricular hypertrophic cardiomyopathy, with maximal wall thickness (MWT) of 12 mm and normal systolic function.In addition to obstetric care, she was seen once every trimester by metabolic and cardiologic team, showing stable parameters. Euglycemia was maintained throughout the labour by a dextrose infusion alone. Both infants were delivered vaginally at term, with an adequate weight for gestational age. Six months after the second childbirth, an increased cardiac MWT (20 mm) with stable ejection fraction was observed.
Conclusion: This case strengthens the evidence that a careful and suitable management strongly concurs to positive outcomes in GSD III pregnancies.

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Four patients with glycogen storage disease type 1b and Crohn's like colitis

Karhan A N[1], Yuce A[1], Saltik-Temizel I N[1], Demir H[1], Gurakan F[2], Hizarcioglu-Gulsen H[1], Berberoglu-Ates B[1], Aydemir Y[1], Ozen H[1]

[1]Hacettepe Univ, Div Pediatr Gastroenterol, Ankara, Turkey, [2]American Hospital, Istanbul, Turkey

Background: Glycogen storage disease type Ib is an autosomal recessive disorder caused by defective translocase that transports glucose-6-phosphatase. Inflammatory bowel disease (Crohn's-like colitis) develops in those patients due to impaired neutrophil function and neutropenia.
Case Reports: Our report describes clinical and laboratory features of four patients with GSD Ib and Crohn's-like colitis. Three patients were two, four and five months of age; one patient was one day old. Two patients' molecular genetic analysis showed heterozygous mutation of G339C and homozygous mutation of C1211-2. Common findings were hepatomegaly, slightly elevated transaminases and triglyceride levels, lactic acidosis, hyperuricemia, and neutropenia. Liver biopsies were compatible with glycogen storage disease. During the follow-up, patients had abdominal pain, fever, diarrhea and perianal lesions. Colonoscopic findings and histopathological examinations of the biopsies showed active colitis and ileitis. All patients used immunosupression with corticosteroids and azathioprine. They are all in remission, and have been undergoing a combination of 5-ASA (5-aminosalicyclic acid), azathioprine and GCSF (Granulocyte colony-stimulating factor) treatments.
Conclusion: If patients with GSD1b have chronic diarrhea, anemia, fever of unknown origin, perianal and oral lesions, Crohn's-like colitis should be considered. GCSF with immunosupressive treatment improves neutropenia and the colitis in the patients.

P-297

A novel mutation in the *ABCC8* gene causing a variable phenotype of impaired glucose metabolism in the same family

Maines E[1], Hussain K[2], Flanagan S E[3], Ellard S[3], Piona C[4], Morandi G[4], Dal Ben S[4], Cavarzere P[4], Antoniazzi F[4], Dianin A[1], Bordugo A[1], Gaudino R[4]

[1]Inherit Metab Dis Unit, Dept of Ped, Verona, Italy, [2]Clin Mol Gen Unit, Child Health Inst, London, United Kingdom, [3]Biomed Clin Science Inst, Univ Exeter, Exeter, United Kingdom, [4]Ped Endocrinol Div, University of Verona, Verona, Italy

Background: Dominantly acting loss-of-function mutations in the *ABCC8* gene, encoding the SUR1 subunit of KATP channel, are usually responsible for mild diazoxide-responsive congenital hyperinsulinism (CHI). An increased risk of diabetes mellitus (DM) in adulthood has been suggested. The mechanism is not yet clear.
Case Presentation: The index patient was born at term to non consanguineous parents. Birth weight was 3900 g. Diagnosis of CHI was performed in the first week of life. The patient was started on diazoxide when he was 3 months as the drug was unavailable in his country. He showed a good response. A novel heterozygous *ABCC8* missense mutation (p.A478T) was found. F-DOPA PET/CT scanning was inconclusive.The patient's mother had gestational diabetes and after delivery she developed type 2 DM. The patient's grandfather developed type 2 DM at 45 years of age. Both had no past history of hypoglycaemia and were heterozygous for the p.A478T mutation.
Conclusion: The novel mutation identified in our patient was not previously reported in diazoxide-responsive forms of CHI; nevertheless a different mutation at the same residue has been reported in a family with CHI. The p.A478T *ABCC8* mutation seems to be associated to an incomplete penetrance of hypoglycaemia in infancy.

P-298

Ketogenic diet in congenital hyperinsulinism: a novel approach to prevent brain damage

Maiorana A[1], Manganozzi L[1], Barbetti F[2], Bernabei S[3], Gallo G[1], Cusmai R[4], Caviglia S[1], Dionisi-Vici C[1]

[1]Div Metab Dis, Bambino Gesù Child Hosp, Rome, Italy, [2]Lab Mendel Diab, Bambino Gesù Child Hosp, Rome, Italy, [3]Clin Nutrition, Bambino Gesù Child Hosp, Rome, Italy, [4]Neurology,Bambino Gesù Child Hosp, Rome, Italy

Background: Neurological sequelae of congenital hyperinsulinism (CHI) are particularly severe because insulin, that primarily causes neuroglycopenia, also suppresses all catabolic pathways, leading to a complete lacking of cerebral energy substrates (glucose, lactate and ketone bodies). In GLUT1 deficiency, where glucose transport is impaired, ketogenic diet (KD) is efficaciously used to provide ketone bodies as alternative cerebral fuel and sustain cerebral development. Similarly, in a patient with CHI we tried to provide ketone bodies as energy substrates alternatively to glucose by administrating a KD.
Methods: An 11 year CHI girl presented severe recurrent symptomatic hypoglycemia, epilepsy and neurological impairment, despite high dose of diazoxide and octreotide and high carbohydrate diet. As a last chance to avoid near-total pancreatectomy, we administered a 3:1 ratio KD aiming to obtain cerebral energy from lipids instead of carbohydrates, thus bypassing the effect of hypoglycemia.
Results: KD was well tolerated over a period of 21 months. Despite persistence of hypoglycemia, epilepsy and neuroglycopenic signs disappeared, EEG normalized, psychological development improved with an evident amelioration in social, cognitive and verbal capacities and a consequent improved quality of life.
Conclusion: KD could represent an effective treatment in severe CHI to support optimal brain growth and development during infancy.

P-299

Glucose tetrasaccharide biomarker in 24 hour urine collections from patients with glycogen storage disease IIIa

Austin S[1], Stefanescu M[1], Werneke A[1], Zhang H[1], Kishnani P S[1], Young S[1]

[1]Duke University Medical Center, Durham, North Carolina, United States

Background and Objective: Deficiency of glycogen debranching enzyme (GDE) causes an accumulation of abnormally structured glycogen in liver and muscle glycogen storage disease (GSD) IIIa), or liver only (GSD IIIb). Urinary glucose tetrasaccharide, Glcα1-6Glcα1-4Glcα1-4Glc (Glc4), a limit dextrin of glycogen, is elevated in patients with GSD III. We investigated Glc4 in 24 hour urines in subjects with GSD IIIa, and compared with muscle and liver disease biomarkers.
Methods: Subjects with GSD IIIa (n =16, aged 3-50 years) were consented to an IRB-approved study. Urine was collected over 24 hours, and aliquots from individual collection time points and pooled 24 hour samples were analyzed.
Results: Urinary Glc4 was elevated in all subjects with GSD IIIa and was not correlated with age. Intraday Glc4 excretion variability was < 25% for 14 of 16 subjects. Glc4 was positively correlated with creatine kinase (CK) and CK-MB in subjects >15 years of age, but not in subjects < 15 years. Glc4 was also positively correlated with aspartate aminotransferase and alanine transaminase in all patients.
Conclusion: Single time point urine collections were representative of 24 hour collections in most cases. Glc4 correlates with skeletal and cardiac muscle and/or liver markers of disease in an age-dependent manner.
Conflict of Interest declared.

P-300

A patient with a novel mutation in the *GALT* gene, and initially misdiagnosed with a Congenital Glycolysation Defect

Gunduz M[1], Ozaydin E[1], Koc N[1], Kirsaclioglu C T[1], Unal S[1], Unal O[1]

[1]Ankara Hematology Oncology Children's, Ankara, Turkey

Background: Untreated classic galactosemia (galactose-1-phosphate uridyltransferase [GALT] deficiency) is known as a secondary congenital disorders of glycosylation (CDG) characterized by galactose deficiency of glycoproteins and glycolipids (processing defect or CDG-II). With galactose restricted diet they become normal. Here we presented a patient who initially misdiagnosed with congenital glycosylation diet was presented.
Case report: The patient was hospitalized because of jaundice from 3 days old and coagulation disorder. She followed with hypoglycemia and neonatal cholestasis in the newborn period. Sugar chromatography was normal. Clinical findings improved with supportive treatment and hypoallergenic elemental diet was commenced. Laboratory findings showed decreased phosphomannomutase enzyme activity and transferrin isoelectric focusing testing was consisted with PMM2-CDG, but no mutation was found. Subsequent investigations revealed novel homozygous c.462G>A (p.Trp154*) stop codon mutation in the *GALT* gene. Clinical and laboratory findings reevaluated while the patient was on liberalized diet, and all parameters were within normal limits, only minimal increased liver parenchyme echogenicity was detected on abdominal ultrasonography.
Conclusion: Our patient emphasizes the importance of suggesting galactosemia in the differential diagnosis of congenital glycosylation defects. Normal liver function tests of the patient on liberalized diet is a rare manifestation of galactosemia except for duarte variant.

P-301

A patient with Fanconi Bickel syndrome and a novel mutation

Gunduz M[1], Gurbuz F[1], Unal O[1]

[1]Ankara Hematology Oncology Children's, Ankara, Turkey

Background: Fanconi Bickel syndrome (FBS) is a rare autosomal recessively inherited glycogen storage disease, characterized by glycogen accumulation in the liver and kidneys, severe renal tubular dysfunction, and impaired glucose and galactose metabolism. It is caused by GLUT2 deficiency and mutations in the *SLC2A2* gene. Signs and symptoms begin in the first few months of life and include failure to thrive, polyuria, rickets, and followed by short stature and hepatosplenomegaly in early childhood.
Case Report: 13 months old girl patient was referred to our hospital with hepatomegaly, glucosuria and fasting hypoglycemia. Parents were first degree cousins. On physical examination she had mild failure to thrive, abdominal distension and 3 cm hepatomegaly. Laboratory findings revealed mildly elevated liver enzyme and triglyceride levels hypoglycemia, hypophosphatemia and generalized aminoaciduria. Clinical and laboratory findings suggested Fanconi Bickel syndrome, and molecular genetic analysis showed homozygous novel p.S169*(c.506C>G) stop codon mutation. The patient was treated and improved with a diet with adequate caloric intake compensating for the renal glucose loss including high amylopectin maize starch.
Conclusion: FBS has a broad phenotypic variability, and many mutations have been described. Here we presented clinical and laboratory findings of a patient with a novel stop codon mutation.

P-302

The diagnosis and monitoring of Galactosemia using NMR spectroscopy

Usurelu N[1], Nicolescu A[5 6], Blanita D[1], Boiciuc C[1], Rotaru D[1], Sacara V[1], Gatcan S[1], Garaeva S[2], Tarcomnicu I[3], Stambouli D[3], Szonyi L[4], Balogh L[4], Deleanu C[5 6]

[1]Institute of Mother and Child, Chisinau, Moldova, Republic of, [2]Inst Physiol Sanocreat ASM, Chisinau, Moldova, Republic of, [3]"CytoGenomic" Med Lab, Bucharest, Romania, [4]1st Dept Ped, Semmelweis Univ, Budapest, Hungary, [5]"P.Poni" Inst Macromol Chem, Roman Acad, Iasi, Romania, [6]"C.D.Nenitescu" Centr Org Chem, Rom Acad, Bucharest, Romania

Background: Three inherited disorders of galactose metabolism (GALT, GALK, GALE deficiency) resulting in galactosemia have been delineated leading to severe life-threatening disease affecting the liver and/or eyes.
Material and methods: We report on 5 children (2M/3F) with impaired galactose metabolism. The screening test for blood Galactose level, GALT activity and NMR spectroscopy of urine (Galactose, Galactitol) were used for evaluation.
Results: In all cases GALT activity was low [0-15%]. Three children with neonatal onset (GALT activity-0%) manifested liver affection and sepsis (E.coli) after breastfeeding during the first week of life. A positive screening test and high urine Galactose [28.7-50.7 mol/mol creat] were appreciated before starting free-lactose/galactose diet; after diet these normalized. A high urine level of Galactitol was found at diagnosis [~10 mol/mol creat] and maintained high concentrations [0.3-1.0 mol/molCrea] in spite of a very strong diet. One late diagnosed child was admitted (15yrs old) with severe hypotonia, growth retardation, liver affection and cataract with GALT activity at 8.5%; blood and urine were negative on free-lactose/galactose diet. Another child (16 months old) was evaluated for cataracts only with repeatedly negative screening tests, but positive galactosuria [0.13-0.22 mol/mol creat] and no urine Galactitol on breastfeeding, GALT activitywas found at 15%.
Conclusion: NMR spectroscopy of urine is very useful in diagnosis and monitoring of galactosemia.

P-303

Nutritional status and body composition of patients with hepatic Glycogen Storage Diseases treated at Hospital de Clínicas de Porto Alegre, Brazil

Nalin T[1], Grokoski K C[1], Dos Santos B B[2], Perry I D S[3], Refosco L F[2], VAIRO F P[2], Souza C F M[2], Schwartz I V D[1 2]

[1]Univ Fed do Rio Grande do Sul, Porto Alegre, Brazil, [2]Hosp de Clínicas de Porto Alegre, Porto Alegre, Brazil, [3]Univ do Extremo Sul Catarinense, Criciúma, Brazil

Objectives: To evaluate the nutritional status and the body composition of patients with hepatic Glycogen Storage Diseases (GSD) through bioelectrical impedance (BIA).
Methods: Thirty-one patients, age mean of 11 ± 6 years (range: 3–32 years), and 31 healthy gender-matched controls, age mean of 14 ± 5 years (range: 4–31 years), were included. Weight and height were verified and body mass index (BMI) was calculated. Body composition data were assessed by performing BIA in both groups.
Results: Regarding BMI classification, among patients, nine (29%) were considered eutrophic, eight (26%) overweight and fourteen (45%) obese. In the control group, nineteen (61%)

were considered eutrophic, one (3%) low weight, seven (23%) overweight and four (13%) obese. As for body composition, the average fat mass in patients was 26.6±8.6%, among the controls this value was 22.0±8.1% (p=0.037).
Conclusion: The results of this study reinforce the literature findings, which indicates that patients with hepatic GSD are frequently overweight. This excess weight also reflected in the fat mass accumulation, which is significantly higher in patients than in healthy controls. These data may be associated with administration of high daily doses of cornstarch, used in the treatment of hepatic GSD. Support: CNPq, FAPERGS.

P-304

Fructose-1,6-bisphosphatase deficiency: natural course of the disease with relevance to diagnosis and treatment in 23 patients

Gokcay G[1], Shin Y S[2], Podskarbi T[2], Balci M C[1], Karaca M[1], Demirkol M[1]

[1]Ped Nutr Metab, Ist Med Faculty Ist Univ, Istanbul, Turkey, [2]Molec Genet Metabol Lab, Munich, Germany

Background: Fructose-1,6-bisphosphatase (FBPase) deficiency (OMIM 229700) can cause life-threatening hypoglycemia and lactic acidemia following fasting.
Objectives: The aim was to evaluate the impact of diagnosis and treatment on the natural course of FBPase deficiency.
Methods: FBPase deficient patients, diagnosed and followed in Istanbul Medical Faculty during 1990-2014 using enzyme activity measurement on white-blood-cells (n=21) or molecular analysis (n=2), were evaluated.
Results: There were 23 patients (14 males, 9 females) with ages (14.8 ± 5.5 years, range: 4.5-26), age at initial symptom (1.0 ± 0.9 years, range: 0-3.5), age at diagnosis (3.7 ± 3.2 years, range: 0-13). Follow-up after diagnosis was 9.8 ± 4.6 years, range: 1-20). Initial symptoms were during neonatal period (n=6), in the first year (n=6) or after the first year (n=11). The number of acute episodes before and after diagnosis were 4.2 ± 4.7,range: 1-20 and 1.3 ± 2.4, range: 0-5 respectively. Nineteen patients had hypoglycemia with lactic acidosis, three had hypoglycemic seizures, one had fructose intolerance. There were six sibling deaths. Prognosis improved after diagnosis. Acute episodes decreased significantly. Mild psychomotor retardation (n=7), autism (n=1), seizures (n=3), attention deficit/hyperactivity (n=1), growth retardation (n=2) and patient loss (n=1) were observed. Thirteen patients followed fructose restricted diet, ten just fed frequently, three reported sensitivity to fructose.
Conclusion: FBPase deficiency must be considered in patients who have episodes of ketotic hypoglycemia, lactic acidemia triggered by infections, fasting or fructose. Early diagnosis can prevent death, improve growth and quality of life.

P-305

Fructose 1,6-biphosphatase (FBP) deficiency in early childhood: 5 Turkish cases

Bulut D[1], Seker Yilmaz B[1], Kor D[1], Ceylaner S[2], Ozgur Horoz O[3], Onenli Mungan N[1]

[1]Div Ped Metab, Cukurova University, Adana, Turkey, [2]Intergen Genetic Centre, Ankara, Turkey, [3]Div Ped Intens Care, Cukurova University, Adana, Turkey

Background: Fructose 1,6-biphosphatase (FBP) deficiency (OMIM 229700) is an autosomal recessive disorder of gluconeogenesis caused by mutations within the *FBP1* gene. FBP deficiency is characterized by recurrent episodes of hypoglycemia and ketoacidosis precipitated by catabolic conditions.
Case Reports: Here we report 5 patients, presented with acute attacks of hypoglycemia and metabolic acidosis in the first year of life. Parental consanguinity was reported in all families. All patients have unremarkable prenatal and perinatal history. Recurrent metabolic decompensation attacks occurred with the triggering factors; fever, infection, fasting or after a fructose load such as complementary feeding. Elevated liver transaminases, hyperuricemia, excretion of urinary glycerol and lactic acidosis were the common laboratory findings. Clinical and biochemical features were consistent with the diagnosis of FBP deficiency. *FBP1* sequencing of genomic DNA confirmed the diagnosis, 4 of the patients have the homozygous exon 1 deletion and one of them has c.472C>T homozygous mutation.
Conclusion: FBP deficiency should be kept in mind for the differential diagnosis of hypoglycemic episodes. Homozygous exon 1 deletion of *FBP1* gene is the most common mutation for Turkish patients, exon 1 should be considered as a hotspot mutation region.

P-306

Serbian patients with early manifestations of hyperinsulinism-hyperammonemia syndrome

Sarajlija A[1], Milenkovic T[1], Djordjevic M[1], Kecman B[1], Grkovic S[1]

[1]Mother and Child Health Institute, Belgrade, Serbia

Background: Reports on manifestations and outcome of hyperinsulinism-hyperammonemia (HI/HA) syndrome vary

between researchers. We present cases with emphasis on genetic basis and neurological outcome.
Case reports: Patient 1 presented at nine weeks of age with generalized seizures. The male infant had hypoglycemia, hyperammonemia (277 μmol/L) and hyperinsulinemia, along with mild hypotonia and macrosomia. Heterozygosity for mutation c.1334C>T in *GLUD1* gene was revealed. Treatment included diazoxide, hydrochlorotiazide and avoidance of protein-only meals. Over years, plasma ammonia gradually decreased with values from 133.4 to 153.4 μmol/L. There is a mild delay of motor milestones and cognitive abilities. Patient 2 manifested hypoglycemic seizures at 10 weeks of age. Six months later, the boy had recurrence of seizures with hypoglycemia, hyperinsulinemia and hyperammonemia (159.2 μmol/L). His mother was diagnosed with nesidioblastosis at 6.5 years of age. She currently receives lamotrigine for epilepsy and also has hyperammonemia. The boy is heterozygous for mutation c.833C>T (p.R221C) in exon 6 of *GLUD1* gene. At 20 months of age there is speech delay with mild hyperactivity.
Conclusion: Early manifestations with hypoglycemic seizures could lead to later neurologic sequelae in HI/HA syndrome. Relation of exon 6 mutations to epilepsy has already been suggested and we reiterate this possibility.

P-307

Heterogeneity in glycogen storage disease type Ia

Derks T G J[1], Reijngoud D J[2], Smit G P A[1], Oosterveer M H[2]

[1]Sect Metab Dis, Beatrix Child Hosp, UMCG, Groningen, Netherlands, [2]Lab Metab Dis, Dept Lab Med, UMCG, Groningen, Netherlands

Background: Case reports and cohort studies defined the classical clinical and biochemical phenotype of glycogen storage disease type Ia (GSD Ia), but clinical heterogeneity between GSD Ia patients is poorly documented.
Patients: Descriptive retrospective study of longitudinal clinical and biochemical data in a selected group of 11 GSD Ia patients. Data were compared between 4 patients with homozygous *G6PC* mutations and affected compound heterozygous GSD Ia siblings originating from 3 different families.
Results: The 4 patients with homozygous *G6PC* genotypes display large differences in terms of clinical (age of clinical presentation, growth, long-term complications) and biochemical (plasma concentrations of lactate, cholesterol, triglycerides) parameters. Heterogeneity was also observed between affected siblings carrying identical *G6PC* genotypes.
Discussion: Differences between the homozygous patients define the large heterogeneity of GSD Ia and indicate that the *G6PC* genotype plays an important role. Differences between affected siblings suggest that there is a role for additional (genetic and/or environmental) modifying factors. These differences emphasize the importance of a personalized therapeutic (dietary and/or pharmacologically) approach and follow-up.

P-308

High prevalence of fractures in glycogen storage disease type I (GSD-I)

Van der Ende R M[1], Martens D H[1], Smit G P A[1], Derks T G J[1], Van der Veer E[2]

[1]Sect Metab Dis, Beatrix Child Hosp, UMCG, Groningen, Netherlands, [2]Sect Lab Med, UMCG, Groningen, Netherlands

Background: Patients with GSD-I have osteoporosis but the prevalence of fractures is not documented.
Methods: In a single center retrospective longitudinal cohort study, 59 GSD-I patients, mean age 28.8 years, were asked to fill in a questionnaire about life-time fractures. Bone mass density (BMD) and bone turnover markers (BTM) were measured.
Results: 36 (61%) patients returned the questionnaire, fourteen (39%) had had one or more fractures. Mean BMD Z-score of all 59 GSD I patients for radius, femur and lumbar spine were respectively -1.60 (±0.32), -1.20 (±0.18) and -1.60 (±0.24). Mean osteocalcin Z-score was -0.98 (±0.21), median PINP Z-score was -0.39 (-2.44-6.38) and mean sCTX Z-score was -0.76 (±0.16). For GSD-I patients with one or more life-time fractures, median or mean BMD z-scores for radius, femur and lumbar spine were respectively -1.95 (-4.90–1,00), -1.40 (±0.31) and -1.76 (±0.36) and for patients with no fractures -0.58 (±0.34), –0.83 (±0.22) and -1.15 (-3.30-0.00). BMD Z-score for radius was significantly lower in patients with one or more life-time fractures (p=0.013).
Conclusion: GSD-I patients display high prevalence of life-time fractures and these patients have lower radius BMD Z-score. Close monitoring of BMD and BTM during follow-up may earlier identify those GSD-I patient with increased risk of fractures.

P-309

Molecular characterization of mutations in Serbian patients with glycogen storage diseases

Skakic A[1], Klaassen K[1], Kostic J[1], Djordjevic M[2], Sarajlija A[2], Kecman B[2], Kotur N[1], Stankovic B[1], Srzentic S[1], Pavlovic S[1], Stojiljkovic M[1]

[1]IMGGE, University of Belgrade, Belgrade, Serbia, [2]Mother and Child Healthcare Institute, Belgrade, Serbia

Background: Glycogen storage diseases (GSD) are group of rare metabolic monogenic diseases with variable clinical symptoms and heterogeneous genetic base.
Methods: In this study, we analyzed 16 Serbian patients diagnosed as GSD according to biochemical data and clinical symptoms. We used Sanger sequencing for targeted analysis of *G6PC* and *SLC37A4* genes.
Results: In 75% of patients, a GSD Ib diagnosis was confirmed by mutation detection in *SLC37A4* gene: c.1042_1043delCT (66.7%) and c.81T>A (33.3%). Interestingly, only two GSD Ia patients were identified. Mutations in *G6PC* gene were c.247C>T and c.441T>C, on 3 and 1 alleles respectively. Further, we used an NGS panel with 4813 genes to detect disease-causing mutations in two patients with ambiguous symptoms. In the first patient, mutations in *AGL* gene (c.655A>G and c.3980G>A) indicated GSD III type. Surprisingly, in the second patient with characteristic neutropenia, we detected mutations in *SBDS* gene (c.258+2T>C and c.184A>T) responsible for Schwachman-Diamond syndrome.
Conclusion: This study provided the first data about molecular genetics of Serbian patients presenting with GSD clinical symptoms, thus enabling molecular genetic diagnostics and genetic counseling of this disease in the country. Furthermore, reaching 100% of mutation detection by high-throughput resequencing approach, set the base for research and clinical utility of NGS in Serbia.

P-310

Identification of key genetic modifiers of neuromuscular synaptogenesis in a *Drosophila* classic galactosemia disease model

Jumbo-Lucioni P P[1], Parkinson W[1], Broadie K B[1]

[1]Dept. Bio Sci, Vanderbilt University, Nashville, United States

Background: Classic galactosemia (CG) results from loss of galactose-1-phosphate uridyltransferase (GALT), which catalyzes conversion of galactose-1-phosphate+UDP-glucose to glucose 1-phosphate+UDP-galactose. UDP-galactose 4'-epimerase (*dGALE*) interconverts UDP-galactose to UDP-glucose, and UDP-N-acetylgalactosamine to UDP-N-acetylglucosamine. UDP-glucose pyrophosphorylase (*UGP*) provides an alternate pathway for galactose metabolism by catalyzing the UTP and galactose-1-phosphate reaction yielding UDP-galactose. These UDP-sugars are essential donors for glycoprotein/glycolipid synthesis, with crucial roles in developing neuronal synapses. Dietary galactose restriction prevents neonatal CG lethality, but patients still show severe neurodevelopmental, motor and cognitive impairments.
Methods: Here, we assess impacts of *dGALE* and *dUGP* manipulation on behavioral output and cellular synaptic development in a new *Drosophila* CG disease model.
Results: Both *dGALE* and *dUGP* were identified as critical genetic modifiers of both behavioral and cellular defects in the CG disease state. Similar to GALT-deficient animals, *GALE* and *UGP* mutants display locomotor deficits and altered neuromuscular junction (NMJ) formation, including synaptomatrix glycosylation losses (e.g. N-acetylgalactosamine residues), increased synaptic bouton/branch numbers and elevated neurotransmission. Phenotypes are exacerbated by co-removal of *dGALT* with *dGALE* and *dUGP*.
Conclusion: Our results suggest UDP-sugar balance as key modifier of neurological outcomes in the CG disease state, and identify two potential therapeutic targets for treatment, GALE and UGP.

P-311

Glycogen storage disease (GSD): Epidemiological data for 106 patients from a single metabolic clinic in Athens with up to 23 years follow up

Drogari E[1], Manolaki N[2], Tsambounis E[3], Laskos O[3], Manessis E[4], Hatjiyannis S[5]

[1]1st Dept Ped,Univ Athens,Agia Sofia Hosp, Athens, Greece, [2]2nd Dept Ped, NHS, Agia Sofia Hosp, Athens, Greece, [3]Dept Sports Med,State Olympic Stadium, Athens, Greece, [4]Emer Prof, Evrocliniki Athinon, Athens, Greece, [5]Emer Prof, Hygeia Gen Hosp, Athens, Greece

Background: GSD is a group of rare IEM. The mode of inheritance is mainly autosomal recessive although in some is sex-linked. These disorders affect mainly the liver, muscle, GI, kidneys and bones.
Methods: 106 patients (clinical, enzymatic, molecular criteria) were followed up from 1 to 23 years (biochemistry, hormones, Height, Weight, ECHO, ECG, MRI, DEXA, etc). Treatment involved NTF, high CHO and protein diets, low fat, cornstarch,glucose polymers and gastrostromy.
Results: Most frequent types were III (27,56%) and V (36, 04%) with the rest to follow (I_a =11,66%, I_b=7,42%, IV=2,12%,VI=14,84%, VII=9,54% and XI=3,18%). Elevated uric acid, liver enzymes and lipid levels were substantially reduced between 3-6 months with very good glucose control. 16 had renal deficiency and 81 had bone disease. None had malabsorption or Crohn's disease. 39% of type III started muscle disease before the age of 10

years and remained steady. The majority of types V and VII were diagnosed because of cramps and raised CK after exercise as adolescents. 5 patients died of heart failure.
Conclusion: GSD causes severe problems. In our experience the "mild" forms of the muscle types are under- diagnosed. A good area to find them early is related to sports and gymnastics.

P-312

Congenital and perinatal Glycogen Storage Disease type IV: clinical, pathological and biological data in 5 cases

Pettazzoni M[1], Streichenberger N[2], Mezin P[3], Clamadieu C[4], Roume J[5], Gelot A[6], Duband S[8], Prieur F[7], Vianey-Saban C[1], Piraud M[1], Froissart R[1]

[1]Metab Dis Lab, Hospices Civils, Lyon, France, [2]Pathology Dept, Hospices Civils, Lyon, France, [3]Pathology Dept, CHU, Grenoble, France, [4]Neonat Intens Care Unit, Hospices Civils, Lyon, France, [5]Pathology Dept CHI Poissy, Poissy, France, [6]Neuropath Dept, Trousseau APHP, Paris, France, [7]Genetic Dept, CHU, Saint Etienne, France, [8]Leg Med, CHU, Saint Etienne, France

Background: Glycogen storage disease type IV (GSDIV) is a highly heterogeneous disorder caused by a deficiency of glycogen branching enzyme (GBE). Several rare variants have been reported, including fatal forms that can present *in utero* or at birth.
Patients and Methods: Clinical, histopathological, biochemical and molecular data of perinatal (2 cases) and congenital (3 cases) GSDIV patients are reported.
Results: Two *in utero* cases presented with hydrops fetalis (at 28 or 18 weeks gestation), akinesia and hydramnios. Three congenital variants presented very severe hypotonia requiring mechanical ventilation, neuronal involvement and death within 2 weeks after birth. All samples showed PAS positive, diastase-resistant inclusions in heart, muscles and liver (when tested), except one perinatal case with a pure muscular form with almost complete absence of limb muscle and without storage in placenta. The diagnosis was confirmed by a markedly decreased GBE activity in cultured amniocytes, fibroblasts or chorionic villi. *GBE1* genotyping revealed six novel mutations.
Conclusion: GSDIV is a probably underdiagnosed cause of severe congenital hypotonia. This disorder should also be considered when pregnancies are complicated by hydrops fetalis and depressed fetal movements. This study underlines the major role of histopathological examination in different tissues including placenta.

P-313

Delay before spontaneous remission in 162 patients with congenital hyperinsulinism medically treated

Arnoux J B[1], Roda C[1], Saint-Martin C[3], Guemann A S[1], Grisel C[1 2], Bellanne-Chantelot C[3], De Lonlay P[1 2]

[1]Dept Metab, Necker Univ Hosp, APHP, Paris, France, [2]Paris-Descartes Univ, Paris, France, [3]Pitié-Salpêtrière Univ Hosp, APHP, Paris, France

Background: Diffuse form of congenital hyperinsulinism (CHI) is a dysregulation of the insulin secretion by the pancreatic ß-cells, leading to recurrent hypoglycaemia. This condition might be severe during infancy, requiring a subtotal pancreatectomy, which leads to an insulin requiring diabetes within a few years. However, conservatively treated patients experience a slow and progressive spontaneous remission of hypoglycaemia over years, but no natural history study comforted this observation.
Patients: We present a retrospective natural history study of 162 patients with medically treated CHI. Among them, 111 were diazoxide-responsive and 51 were diazoxide-unresponsive diffuse CHI.
Results: In diazoxide-responsive patients, 66% were weaned from the diazoxide after an average of 2.9 years of treatment. 50% could stop their treatment within the first year of life and 75% before 5.1 year old.
In diazoxide-unresponsive patients, the medical treatment could be progressively lightened in all, and even totally stopped in 12% of them, at an average age of 3.9 year old.
Conclusion: We observed a progressive remission of CHI permitting a progressive weaning of treatment over years. Finally, if glycaemia is controlled by the medical treatment (diazoxide, somatostatin analogues, enteral feeding), pancreatectomy might not be justified anymore.

P-314

Genotypic profile of Brazilian patients with classic galactosemia and study of the genotype-phenotype correlation

Camelo Jr. J S[1], Garcia D F[1], Turcato M[1], Molfetta G A[1], Marques A A[1], Souza C F M[2], Porta G[3], Steiner C E[4], Silva Jr. W A[1]

[1]Ribeirão Preto School of Medicine - USP, Ribeirão Preto, Brazil, [2]Federal University of Rio Grande do Sul, Porto Alegre, Brazil, [3]Children´s Institute - USP, São Paulo, Brazil, [4]School of Medical Science - Unicamp, Campinas, Brazil

Background: Classic galactosemia (CG) is an autosomal recessive inborn error of galactose metabolism, caused by the deficiency of the galactose-1-phosphate uridyltransferase enzyme (GALT). Objective of this study was to evaluate the profile of mutations in the *GALT* gene of Brazilian CG patients and to perform genotype-phenotype correlation analysis.
Methods: Direct bidirectional sequencing of *GALT* gene and additional studies, such as *GALK1* genotyping, ancestrality study and in silico simulations. Main clinical features were obtained.
Results: Major known CG causing mutations were identified, p.Q188R, p.S135L and p.K285N, Duarte 2 allele and six novel mutations: p.M1T; p.R33S; p.P73S; IVS3+1G>A; IVS4+4A>C and p.Q169P. Patients homozygous for p.Q188R mutation present a more severe phenotype than individuals who had at least one p.S135L mutation. For individuals with new mutations, it was observed a wide range of phenotypes (death due to liver failure, sepsis and asymptomatic case).
Conclusion: This study expands the spectrum of mutations in the *GALT* gene described in the literature and reinforces the importance of early diagnosis and the introduction of dietary treatment; also adds more evidence to the discussion on the introduction of galactosemia in the neonatal screening program of Brazil, where the incidence of the disease is estimated at about 1:20,000.

P-315

A novel mutation of the *GBE1* gene in a patient with the non-progressive hepatic form of type IV glycogen storage disease

Ersoy M[1], Onal Z[2]

[1]Child Metab Dis,Sadi Konuk Res&Tra Hosp, Istanbul, Turkey, [2]Div Ped. Gast&Hepa. Sadi Konuk R&T Hosp., Istanbul, Turkey

Background: Glycogen storage disease type IV (GSD-IV) is an autosomal recessive disorder due to deficiency of glycogen branching enzyme, span a continuum of different subtypes with variable ages of onset, severity, and clinical features. The most common form of GSD-IV presents in the first 18 months of life with failure to thrive, hepatosplenomegaly, liver cirrhosis, leading to death by age 5 years. Patients may have liver dysfunction without liver failure, referred to as 'nonprogressive' hepatic GSD-IV.
Case report: In 3-month-old girl diagnosed with pneumonia high transaminase levels were determined. The liver was 2cm below the costal margin. She was mildly hypotonic. Transaminases elevated four to five times of normal levels.INR and albumine levels were within the normal ranges. Liver biopsy showed enlarged hepatocytes with periodic-acid-Schiff (PAS) positive, diastase-resistant inclusions with intertistitial fibrosis. *GBE1* gene sequencing showed a novel homozygous mutation of p.E498K (c.1492G>A). The patient had been followed-up for five years. In her last assessment, although liver and spleen sizes increased, liver function tests remained within normal ranges.
Conclusion: Patients should be carefully monitored for evidence of progression and hepatocellular carcinome before recommending liver transplantation which is not an innocent treatment. Genotype-phenotype relationship should be examined for the identification of subtypes.

P-316

Detection of common *GALT* mutations through ARMS

Mahmood U[1], Cheema H A[2], Mahmood S[1]

[1]University of Health Sciences, Lahore, Pakistan, [2]The Children's Hospital & ICH, Lahore, Pakistan

Background: Type I galactosemia is an inborn error resulting from mutations on both alleles of *GALT* gene which leads to absence or deficiency of galactose -1-phosphate uridyltransferase (GALT). Classical galactosemia is frequently associated with p.Q188R, p.S135L and p.K285N mutations and p.N314D is associated with Duarte galactosemia and is wide spread among various worldwide populations.
Objectives: The objectives of this study are to: 1) Identify most common mutations p.Q188R, p.S135L, p.K285N and p.N314D for patients with classical and Duarte galactosemia. 2) Correlate genotype with its phenotype.
Methods: The present study aims at detecting p.Q188R, p.S135L, p.K285N mutations and p.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: p.S135L and p.K285N mutations were present neither in galactosemia patients nor in normal subjects. Only one galactosemia patient carried p.Q188R mutation that was in homozygous state. However, p.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 p.D314 might be far more common in Pakistani population than in European and North American ones.

P-317

Hyperinsulinism: a quality of life study

Caviglia S[1], Maiorana A[2], Tonto F[1], Bazzu P[1], Manganozzi L[2], Dionisi-Vici C[2]

[1]Psychology Unit, Bambino Gesu Child Hosp, rome, Italy, [2]Div Metab Dis, Bambino Gesu Hosp, rome, Italy

Background: Congenital hyperinsulinism (CHI) is the most frequent cause of persistent hypoglycemia in children, due to an inappropriate secretion of insulin. CHI patients were investigated for their quality of life (QoL) as they are forced to follow a strict pharmacological and dietary treatment to avoid neurological symptoms and sequelae.
Methods: The PedsQL™ questionnaire was administered to parents of 36 CHI patients aged between 1-22y (mean 8.4y±14.2) aiming to investigate specific domains (physical, emotional, social, school scale) and assess the total QoL score.
Results: A normal QoL score was found in 67% of children with a mean score of 66.5±52.26 (n.v. >50). Patients with QoL< 50 (33%) fell especially in physical and school scales. Remarkably, a third of patients with normal QoL fell in some domains, particularly in the physical and social scales as opposed to area of excellence in emotional domain.
Conclusion: The majority of our CHI patients present a normal QoL score despite frequent monitoring of glycemia and strict adherence to therapy and dietary regimens. The multidisciplinary team composed of pediatrician, nutritionist and psychologist supports these patients and their families in the management of disease. Falls in specific domains are likely due to parents' perception rather than true deficits.

P-318

A new phenotype of glycogen storage disorder associated with brain atrophy, epilepsy, arthrogryposis and polyglucosan body myopathy

Martinelli D[1], Taddei A[2], Bevivino E[1], Manganozzi L[1], Oldfors A[4], Inserra A[2 3], Dionisi-Vici C[1]

[1]Div Metab Dis, Bambino Gesu' Child Hosp, Rome, Italy, [2]Pediatric Surgery Unit, Catholic Univ, Rome, Italy, [3]Surgery Dept, Bambino Gesu' Child Hosp, Rome, Italy, [4]Dept Pathology, Gothenburg Univ, Gothenburg, Sweden

Background: Genetic defects of glycogen metabolism cause glycogen storage diseases (GSDs), characterized histologically by abnormal quantity or quality of glycogen in the cells. Polyglucosan (PG) is an abnormal polysaccharide that, compared with glycogen, has fewer branched points and excessively long peripheral chains. PG bodies react to PAS stain and are partially resistant to diastase digestion. PG accumulation represents the histopathological manifestation of different disorders (Lafora disease, glycogenosis type IV and VII, other polysaccharide storage myopathies).
Methods: Case-study of a patient with a new phenotype of GSD.
Results: This 1 year-old female patient presented at birth with arthrogryposis, respiratory insufficiency, severe hypotonia. She progressively developed epilepsy and central hypoadrenalism. Brain MRI documented brain atrophy. Muscle and liver biopsy showed accumulation of PG bodies, filled with PAS-positive reacting material resistant to diastase digestion. Array-CGH and whole exome sequencing performed on the patient's and her parents' blood followed by a candidate gene approach did not show rearrangements and/or pathogenic variants explaining the phenotype. Whole genome sequencing is ongoing.
Conclusion: We present a new phenotype of GSD with PG body myopathy. This case confirms the fair number of glycogen storage abnormality disorders, which cannot be assigned to one of the known molecular etiologies.

P-319

Exercise-induced lactic acidosis and abnormal phagocytosis in a patient with transaldolase deficiency

Tsiakas K[1], Hempel M[2], Wamelink M M C[3], Kremer L[4], Prokisch H[4], Haack T B[4], Santer R[1]

[1]Dept Pediatr, Univ Med Cent Eppendorf, Hamburg, Germany, [2]Inst Hum Genet, Univ Med Cent Eppendorf, Hamburg, Germany, [3]Dept Clin Chem, VU Univ Med Center, Amsterdam, Germany, [4]Inst Hum Genet, Techn Univ, Munich, Germany

Background: Deficiency of transaldolase (TALDO), an enzyme catalyzing the reversible part of the pentose phosphate pathway, causes a rare disorder with variable presentation including growth retardation, pancytopenia, bleeding tendency, hepatosplenomegaly, hepatic fibrosis, cirrhosis, hepatocellular carcinoma, hepatopulmonary syndrome, cutis laxa, dysmorphic features, tubulopathy, congenital heart disease, and abnormal polyol concentrations in urine. We describe a patient with a phenotype similar to a mitochondrial disorder or an immunodeficiency syndrome, who was eventually diagnosed to suffer from transaldolase deficiency.
Case report: A 17-years-old male with chronic exertion intolerance, exercise-induced muscle pain, dystrophy, hepatomegaly, chronic diarrhea, recurrent infections (predominantly skin abscesses), thin skin with a network of visible blood vessels, chronic renal failure, tubular proteinuria, and aminoaciduria.

During spiroergometry severe lactic acidosis evolved which resolved completely after a phase of recovery. Granulocytes showed an impaired respiratory burst. Diagnosis was established by next-generation sequencing showing homozygosity for a novel *TALDO* mutation and it was confirmed by abnormal polyol concentrations in urine.
Conclusion: This report extends the phenotypic spectrum of TALDO deficiency and demonstrates that next-generation sequencing is a powerful tool (and in some cases the only one) to identify the etiology of rare metabolic disorders, particularly of those with variable presentations and overlapping phenotypes.

14. Disorders of fatty acid oxidation and ketone body metabolism

P-018

Attention deficit-hyperactivity disorder as a dominant clinical presentation in OCTN2 deficiency

Lamhonwah A M[1 2 5], Baric I[3 4], Lamhonwah J[1 2 5], Grubic M[3], Tein I[1 2 5]

[1]Div of Neurology, The Hospital for Sick, Toronto, Canada, [2]Dept.Lab Medicine, University of Toronto, Toronto, Canada, [3]Pediatrics, University Hospital Centre, Zagreb, Croatia, [4]Univ of Zagreb, School of Medicine, Zagreb, Croatia, [5]Genetics & Genome Biology, SickKids, Toronto, Canada

Background: We report a novel OCTN2 mutation (novel in-frame deletion (p.T440-Y449) in a patient with ADHD.
Case report: This boy presented with Attention-Deficit/Hyperactivity Disorder (ADHD) at 3 years and at 8 ½ years was notably hyperactive in the absence of hypoglycemic hypoketotic coma and had myopathy, cardiomyopathy, and very low serum carnitine. Formal psychological evaluation with the standardized ADHD Test, gave a score consistent with severe ADHD. He had elevated aminotransferases. His sister died of sudden infant death. On clinical suspicion of OCTN2 deficiency, he was treated with high dose oral L-carnitine (100 mg/kg/day) which led to significant improvements in his cardiomyopathy, exercise intolerance and behavioural problems.
Studies and Results: [^{3}H]-L-carnitine uptake studies in his cultured skin fibroblasts confirmed OCTN2 deficiency. Molecular analysis of *SLC22A5* gene in genomic DNA from the proband and his parents by PCR and Sanger sequencing revealed heterozygosity for a premature stop codon (p.R282X) (paternal inheritance) and a novel in-frame deletion (p.T440-Y449) (maternal inheritance) in a highly conserved putative caveolin-1 binding site. Immunoblot of fibroblasts with anti-mOctn2 antibody revealed a reduced truncated protein.
Conclusion: L-carnitine therapy not only reverses the myopathy and cardiomyopathy of OCTN2 deficiency, but may improve the neurological phenotype including ADHD.

P-320

Identification of novel and recurrent *ACADS* mutations and phenotypic characterization of Korean patients with short-chain acyl-CoA dehydrogenase deficiency

Cheon C K[1], Ko J M[2]

[1]Dep Ped, PNU Child Hosp, Yangsan, Korea, Republic of, [2]Dep Ped, SNU Child Hosp, Seoul, Korea, Republic of

Background and objectives: Short-chain acyl-CoA dehydrogenase (SCAD) catalyzes the first step in mitochondrial short-chain beta-oxidation, and its deficiency is caused by mutations in the *ACADS*. The spectrum *ACADS* mutations and clinical manifestations in Korean patients with SCAD deficiency were investigated.
Methods: The study included 9 patients with SCAD deficiency from 7 unrelated families as diagnosed by biochemical and mutation analyses. Clinical features, biochemical and molecular genetic data were reviewed retrospectively.
Results: Seven patients were found during newborn screening, and 2 were diagnosed by family screening. Their mean age at diagnosis was 14.1 ± 31.9 months. During follow-up period, no hypoglycemic event was noted, and the development and growth of the patients was normal, except in 2 siblings showing hypotonia and delayed gross motor development. One girl showed cyclic vomiting until the age of 2 years. *ACADS* genotype in all patients showed 7 different mutations. Of these, p.E344G was the most frequent mutation. Four novel mutations were identified: p.L93I, p.E228K, p.P377L, and p.R386H.
Conclusion: Patients with SCAD deficiency generally showed mild clinical manifestation. However, the *ACADS* genotype was heterogeneous. Our data contribute to a better understanding of the clinical and molecular genetic characteristics of SCAD deficiency in Korean patients.

P-321

Synergistic effects of low doses of resveratrol and bezafibrate for correction of CPT2 and VLCAD deficiencies in patient fibroblasts

Djouadi F[1], Le Bachelier C[1], Habarou F[1], Bastin J[1]

[1]INSERM UMR-S 1124, Univ Paris Descartes, Paris, France

Background: Disorders of mitochondrial long-chain fatty acid beta-oxidation (FAO) form a large group of rare diseases with few treatments to date. We previously showed that exposure to bezafibrate (BZ; 400 μM, 48h), or resveratrol (RSV; 75 μM, 48h) could improve or restore FAO values in fibroblasts from patients with the myopathic form of carnitine palmitoyl transferase 2 (CPT2) or very-long-chain acyl-CoA dehydrogenase deficiency.
Method: Here, we investigated the effects of treatments by various combinations of BZ+RSV in control and FAO-deficient fibroblasts. Tritiated palmitate oxidation experiments after treatment with BZ+RSV at 400+75 μM for 48h first demonstrated additive effects of these compounds in 5 CPT2- and 9 VLCAD-deficient cell lines. We then focused on analyzing the effects of low doses of BZ (15, 25, 35, 50 μM), of RSV (10, 20, 30 μM), and of various combinations of low doses.
Results: Interestingly, the results revealed marked stimulations of FAO in VLCAD- and in CPT2-deficient fibroblasts after treatment with combinations of BZ+RSV at 15+10, 25+20, or 35+30 μM for 48h, whereas each compound alone had little or no effect at these low concentrations.
Conclusion: This clearly suggests possible synergistic effects of BZ and RSV in mediating bioenergetic stimulation, eventually leading to correction of FAO capacities in patient cells.

P-322

Mitochondrial trifunctional protein deficiency in human cultured fibroblasts: effects of bezafibrate

Djouadi F[1], Habarou F[1], Le Bachelier C[1], Ferdinandusse S[2], Schlemmer D[3], Benoist J F[3], Boutron A[4], Andresen B S[5], Visser G[6], De Lonlay P[7], Olpin S[8], Fukao T[9], Yamaguchi S[10], Strauss A W[11], Wanders R J A[2], Bastin J[1]

[1]INSERM UMR-S 1124, Univ Paris Descartes, Paris, France, [2]Lab Gen Metab Dis, Acad Medical Center, Amsterdam, Netherlands, [3]Ref Cent Inh Metab, Dis Rob Debré Hosp, Paris, France, [4]Service de Biochimie, Hôpital Bicêtre, Le Kremlin Bicêtre, France, [5]Dept Biochem, Univ South Denmark, Odense, Denmark, [6]Wilhelmina Child Hosp, Univ Med Center, Utrecht, Netherlands, [7]INSERM U781, Instit Imagine, Hop Necker, Paris, France, [8]Dept of Clin Chem Sheff Children's Hosp, Sheffield, United Kingdom, [9]Dept Pediatr, Grad Med School,Gifu Univ, Gifu, Japan, [10]Dept Pediatr, Shimane Univ School Med, Izumo, Japan, [11]Cincinnati Children Hosp Med Center, Cincinnati, United States

Background: Mitochondrial trifunctional protein (MTP) deficiency caused by *HADHA* or *HADHB* gene mutations ranks among the more severe fatty acid oxidation (FAO) disorders, without pharmacological treatment to date.
Method: We analyzed the response to bezafibrate, a hypolipidemic drug shown to potentially correct other FAO disorders in patient cells, in a panel of control or MTP-deficient patient fibroblasts. In control fibroblasts, exposure to bezafibrate (400μM, 48h) increased *HADHA* and *HADHB* mRNA abundance, immunodetectable α- and ß-subunit proteins, activities of LCHAD and LCKAT, and stimulated tritiated palmitate oxidation, indicating that MTP is pharmacologically up-regulated by bezafibrate in human fibroblasts.
Results: Patients' cell lines exhibited variable pharmacological responses. HADHA-deficient fibroblasts showed markedly reduced α-subunit and decreased ß-subunit protein levels, exhibited a profound defect in LCHAD activity, and produced large amounts of C14 and C16 hydroxyacylcarnitines, MTP-deficient fibroblasts were markedly FAO-deficient, and bezafibrate improved FAO in 6 of 26 (23%) cases, including 3 cell lines heterozygous for the common c1528G>C mutation.
Conclusion: Altogether, our results suggest that, due to variable effects of *HADHA* and *HADHB* mutations on MTP abundance and residual activity, pharmacological improvement of MTP deficiency in response to bezafibrate could not occur in all the cell lines, but was achieved in a subset of responsive genotypes.

P-323

Short-chain acyl-CoA dehydrogenase deficiency is not a disease: common *ACADS* variants and mutations have no clinical significance

Rhead W J[1]

[1]Med Gen, Med. Coll. of WI, Milwaukee, United States

Short-Chain Acyl-CoA Dehydrogenase Deficiency (SCAD) was considered a clinically significant fatty acid oxidation disorder in the 1980s. However, 4 categories of biochemical, molecular, epidemiologic, and clinical evidence argue strongly for SCAD being clinically benign:
1: Homozygous "Inactivating" SCAD variants only lower butyrate oxidation/pathway flux and butyryl-CoA dehydrogenation by 40-60% *in vivo*, due to Medium Chain Acyl-CoA Dehydrogenase's activity towards butyryl-CoA ;
2: ExAC data reveals published "Inactivating" *ACADS* variants have a heterozygote frequency of at least 1/125. Furthermore, ~53% of the general population are heterozygotes or double/compound heterozygotes or homozygotes for 2 common, completely benign *ACADS* variants (c.625G>A/

p.Gly209Ser;c.511C>T/p.Arg171Trp). In addition, at least 2, 130,000 EU individuals with these genotypes also carry a published "Inactivating" *ACADS* variant, a putative "Pathogenic" genotype;
3: Homozygosity for an "Inactivating" variant (c.319C>T/p.Arg107Cys) is very common in the US and Israeli Ashkenazi Jewish populations, where 2-3,000 clinically normal individuals carry this genotype;
4: Published clinical phenotypes of SCAD include normal individuals, are otherwise nonspecific, and are very often due to other confirmed causes. Virtually all SCAD infants identified by newborn screening worldwide appear clinically well.
Conclusion: SCAD is not a metabolic disease, does not meet NBS criteria, and should be disregarded when discovered coincidentally during laboratory evaluations.

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Basal ganglia involvement in mitochondrial acetoacetyl-CoA thiolase deficiency

Paquay S[1], De Lonlay P[2], Dobbelaere D[3], Fouilhoux A[4], Guffon N[4], Labarthe F[5], Mention K[3], Touati G[6], Valayannopoulos V[2], Vianey-Saban C[4], Schiff M[1]

[1]Div Metab Dis, Robert Debré, APHP, Paris, France, [2]Div Metab Dis, Necker, APHP, Paris, France, [3]Div Metab Dis, CHU, Lille, France, [4]Div Metab Dis, CHU, Lyon, France, [5]Div Metab Dis, CHRU, Tours, France, [6]Div Metab Dis, CHU, Toulouse, France

Background & Objectives: Mitochondrial acetoacetyl-CoA thiolase (T2) deficiency affects both ketone bodies and isoleucine catabolism. Neurological impairment can occur as a result from ketoacidotic episodes. However, it has been observed without ketoacidotic events in two T2-deficient families from our hospital. We reviewed the French cohort to document this neurological involvement.
Methods: Twenty six cases were retrospectively analyzed. Clinical, biological and radiological data were collected.
Results: Neurological signs were observed in 6/26 patients: 2 had never experienced ketoacidotic episodes and developed extrapyramidal signs with putamen involvement, 2 developed neurological abnormalities before the first ketoacidotic crisis and putamen was involved in one case, 1 developed extryramidal symptoms more than 10 years after the initial decompensation with involvement of pallidi and 1 experienced extrapyramidal signs with lesions in putamen immediately after a severe ketoacidotic episode.
Conclusion: Due to the key role of T2 in isoleucine catabolism, T2-deficient patients are exposed to accumulation of toxic isoleucine-derived acyl-CoA esters in brain mitochondria suggesting that T2 deficiency should be reconsidered in clinical practice not only as a ketolysis defect but also as an organic aciduria giving rise to progressive and chronic intoxication. The impact of protein restriction as a preventive effect on neurological symptoms remains to be studied.

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Fatty acid oxidation flux data from 304 symptomatic patients diagnosed with a range of fatty acid oxidation disorders facilitates the prediction of phenotype in screen positive babies from Newborn Screening programs

Olpin S E[1], Webb J M[1], Clark S[1], Dalley J[1], Hind H[1], Croft J[1], Colyer S[1], Manning N[1], Scott C[1], Kirk R[2], Bonham J[1], Dowling M[1], Yap S[3], Glamuzina E[4], Sharrard M[3]

[1]Dept Clin Chem, Sheffield Child Hosp, Sheffield, United Kingdom, [2]Sheff Diag Gen Service, Sheff Child Hosp, Sheffield, United Kingdom, [3]Dept Inher Metab Med, Sheff Child Hosp, Sheffield, United Kingdom, [4]National Metab Serv, Starship Child Hosp, Auckland, New Zealand

Background: Newborn screening programs are used widely to detect fatty acid oxidation disorders FAOD. Most screen positive patients are well at diagnosis. Biochemical and molecular findings are in a significant number of cases insufficient to either confirm/ exclude a diagnosis or fail to predict the likely phenotype. This has important implications for treatment and parental counselling. As a consequence functional assays remain as a vital tool.
Method: We have confirmed by specific enzyme assay and/or molecular analysis 304 clinically presenting patients spanning 9 different fatty acid oxidation disorders. Fibroblast FAO flux data using substates [9,10-H^3]myristate, palmitate and oleate are available for each patient. On analysis characteristic disease patterns emerge. Using age, clinical outcome, biochemical features, and molecular data we have grouped patients into phenotypes for each disorder.
Results: Our data is expressed as bar graphs representing % residual flux compared to controls for each FAOD e.g. palmitate flux for severe neonatal CPT2 (n=5) vs infantile CPT2 (n=7) vs myopathic CPT2 (n=36) is 4.2±3.5, vs 13.4±9.4, vs 60.5±28.8, respectively. Similarly for neonatal MADD (32) vs infantile/childhood MADD (15) palmitate is 6±4.1 vs. 34.4±15.6. Fibroblast flux results from specific screen positive "difficult to predict" patients are characterised by comparing them with our established flux data.

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Accumulation of long-chain acylcarnitines impairs lung function by inhibiting pulmonary surfactant

Otsubo C[1], Bharathi S[1], Uppala R[1], McHugh K[1], Ilkayeva O[2], Wang D[2], Zou Y[3], Wang J[1], Alcorn J[1], Zuo Y[3], Hirschey M[2], Vockley J[1], Goetzman E[1]

[1]Dep Ped, Univ Pitt Sch Med, Pittsburgh, United States, [2]SWS Nutr & Metab Ctr, Duke Univ Med Ctr, Durham, United States, [3]Dep Mech Eng, Univ HI, Honolulu, United States

Background: We previously observed reduced lung function in mice lacking the fatty acid oxidation enzyme long-chain acyl-CoA dehydrogenase (LCAD), but the mechanism behind these changes remain unknown. Here, we tested the hypothesis that accumulation of acylcarnitines may inhibit lung function.
Methods: Bronchoalveolar lavage fluid from $LCAD^{-/-}$ mice was subjected to acylcarnitine profiling by mass spectrometry. Acylcarnitine secretion was manipulated by using the drug mildronate to render the mice carnitine-deficient. Lung function was measured with a small animal ventilator. The effect of acylcarnitines on surfactant was studied *in vitro* with a constrained-drop surfactometer.
Results: Long-chain acylcarnitines were elevated several-fold in lavage fluid from $LCAD^{-/-}$ mice while short-chain species were reduced. Acylcarnitines greater than 12 carbons long were observed to co-localize with pulmonary surfactant isolated from mouse lavage fluid. *In vitro*, palmitoylcarnitine directly inhibited the surface adsorption of pulmonary surfactant as well as its ability to reduce bubble surface tension during dynamic cycling on a surfactometer. Treating $LCAD^{-/-}$ mice with mildronate eliminated acylcarnitines in bronchoalveolar lavage fluid and improved lung function.
Conclusion: Long-chain acylcarnitines inhibit pulmonary surfactant, thereby reducing lung function in the $LCAD^{-/-}$ mouse.

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Correlation between genotype and residual enzyme activity: does this concept work in VLCAD-deficiency?

Tucci S[1], Braun C[1], Stein U[1], Behringer S[1], Spiekerkoetter U[1]
[1]Div Metab Dis, Univ Child Hosp, Freiburg, Germany

Background: Newborn screening programs allow the identification of a constantly increasing number of patients with VLCAD-deficiency. A phenotype-genotype correlation has been proposed in the past with null and missense mutations. However, clinical characterization of siblings carrying the same mutations demonstrates heterogeneous clinical phenotypes also within the same family.
Results: We here report the results of the VLCAD enzyme testing performed in 200 samples from newborns identified by screening in the last 16 months. 22 out of 200 individuals showed a residual activity in the range 0% to 20% confirming VLCAD-deficiency. A high degree of correlation between residual enzyme activity and genotype was found. Homozygous patients for the mutation c.1376G>A/c.1376G>A (n=3) displayed a median residual activity of 4.7%±0.47 of healthy controls. When this mutation was combined with the c.848T>C mutation the residual activity increased up to 9.5%±0.91. Patients homozygous for c.848T>C/c.848T>C (n=4) showed an even higher residual activity with 12.3%±0.47. Similar correlations have been found also in other ranges of residual activity, although the number of individuals in the groups is still low.
Conclusion: We speculate that there is a high degree of correlation genotype-residual enzyme activity. However, the phenotype may differ since environmental factors or other modifiers can affect the development of the disease.

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Hyperprolinemia in primary and secondary MAD deficiencies

Pontoizeau C[1 2], Habarou F[1 2 3], Brassier A[1], Grisel C[1], Arnoux J B[1], Barouki R[1 2 3], Chadefaux-Vekemans B[1 3 4], Acquaviva C[4], De Lonlay P[1], Ottolenghi C[1 2 3]

[1]Metab Dis Ref Center, Necker Hosp, APHP, Paris, France, [2]Metab Biochem Lab, Necker Hosp, APHP, Paris, France, [3]INSERM UMR-S 1124, Univ Paris Descartes, Paris, France, [4]Metab Biochem Lyon University Hospital, Lyon, France

Background and objectives: Early studies reported that hyperprolinemia is common in classical neonatal-onset multiple acyl-CoA dehydrogenase deficiency (MADD). We investigated here the potential clinical interest of amino acid analyses during severe neonatal onset of primary MADD and of secondary dysfunction of acyl-CoA dehydrogenases presumably resulting from deficiency for riboflavin transporters or iatrogenic riboflavin deficiency.
Patients: Nine newborns with biochemical features consistent with MADD were identified in the last decade, five of whom died during the neonatal period. Two premature newborns on parenteral nutrition probably suffered from iatrogenic riboflavin deficiency. Mutations in genes encoding ETF or ETF-QO flavoproteins were detected in four patients, whereas the remaining three patients presumably had secondary deficiencies of unknown mechanism.

Results: Hyperprolinemia was observed in 6/7 newborns tested for plasma amino acids and in one case suggested the diagnosis as the initial diagnostic workup did not include organic acids and acylcarnitines profiling. The proline/alanine ratio may be a good marker of MADD as suggested by the analysis of our full cohort of >50000 samples from >30000 patients.
Discussion and conclusion: Plasma amino acid analysis with the proline/alanine ratio could contribute to a more effective management of primary or secondary MADD, in particular of treatable forms.

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Phospholipids remodelling in liver of a long chain acylCoA dehydrogenase mouse model

Imbard A[1 2], Goetzman E[4], Thiemele A[1 2], Otsubo C[4], Schlemmer D[1], Schiff M[3], Vockley J[4], Benoist J F[1 2]

[1]Biochem Lab, RDB Hosp, APHP, Paris, France, [2]Paris Sud Univ, Chatenay Malabry, France, [3]Ref Cent for Metab Dis, RDB Hosp, Paris, France, [4]Div of Med Genet, Child Hosp, Pittsburg, United States

Background and objectives: Long chain acyl-CoA dehydrogenase (LCAD) is the first step of long chain fatty acids mitochondrial ß-oxidation in mice. Liver steatosis has been reported in various ß-oxidation defects including LCAD deficiency and has recently been related to phospholipids (PLs) metabolism abnormalities. Docosahexaenoic acid (DHA) supplementation especially as DHA-PLs has been showed to improve liver steatosis. We hypothesized that LCAD could interfere with the PLs and DHA metabolism in the liver.
Materials and methods: Total lipids were extracted by Folch method from LCAD -/- mice liver homogenates and controls (WT). PLs profile by ESI-LC-MS/MS and fatty acid methyl ester (FAME) measurement by GC-MS were performed.
Results: There were no differences in total or polar FAME concentrations including for DHA but PLs profile revealed qualitative differences in LCAD -/- compared to WT. Specifically, PLs profile showed the decrease of some phosphatidylcholine, phosphatidylethanolamine and phosphatidylglycerol species containing docosahexaenoic acid (DHA) (eg. PC 16:0-22:6, PE 16:0-22:6, PE 18:0-22:6…).
Discussion/conclusion: These are the first data showing an abnormal DHA distribution among PLs in LCAD deficiency, which could be involved in the clinical phenotype.

P-330

Biochemical and molecular study of carnitine-acylcarnitine translocase deficiency in a neonate with hyperammonemic encephalopathy

Leong H Y[1 5], Ngu L H[1], Abd Azize N A[2], Yakob Y[2], Habib A[3], Md Yunus Z[3], Catchpole A[4], Yap S[5], Olpin S[4]

[1]Genet Dept, Kuala Lumpur Hosp, Kuala Lumpur, Malaysia, [2]Molec Diag Prot Unit, Inst Med Res, Kuala Lumpur, Malaysia, [3]Biochem Unit, Inst Med Res, Kuala Lumpur, Malaysia, [4]Dept of Clin Chem, Sheffield Child Hosp, Sheffield, United Kingdom, [5]IMD Dept, Sheffield Child Hosp, Sheffield, United Kingdom

Background: Carnitine-acylcarnitine translocase (CACT) deficiency is a rare autosomal recessive disorder of long chain fatty acid transport at the mitochondrial inner membrane. We report a Malaysian patient who presented with hyperammonemic encephalopathy.
Case Report: A female term infant presented at day 3 with encephalopathy, seizures, hypothermia and hyperammonemia (342 μmol/L). Her parents were consanguineous of indigenous Bajau ethnic group and four older siblings died in early infancy. Investigations showed increased C16 (6.91 μmol/L; ref 0.2–5.5) and C16+C18/C2 ratio with low normal free carnitine and dicarboxylic aciduria. Fibroblast fatty acid oxidation studies showed marked reduced activity compared to controls, [9,10^{3}H]-myristate 6%, [9,10^{3}H]-palmitate 5% and [9,10^{3}H]-oleate 5%, with upregulation of octanoate oxidation [2,2,3,3^{3}H] to 208%. CACT activity was markedly reduced, 0.011 nmol/mg protein/min (normal control 0.485 ± 0.097). *SLC25A20* gene analysis revealed homozygous mutation c.199-10T>G. She was started on low fat high carbohydrate diet but she died at 2½ months old.
Conclusion: Homozygozity for the splicing mutation c.199-10T>G was previously associated with a severe clinical phenotype. This mutation resulted in a severe impairment of the CACT enzyme activity and mitochondrial fatty acid oxidation in our patient.

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Characterizing the molecular architecture of mitochondrial energy metabolism

Vockley J[1], Wang Y[1], Palmfeldt J[2], Zeng X[1], Yates N[1], Gregersen N[2]

[1]Univ. Pittsburgh, Medical Genetics, Pittsburgh, United States, [2]Åarhus University, Åarhus, Denmark

Background: Efficient cellular function demands coordination of multiple physiologic processes. Metabolic channeling in protein complexes allows multiple enzymatic reactions to occur without release of intermediates, increasing metabolic efficiency. Mitochondrial energy metabolism is comprised of three major biochemical pathways: oxidative phosphorylation (OXPHOS), fatty acid oxidation, and the tricarboxylic acid cycle. OXPHOS is organized into functional enzymatic complexes that in turn assume higher order structures called super complexes.
Method: We have used 2D western blots, proteomic techniques, and immune-electron microscopy studies to show that fatty acid oxidation proteins physically interact with each other and OXPHOS super complexes.
Results: The mitochondrial trifunctional protein, an NADH generating enzyme, directly interacts with complex I matrix NADH binding domain, as well as with very long chain acyl-CoA dehydrogenase of fatty acid oxidation. Electron transfer flavoprotein dehydrogenase, which funnels reducing equivalents from acyl-CoA dehydrogenases in fatty acid oxidation to OXPHOS chain complex III, was found to interact with several subunits of complex III and complex I, consistent with the known interaction of these two enzymes in super complexes.
Conclusion: Our results provide a novel first look at the molecular architecture of mitochondrial energy metabolism and its potential impact on disease.

P-332

Thermolability of fat oxidation flux in a medium chain acyl-CoA dehydrogenase deficient (MCADD) patient homozygous for the c.199T>C mutation

Hulley S L[1], Olpin S E[2], Clarke S[2], Kirk R[3], Downing M[4], Sharrard M J[1]

[1]Paediatric med, Sheffield Child Hosp, Sheffield, United Kingdom, [2]Clin Chem, Sheffield Child Hosp, Sheffield, United Kingdom, [3]Sheffield Diagnostic Genetics Service, Sheffield, United Kingdom, [4]Newborn Screening, Sheffield Child Hosp, Sheffield, United Kingdom

Background: Newborn screening programmes for MCADD have identified individuals with previously unreported genotypes. Fat oxidation studies in cultured fibroblasts are used to evaluate the significance of these changes to assist in making a diagnosis of MCADD or confirming normality.
Case report: A healthy term baby breast feeding well was screened for MCADD on day 6 of life. The initial dried blood spot (DBS) octanoyl carnitine (C8) was 0.56 μmol/L (< 0.5) with a C8:C10 ratio of 0.93 (< 1.0). Day 12 samples when the baby was well showed a DBS C8 of 0.38 μmol/L (< 0.3) and plasma C8 of 0.67 μmol/L (< 0.22) and acylcarnitine profile consistent with MCADD. Urine hexanoylglycine was 3.4 mmol/mmol creat, the MCADD range being >3.4. *ACADM* sequencing detected homozygosity for the c.199T>C variant. Fat oxidation flux in fibroblasts was normal at 37°C but reduced at 41°C: myristate oxidation 52%, palmitate 44%, oleate 67%, consistent with a diagnosis of MCADD.
Conclusion: Persistent abnormal biochemistry is associated with normal fat oxidation at 37° in the previously unreported homozygous *ACADM* c.199T>C individual. Abnormal fat oxidation consistent with MCADD is found at 41°C emphasising the importance of determining temperature sensitivity in unreported genotypes.

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Report of five Turkish patients with ketolysis defects and four novel mutations

Onenli Mungan N[1], Şeker Yılmaz B[1], Kör D[1], Bulut D[1], Ökten M[2], Yıldızdaş D[1], Ceylaner S[3], Fukao T[4]

[1]Div Metab Dis, Univ Cukurova, Adana, Turkey, [2]Duzen Laboratory, Ankara, Turkey, [3]Intergen Genetics Laboratory, Ankara, Turkey, [4]Dept Pediatr, Grad Sch Med, Gifu Univ, Gifu, Japan

Background and Objectives: Succinyl-CoA-3-oxoacid CoA transferase(SCOT) and beta-ketothiolase(T2) deficiencies are two defects in ketolysis. The clinical picture mimics organic acidurias with attacks of severe ketoacidosis, accompanied with loss of conscious and dehydration.
Patients and Methods: The age of first symptom was 1-9 months. The most frequent signs-symptoms were attacks of severe ketoacidosis, dehydration and change in conscious after a mild infection/gastroenteritis. Four of them were diagnosed as organic aciduria before admission and were on protein-restricted diet. Parental consanguinity was positive in four families. Six siblings from three families died with same symptoms without a diagnosis. Patients had 2-10 attacks before and 1-3 attacks after the specific diagnosis. The age of diagnosis was 8-42 months. Need for invasive therapeutic approaches was 12 for 3 patients. The patient with SCOT deficiency had the most severe clinic. Mutation analysis revealed four homozygous novel mutations p.C1265(c.370T>A), p.G53E(c.158G>A), IVS4+1G>A, a known homozygous mutation IVS11+2T>XC(c.1163+2T>C in ACAT1 gene, and a novel mutation p.G422V in OXCT1 gene. Parents are heterozygous for mutations. Except one patient none of them had neurological impairment.
Conclusion: Here, we report these cases as four novel mutations detected and to emphasize the importance of thinking ketolysis defects in differential diagnosis of organic acidurias.

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Serum C14:1/C12:1 ratio is a sensitive diagnostic marker for VLCAD deficiency

Yamada K[1], Bo R[1 2], Kobayashi H[1], Hasegawa Y[1], Yamaguchi S[1]

[1]Dept of Pediatr, Shimane Univ, Izumo, Japan, [2]Kobe Univ Grad Sch of Med, Kobe, Japan

Background: C14:1 acylcarnitine (AC) is a diagnostic marker for very-long chain acyl-CoA dehydrogenase deficiency (VLCADD) in newborn mass screening using tandem mass spectrometry, but there are a considerable number of false positive cases. It is often difficult to distinguish the milder form of VLCADD from the carrier. We report that the ratio of C14:1 to C12:1 (C14:1/C12:1) in serum is highly sensitive for detection of VLCADD.
Materials and Methods: Cs in dried blood spot (DBS) and serum from 24 patients with VLCADD and 13 carriers were analyzed after butyl-derivatization.
Results: C14:1/C12:1 of DBS in 4 of 24 patients was below the cutoff (< 4.0), while 2 of 13 carriers exceeded the cutoff. The above 4 patients were non-symptomatic cases. In serum, C14:1/C12:1 in 2 of 24 patients was under cutoff, however, both these patients showed the ratio was above the cutoff in re-examination.
Discussion: Serum C14:1/C12:1 can be a sensitive diagnostic marker for VLCADD, while an overlap of the ratio was seen in DBS. Previously, usefulness of serum C14:1/C12 was reported, but it has not been complete. False positive for VLCADD by the elevation of C14:1 is most likely to be prevented by combination use of C14:1/C12:1.

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Autosomal dominant monocarboxylate transporter-1 (MCT1, *SLC16A1*) deficiency as a cause of recurrent ketoacidoses

Sass J O[1], Lewis B[2], Greed L[2], Meili D[5], Flier A[5], Yamamoto R[6], Bilić K[7], Till C[1], Balasubramaniam S[3 4]

[1]Bonn-Rhein-Sieg Univ Appl Sciences, Rheinbach, Germany, [2]Princess Margaret Hosp, PathWest Lab WA, Perth, Australia, [3]Princess Margaret Hosp, Metab Unit, Perth, Australia, [4]Univ West Australia, Paed Child Health, Perth, Australia, [5]Univ Child Hosp, Clin Chem Biochem, Zürich, Switzerland, [6]MVZ Dr Eberhard & Partner, Dortmund, Germany, [7]Univ Hosp Ctr Zagreb,Clin Inst Lab Diagn, Zagreb, Croatia

Background: We describe two half-siblings with autosomal dominant monocarboxylate transporter-1 (MCT1, *SLC16A1*) deficiency, which has only recently been identified (van Hasselt et al. NEJM 2014; 371:1900-7) as a novel cause for recurrent ketoacidoses.
Case report: Our index patient was a boy with non-consanguineous British parents. He had presented acutely with severe metabolic acidosis and impaired consciousness following a 3 day history of gastroenteritis with ketoacidosis at age 5 years. A 12.5 year-old half-brother who shared the proband's mother also had a previous history of recurrent ketoacidoses.
Results: Results of mutation and enzyme activity analyses in proband samples advocated against beta-ketothiolase and SCOT deficiencies. A single heterozygous c.982C>T transition in the *SLC16A1* gene resulting in an Arg328-to-Ter (p.R328X) substitution was detected in both boys. It was shared by their healthy mother and by the proband's half-sister, but was absent in the proband's father. Western blot analysis suggested a decreased amount of MCT1 protein in the proband's fibroblasts.
Conclusion and Discussion: MCT1 may be more prevalent than is apparent and is an important differential diagnosis in recurrent ketoacidosis with or without hypoglycemia. Clinical severity in the cohort of patients reported by van Hasselt et al showed correlations with mutational status (homozygous *versus* heterozygous). Early diagnosis may enable improved disease management.

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Clinical and genetic investigation of Japanese 16 patients with trifunctional protein deficiency

Bo R[1], Purevsuren J[1], Fukao T[2], Yamada K[1], Kobayashi H[1], Hasegawa Y[1], Yamaguchi S[1]

[1]Department of Pediatrics, Shimane Univ, Izumo, Shimane pref, Japan, [2]Department of Pediatrics, Gifu Univ, Gifu, Gifu pref, Japan

Background: Trifunctional protein (TFP), consisted of hetero-octamer of alfa (TFP-α)and beta (TFP-β) subunits, plays a significant role of the last 3 steps of long-chain fatty acid oxidation cycle. TFP deficiency is a target of the expanded neonatal screening in Japan, but the clinical and genetic aspects have not been characterized sufficiently in Japanese patients. We investigated the clinical and genetic features in 16 Japanese patients with TFP deficiency.
Patients and Methods: 16 Japanese patients in 14 families, all of whom were detected based on acylcarnitine measurement and gene analysis during the period between 2001 and 2015, were investigated.

Results and Discussion: TFP-β deficiency was identified in 13 of the 16 cases, suggesting that TFP-β deficiency is predominant in Japan. In contrast, LCHAD deficiency (1528G>C), common in Caucasian, was not identified. There were 7 cases with neonatal form (44%), 2 with infantile form (12%), and 7 with myopathic form (44%). In the 2 cases, HELLP syndrome and acute fatty liver of pregnancy were observed in their respective mother. Gene mutations were heterogeneous. Sibling cases with identical genotype showed similar age at onset and clinical course, suggesting presence of genotype/phenotype correlation.

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Does lipoic acid alleviate pathophysiology in medium-chain acyl-CoA dehydrogenase deficiency?

Nochi Z[1], Birkler R I D[1], Olsen R K J[1], Corydon T J[2], Gregersen N[1]

[1]Dept Clin Med, Aarhus University, Aarhus, Denmark, [2]Dept Biomed, Aarhus University, Aarhus, Denmark

Background: Medium-chain acyl-CoA dehydrogenase deficiency (MCADD) is the most frequent fatty acid oxidation (FAO) defect. MCAD deficient patient fibroblasts are more resistant to oxidative stress than other FAO defects. The enzymatic defect results in accumulation of octanoic acid, the precursor of lipoic acid (LA). Therefore LA increment and its protective role in MCADD are hypothesized. LA is an essential cofactor of E2 components of pyruvate (PDH), 2-oxoglutarate (OGDH) and branched-chain alpha-keto acid dehydrogenase (BCKAD) complexes.
Method: The mRNA expression, total and lipoylated protein content of LA-dependent enzymes were determined in fibroblasts from 3 MCAD deficient patients (c.985A>G/c.985A>G) and 3 controls.
Results: Patient fibroblasts displayed significantly increased PDH-E2 and lipoylated-PDH-E2 protein levels compared to healthy fibroblasts. E2 subunits of both OGDH and BCKAD complexes showed no significant difference of protein content in patient and healthy fibroblasts. There was no significant difference between patient fibroblasts and healthy controls at the mRNA level of the E2 components.
Conclusion: Preliminary results could suggest that accumulated octanoic acid in MCAD deficiency increases the level of LA, keeping the PDH-E2 at the steady-state level, facilitating citric acid cycle to produce more acetyl-CoA from pyruvate, and thus alleviating the bottleneck in FAO and limiting the devastating production of

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Molecular characterization of new *ACADS* gene alterations in SCADD patients

Tonin R[1 2], Caciotti A[1], Funghini S[3], Pasquini E[4], Procopio E[4], Donati M A[4], Mooney S D[5], Baronio F[6], Bettocchi I[6], Cassio A[6], Biasucci G[7], Bordugo A[8], Guerrini R[1 2], Morrone A[1 2]

[1]Clin Paed Neurol & Lab, Meyer Child Hosp, Florence, Italy, [2]Dept of Neurofarba, Univ of Florence, Florence, Italy, [3]Newborn Screen Biochem & Pharm Lab, Meyer Child Hosp, Florence, Italy, [4]Metabolic Unit, Meyer Child Hosp, Florence, Italy, [5]Dept of Biomed Informatics and Med Educ, Univ of Washington, Seattle, United States, [6]Dept of Paed, Univ of Bologna, Bologna, Italy, [7]Dept of Paed and Neonat, Piacenza Hosp, Piacenza, Italy, [8]Dept of Paed and Inher Met Dis Unit, AOUI Verona, Verona, Italy

Background: Short-chain acyl-coA dehydrogenase deficiency (SCADD) is an autosomal recessive disorder of mitochondrial fatty acid oxidation caused by mutations in the *ACADS* gene. SCADD is a heterogeneous condition that has been associated with various clinical phenotypes ranging from fetal metabolic decompensation in infancy to asymptomatic individuals.
Methods: Here we report the characterization of the *ACADS* gene in 80 patients with mild to elevated increased levels of plasmatic C4-acylcarnitine and of urinary ethylmalonic acid.
Results: We identified 13 new variants: 8 missense, 2 nonsense, 1 deletion, 1 splicing and a silent variant (p.Gly255Gly) that was proven to cause a splicing error. Phenotype-genotype correlations of the new missense variants were assessed by computational analysis based on phylogenetic conservation of amino acids and functional algorithms. In silico data and RNA studies demonstrated that this silent variant leads to a disease causing allele.
Conclusions: We would like to stress the importance of the accurate characterization of patients at both molecular and biochemical level. We underline that silent variants have to be further investigated in the concept of mRNA evaluation/quantization to provide accurate genotype-phenotype correlations in SCADD patients.

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An attempt to rescue medium-chain acyl-CoA dehydrogenase (MCAD) disease-causing variants by small molecules

Nunes J[1], Palir N[1], Bonito C A[1 2 3 4], Louro F[1], Camilo J[1], Leandro P[1 2], Ventura F V[1 2]

[1]Dep Biochem Hum Biol, Fac Pharm, ULisboa, Lisboa, Portugal, [2]Met&Gen, iMed.ULisboa, Lisboa, Portugal, [3]Dep Pharm Chem Ther, Fac Pharm, ULisboa, Lisboa, Portugal, [4]Medicinal Chemistry, iMed.ULisboa, Lisboa, Portugal

Background: Medium-chain acyl-CoA dehydrogenase deficiency (MCADD, OMIM 201450) is the most frequent inborn error of mitochondrial fatty acid beta-oxidation. No pharmacological strategies are available for MCADD treatment and the recommended preventive measures are sometimes ineffective, reflecting the urgency for novel therapeutic approaches. MCADD results from the impairment of MCAD (EC 1.3.8.7), a homotetramer flavoprotein with affinity for C6-C10 fatty acids. Most patients carry the common missense mutation p.K304E (c.985A>G). This and many other *hMCAD* variants have been associated with protein misfolding. MCADD can thus be considered a conformational disorder with loss of function and a target for small molecules acting as pharmacological chaperones.
Method: Using various recombinant *hMCAD* variants, we tested the potential stabilizing effect of FAD and some osmolytes (glycerol, TMAO, arginine and taurine) by differential scanning fluorimetry. FAD increased the thermostabiliy of most of the proteins tested, including the wild-type (ΔTm: 7-27°C).
Results: Glycerol and TMAO had contradictory effects: stabilization of p.K304E but destabilization of the p.A140T, p.Y372N and p.G377V proteins. Arginine also induced instability in many *hMCAD* variants including p.K304E.
Conclusion: These preliminary studies may contribute to the understanding of the mechanisms underlying *in vitro* rescue of MCAD misfolded proteins guiding the design of small molecules with potential interest for MCADD treatment.

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The value of combined biochemical and genetic testing in suspected SCAD deficiency; case reports of three families

Kirk R J[1], Leong H Y[3], Croft J M[2], Gregersen N[4], Watkinson J[2], Nesbitt I M[1], Olpin S E[2], Yap S[3]

[1]Sheff Diag Genet Serv, Sheff Child Hosp, Sheffield, United Kingdom, [2]Dept Clin Chem, Sheff Child Hosp, Sheffield, United Kingdom, [3]Dept Inherit Metab Dis, Sheff Child Hosp, Sheffield, United Kingdom, [4]Res Unit Mol Med, Aarhus Univ Hosp, Aarhus, Denmark

Background: Short-chain acyl-CoA dehydrogenase (SCAD) deficiency is a mitochondrial fatty acid oxidation disorder. Its clinical relevance and the relationships between clinical phenotype, environmental factors, biochemical findings and genetic status are still unclear.
Case reports: Case 1 presented with cardiac arrest but otherwise was developmentally normal. Case 2 displayed developmental delay, hearing loss and failure to thrive. Case 3 had an undiagnosed syndrome of kinky hair, peg-like teeth, developmental delay and failure to thrive.
Methods: Urine organic acid and plasma acylcarnitine analyses were carried out on index cases and other family members. Sequencing of the *ACADS* gene was carried out using either next generation sequencing, as part of a fatty acid/ketone body metabolism panel, or Sanger sequencing.
Results: In index cases and relatives with raised ethylmalonic acid (EMA) and C4, those with two severe mutations showed higher levels than those who were compound heterozygous or heterozygous for a severe mutation and/or the common c.625G>A variant. Unexpectedly, the asymptomatic mother in case 3 showed levels above those expected of a heterozygous carrier, prompting full sequencing; two severe mutations were detected.
Conclusion: Biochemical and genetic testing together provide the optimal evaluation of individuals and families with suspected SCAD deficiency.

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Carnitine palmitoyltransferase 1A deficiency associated with acute tubular necrosis, hepatic failure and loss of acquired functions after the H1N1 infection

Ersoy M[1], Bursal Duramaz B[2], Yesilbas O[3], Kıhtır H S[3], Kıhtır H S[3], Sevketoglu E[3]

[1]Child Metab Dis,Sadi Konuk Res&Tra Hosp, Istanbul, Turkey, [2]Depart Pedia. Sadi Konuk Res&Tra Hosp, Istanbul, Turkey, [3]Div Ped İnten Car, Sadi Konuk Res&Tra Ho, Istanbul, Turkey

Background and objectives: Carnitine palmitoyltransferase 1A (CPT1A) is required for the import of fatty acids into the matrix in the liver and kidney. CPT1A deficiency presents with a variety of symptoms including hypoketotic hypoglycemia, Reye-like syndrome and acute renal failure. Cases can show atypical presentations which haven't been elucidated yet.
Case Report: A 10-year-old girl admitted with vomiting and lethargy proceeding the flu symptoms. Her elder sister died with hepatic failure at the age of 8-month-old. At the age of three, she had a Reye-like syndrome episode. She had hepatomegaly. Glycose was 34 mg/dl, transaninases were elevated

(AST:8599, ALT:6294 IU/L), ammonium was 263 mcg/dl (n< 60), lactate was 12 mmol/L (n< 2,2) and ketone was negative. INR was 3,3 (n:0,8-1,2). Hemofiltration and plasmapheresis was started. Abdominal ultrasound showed hepatosteatosis. H1N1 was positive in tracheal aspirates. Carnitine/acylcarnitine profile at the metabolic attack was consistent with CPT1A deficiency [C0/C16+C18=84 (N< 70)]. Despite the proper therapy conjugated was elevated continuously. Renal biopsy revealed acute tubuler necrosis. At the end of 3-weeks,liver and renal function tests were normalized. She lost of her ability of sitting without support.
Discussion: CPT1A deficiency can have atypical manifestations as not seen in other fatty acid oxidation disorders.

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Acylcarnitine isomers differentiation to help in beta-oxidation disorders diagnosis

Jeoual A[1 3], Moreau C[1], Dobbelaere D[2 3], Dessein A F[2 3], Mention K[2 3], Deprez B[1 4], Balduyck M[2 3], Briand G[2 3], Beghyn T[1]

[1]Apteeus, Lille, France, [2]CRMHM-CHRU, Lille, France, [3]RADEME - Université de Lille, Lille, France, [4]U1177 Inserm - IPL - Univ Lille, Lille, France

Introduction The diagnosis of β-oxidation disorders is based on the abnormal fatty acid profile that occurs in the organism. The usual method for analyzing carnitine and acylcarnitines in plasma or dried blood spots, uses butyl ester formation and tandem mass spectrometry. Among detected molecules, some are isomers or isobaric contaminants that are not differentiated from each other. This lack of specificity can lead to false positive or misdiagnosis.
Methods We developed a method based on C18 reverse chromatography coupled to mass spectrometry in MRM mode in order to differentiate isomers and isobaric contaminants for a more precise diagnosis. The chromatographic gradient, using water and acetonitrile with 0,1% formic acid, has been optimized to obtain the best retention conditions of all isomers from C0 to C20 acylcarnitine. Some unavailable isomers have been synthesized and characterized.
Results As an example, the isovaleryl carnitine, stemming from the degradation of leucine, can be differentiated from its exogenous isomer valeryl carnitine. To date, we characterize 54 acylcarnitines in a 3 hour-protocol including 14 minutes of analysis.
Conclusion The new method is being validated using plasma, dried blood spots and even fibroblast lysates from patients. This new methodology should help in case of uncertainty in the diagnosis.

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OXCT1 heterozygous carriers could develop severe ketoacidotic episodes in conjunction with ketogenic stresses

Sasai H[1], Aoyama Y[2], Ohtsuka H[1], Ohara O[3], Fukao T[1]

[1]Dep of Pediatr, Gifu Univ, Gifu, Japan, [2]Dept Biomed Sci, Chubu Univ, Kasugai, Japan, [3]Kazusa DNA Res Inst, Kisarazu, Japan

Background: *OXCT1* deficiency is an autosomal recessive disorder in ketone body utilization and results in severe recurrent ketoacidotic episodes in infancy including neonatal periods. More than 30 patients have been reported and to our knowledge, their heterozygous parents and siblings have had no apparent ketoacidotic episodes.
Results: In the 5 years (2008 – 2012), we found four patients with only heterozygous *OXCT1* mutation among patients who had severe ketoacidosis and were suspected as having *OXCT1* deficiency. MLPA analysis excluded the presence of large deletions and insertions in another allele. The possibility that heterozygous *OXCT1* mutation caused ketoacidotic episodes was raised. Therefore, in these two years (2013 – 2014), we analyzed *OXCT1* mutations in 6 patients who presented severe ketoacidosis (blood pH < 7.25 and TKB (total ketone body) >10 mmol/L) following ketogenic stresses such as gastroenteritis. Their urinary organic acid analysis showed non-specific pattern. Heterozygous *OXCT1* mutations were found in two of six cases.
Discussion and Conclusion: Heterozygous *Oxct1*-knockout mice were reported to show higher blood TKB level than wild-type ones after 24 hours fast. Recently among patients with keto(acido)sis heterozygous *MCT1* carries were also identified. Taken together, *OXCT1* heterozygous carriers could develop severe ketoacidotic episodes in conjunction with ketogenic stresses.

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Metabolic investigation of very long chain acyl-CoA dehydrogenase deficiency

Lund M[1], Nielsen C B[2], Johannsen M[2], Gregersen N[1]

[1]Res. unit for mol. med, Aarhus Uni., Aarhus, Denmark, [2]Dept. of forensic chem., Aarhus Uni., Aarhus, Denmark

Pathogenesis from inborn fatty acid oxidation (FAO) disorders result from specific gene defects, which lead to the production of dysfunctional and unstable proteins or production of nonsense mRNA. The resultant reduction of FAO capacity may give rise to three possible interacting pathogenic factors

in FAO disorders namely: insufficient ATP production, toxicity from excessive concentrations of specific lipids and their metabolites (lipotoxicity) or cellular disturbance resulting from intracellular stress induced by misfolded proteins or nonsense mRNA. In this mass spectrometry based metabolomic investigation, cultured fibroblast cell-lines from 3 mild and 3 severe phenotype patients with very long chain acyl-CoA dehydrogenase (VLCAD) deficiency were challenged with palmitate, a C16:0 saturated long chain fatty acid, under imitated mild fasting conditions. Several metabolic markers of oxidative stress, such as oxidized glutathione, were significantly more abundant in patient fibroblasts compared to control fibroblasts without inborn metabolic disorders. Interestingly this was the case both with palmitate treatment and when cells were cultured without the addition of palmitate. This indicates that lipotoxicity may not be a primary pathogenic factor under mild fasting conditions.

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Triheptanoin lowers cardiac workload compared to medium-chain triglyceride (MCT) in patients with long-chain fatty acid oxidation (FAO) disorder

Gillingham M B[1], Harding C O[1], Goldstein A[2], El-Gharbaway A[2], McCracken E[2], Martin J[1], Vockley J[2]

[1]Oregon Health & Science University, Portland, United States, [2]University of Pittsburgh, Pittsburgh, United States

Background: Observational reports have suggested the anaplerotic substrate triheptanoin may have a therapeutic advantage over MCT for long-chain FAO disorders.
Methods**:** Subjects with CPT2, VLCAD, or LCHAD/TFP with at least one episode of rhabdomyolysis who were 7 yrs. old or greater participated. Energy expenditure, exercise tolerance, and cardiac function were measured at baseline. Subjects were randomly assigned to consume MCT or triheptanoin at 20% of their total energy needs for 4 months followed by repeating the baseline measures.
Results: Eleven subjects with CPT2, 9 subjects with VLCAD, and 12 subjects with LCHAD/TFP were enrolled; 16 subjects were randomized to each group. Most subjects had mild gastrointestinal adverse effects but no subject dropped out of the study. There was no difference in resting energy expenditure or body composition between groups. Metabolic control and CPK were also similar between groups. Subjects randomized to triheptanoin had a significantly lower heart rate for the same work performed compared to subjects randomized to MCT.
Conclusions**:** Triheptanoin lowered cardiac workload for the same work performed in a controlled treadmill exercise study. We observed no differences in the incidence of rhabdomyolysis or adverse events between groups. Funding provided by the Food and Drug Administration of the USA.

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A survey of UK usage of L-carnitine in children with medium chain acyl CoA dehydrogenase deficiency (MCADD)

Sreekantam S[1], Santra S[1], Vijay S[1]

[1]Dept Of IMD, Birmingham Children's Hosp, Birmingham, United Kingdom

Introduction: There is considerable international variation in the use of L-carnitine in MCADD with relatively few published studies to guide a consistent approach. The resulting differences in management could heighten anxiety amongst parents and families. This survey of current practice in metabolic centres in UK, aims to understand the variations in the use of L-carnitine.
Methods: A standard web-based questionnaire was sent to all UK metabolic clinicians. We analysed the responses to specific questions regarding their current practice, received between October 2014 and March 2015.
Results: 17 responses (17/22) were received from all centres in UK. 52.9% (9/17) of respondents measured plasma free carnitine concentration in MCADD; 67% (6/9) tested only symptomatic patients while the rest tested all.70% (7/10) initiated treatment in symptomatic patients; 30% (3/10) in patients with low levels. Treatment decisions were made based on measured plasma free carnitine concentration by some centres and on clinical response by others.
Conclusion: The results of this survey confirm the variation that exists in MCADD management. There is a need for robust clinical studies to support or refute the argument to use L-carnitine in MCADD. In the absence of such prospective studies, consensus guidelines are required to bring some uniformity into clinical practice.

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A case of late onset riboflavin-responsive multiple acyl-CoA dehydrogenase deficiency manifesting as recurrent vomiting

Kasapkara Ç S[1], Şenel S[2], Kılıç M[1], Acar M[2], Özbay-Hosnut F[2], Aydın H I[3], Ceylaner S[4]

[1]Div Metab Dis, Dr Sami Ulus Child Hosp, Ankara, Turkey, [2]Div Ped Unit, Dr Sami Ulus Child Hosp, Ankara, Turkey, [3]Div Metab Dis, Turgut Özal Univ Hosp, Ankara, Turkey, [4]İntergen Genetics Center, Ankara, Turkey

Background: Multiple acyl-CoA dehydrogenase deficiency (MADD), also known as glutaric aciduria type 2, is an autosomal recessive inherited organic acid disorder. MADD is caused by defects in electron transfer flavoprotein (ETF) or ETF-ubiquinone oxidoreductase (ETF-QO), which are indispensable in the final process of fatty acid oxidation, leading to impaired ATP biosynthesis from fatty acid, excessive lipid accumulation and insufficient gluconeogenesis.The clinical phenotypes of MADD have been classified into three groups, namely, neonatal onset form with (type 1) or without (type 2) congenital anomalies, and mild and/or late onset form (type 3). Mild and/or late onset cases manifest their first symptoms till early adulthood, such as intermittent vomiting, abdominal pain, hypoketotic hypoglycemia, hepato/cardiomegaly, metabolic acidosis, and/or hyperammonemia/ lactatemia, which are often preceded by general infection or a catabolic condition.
Case report: Herein, we present a case of late onset MADD characterized by episodic recurrent vomiting harboring homozygous novel mutation, c.1790C>T (p.L597P), in *ETFDH* gene with dramatic response to riboflavin.She has shown significant improvement after starting oral riboflavin supplementation
Conclusion: In case of non-ketotic hypoglycaemia with vomiting the patient should be urgently referred to a specialist in metabolic diseases.Molecular analysis is useful to confirm the diagnosis, and early diagnosis is important because riboflavin treatment has been effective in a significant number of patients with MADD.

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LCHAD deficiency diagnosed in 42-year-old woman: case report

Taybert J[1], Sykut-Cegielska J[2], Kowalik A[1], Boczoń K[4], Ołtarzewski M[2], Jabłońska E[2], Sobczyńska-Tomaszewska A[3]

[1]Metab Outpat Clinic, Inst Mother&Child, Warsaw, Poland, [2]Screening Dept, Institute Mother&Child, Warsaw, Poland, [3]Medgen, Genetic Laboratory, Warsaw, Poland, [4]Metab Outpat Clinic, Regional Hosp No2, Rzeszów, Poland

Case report: A 43-year-old woman with a long complicated medical history was diagnosed as LCHAD deficient at the age of 42 years due to a typical acylcarnitine profile in dry blood spot by MS/MS. DNA analysis showed the frequent c.1528G>C mutation on one allele in the *HADHA* gene. In her history: at 6 months of age – myocarditis, at 1.5 years – fatigability and hypoglycaemia, at 7 years – hypoglycaemic coma. Since childhood she has had recurrent episodes of hypoglycaemia, myalgia and vomiting and since 6 years of life retinopathy with progressive amblyopia. Additionally iritis was found. Biopsies revealed liver steatosis at 10 years of age and lipid myopathy at 19 years. Due to dilatated cardiomyopathy with chronic cardiac insufficiency and paroxysmal tachycardia she has been treated with several cardiac drugs. Pulmonary hypertension was diagnosed two years ago. Nine years ago she had acute episode of rhabdomyolysis followed by renal insufficiency. However she gave birth to two healthy sons. Since diagnosis was established, prevention of prolonged fasting (uncooked cornstarch in the evening), low LCT diet with MCT oil and an emergency regimen with intravenous glucose administration, were recommended.
Conclusion: The patient still has had recurrent episodes of myalgia but less frequent and severe, her quality of life has improved.

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Response to compassionate use of triheptanoin in infants with cardiomyopathy due to long chain fatty acid oxidation defects (LC-FAODs)

Vockley J[1], Charrow J[4], Ganesh J[5], Eswara M[7], Diaz G A[6], Enns G[3], Marsden D L[2]

[1]Med Genetics Child Hos UPMC, Pittsburgh, United States, [2]Ultragenyx, Novato, United States, [3]Div Medical Genetics, Stanford Univ, Stanford, United States, [4]Div Genetics, Lurie Children's Hospital, Chicago, United States, [5]Div Medical Genetics, Cooper University, Philadelphia, United States, [6]Div Med Genetics, Icahn School of Med, Mt Sinai, United States, [7]Ped and Metab Genetics, Sutter Med Ctr, Sacramento, United States

Background: Severe LC-FAODs may present in early infancy, when heart energy demands are high, with cardiomyopathy, hypoglycemia, hepatic dysfunction. Mortality is high despite standard treatment with even medium chain triglycerides (MCT). Triheptanoin, an odd medium chain triglyceride, has been shown in a mouse model to be ketogenic and also gluconeogenic, by providing anaplerotic substrates for the depleted TCA cycle.
Case Reports: Five patients (TFP, VLCAD(2), CACT, LCHAD) presented with moderate (1/5) or severe, life-threatening (4/5) cardiomyopathy in early infancy, despite maximal treatment with MCT and cardiac support (ventilation/ECMO, pressors). They were treated with triheptanoin (4g/kg) on emergency protocols (FDA eINDs). Rapid improvement occurred within 48 hours in 2 patients near death, (CACT, VLCAD), or within 2-3 weeks (LCHAD) of starting treatment. 2D-echo ejection fractions ranged from 21, 22, N/A, 39, 44% pretreatment to 71, 33, 71, 69, 53% post- treatment, respectively. The severe patients were able to be weaned from support and remain stable; the moderately severe TFP patient remains

clinically well. Adverse events were GI distress; 4 patients continue on treatment.
Conclusion: These data demonstrate the potential therapeutic benefit of triheptanoin treatment in infants with cardiomyopathy. Further studies are warranted to confirm these initial promising findings.
Conflict of Interest declared.

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First two patients with mitochondrial HMG-CoA synthase deficiency in Asia

Nakajima Y[1], Fukao T[2], Nakano Y[3], Sasai H[2], Aoyama Y[4], Kato S[3], Hasegawa Y[5], Sakai Y[6], Yoshikawa T[1], Ito T[1]

[1]Dept, Pediat, Fujita Health Univ Sch Med, Toyoake, Japan, [2]Dept Pediatr, Grad Sch Med, Gifu Univ, Gihu, Japan, [3]Dept Pediatr, Grad Sch Med, Nagoya City, Nagoya, Japan, [4]College Life & Health Sci, Chubu Univ,, Kasugai, Japan, [5]Dept Pediatr, Shimane Univ Sch Med, Izumo, Japan, [6]Aichi Health Promotion Pub Foundation, Nagoya, Japan

Background: Mitochondrial HMG-CoA synthase (mHMGCS) deficiency is a rare autosomal recessive genetic disorder characterized by hypoketotic hypoglycaemia during prolonged fasting, and has been reported in 12 patients worldwide. Here, we report the first two patients with mHMGCS deficiency in Asia.
Case report: The first patient is an 8 month old girl who presented with vomiting, hyperpnea, retraction and hypoglycaemia after 16 hours of fasting due to appetite loss. The second patient is a 6 month old boy who presented acute hypoglycaemic coma with hyperpnea following a 2-day history of gastroenteritis. In both patients, hypoketotic hypoglycemia, profound metabolic acidosis, elevated transaminases with mild hepatomegaly, and dicarboxylic aciduria with markedly increased glutaric acid excretion, were observed. Acylcarnitine profile in serum showed low free carnitine with elevated acetylcarnitine. In spite of immediate correction of hypoglycaemia, metabolic acidosis progressed (pH; 6.796, BE; -29.6mmol/L, and pH; 6.861, BE; -27.2, respectively). L-carnitine administration was started immediately. CHDF was performed within 24 hours, resulting in stabilization in 2-3 days. Both patients fully recovered and have remained well with avoidance of prolonged fasting. DNA analysis of *HMGCS2* identified c.1175C>T (p.S392L)/c.1499G>A (p.R500H) and c.1656G>A (p.G219E)/c.1498C>T (p.R500C), respectively.
Conclusion: Life-threatening metabolic acidosis with hypoketotic hypoglycemia occurred in mHMGCS deficient patients. Glucose infusion given concomitantly with L-carnitine supplementation and the early introduction of hemodiafiltration were effective.

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SCOT deficiency: the role of peritoneal dialysis in treatment of attack

Canda E[1], Köse M[1], Kağnıcı M[1], Yazıcı H[1], Kabasakal C[2], Habif S[3], Kalkan Ucar S[1], Çoker M[1]

[1]Div Metab Dis, Ege Univ Med Faculty, Izmir, Turkey, [2]Div Ped Nephrol, Ege Univ Med Faculty, İzmir, Turkey, [3]Div Clin Biochems, Ege Univ Med Faculty, İzmir, Turkey

Background: The deficiency of succinyl-CoA:3-ketoacid CoA transferase (SCOT) enzyme involved in degradation of ketone bodies is an autosomal recessively inherited congenital metabolic disease.
Case report: We presented a 19 month old male patient with SCOT deficiency who received peritoneal dialysis for acute decompensation attack.
The patient had a history of metabolic acidosis for the first time when he was 7 months old. Examination was made during acute attack; blood ketone was found 7 mmol/L and high anion gap metabolic acidosis in blood gas. The patient's urine organic acid analysis revealed a very high 3-OH-butyrate concentration (336.000 mmol/mol of creatinine). Blood spot carnitine profile was normal. Following administration of peritoneal dialysis for two days, notable decrease was observed in blood ketone with 0.3 mmol/L and urine 3-OH-butyrate with 116 mmol/mol of creatinine and full recovery was achieved in his clinical manifestations. SCOT enzyme activity was found low with 2.5 nmol/(min/mg of protein) (Normal: 5.1 - 19.1).
Conclusion: Ketolysis defects must be considered in the presence of persistent ketosis and repetitive ketoacidosis attacks. Peritoneal dialysis must be administered in patients not responding to relevant symptomatic treatment.

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HMG CoA Lyase deficiency and Kaposiform lymphangiomatosis: Bad luck or increased risk?

Chapman K A[1], Yang S[1], Cusmano-Ozog K[1]

[1]Div Genet Metab, Children's National, Washington, United States

Background: Several biochemical enzymes, especially Krebs cycle related, have been associated with rare cancers and with congenital heart and vascular malformations. To our knowledge, cancers have not been reported in individuals with 2-Hydroxy-3-methylglutaryl CoA Lyase (HMG CoA Lyase)

deficiency; however there are scattered reports of congenital heart differences. HMG CoA lyase is at the intersection of ketogenesis and leucine metabolism and can present with hypoketotic hypoglycemia at young ages.
Case Report: Here we present a now 15 year old diagnosed with HMG CoA lyase deficiency at 11 months of age by biochemical markers who at 11 years was admitted with abdominal pain and identified to have Kaposiform lymphangiomatosis. The patient had been lost to follow up for more than 6 years and no longer followed any diet recommendations.
Discussion: Studies by others in animal models demonstrate that 2-hydroxy-3-methylglutarate can deplete cytosolic anti-oxidant defenses. Although we will never know, one has to wonder whether this combination of disorders is caused by decreased anti-oxidant defenses and whether additional patients are at risk.

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Neuropsychological outcome in patients with long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency

Strandqvist A[1 2], Bieneck Haglind C[1 2], Zetterström R H[1 2], Nemeth A[1 2], Von Döbeln U[1 2], Halldin Stenlid M[3], Nordenström A[1 2]

[1]Karolinska Institutet, Stockholm, Sweden, [2]Karolinska University Hospital, Stockholm, Sweden, [3]University Children's Hospital, Uppsala, Sweden

Background: Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency is a defect in mitochondrial beta-oxidation of long chain fatty acids. There are only few reports on cognitive outcome due to the rarity of the disease and to high morbidity and mortality among the patients. We assessed neuropsychological outcome for eight patients with LCHAD in relation to clinical disease severity in patients diagnosed prior to newborn screening.
Methods: Intellectual ability, adaptive and executive functions were assessed with age appropriate Wechsler scales, Adaptive Behavior Assessment Scales (ABAS) and Behavior Rating Inventory of Executive function (BRIEF).
Results: Five patients performed in the normal range on IQ tests with lower results on verbal working memory. They had somewhat impaired parent rated adaptive and executive functions.Three patients had intellectual disabilities with IQ below normal and/or autism spectrum disorder. In addition they had low results on parent rated adaptive functions. Two of these patients had epilepsy.Patients with early diagnosis, and adherence to treatment seemed to have better outcome.
Conclusion: In this group of LCHAD deficient patients, 5/8 had normal IQ but lower verbal working memory, 3/8 patients (37.5%) had autistic type behaviour, in addition to general intellectual disability. Parents reported impaired executive and adaptive functions.

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Multidisciplinary retrospective study of homocystinuria patients followed at Reference Center for Inborn Errors of Metabolism (CREIM) of Universidade Federal de São Paulo (UNIFESP)

Frangipani B J[1], Mendes C S C[1], Curiati M A[1], Silva J A O[1], Oliveira R B[1], Kyosen S O[1], D'Almeida V[1], Martins A M[1]

[1]Universidade Federal de São Paulo, São Paulo, Brazil

Objective: Describe the clinical and nutritional profile of homocystinuria patients followed at CREIM.
Method: Retrospective study about clinical outcome and laboratory features of 20 patients followed at CREIM.
Results: Thirteen male and seven female, medium age 21,7y (8-33y). 30% referred to our department due to *ectopia lentis*, 25% previous thromboembolic event, 15% *ectopia lentis* and developmental delay, 10% familial screening and 15% miscellaneous. Medium gap between first symptoms and diagnostic 4,8y. At diagnosis 70% presented myopia, astigmatism and *ectopia lentis*, 20% myopia and *ectopia lentis*, 5% myopia and 5% *ectopia lentis.* Skeletal abnormalities: 75% scoliosis, 10% scoliosis and osteoporosis, 5% osteoporosis, 10% normal. Treatment: 45% using methionine-free amino acid mixture as prescribed, 35% irregular use, 10% interrupted the treatment and 10% responsive to pyridoxine. Homocysteine levels: medium of 117,3 umol/L, ranging from 18,7 to 329, a gap showing the difference between non-compliant patients and the treatment compliant or pyridoxine responsive.
Conclusion: Early diagnosis and treatment compliance are essential to avoid complications such as intellectual and visual disability, reducing patients' QoL, and also to prevent life-threatening conditions like thromboembolic events. Despite the low compliance, our casuistic shows that multidisciplinary workup aiming patient awareness is imperious.

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Prenatal diagnosis of brain malformations in patients with Multiple AcylCoA Dehydrogenase Deficiency

Gomez M[1], Macaya A[6], Camba F[4], Delgado I[3], Vazquez E[3], Arranz J A[6], Carnicer C[2], Ribes A[5], Navarro-Sastre A[5], Del Toro M[6]

[1]Pediatric Neurol, Hospital Vall d'Hebron, Barcelona, Spain, [2]Metabolic Lab, Hospital Vall d'Hebron, Barcelona, Spain, [3]Radiology Dep, Hospital Vall d'Hebron, Barcelona, Spain, [4]Neonatology Unit, Hospital Vall d'Hebron, Barcelona, Spain, [5]Hospital Clinic, IDIBAPS, CIBERER, Barcelona, Spain, [6]Hospital Vall d'Hebron, CIBERER, Barcelona, Spain

Background: Multiple acyl-CoA dehydrogenase deficiency (MADD) is a metabolic condition caused by impaired electron transfer, which in the neonatal form can be associated with kidney and brain malformations. We report two patients with neonatal onset and brain abnormalities, one of them detected on fetal MRI.
Clinical cases:
1. First son of healthy non related parents. Prenatal MRI revealed kidney malformations and brain abnormalities consistent with glutaric aciduria. After birth he showed macrocephaly, dysmorphic features, respiratory distress, metabolic acidosis and hypoglycemia. MRI study showed opercularization deficit, simplified frontal gyral pattern and hypomyelination. Metabolic profile was consistent with MADD. Despite the treatment he died at the age of 2 months.
2. Third child from healthy consanguineous parents with a sibling who died in the second day of life. He had no dysmorphic features but presented hypotonia and respiratory distress at birth with lactic acidemia, hypoglycemia and hyperammonemia. MRI showed perisylvian polymicrogyria, hypoplasic temporal lobes and increased subarachnoid spaces. He was diagnosed as MADD and died at the age of 7 days.
Conclusion: Neuroradiologic findings such as opercularization deficit in fetal MRI should lead to suspect MADD and can help to an early diagnosis and treatment.

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Functional and molecular evaluation of patients with primary carnitine deficiency

Frigeni M[1], Yin X[1], Pasquali M[1 2 3], Longo N[1 2 3]

[1]Dept Pediatrics, Univ Utah, Salt Lake City, United States, [2]Dept Pathology, Univ Utah, Salt Lake City, United States, [3]ARUP Laboratories, Salt Lake City, United States

Background: Primary carnitine deficiency (PCD) is caused by mutations in the *SLC22A5* gene encoding the OCTN2 carnitine transporter. It causes low carnitine levels detectable at birth by newborn screening and confirmed by measuring reduced carnitine transport in fibroblasts or by *SLC22A5* sequencing.
Methods: Carnitine (1 mM) transport was measured in skin fibroblasts. Each exon of the *SLC22A5* gene was sequenced and missense mutations generated in the OCTN2-EGFP expression vector by site-directed mutagenesis prior to stable transfection of CHO cells. Gene expression was verified by RT-PCR or sequencing of PCR products.
Results: Carnitine transport was reduced to 20% or less of normal in fibroblasts of 114/325 subjects referred for possible PCD. *SLC22A5* gene sequencing in 66/114 affected subjects identified causative variants in about 80% of the alleles. Expression of 90 missense variants in CHO cells failed to reproduce defective carnitine transport for 3 putative mutations. RNA studies revealed that these variant affected OCTN2 RNA splicing and/or abundance.
Conclusions: PCD is caused by heterogeneous variations in the *SLC22A5* gene. Measurement of carnitine transport in fibroblasts remains the best strategy to confirm or exclude a diagnosis, since DNA analysis fails to identify causative variations in about 20% of the alleles.

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Late-onset of Multiple Acyl-CoA Dehydrogenase Deficiency (MADD) and rhabdomyolisis: A case report

Donis K C[1], Lorea C F[1], Silva A A[1], Refosco L F[1], Vairo F P[1], Souza C F M[1], Vilarinho L[2], Sitta A[1], Vargas C R[1], Schwartz I V D[1]

[1]Med Genet Serv, Hosp Clin Porto Alegre, Porto Alegre, Brazil, [2]Instituto Nacional de Saúde Dr. Ricardo, Porto, Portugal

Background and objectives: MADD is an autosomal recessive disorder caused by deficiency of electron transfer flavoprotein dehydrogenase. The late-onset form is rare and shows wide clinical heterogeneity.
Case report: A 35-year-old man presented with progressive myopathy, chronic pain in lower limbs and weight loss. Acutely, he presented with hypoglycemia, rhabdomyolysis, respiratory and renal failure, and needed to be seen as an emergency. He reported a previous similar episode, which happened at the age 15. His sister died with similar symptoms at 22 years old. Urinary organic acids analysis showed methyl succinic acid and the acylcarnitines profile was compatible with MADD. He presented clinical improvement after treatment. Molecular analyses showed two *ETFDH* mutations (p.P27S/P534L), the p.P27S being a novel mutation. Nowadays, he takes L-carnitine (3g/day), riboflavin (150 mg/day), a low-fat and high caloric diet, and presents with normal strength and no new metabolic descompensations.
Discussion/Conclusion: Late-onset MADD should be considered in patients with muscular symptoms, and acute metabolic decompensation.

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VLCAD deficiency leads to chronic inflammation with an atypical cytokine and activated monocyte signature

Mroczkowski H J[1], Mihalik S J[2], Swigonova Z[3], Michel J J[1 4], Ralphe J C[5], Vallejo A N[1 4], Vockley J[1 6]

[1]Dept Pediat, Child Hosp Pittsburgh, Pittsburgh, PA, United States, [2]Dept Path, Univ Pittsburgh Sch Med, Pittsburgh, PA, United States, [3]Dept Biol Sci, Univ Pittsburgh, Pittsburgh, PA, United States, [4]Dept Immunol, Univ Pittsburgh Sch Med, Pittsburgh, PA, United States, [5]Dept Pediat, Univ Wisconsin Sch Med PH, Madison, WI, United States, [6]Dept Hum Genet, Univ Pittsburgh Grad Sch, Pittsburgh, PA, United States

Background & Objectives: Very long-chain acyl-CoA dehydrogenase deficiency (VLCADD) is a life-threatening condition. Newborn screen with early intervention provides the best opportunity to prevent morbidity and mortality. Anaplerotic energy supplementation therapy is experimental and seems to be effective in treating hypoglycemia; however, rhabdomyolysis episodes often persist. We hypothesize that susceptibility to rhabdomyolysis is associated with atypical inflammation that is independent of energy deficiency.
Materials, Patients & Methods: Cytokine profiles of VLCA DD patients and mice were analyzed by Luminex. Of the patients, 9 female / 8 male, ages 1 week to 34 years old, including 14 serial samples obtained during rhabdomyolysis episodes in 3 individuals, were examined. Monocyte phenotypes were examined by multicolor flow cytometry.
Results: Multiple cytokines were elevated in VLCADD mice not on dietary therapy, including IL-1β, IL-3, IL-6, IL-10, IL-12, IL-17, INFγ, MIP-1β when compared to WT mice. Similarly, elevations of IL-8, IL-12, IL-17, INFγ, MCP-1, and MIP-1β were observed in VLCADD patients regardless of dietary therapy or hospitalization. Monocytes of VLCADD patients displayed activation markers including cytoplasmic stores of cytokines.
Discussion & Conclusion: VLCADD has underlying chronic inflammation that may predispose patients to rhabdomyolysis. These findings offer new therapeutic options with the potential to significantly improve patient quality of life.

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Management of HMG-CoA lyase deficiency: a review of five cases from a single centre

Petkovic Ramadza D[1], Abulhoul L[2], Grünewald S[2], Cleary M[2], Dixon M[2]

[1]Dep of Pediatr, Univ Hosp Centr Zagreb, Zagreb, Croatia, [2]Gt Ormond St Hosp for Children, NHS FT, London, United Kingdom

Background: HMG-CoA lyase deficiency is a disorder of ketone synthesis and leucine degradation. Acute treatment consists of reversing catabolism with carbohydrates and stopping protein intake. Tenets of long term management are less clear; in addition to avoidance of fasting and carnitine supplementation, some recommend protein restricted diet.
Objectives: to review treatment and outcome of our cohort.
Patients/methods: Retrospective data collection of five HMG-CoA lyase deficient patients followed-up since 2000. All diagnosed as newborns. Current age range: 2-7 years, median 5 years.
Results: Two patients on moderate protein restriction (1.8-2 g/kg), three on normal diet. All had glucose polymer based emergency regimen and carnitine supplementation (100 mg/kg, later reduced to 50 mg/kg). All achieved a good outcome except one who had severe crisis as newborn, later recurrent hypoglycaemias, and now has developmental delay. Two patients had recurrent hypoglycaemia and low fasting tolerance confirmed by CGM which resolved after introduction of uncooked cornstarch (1 and 2g/kg/body weight) at bedtime.
Conclusion: The major aim of long term treatment is avoidance of prolonged fasting and rapid intervention during crises. In our experience there is no difference in outcome between patients on moderate protein restriction and normal diet. UCCS is useful to prevent overnight hypoglycaemia.

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Brain and muscle redox imbalance elicited by acute ethylmalonic acid administration

Schuck P F[1], Galant L S[2], Ferreira B K[1], Malgarin F[1], Petronilho F[3], Dal-Pizzol F[2], Streck E L[1], Ferreira G C[4]

[1]Lab Inb Err Metab, Univ Extr Sul Catarin, Criciúma, Brazil, [2]Lab Fisiopat Exper, Univ Extr Sul Catari, Criciúma, Brazil, [3]Lab Imunopat Clin Experim, Univ Sul SC, Tubarão, Brazil, [4]Instit Bioq Med, Univ Fed RJ, Rio de Janeiro, Brazil

Background and objectives: Ethylmalonic acid (EMA) accumulates in tissues of patients affected by short-chain acyl-CoA dehydrogenase deficiency (SCADD) and ethylmalonic encephalopathy, illnesses characterized by neurological and muscular symptoms. Considering that the mechanisms responsible for the brain and skeletal muscle damage in these diseases are unknown, we investigated the effects of acute EMA administration on redox status in rat cerebral cortex and skeletal muscle.
Materials and Methods: Animals received three subcutaneous injections of EMA (6 mmol/g; 90 min interval) and were

killed 1 h after the last administration. Control animals received saline in the same volumes.
Results: EMA administration increased thiobarbituric acid-reactive substances levels in cerebral cortex and skeletal muscle, indicating increased lipid peroxidation. In addition, carbonyl content was increased and glutathione concentrations were decreased in EMA-treated animal skeletal muscle. EMA administration also significantly increased 2,7-dihydrodichlorofluorescein oxidation, and superoxide production and decreased glutathione peroxidase activity in cerebral cortex. Respiratory chain complex I-III activity was not altered in both tissues.
Conclusions: Our results showed that EMA administration elicits oxidative stress in rat brain and skeletal muscle, suggesting that oxidative damage may be involved in the pathophysiology of neurological and muscle symptoms found in patients affected by SCADD and ethylmalonic encephalopathy.

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Obesity and overweight in a cohort of patients with fatty acid oxidation disorders

Luz I R[1], Garcia P[1], Diogo L[1], Faria A[1]

[1]Metab Dis Unit, Ped Hosp, CHUC, Coimbra, Portugal

Background: Nowadays childhood obesity is a major public health problem. Patients with fatty acid oxidation (FAO) disorders should avoid prolonged fasting and are submitted to long-term dietary treatment. Consequently, they are susceptible to nutritional imbalances.
Objectives: Estimate the prevalence of obesity/overweight in a cohort of FAO disorders patients in central region of Portugal.
Materials & Methods: Clinical data of paediatric patients with FAO disorders followed in a single tertiary centre was retrospectively reviewed.
Results: Twenty one patients (57% female; aged 15 months to 19 years) were analysed: 1 CPT2, 1 VLCAD, 4 LCHAD and 15 MCAD deficiencies. According to body mass index, overweight was found in 7 (3 LCHAD, 4 MCAD) and obesity in 3 (1 CPT2, 1 LCHAD, 1 MCAD). No patients presented underweight. Concerning obese patients, dyslipidemia and sleep obstructive apnoea were the only co-morbidities, found in a LCHAD and a CPT2 patient, respectively.
Conclusion: In Portugal, overweight/obesity affect 30,3% of paediatric population. In this cohort, overweight or obesity were present in 47,6%, which is significantly above the general population. Anthropometry and body composition evaluation, as well as nutritional and "healthy" lifestyle recommendations should be routinely applied in the follow-up of these patients, in order to reduce co-morbidity.

15. Disorders of pyruvate metabolism and the Krebs cycle

P-362

Lactic acidosis and neonatal death in a patient with deficiency of the E2 subunit of the pyruvate dehydrogenase complex

Mayr J A[1], Burnyte B[2 3], Snariene R[4], Feichtinger R G[1], Zimmermann F A[1], Lunzer V[1], Wortmann S B[1], Baskirova I[2], Drazdiene N[4], Algirdas U[2 3], Sperl W[1]

[1]Dept Pediatr, Paracelsus Med Univ, Salzburg, Austria, [2]Center f Med Genet, Santariskiu Klinikos, Vilnius, Lithuania, [3]Dept Human Genet, Vilnius University, Vilnius, Lithuania, [4]Neonatology Centre of Vilnius University, Vilnius, Lithuania

Background: Deficiency of the E2 subunit of the pyruvate dehydrogenase complex (PDHC) is a rare inborn error of pyruvate oxidation, only six patients with *DLAT* mutations have been published. Five patients presented with relatively mild symptoms predominately dystonia, but also hypotonia, ataxia, and bilateral lesions in the globus pallidus. Lactate was normal or slightly elevated.
Case report: We report a girl from non-consanguineous Lithuanian parents born at term, Apgar scores 8-9. Immediately after birth, she developed failure to thrive, respiratory insufficiency, CNS irritability and on the third day of life she died due to progressive lactic acidosis. Lactate and alanine were severely elevated in blood. In urine lactate, pyruvate and malate were increased.
Results: Investigation of the mitochondrial energy metabolism in fibroblasts revealed PDHC deficiency, western blot and immunohistochemical staining a deficiency of the E2 subunit. Sequencing of *DLAT* showed a heterozygous mutation c.436G>C (p.Ala146Pro), which was the only detectable form in the cDNA. No other mutation was found in the coding exons and adjacent intronic regions, pointing to either a regulatory or deep intronic mutation on the second allele.
Conclusion: DLAT deficiency has to be considered in patients with severe and fatal lactic acidosis.

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Phenotype and genotype analysis of Indian patients with Pyruvate Carboxylase deficiency

Gupta N[1], Verma G[1], Sharma S[2], Shastri S[1], Valayannopoulos V[3], Monnot S[3], Kabra M[1], Kaur P[1]

[1]All India Institute of Medical Sciences, New Delhi, India, [2]Lady Harding Medical college, New Delhi, India, [3]Materials and Necker-Enfants Malades Hos, Paris, France

Background: Pyruvate carboxylase deficiency (PCD) is an autosomal recessive disorder with three clinically well characterized types caused by defect in the pyruvate carboxylase gene. We retrospectively studied 3 families with PC deficiency to evaluate the clinical spectrum and genotype phenotype correlation in Indian patients.
Material and Methods: We evaluated four patients from three families with PCD. Two of the families had the severe neonatal type whereas one family had the American Indian form. All 3 families had more than one affected child. Diagnosis was based on clinical and metabolic workup and later confirmed by *PC* gene analysis.
Results: *PC* gene analysis revealed 3 novel mutations in family 1 and 3 (c.2514G>A in Exon 16 ; c.3514G>A in Exon 23 and c.616G>T in Exon 7 respectively). Parents in all three families were confirmed as carriers. Prenatal diagnosis was offered in family 2 and 3 respectively. In family 2, the fetus was unaffected, whereas in family 3 the fetus was affected and the pregnancy was terminated.
Discussion and conclusion: PC deficiency is an uncommon metabolic disorder. Precise diagnosis needs stepwise and timely evaluation of the patient due to clinical overlap. Molecular analysis of the *PC* gene is confirmatory and is helpful in providing accurate prenatal diagnosis.

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Fumaric aciduria: is arginine aspartate a treatment option?

Janeiro P[1], Moreno T[2], Jardim I[3], Neiva R[5], Vilarinho L[5], Tavares de Almeida I[4], Gaspar A[1]

[1]Metabolic Unit, Ped Dep, HSM, CHLN, Lisbon, Portugal, [2]Neuroped Unit, Ped Dep, HSM, CHLN, Lisbon, Portugal, [3]Dietetic Nutrit Unit Dep, HSM, CHLN, Lisbon, Portugal, [4]Met&Gen, iMed.UL, Fac Farmácia, UL, Lisbon, Portugal, [5]ENS Metab Gen Unit, INSA, Oporto, Portugal

Background: Fumaric aciduria is an exceptionally rare metabolic disease characterised by brain abnormalities, developmental delay and increased urinary fumaric acid excretion. No medical treatments currently exist and most patients do not survive beyond early childhood. In a model of fumarase deficiency, Smith and Robinson have shown that ATP production is improved by addition of amino acids involved in the malate-aspartate shuttle.
Case report: A 25 month old boy is the first child of a nonconsanguineous couple, with unremarkable family background. Gestation was normal, however he developed tachypnea and asphyxia from birth. Poor weight gain was reported at 2 months, motor developmental delay at 6 months, hypotonia and pseudo-strabismus from 9 months. He also had failure to thrive, microcephaly, marked hypotonia, hyperlaxity and distal dystonic posturing. Metabolic profiling revealed high urinary fumaric acid excretion and slightly elevated plasma/CSF lactate and pyruvate concentrations. Molecular genetic studies of the *FH* gene confirmed the diagnosis.
He was treated with a low glycaemic index diet supplemented with MCT oil, DHA and L-carnitine. Two months after introduction of arginine aspartate 5g/day, he has better cervical control and prolonged sitting without support.
Discussion: No effective specific treatment has been reported for fumaric aciduria. In the present case, aspartate and arginine supplements have led to some clinical improvement. but further studies are necessary to prove whether they are of long term benefit.

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Variable phenotypes in nine Arab patients with dihydrolipomide dehydrogenase deficiency due to homozygous c.685G>T mutation in the *DLD* gene

Makhseed N[1], Almureikhi M[2], Shahbeck N[2], A.Rahman R[2], Mahmoud L[2], Rodenburg R J[3], Ben-Omran T[2]

[1]Pediatric department,Jahra Hospital,, Jahra, Kuwait, [2]Section of Clinical and Metabolic Genet, Doha, Qatar, [3]Department of Paediatrics, Nijmegen Cen, Nijmegen,, Netherlands

Introduction: Dihydrolipoamide dehydrogenase; DLD; (EC 1.8.1.4), is the E3 subunit, of multi-enzyme complexes: PDH, KGDHC, BCKDH and it is the L protein in glycine cleavage system. DLD deficiency (OMIM 246900) is a rare autosomic recessive disease of variable phenotypes; severe neonatal presentations with neurological impairment, intermittent lactic acidosis, and ketoacidemia with fatal outcome, late onset presentation with exertional fatigue, liver failure, Some have increased branched-chain amino acids with increased excretion of α-ketoacids.
Materials & Methods: We report clinical, biochemical and molecular data of nine patients with DLD deficiency (4 males and 5 females).
Results: All patients presented with recurrent episodes of anicteric hepatitis preceded by epigastric pain, nausea, vomiting and occasional hypoglycemia. One patient had jaundice with normal synthetic liver functions, another had acute liver failure, and one had hypotonia and motor delay triggered by episodes of vomiting with fever. All patients have a homozygous mutation (c.685G>T) (p.Gly229Cys) in the *DLD* gene.

Conclusions: Phenotypic variability seen in our patients highlights the importance of considering DLD deficiency in patients with atypical clinical manifestations and poses a diagnostic challenge. DLD deficiency exists among Arabs, and the founder mutation may be an ancient Arab mutation.

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Deficiency of the mitochondrial pyruvate carrier subunit MPC1 in a patient with splenomegaly, epilepsy and diabetes mellitus

Makhseed N[2], Assmann B E[3], Kotzaeridou U[3], Feichtinger R G[1], Matakovic L[1], Wortmann S B[1], Haack T B[4 5], Prokisch H[4 5], Hoffmann G F[3], Sperl W[1], Mayr J A[1]

[1]Dept Pediatr, Paracelsus Med Univ, Salzburg, Austria, [2]Ped Dept, Jahra Hosp, Ministry of Health, Kuwait, Kuwait, [3]Dept Pediatr, University of Heidelberg, Heidelberg, Germany, [4]Technische Universität München, Munich, Germany, [5]Helmholtz Zentrum München, Munich, Germany

Background: MPC1 and MPC2 form a heteromeric pyruvate carrier in the inner mitochondrial membrane. Defects of MPC1 have been reported in five individuals from three families. One patient presented with neonatal encephalopathy, the other with psychomotor retardation and either epilepsy or peripheral neuropathy.
Case report: Our patient, a male of consanguineous Kuwaiti descent, developed splenomegaly with normal developmental milestones. At six years he presented with generalized tonic-clonic seizures, which were successfully controlled with valproate. A few years later, he presented with insulin dependent diabetes (antibody negative). He had significant learning disabilities and at 12 years he showed microcephaly, growth retardation (length < 2P) and his IQ was 56. The MRI/MRS of the brain at the same age was unremarkable as were sensory evoked potentials of the N. medianus. Serum and urinary lactate levels were mildly elevated.
Results: Investigation of the mitochondrial energy metabolism in fresh muscle showed decreased pyruvate oxidation, however, PDHC activity was normal. Sequence analysis revealed a homozygous mutation c.95C>G (p.Ala32Gly) in MPC1, affecting a conserved position.
Conclusions: Our patient broadens the clinical spectrum of MPC deficiency. Association of MPC deficiency with diabetes mellitus is in line with a recent Mpc2 mouse model demonstrating impaired glucose-stimulated insulin secretion.

16. Mitochondrial disorders: nuclear encoded

P-367

Resveratrol attenuates oxidative stress in complex I-deficient fibroblasts: involvement of SIRT3, ER and ERRalpha

Mathieu L[1], Lopes Costa A[1], Le Bachelier C[1], Slama A[2], Lebre A S[3], Taylor R W[4], Bastin J[1], Djouadi F[1]

[1]INSERM UMRS 1124, Univ Paris Descartes, Paris, France, [2]Biochimie, AP-HP Kremlin Bicêtre, Paris, France, [3]CHU de Reims, Service de Génétique, Reims, France, [4]Wellcome Trust Centre for Mitoch Res, Newcastle upon Tyne, United Kingdom

Background: The pathophysiological mechanisms underlying complex I (CI) deficiencies are only partially understood, making the treatment of this common mitochondrial disorder very limited. Recently, we showed that resveratrol (RSV), a natural polyphenol, could have beneficial effects on CI deficiency from nuclear origin.
Methods & Results: We confirmed this result by testing the effects of RSV on oxygen consumption in several CI-deficient patients' fibroblasts and showed that RSV corrected the defect in 5 out of 14 cell lines. Other beneficial effects of RSV are illustrated by the decrease of total intracellular ROS and the up-regulation of mitochondrial superoxide dismutase (SOD2) protein level, a key antioxidant defense enzyme. However, surprisingly, RSV failed to subsequently increase SOD2 enzyme activity in patients' fibroblasts while a clear up-regulation was measured in control cells. This led us to hypothesize a post-translational regulation of SOD2 enzyme activity and we demonstrated the involvement of SIRT3, a mitochondrial NAD-dependent deacetylase. Moreover, we deciphered the molecular mechanisms leading to the up-regulation of SOD2 expression by RSV, which required ER (estrogen receptor) and ERRα (estrogen-related receptor alpha).
Conclusion: Altogether, we show that the metabolic effects of RSV combined with its antioxidant capacities makes RSV particularly interesting as a candidate molecule for therapeutic approach of CI deficiencies.

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A preterm infant who had hemophagocytic lymphohistocytosis (HLH) caused by mitochondrial respiratory chain disorders (MRCD)

Fuwa K[1], Kanno M[1], Miyabayashi H[1], Kawabata K[1], Kanno K[1], Kubota M[2], Shimizu M[1]

[1]Div neonatology,Saitama Child Hosp, Saitama, Japan, [2]Div general pediatr,Saitama Child Hosp, Saitama, Japan

Case report: We report a case of a premature female infant born at 34 weeks of gestation who had fetal hydrops, respiratory insufficiency, lactic acidosis, thrombocytopenia, coagulation disorder, and hepatic steatosis. Hemophagocytic lymphohistiocytosis (HLH) was revealed by bone marrow aspiration. She died on day 10 due to liver failure despite the absence of icterus, similar to the presentation of Reye syndrome. An autopsy was performed, and we measured the activity of the mitochondrial respiratory chain enzyme in the liver, muscle and heart. The activity of complex I was decreased in all tissues. According to the above results, she was diagnosed as a mitochondrial respiratory chain disorder (MRCD) with HLH.
Discussion: The incidence of MRCD is 1 in 5,000 births in Japan, and 60% of neonates with MRCD will die. The incidence of primary familial HLH is 1 in 50,000 births. As we could not detect a genetic mutation in this case, which is the primary cause of HLH, the infant's HLH might be secondary to the MRCD.
Conclusion: Although patients who have both MRCD and HLH are very rare, we recommend an assay of mitochondrial respiratory chain enzyme be performed if patients have HLH and liver failure that clinically resemble Reye syndrome.

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AIFM1 deficiency with cardiac involvement: description of three new cases

Kulhanek J[1], Tesarova M[1], Vondrackova A[1], Dvorakova V[1], Hansikova H[1], Zeman J[1], Honzik T[1], Magner M[1]

[1]Dep Pediatr, 1st Facult Med, Charles Uni, Prague, Czech Republic

Background: Mutations in *AIFM1* gene, encoding the Apoptosis-Inducing Factor Mitochondrion-associated 1, cause a very rare X-linked mitochondrial disorder. 12 hitherto described patients manifested either with Leigh syndrome, hypotonia and epilepsy, or progressive axonal neuropathy (Cowchock syndrome; CMTX4). Hypertrophic cardiomyopathy (HCMP) has been described in only one patient, so far. Here, we present three new patients with HCMP in two of them and with two novel mutations.
Results: Two brothers (P1, P2) and one unrelated boy (P3) manifested in neonatal period with hypotonia, respiratory failure and lactic acidosis in P1, P2, and myoclonic epilepsy in P3. P1 had ileal volvulus, P2 glandular hypospadias, P3 syndactyly of the 2nd and 3rd toes. No developmental progress occurred. Severe hypertrophic cardiomyopathy leading to heart failure was documented in P1, P2. Leigh syndrome presented only in P3. The prognosis was unfavourable – all patients died before the age of 18 months. Muscle biopsy showed decreased activities of complex IV. Molecular analyses revealed two novel mutations, c.506C>T (p.Pro169Leu) in P1, P2 and c.1391T>G (p.Leu464Trp) in P3.
Conclusion: AIFM1 deficiency is probably an underdiagnosed mitochondrial disease with Leigh syndrome, myoclonic epilepsy or axonal neuropathy. Cardiomyopathy may be its leading clinical symptom. *Supported by grants IGA NT14156/3, IGA NT13114/4 and RVO-VFN64165/2012.*

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Three families sharing acyl-coA dehydrogenase 9 deficiency: from severe neonatal cardiomyopathy to ventricular hypertrophy diagnosed at 12 years

Dewulf J[1], Barrea C[4], Vincent M F[1], De Laet C[3], Van Coster R[5], Seneca S[6], Marie S[1], Nassogne M C[2]

[1]Biochem Genetics, Clin univ Saint-Luc, Brussels, Belgium, [2]Pediatric Neurology, Clin univ Saint-Luc, Brussels, Belgium, [3]Nut & Metab Lab, Hospital Qeen Fabiola, Brussels, Belgium, [4]Pediatric Cardiology Clin univ Saint-Luc, Brussels, Belgium, [5]Pediatric Neurology, University Hospital, Gent, Belgium, [6]Center for Medical Genetics, UZ VUB, Brussels, Belgium

Acyl-CoA dehydrogenase 9 (ACAD9) is a mitochondrial enzyme involved in fatty acid oxidation and in complex I biogenesis. ACAD9 deficiency usually presents with lactic acidosis, complex I deficiency and normal acylcarnitine profile. We report 9 ACAD9 deficiency patients from 3 families sharing complex I deficiency with lactic acidosis and very heterogeneous cardiac involvement at diagnosis. In the first family we observed 3 patients severely affected who died before one year while the fourth affected child is now seven years and has electric left ventricular hypertrophy with growth retardation. In the second family, the two affected patients were diagnosed during teenage with exercise intolerance and hypertrophic cardiomyopathy. Two affected children from the third family died before the age of ten days in a context of severe lactic acidosis and multiorganic failure while the third died at the age of 6 months following a decompensation of his hypertrophic cardiomyopathy. These observations underline the complex pathophysiology of ACAD9 deficiencies. Clinical expression of ACAD9 deficiency can occur between birth and teenage with heterogeneous cardiac involvement. Furthermore, within a same family, the deficiency may result in varying degree of severity. Since some ACAD9 deficiency patients respond to riboflavin, this treatment should be tried.

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Leigh syndrome due to mutations in *ECHS1* gene

Gort L[1 2], Ferrer-Cortès X[1 2], Ugarteburu O[1 2], Narbona J[3], Aldamiz-Echevarria L[4], Briones P[1 2 5], Montoya J[6 7], Morales-Romero B[1], Pajares S[1], Arias A[1], Garcia-Villoria J[1 2], Tort F[1 2], Ribes A[1 2]

[1]ECM-BGM-Hospital Clínic, IDIBAPS, Barcelona, Spain, [2]CIBERER, Barcelona, Spain, [3]Hospital Univ de Navarra, Pamplona, Spain, [4]Hospital de Cruces, Bilbao, Spain, [5]CSIC, Barcelona, Spain, [6]Dep Bioq Biol Mol Cel, Univ Zaragoza, Zaragoza, Spain, [7]CIBERER, Zaragoza, Spain

We present a 10 months old male patient presenting psychomotor and growth retardation, hypotonia, dystonia, deafness, bilateral pallidal necrosis, altered auditory evoked potentials, and high lactic acid in blood and CSF. Organic acids showed a slight increase of 3-methylglutaconic acid. Acylcarnitines and aminoacids in plasma and urine were normal. Activities of the mitochondrial respiratory chain complexes were normal but they were low when referred to citrate synthase. Pyruvate dehydrogenase activity was low, 4mU/U CS (CV 9-26) but no mutations in *PDHA1*, *PDHB* and *DLAT* genes were found. Due to the 3-methylglutaconic aciduria we also excluded mutations in *ATP5E*, *ATP6*, *ATP8*, *TMEM70* and *SERAC1 genes.* No deletions in mtDNA or changes in mitochondrial $tRNA^{Lys}$ were found. Whole exome sequencing allowed us to identify two mutations in heterozygosity in *ECHS1*: c.123_124delAG (p.Gly42Glu*fs**3) and c.371C>T (p.Thr124Ile). Recently, two siblings with mutations in this gene were reported (Peters 2014. Brain 137:2903). An increase of 2-methyl-2,3-dihydroxybutyric acid in urine of the two siblings was found. This acid was also found in the present patient and in two more patients studied retrospectively among undiagnosed Leigh patients (under molecular study).
Conclusion: *ECHS1* should be studied in patients presenting Leigh syndrome and high excretion of 2-methyl-2,3-dihydrobutyric acid.

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Massive exome sequencing identifies a novel heterozygous-compound in ACAD9 gene in affected siblings with severe lactic acidosis

Merinero B[1], Bravo-Alonso I[1], Navarrete R[1], Rausell L[2], Vitoria I[2], Blázquez A[3], García-Consuegra I[3], Martín M A[3], Ugarte M[1], Rodríguez-Pombo P[1]

[1]CEDEM, CBM-SO, UAM, CIBERER, IDIPAZ, Madrid, Spain, [2]Unit Metab, Hosp La Fe, Valencia, Spain, [3]Inst Invest Hosp 12 Octubre, CIBERER, Madrid, Spain

Acyl-CoA dehydrogenase 9 (ACAD9) is an assembly factor for mitochondrial respiratory chain (MRC) complex I. This study describes the identification of two novel variants in the *ACAD9* gene in a non-consanguineous family with diamniotic twins suffering severe neonatal lactic acidosis, and one previous child that died soon after birth with progressive multi-organ failure. Twin A presented a neonatal fatal outcome, and twin B died at 3 months despite different therapeutic approaches (including riboflavin) after sudden onset of respiratory distress by severe dilated cardiomyopathy. Plasma acylcarnitines in both twins were normal. Genetic analysis of nuclear genes related to mitochondrial disorders, using a clinical targeted-exome sequencing panel, identified two novel variations in the *ACAD9* gene: p.Phe120Serfs*9 and p.Thr158Ile. Segregation analysis in mother's DNA confirmed her carrier condition. Reflecting the impact of mutations on complex I activity, the analysis of MRC activities in muscle showed a diminished activity of complex I, and western blot analysis of OXPHOS complexes on isolated mitochondria from fibroblasts showed a decrease of complex I levels using anti-NDUFAB8 subunit. Application of massive exome sequencing to a genetically heterogeneous condition such as lactic acidosis can be fruitful to yield the molecular diagnosis when a well-sustained clinical suspicion has been established.

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GRACILE syndrome: a severe neonatal mitochondrial disorder

Eminoglu F T[1], Akduman H[2], Atasay B[2], Erdeve O[2], Arsan S[2], Ceylaner S[3]

[1]Ankara Univ Hosp, Dept Ped Metab Dis, Ankara, Turkey, [2]Ankara Univ Hosp, Div of NICU, Ankara, Turkey, [3]Intergen, Dept Mol Biology and Genetics, Ankara, Turkey

Background: GRACILE syndrome is a rare autosomal recessive disease characterized by fetal growth retardation, Fanconi type aminoaciduria, cholestasis, iron overload, profound lactic acidosis, and early death. Here we report that a homozygous mutation c.296C > T (p.P99L), in the first exon of the *BCS1L* gene, found in an affected six-day-old boy of consanguineous parents, results in GRACILE syndrome. This genotype is associated with a severe clinical presentation.
Case report: Six-day-old male newborn was referred to our clinic because of intractable acidosis. Physical examination

revealed severe hypotonia, and hepatomegaly. The laboratory examinations revealed lactic acidosis, increased blood alanine, ALT and AST levels, generalized aminoaciduria and glucosuria. The tubular reabsorption of phosphate was reduced. Because of multisystem involvement, mitochondrial disease was suspected and the mutational analysis of the *BCS1L* gene revealed a homozygous P99L mutation.
Conclusion: So far no available treatments have changed the fatal course of the disease, and the metabolic disturbance responsible is still not clearly identified. Therefore, providing prenatal diagnosis in families with previous affected infants is of major importance. Although the first patients with GRAC ILE syndrome were recognised by the deficiencies of CIII in different tissues these invasive procedures may be bypassed by the molecular analysis of *BCS1L* gene in clinically suspected patients.

P-374

A novel *AIFM1* mutation expands the phenotype to an infantile motor neuron disease

Diodato D[1 2], Tasca G[2], Verrigni D[2], D'Amico A[2], Rizza T[2], Tozzi G[2], Martinelli D[1], Verardo M[2], Invernizzi F[3], Nasca A[3], Bellacchio E[4], Ghezzi D[3], Piemonte F[2], Dionisi-Vici C[1], Carrozzo R[2], Bertini E[2]

[1]Metab Div, Child Hosp Bambino Gesù, Rome, Italy, [2]Neuromusc Div, Child Hosp Bambino Gesù, Rome, Italy, [3]Mol Neurogenetics, Besta Institute, Rome, Italy, [4]Res Lab, Child Hosp Bambino Gesù, Rome, Italy

Background: *AIFM1* is a gene located on the X chromosome, coding for AIF (Apoptosis Inducing Factor), a mitochondrial flavoprotein involved in caspase-independent cell death. *AIFM1* mutations have been associated with different clinical phenotypes: a severe infantile encephalopathy with combined oxidative phosporylation deficiency (COXPD6) with or without ventriculomegaly, and the Cowchock syndrome, an X-linked Charcot-Mary-Tooth disease (CMTX4) with axonal sensorimotor neuropathy, deafness and cognitive impairment.
Case report: Two males presented a phenotype characterized by global developmental delay with marked hypotonia and early onset, sub-acute weakness of limbs followed by very poor spontaneous motility, epilepsy and lactic acidosis.
Results: muscle biopsies showed signs of severe denervation that was particularly severe in one of them, where the presence of large groups of markedly atrophic fibers and clusters of hypertrophic fibers resembled the picture of spinal muscular atrophy (SMA); COX deficiency was present in fibroblasts and muscle.
Conclusions: Our patients manifested a phenotype that included signs of both cortical and motor neuron involvement; the severe neurogenic pattern at muscle biopsy emphasizes the role of AIF in development and function of motor neurons.

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Molecular genetic basis of an unusual biochemical phenotype associated with *NFU1* mutations

Ferrer-Cortès X[1], Narbona J[2], Bujan N[1], Matalonga L[1], Del Toro M[3], Arranz J A[3], Riudor E[3], Garcia-Cazorla Á[4], O'Callaghan M[4], Pineda M[4], Jou C[4], Montero R[4], Alston C L[5], Taylor R W[5], Briones P[6], Ribes A[1], Tort F[1]

[1]Hosp. Clínic, IDIBAPS, CIBERER, Barcelona, Spain, [2]Clinica Univ de Navarra, Fac Med, Pamplona, Spain, [3]Hosp. Vall d'Hebron, UAB, Barcelona, Spain, [4]Hosp. Sant Joan de Deu, CIBERER, Barcelona, Spain, [5]Wellc Trust Cent Mit Res,Newcastle Univ, Newcastle, United Kingdom, [6]Hosp. Clínic, IDIBAPS, CSIC, Barcelona, Spain

Background: NFU1 is a mitochondrial protein necessary for the activation of lipoic acid synthase and the assembly of mitochondrial [4Fe-4S] proteins. Mutations in *NFU1* have been reported in patients with a biochemical phenotype consistent with defects in lipoic acid-dependent enzymes and respiratory chain complexes I and II.
Objectives & Methods: The aim of our study was to provide a better understanding of the molecular basis of this disease and of a newly identified unusual biochemical phenotype, characterised by normal mitochondrial complex I and lipoic acid-dependent enzymatic activities in fibroblasts from two individuals harbouring compound heterozygous (c.[622G>T];[545+5G>A]) *NFU1* mutations.
Results: Surprisingly, we demonstrated that both individuals had detectable levels of lipoylated proteins. Interestingly, brain necropsy analysis of one NFU1 patient demonstrated a specific protein lipoylation defect in the white but not in grey matter. Molecular studies showed that the allele harbouring the splice site mutation was subjected to a peculiar regulation, leading to small amounts of normally-spliced transcripts producing sufficient levels of active NFU1 protein, to normalize the activities of PDHC, ^{14}C-substrate oxidation rates and the activity of complex I.
Conclusions: That peculiar biochemical phenotype allowed us to gain insights into the molecular basis underlying NFU1 disease and should be taken into account for the diagnosis of these patients.

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Provision of a national diagnostic service for Barth syndrome: a five year review

Bowron A[1 2], Honeychurch J[3], Williams M[3], Powers V[1], Thorpe T[1], Thomas P H[1], Steward C G[2 4]

[1]Clin Biochem, UHBristol NHSFT, Bristol, United Kingdom, [2]Cell and Mol Med, Uni Bristol, Bristol, United Kingdom, [3]Mol Gen, North Bristol NHS Trust, Bristol, United Kingdom, [4]Dept Haem, Onc and BMT, UHBristol NHSFT, Bristol, United Kingdom

Background & Objectives: The NHS Specialist Service for Barth syndrome (BTHS) started in Bristol in April 2010. A diagnostic service comprising leukocyte cardiolipin and *TAZ* gene mutation analysis was provided at no cost. Data from five years' provision of this service was reviewed.Cardiolipin (CL_4) and monolysocardiolipin (MLCL) were analysed by LC-MS/MS.
Methods & Results: Patients with low CL_4 or elevated MLCL/CL_4 ratio underwent *TAZ* gene analysis to confirm a diagnosis of BTHS. Two hundred samples were referred for cardiolipin analysis: 12 (6%) had abnormal results, and all had a *TAZ* mutation identified, of which 5 had not been previously reported. At least 3 patients had undergone excessive metabolic investigations before cardiolipin analysis was performed, delaying their diagnosis. Two had normal CL_4 but increased MLCL/CL_4 ratio, consistent with a newly identified intermediate biochemical variant of BTHS.The rate of diagnosis of BTHS is relatively high for a rare disorder. Many patients have new mutations.
Conclusions: Measurement of MLCL/CL_4 ratio is essential as CL_4 alone may miss cases. Cardiolipin analysis should be included in a metabolic screen for a patient with cardiomyopathy; normal urine 3-methylglutaconic acid does not exclude the disorder.

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New insights into mitochondrial structure in Barth syndrome

Bowron A[1 2], Groves S J[2], Verkade P[4], Mantell J[4], Tilly G[4], Thomas P H[1], Heales S J R[5], Steward C G[2 3]

[1]Clin Biochem, UHBristol NHSFT, Bristol, United Kingdom, [2]Cell and Mol Med, Uni Bristol, Bristol, United Kingdom, [3]Dept Haem, Onc and BMT, UHBristol NHSFT, Bristol, United Kingdom, [4]Wolfson Bioimaging Facility, Uni Bristol, Bristol, United Kingdom, [5]Chem Path, Gt Ormond St Hosp, London, United Kingdom

Barth syndrome (BTHS) is a disorder of synthesis of cardiolipin, a major mitochondrial membrane phospholipid. Grossly abnormal mitochondrial structure has been demonstrated in BTHS EBV-transformed lymphoblasts using electron microscopy (EM) of chemically fixed samples.This study aimed to investigate BTHS mitochondrial structure in fresh lymphocytes prepared by cryofixation, a technique giving improved preservation of organelle ultrastructure, and to compare this with appearance in EBV-transformed lymphocytes.Fresh lymphocytes and EBV-transformed lymphoblasts from controls and BTHS patients were prepared by high pressure freezing and freeze-substitution and examined by EM. Measurements were made of mitochondrial number, area and shape. Fresh BTHS lymphocytes had larger mitochondria than controls, but no other significant differences. EBV-transformation caused significant mitochondria structural changes including increased number, size and roundness. These changes were greater in BTHS cells. Almost all BTHS EBV cells had mitochondria with abnormal cristae structures. Changes were less than those shown with previous studies using chemically-fixed cells. Cryofixation produces samples with fewer artefacts than chemical methods. Using this technique we demonstrated that BTHS patients have enlarged lymphocyte mitochondria but otherwise no structural changes. EBV transformation results in greater changes to mitochondrial structure in BTHS cells, possibly demonstrating altered mitochondrial membrane dynamics in response to stress.

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Target sequencing for mitochondrial disorders

Itkis Y S[1], Krylova T D[1], Tsygankova P G[1], Zakharova E Y[1], Ilyina E S[2], Fedonyuk I D[2], Mikhailova S V[2]

[1]Research Centre for Medical Genetics, Moscow, Russian Federation, [2]Russian Pediatric Regional Hospital, Moscow, Russian Federation

To date NGS methods are becoming the main diagnostic tool for searching genetic substitutions in cases of rare hereditary diseases. We applied target sequencing for mitochondrial disorders (MD) as it is a group of highly heterogeneous disorders with variety of clinical features.
We established a target sequencing of 62 nuclear mitochondrial genes, substitutions in which are associated with ETC complexes deficiencies, to reveal mutations in patients suspected for MD. The main inclusion criteria were typical MRI features and/or high level of

protein FGF21 (potential biochemical marker for myopathic MD). All these patients have no pathogenic mtDNA variants.
So far we've investigated patients from 18 unrelated families (retrospective analysis) and have revealed mutations in 5 cases in genes *NDUFS2*, *SCO2*, *C10orf2*. Interestingly, twice we have found the homozygous mutation p.E140K in *SCO2* gene in patients with fatal myopathic form of MD. Also twice we've found a new mutation p.R400L in *C10orf2* gene in compound heterozygous state; one patient had classical Leigh syndrome and the other one has polyneuropathy, ophthalmoplegia and myoclonus. Perhaps, these mutations are frequent in Russian patients with MD.
Our results show that NGS is effective methodology for diagnostic as well as for investigation of mutations distribution in different populations.

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High prevalence of complementary and alternative medicine use in patients with genetically proven mitochondrial disorders

Franik S[1], Huidekoper H H[2], Visser G[3], De Vries M[1 4], De Boer L[1], Hermans-Peters M[1], Rodenburg R J[1], Verhaak C[4], Vlieger A M[5], Smeitink J A M[1], Janssen M C H[6], Wortmann S B[1]

[1]NCMD, Dep of Ped, Child Hosp, Radboudumc, Nijmegen, Netherlands, [2]Dep of Ped, AMC, Amsterdam, Netherlands, [3]Dep of Ped, Wilhelmina Child Hosp, UMC U, Utrecht, Netherlands, [4]Dep of Psych, Radboudumc, Nijmegen, Netherlands, [5]St Anton Hosp, Nieuwegein, Netherlands, [6]Dep of Int Med, Radboudumc, Nijmegen, Netherlands

Background and objective: Despite advances in understanding the pathophysiology of mitochondrial diseases, clinical management remains largely supportive, no effective treatment is available. We therefore assumed that this combined with the burden of disease leaves open a big market for complementary and alternative medicine (CAM) use in these patients.
Patients and Methods: 38 pediatric and 46 adult patients with a genetically proven mitochondrial disorder regularly attending the outpatient clinics were requested to complete a questionnaire evaluating the use and effectiveness of CAM.
Results: Questionnaires of 24 (63%) pediatric and 33 (72%) adult patient's were returned. The reported use was surprisingly high, with 88 % of children and 91% of adults having used some kind of CAM in the last 2 years. Also, the mean cost of these treatments was impressive (children €489/year, adults €359/year). Over-the-counter remedies (e.g., food supplements, homeopathy) and self-help techniques (e.g., Reiki, yoga) were the most frequently used CAM therapies. 54 % of children and 60 % of adults reported the various CAM therapies to be effective.
Conlusion: Based on the reported high use, high cost of CAM and subjectively reported efficacy, further research into different CAM therapies is needed, and highly sought after by affected patients.

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New features for Twinkle mutations: high alpha-fetoprotein and abnormal CDG profile

Bouchereau J[4], Vuillaumier Barrot S[1 6], Dupré T[1 6], Cardas R[3], Capri Y[7], Slama A[2], Seta N[1], Schiff M[4 5], Servais L[3]

[1]Bioch métabo, Hop Bichat-Claude Bernard, Paris, France, [2]Biochimie, Hôpital Kremlin-Bicêtre, APHP, Paris, France, [3]Institut de Myologie, APHP, Paris, France, [4]Inserm U1141, Hôpital Robert Debré, Paris, France, [5]Ref Cent Metab Dis, hop Robert Debré, Paris, France, [6]CRB3, Inserm U773, Paris, France, [7]Génétique clinique, Robert Debré, Paris, France

Background: *C10orf2* gene encodes for Twinkle, a protein involved in mitochondrial DNA (mtDNA) replication. *Twinkle* mutations cause mtDNA deletion or depletion and have been associated with a large spectrum of clinical symptoms, including dominant progressive external ophtalmoplegia (adPEO), infantile-onset spinocerebellar ataxia (IOSCA) and early-onset encephalopathy. The diagnosis remains difficult, because such a clinical spectrum is nonspecific and is not associated with specific biochemical changes.
Case report: We report herein a child with early-onset encephalopathy, unusual abnormal movements, deafness and axonal neuropathy. All laboratory investigations were normal except from high alphafetoprotein and an abnormal glycosylation profile that misled the diagnosis for years towards a yet-unidentified CDG type I syndrome.
Results: Whole exome sequencing revealed two new pathogenic point mutations in *C10orf2* confirmed by Sanger sequencing.
Conclusion: This report enlarges the clinical phenotype of *Twinkle* mutation and suggest that abnormal glycosylation profile suggestive of CDG type I or high blood alpha fetoprotein without obvious cause should prompt *Twinkle* sequencing.

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Insulin-responsive hyperglycemia and ketoacidosis: neonatal diabetes as a red herring for mitochondrial complex III deficiency

Anastasio N[1], Drogemoller B[4], Tarailo-Graovac M[3], Al Khalifah R[5], Legault L[2], Van Karnebeek C[3], Buhas D[1]

[1]Dept Med Genet, McGill Univ, Montreal, Canada, [2]Dept Ped, Div Endocrin, McGill Univ, Montreal, Canada, [3]Centre Mol Med and Therap, Univ of B.C., Vancouver, Canada, [4]Child & Family Res Inst, Univ of B.C., Vancouver, Canada, [5]Div Gen Peds, King Saud Univ, Riyadh, Saudi Arabia

Hyperglycemia, as opposed to hypoglycemia, is a rare presenting symptom in mitochondrial disorders. Here, we report a young girl who presented shortly after birth with ketoacidosis, hyperlactatemia, hyperammonemia and insulin responsive-hyperglycemia. The patient was born at 34 weeks gestation to non-consanguineous Sri Lankan parents. Pregnancy was complicated by oligohydramnios and severe intra-uterine growth restriction. At 8 months of age, she presented with a similar episode, requiring admission to the ICU. She recovered very well and did not require long-term insulin treatment. Now, at 1 year of age, she has mild feeding problems, mild motor delay and normal cognitive development.
Initial metabolic work-up suggested mitochondrial dysfunction. Given the unusual presentation, whole exome sequencing was performed on the parent-offspring trio (mean coverage 50X). The patient was found to be homozygous for the c.643C>T (p.Leu215Phe) mutation in *CYC1*. This nuclear gene encodes cytochrome C_1, a subunit of respiratory chain complex III. Mutations in this gene have only been previously reported in two patients with similar presentation, one of which carries the same mutation as our patient and is also of Sri Lankan origin.
Primary complex III deficiencies are infrequent and strikingly different in their clinical presentation, as evidenced by this report.

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A case with *SURF-1* mutation and hypertrophic olivary nuclear degeneration

Kalkan Ucar S[1], Köse M[1], Canda E[1], Eraslan C[3], Kagnici M[1], Ceylaner S[2], Çoker M[1]

[1]Div Metab Dis, Ege Univ Med Faculty, İzmir, Turkey, [2]Intergen Molec Gen Lab, Ankara, Turkey, [3]Div Neuroradiology, Ege Univ Fac, İzmir, Turkey

Mutations in the nuclear *SURF-1* gene lead directly to cytochrome-c oxidase deficiency, the most common respiratory chain defect in Leigh syndrome. We describe a patient with *SURF-1* mutations presenting with hypertrophic olivary nuclear degeneration (HOD) adding to the growing number of cases of mitochondrial syndromes presenting with different radiological findings. Our patient is a 16 year old girl with ataxia and generalised tremor as the presenting manifestation. As an interesting neurological symptom, palatal tremor was observed. Hypertrichosis and facial dysmorphism were additional phenotypic features. In laboratory analyses profound metabolic acidosis and proximal renal tubular acidosis was detected. On magnetic resonance imaging she had brainstem, cerebellar involvement and basal ganglia involvement. The bilateral hypertrophic olivary nuclear degeneration was observed as a striking sign. *SURF-1* analysis identified a previously reported mutation as p.199delG (c.595_597delGGA)/IVS7 + 1G > A.
We report a patient with a relatively stable course and aim to emphasize that the presence of olivary nuclear degeneration, in the appropriate clinical setting, should alert the clinician to the possibility of a mitochondrial disorder and the need to screen for mutations in *SURF-1* gene.

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Diagnosis and management of drooling in children with progressive dystonia - A case series of patients with MEGDEL syndrome

Blommaert D[1], Van Hulst C C M[3], Van den Hoogen F J A[4], Erasmus C E[2], Wortmann S B[1 5]

[1]Dep Ped, RadboudUMC, Nijmegen, Netherlands, [2]Dep Neurol, RadboudUMC, Nijmegen, Netherlands, [3]Dep Rehab, RadboudUMC, Nijmegen, Netherlands, [4]Dep ENT, RadboudUMC, Nijmegen, Netherlands, [5]Dep Ped, Salzburger Landeskliniken, Salzburg, Austria

Background: Progressive dystonia is seen in different paediatric neurological disorders of which MEGDEL syndrome is an example. Swallowing problems are common in these patients, which can lead to excessive pooling of saliva in the oral cavity, resulting in drooling. Drooling is divided into anterior and posterior drooling, both affecting quality of life, the latter causing recurrent pneumonia.
Patients and results: Four patients with MEGDEL syndrome were evaluated at the multidisciplinary saliva control outpatient clinic. They all suffered severe anterior and posterior drooling with subsequent respiratory problems. One patient improved on anti-reflux medication, in one side effects of

medication necessitated switching and two patients successfully underwent salivary gland surgery.
Discussion: Based on our experience and as illustrated by the presented patients, we propose a practical and stepwise treatment approach. This takes into account all factors contributing to drooling, starting with non-invasive treatment options (for constipation, scoliosis and reflux disease). Medication that induces saliva secretion has to be replaced if possible. When a risk for chronic salivary aspiration remains, more invasive treatment is necessary (anticholinergic medication, botulinum toxin injections and surgery). This approach improved quality of life and reduced pulmonary problems in our patients.

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Case report: atypical juvenile parkinsonism and basal ganglia calcifications due to HSD10 disease

Lorea C F[1 2], Sitta A[1], Yamamoto R[6], Paulo P A F[5], Vairo F P[1], Rieder C R M[5], Wajner M[1 4], Schwartz I V D[1 3], Giugliani R[1], Jardim L B[1], Sass J O[7], Saute J A[1]

[1]Med Genet Serv, Hosp Clin Porto Alegre, Porto Alegre, Brazil, [2]Post Grad Prog Child Adol, UFRGS, Porto Alegre, Brazil, [3]Gen Mol Biol Department, UFRGS, Porto Alegre, Brazil, [4]Biochemistry Department, UFRGS, Porto Alegre, Brazil, [5]Neurol Serv, Hosp Clin Porto Alegre,, Porto Alegre, Brazil, [6]MVZ Dr. Eberhard & Partner Dortmund, Dortmund, Germany, [7]Univ Applied Sciences, Rheinbach, Germany

Background: Basal ganglia calcifications (BGC) are well described in a limited number of genetic conditions. We report the case of a patient with BGC due to X-linked HSD10 disease, a feature not previously described.
Case report: MSC, 27-year-old male, was referred for neurogenetic evaluation with a hypothesis of mitochondrial disease due to BGC and parkinsonism. He was born from a non-consanguineous couple and has 5 brothers, one of them with juvenile-onset epilepsy. The patient had a history of motor and cognitive milestones delay. Seizures started at 8 years-old. After the age of 20, rest tremor, gait abnormality and dysarthria became evident. On physical examination, symmetric parkinsonism, pyramidal findings and intellectual disability were observed. Urinary organic acids profile showed elevated levels of 2-methyl-3-hydroxybutyric acid and tiglylglycine, compatible with HSD10 disease or beta-ketothiolase deficiency. His mother has mild intellectual deficiency and a compatible, but less pronounced urinary organic acid profile, as MSC. Sanger sequencing revealed the novel p.A158V mutation in *HSD17B10* gene - probably pathogenic by our *in silico* analysis - in MSC (hemizygous) and his mother (heterozygous), confirming HSD10 disease diagnosis.
Conclusion: We suggest HSD10 disease as a rare but important differential diagnosis for BGC and/or atypical juvenile parkinsonism in males.

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Diagnosis and molecular basis of mitochondrial respiratory chain disorders in Japan: comprehensive genomic analysis for searching disease causes

Tajika M[1], Murayama K[1], Fushimi T[1], Taniguchi M[1], Ishii M[1], Ajima M[1], Shimura M[1], Ichimoto K[1], Tsuruoka T[1], Matsunaga A[1], Yamazaki T[4], Mori M[3], Kishita Y[3], Tokuzawa Y[3], Kohda M[3], Takayanagi M[1], Okazaki Y[2], Ohtake A[4]

[1]Dep Metab,Chiba Children`s Hospital, Chiba, Japan, [2]Res Center for Genom Med, Saitama Med U, Saitama, Japan, [3]Dep Pediatr, Matsudo City Hosp, Matsudo, Japan, [4]Dep Pediatr, Saitama Med U, Saitama, Japan

Background: Mitochondrial respiratory chain disorders (MRCDs) are the most frequent inherited metabolic diseases caused by a defect of the nuclear or mitochondrial DNA. The aim of our study is to make the correct diagnosis of MRCDs and identify the genetic causess.
Methods: MRCDs were diagnosed biochemically, using in vitro enzyme assay and BN-PAGE analysis. Subsequently, we performed comprehensive genomic analyses that include mtDNA and exome analysis using high-throughtput sequencer, and chromosomal aberration analysis using high-density oligonucleotide array.
Results: 406 of 1291 (31%) candidates were diagnosed by the enzyme analysis. Of the 213 patients analyzed, 62 had mtDNA pathogenic mutations (30%). By whole exome sequencing, a total of 142 cases were analyzed. We identified 36 known genes, and 6 novel candidate genes. Several of them are involved in mtDNA processing (*GTPBP3*, *KARS*, *QRSL1*), a component of complex I (*NDUFB11*), a component of the mitochondrial ribosome small subunit (*MRPS23*) and biosynthesis of CoQ_{10} (*COQ4*). We also identified 3 non-mitochondrial related disease genes and 3 chromosomal aberration regions as causes of MRCD.
Discussion: We demonstrated the comprehensive genomic analysis for MRCDs was effective. However, we have analyzed only half of patients by these method, further analysis is required to identify the disease mutations.

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Isolated complex III deficiency due to LYRM7 deficiency in two siblings

Tsiakas K[1], Hempel M[1], Haack T[2], Kremer L[2], Feichtinger R[3], Sperl W[3], Mayr J[3], Prokisch H[2], Santer R[1]

[1]Dept Pediatr, Univ Med Cent Eppendorf, Hamburg, Germany, [2]Inst Hum Genet, Helmholtz Cent, Munich, Germany, [3]Dept Pediatr, Paracelsus Med Univ, Salzburg, Austria

Background: LYRM7 is necessary for the assembly of the Rieske-Fe/S protein in complex III. To date, a *LYRM7* mutation has only been reported in a single patient (Invernizzi 2013). We report signs, symptoms, and a novel *LYRM7* mutation in siblings with clinical features of a mitochondrial disease.
Case Study: In a 3-year-old girl and her older sister (died at 2 years), we found muscular hypotonia, psychomotor delay, and severe episodic lactic acidosis. MRI showed severe leukoencephalopathy with enhanced intramedullary T2 signal intensity and massively disturbed diffusion. *Globi pallidi* were only mildly affected.
Methods & Results: Exome sequencing in one sibling revealed homozygosity for a 4-bp deletion within the *LYRM7* gene affecting a splice site. Sanger sequencing confirmed homozygosity in both siblings and heterozygosity in both healthy consanguineous parents. Complex II+III activity in patients' fibroblasts was reduced. Diminished activity of complex III was also found in muscle. Western blot of muscle and immunohistochemistry in fibroblasts showed a decreased amount of the Rieske protein while core-2 protein (CIII-subunit), NDUFS4 (CI-subunit) and COX-I were normal.
Conclusion: LYRM7 deficiency is a novel cause of isolated complex III deficiency. Next generation sequencing plays a pivotal role in the diagnosis of such rare and novel genetic defects underlying mitochondrial diseases.

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***NDUFAF4* mutations in two siblings with dysmorphic features, cardiomyopathy and 3-methylgutaconic aciduria**

Ugarteburu O[4], Tort F[1], García-Silva M T[2], Sanjurjo P[5], García-Villoria J[4], Ferrer-Cortès X[1], Arias A[4], Briones P[3], Ribes A[1]

[1]Hosp. Clínic, IDIBAPS, CIBERER, Barcelona, Spain, [2]Serv. de Pediatría, Hosp. 12 de Octubre, Madrid, Spain, [3]Hosp. Clinic, IDIBAPS, CSIC, Barcelona, Spain, [4]Hosp. Clinic, IDIBAPS, Barcelona, Spain, [5]Hosp. de Cruces, Bilbao, Spain

We report two siblings from healthy consanguineous parents. They presented during the neonatal period with convulsions, irritability, facial dysmorphia, leucodystrophy and hypertrophic cardiomyopathy. Metabolic studies showed high plasma levels of lactate and α-alanine. The organic acid profile showed increased excretion of lactate, succinate, fumarate, 2-ketoglutarate and 3-methylglutaconate (3-MG). Respiratory chain activities in muscle were normal and PDH activity was also normal. The excretion of 3-MG together with the dysmorphic features of the affected children made the rationale for the analysis of genes associated to 3-MGA-uria. Analysis by self-designed targeted exome sequencing excluded mutations in *TMEM70*, *OPA3*, *TAZ*, *ATP5E*, *ATPF2*, *SERAC1* and *DNAJC19*. Whole-exome sequencing in the two affected siblings identified a homozygous nonsense mutation in *NDUFAF4* (c.558G>T). Since NDUFAF4 is an assembly factor of the mitochondrial respiratory chain complex I BN-PAGE in patient's fibroblasts was performed and demonstrated an isolated deficiency in the assembly of complex I, that was consistent with the genetic data. Since the first description in 2009 (Saada Am J Hum Genet. 2009) no other patients have been reported. We highlight the importance of a precise biochemical characterization to help next generation sequencing approaches in the elucidation of the genetic causes of rare disorders.

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The first male case with cardiomyopathy and isolated complex I deficiency caused by hemizygous mutation in *NDUFB11* gene

Ounap K[1 2], Reinson K[1 2], Pajusalu S[2 3], Noukas M[4 5], Oiglane-Shlik E[1 6], Rodenburg R J[7], Van den Heuvel L P[7], Reimand T[1 2 3]

[1]Dept of Pediatrics, University of Tartu, Tartu, Estonia, [2]Dept of Genetics, Tartu Univ Hospital, Tartu, Estonia, [3]Inst of Biomed&Transl Med, Univ of Tartu, Tartu, Estonia, [4]Inst of Mol & Cell Biol, Univ of Tartu, Tartu, Estonia, [5]Estonian Genome Center, Univ of Tartu, Tartu, Estonia, [6]Children's Clinic, Tartu Univ Hospital, Tartu, Estonia, [7]Ctr for Mitoch Dis, Radboud Univ Med Ctr, Nijmegen, Netherlands

Mutations in the X-linked *NDUFB11* gene were recently shown to cause microphthalmia with linear skin defects syndrome (MIDAS). Additional clinical features include neurological and cardiac abnormalities. Presently only two females have been described and this condition has thus far been assumed to be lethal in males. The

NDUFB11 gene encodes a subunit of the mitochondrial respiratory chain complex I and is essential for its activity.

We present a male patient born at term by vacuum extraction due to fetal asphyxia. Soon after birth lactic acidosis developed (20 mmol/L). Ultrasound investigation showed hypertrophic cardiomyopathy and periventricular cysts of the white matter. Urinary organic acid analysis showed increased excretion of the Krebs cycle metabolites and 3-methyl glutaconic acid. Enzyme analysis in skin fibroblasts showed a reduced activity of the mitochondrial complex I. A *de novo* hemizygous NM_019056.6:c.328C>T (p.Pro110Ser) mutation was detected in *NDUFB11* gene by exome sequencing analysis. *In silico* analysis predicted the variant to be pathogenic. Mitochondrial DNA sequencing did not exhibit any pathogenic mutations.

This is the first report of a male patient with mutation in *NDUFB11* gene showing novel non-lethal genotype-phenotype association without signs of previously reported MIDAS. This study was supported by the Estonian Research Council grant PUT355.

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A new patient with mutations in *NADK2* and prolonged survival

Ruiz M A[4], García-Villoria J[3], Tort F[1], Ugarteburu O[3], Ferrer-Cortès X[1], Matalonga L[1], Arias A[3], Girós M[1], Pajares S[3], Briones P[2], Ribes A[1]

[1]Hosp. Clínic, IDIBAPS, CIBERER, Barcelona, Spain, [2]Hosp. Clinic, IDIBAPS, CSIC, Barcelona, Spain, [3]Hosp. Clinic, IDIBAPS, Barcelona, Spain, [4]Hosp. Son Dureta, Palma de Mallorca, Spain

We report a nine year old Spanish female with mutations in *NADK2*. Antenatal CNS abnormalities showed ventriculomegaly, colpocephaly and hypoplasia of the corpus callosum. At birth, central hypotonia along with uncoordinated movements, microcephaly and generalized cerebellar atrophy were detected. Metabolic investigations revealed high lysine, lactate and pipecolic acid levels in blood and CSF. PDH activity in fibroblast and complexes III and IV in muscle were slightly low. At 3 months of age she had frequent vomiting and refusing to feed, that resolved with lysine restricted diet. Later on, biotin, pyridoxal phosphate, thiamine, vitamin D and ubidecorenone were added to treatment with good clinical and biochemical response. At 8 years of age plasma acylcarnitines showed high levels of C10:2. Whole exome sequencing identified a homozygous splice site mutation in *NADK2* (c.468+6A>G). This substitution generates a skipping of exon 9, leading to a protein with a PTC at residue 167. In fact, *NADK2* mRNA and the corresponding protein were almost absent. At 9 years of age she presents with ataxia and incoordination. She has oromotor dysphasia but is able to understand fluid language and is a very friendly girl. We highlight the better clinical evolution of this patient with respect to the first *NADK2* description.

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Two cases of ECHS1 deficiency with mitochondrial encephalopathy and cardiomyopathy

Matsunaga A[1], Murayama K[2], Matsubayashi T[3], Nagatomo T[4], Mori M[5], Kishita Y[6], Tokuzawa Y[6], Kohda M[6], Okazaki Y[6], Ohtake A[7]

[1]Dep Cri Care Med, Chiba Children's Hosp, Chiba, Japan, [2]Dep Metab, Chiba Children's Hosp, Chiba, Japan, [3]Dep Ped, Hamamatsu Med Univ, Hamamatsu, Japan, [4]Dep Neo, Ehime Prefe Central Hosp, Ehime, Japan, [5]Dep Ped, Matsudo City Hosp, Matsudo, Japan, [6]Res Cen Genomic Med, Saitama Med Univ, Saitama, Japan, [7]Dep Ped, Saitama Med Univ, Saitama, Japan

Background: The ECHS1 is a multifunctional mitochondrial matrix enzyme that is involved in the oxidation of fatty acids and amino acids. Recently, a few cases of Leigh syndrome due to ECHS1 deficiency were reported. We report here the two girls who had ECHS1 deficiency, one patient presenting with neonatal lactic acidosis and another with Leigh-like syndrome.

Case reports:

Case1: A girl presented with hypertrophic cardiomyopathy, deafness and lactic acidosis soon after birth. MRI showed low intensity in cerebral white matter and brain atrophy. She died at the age of 4 months. Liver autopsy showed deficiency of complex I.

Case2: A girl had epileptic seizures at 2 days of age and developed respiratory failure and unconsciousness with lactic acidosis at the age of 8 months. Brain CT and MRI showed Leigh syndrome. At the age of 1 year, she developed deafness, and at the age of 3 years dilated cardiomyopathy. Muscle biopsy showed deficiency of complex IV. Immunoblotting showed the decreased protein of ECHS1 and gene analysis showed the compound heterozygous mutation in both cases.

Conclusion: ECHS1 deficiency has not been recognized as a FAOD. It's important that FAOD is occasionally associated with mitochondrial respiratory chain disease.

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A case of coenzyme Q10 deficiency diagnosed by next-generation sequencing

Racz G Z[1], Kalmar T[1], Ivanyi B[2], Bereczki C[1], Maroti Z[1]

[1]Dept Ped, Univ Szeged, Szeged, Hungary, [2]Dept Pathol, Univ Szeged, Szeged, Hungary

Background: Congenital non-Finnish type nephrotic syndrome may be caused by mutations in genes involved in coenzyme Q10 (CoQ10) biosynthesis. CoQ10 deficiency has been associated with two infantile onset clinical phenotypes: severe infantile multisystem disease or isolated nephropathy.
Case report: A 7-month-old infant presented with lower limb oedema and developmental delay. Detailed history revealed that he had various, although mild, symptoms since the newborn period, including poor weight gain, poor visual contact, and gastrointestinal problems. Further investigations revealed elevated lactate and pyruvate level, hypothyreosis, hypertrophic cardiomyopathy, hypoproteinemia due to massive proteinuria. Electroencephalogram registered non-specific encephalopathic signs. Ophthalmology and visual evoked potential study confirmed retinitis pigmentosa. Otoneurology found no outer haircell activity. A mitochondrial disorder, more specifically, based on the presence of nephrosis, severe CoQ10 deficiency, was suspected. High-dose coenzyme Q10 treatment was started. However, despite therapy, the infant's status deteriorated quickly, due to intractable protein loss and pulmonary hypertension.
Results: Next generation sequencing (NGS) of all disease-related genes detected compound heterozygous point mutation and large deletion in the prenyl-diphosphate synthase, subunit 2 (*PDSS2*) gene.
Conclusion: This rare CoQ10 deficiency case shows that NGS may be powerful diagnostic tool for patients with multisystem disesase, including rare mitochondrial disorders.

P-392

***SUCLA2* deficiency, a deafness dystonia syndrome with distinctive metabolic findings - report of a new patient and review of the literature**

Maas R R[1], Della Marina A[2], De Brouwer A P M[3], Wevers R A[4], Rodenburg R J[1], Wortmann S B[5]

[1]Child Hosp RadboudUMC, Nijmegen, Netherlands, [2]Dep Neuroped, Univ of Essen, Essen, Germany, [3]Dep Hum Gen RadboudUMC, Nijmegen, Netherlands, [4]Dep Lab Med RadboudUMC, Nijmegen, Netherlands, [5]Dep Ped Paracelsus Med Univ, Salzburg, Austria

Background and objectives: *SUCLA2* encodes for a subunit of succinyl-coenzyme A synthase, the enzyme that reversibly synthesizes succinyl-coenzyme A and ATP from succinate, coenzyme A and ADP in the Krebs cycle. Disruption of *SUCLA2* function leads to mitochondrial DNA depletion clinically presenting as a mitochondrial encephalopathy with dystonia and deafness. Additionally, patients show a characteristic metabolic pattern: methylmalonate, C4-dicarboxylic carnitine and lactate are increased in both plasma and urine.
Patients and Methods: Thus far, eight different disease-causing *SUCLA2* mutations, of which six missense mutations and two splice site mutations, have been described in the 28 patients reported in literature. New is the encounter of the first patient with an intragenic deletion in *SUCLA2*, showing the typical deafness-dystonia syndrome with similar disruption of biochemical markers.
Discussion/Conclusion: Patients with SUCLA2 deficiency present with a rare, but distinctive deafness-dystonia syndrome. The clinical phenotype together with the metabolic findings in blood and urine is so specific that careful description without further investigations (e.g. brain MRI) would be enough to classify patients. If Sanger sequencing of *SUCLA2* of such a patient does not show disease-causing variants, MLPA analysis should follow to detect an intragenic deletion as in our patient.

P-393

Mutations in mitochondrial fission factor *MFF*, a new cause of pediatric mitochondrial disorders

Freisinger P J K[1], Koch J[2], Haack T[3], Feichtinger R[2], Mayr J A[2], Ahting U[3], Pies M[4], Scheffner T[1], Prokisch H[3], Sperl W[2]

[1]Metab Dis Center, Childrens Hospital, Reutlingen, Germany, [2]Dep Ped Paracelsus Medical University, Salzburg, Austria, [3]Inst Human Genetics Techn Univ, München, Germany, [4]Sozialpäd. Zentrum, Klinikum Höchst, Frankfurt, Germany

Mitochondria morphology is controlled by fission and fusion involving different proteins (OPA1, GDAP1, MFN1/2, DNM1L, mitochondrial fission factor MFF). Mutations in *OPA1* (dominant optic atrophy) and *GDAP1* (Charcot-Marie-Tooth-Disease) are detected mainly in adults and are frequent, but mutations in the other genes are rare. Only one patient with mutations in *MFF* is known. Interestingly MFF is also involved in peroxisomal fission. We present 3 patients from 2 families (Austrian, Turkish) with 3 different mutations in

MFF revealed by WES. Patient 1 and 2 showed global developmental delay, muscular hypotonia, microcephaly, optic atrophy and epilepsy in the first year, MRI revealed bilateral abnormalities of the basal ganglia and brain atrophy. Until age 4 and 7 years the clinical course was stable with minimal developmental progress. Lactate was mildly elevated (plasma) but normal in CSF. VLCFA plasma-profile and respiratory chain function in muscle were normal. Fibroblasts showed abnormal tubular appearance of mitochondria and peroxisomes. Patient 3, elder brother of 2, suffered from severe birth asphyxia and died at age 18 months. These observations demonstrate that defects in mitochondrial morphogenesis must also be considered in pediatric patients especially with normal respiratory chain analysis. Defects in MFF might be a common cause of mitochondrial and peroxisomal dysfunction.

P-394

PUS1 and *COX10* mutations in three Czech patients with cytochrome c oxidase deficiency and haematological symptoms

Tesarova M[1], Vondrackova A[1], Kratochvilova H[1], Dvorakova V[1], Stranecky V[1], Berankova K[1], Langer J[1], Honzik T[1], Hansikova H[1], Zeman J[1]

[1]Dpt Pediatr, Charles Univ, Gen Univ Hosp, Prague, Czech Republic

Anaemia represents a heterogeneous group of hematologic diseases, which can be induced by plenty of genetic and risk factors. Although the association of mitochondrial dysfunction and hematologic pathology is generally known, the underlying pathologic factors with regard to evolving anaemias are poorly characterized. In this study, we report on three patients, who manifested profound cytochrome c oxidase deficiency combined with anaemia. Genetic causes of their OXPHOS deficiency were found with the use of targeted sequencing of mitochondrial exome and SNP microarray analysis. A rarely occurring pathological 6-kbp homozygous deletion was identified in two unrelated patients affecting the *PUS1* gene, which remarkably leads to different disease-phenotype in both patients. Two previously characterized deleterious missense sequence variations of *COX10* gene (p.Asn204Lys; p.Pro225Leu) were identified in the third patient, however, their combination has not been reported yet, which may imply the variant patient disease-phenotype. Based on our results and current knowledge, we suggest the infantile deficiency of *PUS1* and *COX10* to be classified as the early fatal and slowly progressive forms. To conclude, mitochondrial disorders manifest poor phenotype-genotype correlation even in the patients with the same causal mutations. This study was supported by grants RVO-VFN64165/2012, IGA NT13114-4, IGA NT14156–3 and GAČR 14-36804G.

P-395

Chromosomal aberrations mimicking mitochondrial disorders

Tumiene B[1 2], Tumiene B[1 2], Songailiene J[1 2], Preiksaitiene E[1 2], Ciuladaite Z[1 2], Bandanskyte A[3], Kucinskas V[1 2], Utkus A[1 2]

[1]Dep of Hum and Med Gen, Vilnius Univ, Vilnius, Lithuania, [2]Vilnius Univ Hosp Santariskiu Clin, Vilnius, Lithuania, [3]State hosp of Panevezys, Panevezys, Lithuania

Case report: two patients (1st: 3,5-years-old girl; 2nd: 2,5-years-old boy) with aberrations of chromosome 10 mimicking mitochondrial disorders are presented. Psychomotor retardation, muscular hypotonia, hypomimia, ptosis, lactic acidosis in both of them prompted for further analyses of mitochondrial function. Urine organic acid analysis at the time of hyperlactatacidemia revealed increased alpha-ketoadipic acid and 3-methylglutaconic acid/ 3-methylglutaric acid in patient 1 and patient 2, respectively.

Chromosomal microarray analysis (CMA) in patient 1 revealed interstitial 5,1 Mb deletion of 10q22.1-q22.3, including 3 genes with mitochondrial functions (*MICU1* and *MCU*, regulation of mitochondrial Ca uptake, *MRPS16*, mitochondrial ribosomal protein S16; homozygous loss-of-function mutations in *MICU1* and *MRPS16* were previously reported).

CMA in patient 2 revealed 4,4Mb deletion of 10p15.1-pter/13,4Mb duplication of 10q26.12qter. *ZMYND11* and *DIP2C* genes were proposed as responsible for clinical features. Recently described patient with de-novo heterozygous *ZMYND11* mutation shares clinical features with 10p15 deletion patients. *ZMYND11* encodes H3K36 trimethylation-dependent transcription repressor, according to functional protein association network database, http://string-db.org, it interacts with *SIRT1*, (PGC-1α)-dependent histone-deacetylase promoting mitochondrial biogenesis, oxidative phosphorylation and energy production.

Conclusion: patients with chromosomal aberrations can present with mitochondrial dysfunction; molecular mapping and gene-interaction studies can pinpoint genes involved in the pathogenesis of mitochondrial dysfunction.

P-396

Two new Turkish siblings with MEGDEL syndrome and novel mutation

Unal O[1], Gunduz M[1], Unal S[1], Yucel D[2], Ozgul R K[2]

[1]Ankara Hematology Oncology Children's, Ankara, Turkey, [2]Institute of Child Health, Hacettepe, Ankara, Turkey

Background: Mutations in *SERAC1* gene, encoding a phosphatidylglycerol remodeling enzyme cause MEGDEL syndrome characterized by deafness, progressive spasticity, dystonia, and Leigh-like lesions. Here, we present two new Turkish siblings with MEGDEL syndrome due to novel *SERAC1* gene mutation.
Case report: Three day-old patient was admitted with lethargy, lactic acidosis and hyperammonemia. He was born at term and birth weight was 2400 g. A mitochondrial disorder was suggested initially. Cranial MRI showed T1A signal changes on temporal and posterior parietal lobes. DWI revealed restricted diffusion areas on posterior corpus callosum and occipital lobes. Basal ganglia were normal. It was learned that first sibling had hearing loss and 3-methylglutaconic aciduria. Cranial MRI of first sibling was consistent with Leigh-like syndrome. MEGDEL syndrome was suggested, but, there was no 3-methylglutaconic acid on urine organic analysis. It was reevaluated at 2 months old and showed one fold increased 3-methylglutaconic acid. Sensorineural hearing loss was detected. Sequencing analysis of *SERAC1* gene showed a novel homozygous c.1015G>C;p.Gly339Arg mutation in exon 10. Mutation prediction software tools PolyPhen-2 and SIFT both indicate that the novel mutation is likely to be pathogenic.
Conclusion: Cranial imaging and urine organic acid analysis may not be typical for MEGDEL syndrome in the newborn period.

P-397

Mutations in *C10orf2* gene mimic clinical picture of Niemann–Pick disease type C

Mikhailova S V[2], Proshlyakova T Y[1], Zakharova E Y[1]

[1]FSBI Res Cent Med Gen, Moscow, Russian Federation, [2]Dep Med Gen, Rus Child Clin Hosp, Moscow, Russian Federation

Niemann–Pick disease type C (NPC) is an inherited neurovisceral lysosomal disease. Clinical features may be nonspecific and overlap with other metabolic disorders. We report a patient whose diagnosis was initially suspected for NPC, but was changed to mitochondrial disease (MD) after additional diagnostic investigations.
Case report: A 9 y.o. Russian girl was suspected for NPC due to combination of neurological symptoms: mild cognitive delay, cataplexy, ophthalmoplegia, dystonia, and ataxia. Urine organic acids, blood lactate, activity of lysosomal enzymes were normal. Score by NPC Suspicion Index was 112 – high probability of NPC. *NPC1*, *NPC2* genes were analyzed by direct sequencing. The variant c.441+1G>A in *NPC2* gene was found in heterozygous state. This variant was previously described, but with different opinions of its pathogenicity. *In silico* analysis predicted altered splicing, but clinical features of the patients with that variant are extremely various. To determine the approximate frequency of the c.441+1G>A among Russian population, 103 control samples were analyzed leading to 2 positive alleles (0,97%). Laboratory testing of the patient were continued. Finally, by using whole exome sequencing, we have found two novel mutations – c.1196A>G (p.N399S) and c.1199G>T (p.R400L) in compound-heterozygous state in *C10orf2* gene, that was causing mitochondrial DNA depletion syndrome 7.

P-398

GDF-15 and *FGF-21* are comparably sensitive and specific biomarkers of mitochondrial diseases

Ramos F J[2], Montero R[1 3], Yubero D[1 3], Henares D[2], Ortez C I[2], O'Callaghan M[2], Rodriguez M A[2], Nascimento A E[2 3], Garcia Cazorla A[2 3], Montoya J[3 4], Meznaric M[5], De Meirleir L[6], Kalko S[7], Artuch R[1 3], Jimenez-Mallebrera C[2 3]

[1]Clin Bioch Dep,Sant Joan de Deu Hosp, Barcelona, Spain, [2]Neuropaed Dep,Sant Joan de Deu Hosp, Barcelona, Spain, [3]CIBERER,Inst de Salud Carlos III, Spain, Spain, [4]Bioch and Mol Biol Dep,Univ of Zaragoza, Zaragoza, Spain, [5]Inst of Anat. Fac of Med,Univ Ljubljana, Ljubljana, Slovenia, [6]Lab for Neuropath, Ghent Univ, Ghent, Belgium, [7]IDIBAPS, Clinic Hospital., Barcelona, Spain

Background: We recently described increased levels of growth and differentiation factor 15 (GDF-15) in muscle and serum from patients with mitochondrial disease suggesting that it could represent a novel biomarker for this group of complex disorders.
Aims: To evaluate the use of GDF-15 in the diagnosis of mitochondrial diseases relative to fibroblast-growth-factor 21 (FGF-21) and other biochemical parameters.
Methods: Circulating GDF-15 and FGF 21 were measured by ELISA. Statistical analysis was performed using SPSS v.20.
Results: We have studied 59 samples from patients with confirmed mutations in nuclear or mitochondrial DNA and with a definitive or probable diagnosis of mitochondrial disease

according to Morava criteria. As controls we studied 19 samples from other myopathies and 34 healthy controls. We found that GDF-15 and FGF-21 were significantly increased in patients relative to healthy and myopathic controls. There was a significant correlation between GDF-15 and FGF-21 levels in patients and between the factors and hepatic enzymes. ROC analysis indicates that both soluble factors have a good discriminatory power.
Discussion: Diagnosis of mitochondrial diseases is challenging. Our data show that GDF-15 is a valuable marker for such purposes. We propose to include both GDF-15 and FGF-21 in the diagnostic workup of these diseases.

P-399

A new pathogenic mutation in the iron-sulfur cluster assembly gene *IBA57* causes impaired protein function leading to massive early leukoencephalopathy

Debray F G[2], Stumpfig C[3], Vanlander A V[1], Smet J[1], Dideberg V[2], Josse C[2], Caberg J H[2], Boemer F[2], Bours V[2], Stevens R[4], Lill R[5], Van Coster R[1]

[1]Div Ped Neur, Univ Hosp Ghent, Ghent, Belgium, [2]Med Genet, Sart-Tilman Univ Hosp, Liège, Belgium, [3]Inst Zytobiol u Zytopath, Philipps Univ, Marburg, Germany, [4]Dept Ped, Clin Espérance, Liège, Belgium, [5]LOEWE Zent Synth Mikrobiol,Philipps Univ, Marburg, Germany

Objective: Unraveling the molecular cause of a progressive leukoencephalopathy and combined OXPHOS deficiency.
Methods: Homozygosity mapping and whole exome sequencing (WES) has been achieved. Pathogenicity has been assessed by means of functional studies in HeLa cells and western blotting in patient material.
Results: The patient, presenting a psychomotor retardation and white matter alterations with high lactate, was identified with a combined complex I and II deficiency in skeletal muscle. This combination is suggestive for a defective iron-sulfur cluster (ISC) biosynthesis, which could be confirmed by an absence of lipoic residues on αKGDH and PDH. Homozygosity mapping revealed several candidate genes involved in ISC biogenesis (*NFU1*, *BOLA3*, *IBA57*, *ABCB10*). WES identified a homozygous variant in *IBA57* [c.436C>T (p.Arg146Trp)]. Complementation studies in HeLa cells depleted of wild type *IBA57* and transfected with mutated *IBA57* showed deficient lipoylation and lowered expression of a complex II subunit.
Conclusion: Combining a biochemical and molecular approach, the genetic cause could be unraveled. This patient constitutes the third report of a pathogenic variant in *IBA57* and discloses the clinical and neuroradiological heterogeneity of this defect. At the biochemical level a combined complex I and II deficiency and absence of lipoylation seems to be a uniform finding.

P-400

Utilizing next-generation sequencing for diagnosis of nuclear-gene encoded mitochondrial disorders

Bijarnia-Mahay S[1], Puri R D[1], Dash P[1], Kotecha U H[1], Kohli S[1], Saxena R[1], Balakrishnan P[1], Verma I C[1]

[1]Center Med Genet, Sir Ganga Ram Hosp, New Delhi, India

Background: Mitochondrial disorders of the nuclear genome mainly present in childhood with wide-ranging phenotype, majority of them being recessively inherited. Diagnosis requires invasive and expensive investigations (muscle, liver and skin biopsies with immunostaining and enzymology), largely unavailable in India. With advent of next-generation sequencing technology (NGS), patients have benefitted greatly by receiving an accurate diagnosis in a timely and cost-effective manner. This has enabled prenatal diagnosis and prevention of the burden of mitochondrial disease in affected families.
Method: Eight families were enrolled, including one couple without proband's DNA, for whom a mitochondrial diagnosis was made using NGS (targeted panel and whole exome sequencing in 4 cases each).
Results: Mutations were detected in *NDUFS6*, *MPV17*, *SCO2*, *AGK*, *LRPPRC*, *NDUFAF3*, *MTO1* and *RMND1* genes. Three novel mutations were identified - c.286C>T – *NDUFS6*, c.2770C>T – *LRPPRC*, c.99+1G> C – *NDUFAF3* gene. In 2 cases, only heterozygous mutations were detected. Prenatal diagnosis was performed in 3 families with *MPV17*, *NDUFS6* and *MTO1* gene mutations, resulting in unaffected fetuses in two and affected fetus in one, where pregnancy was terminated.
Conclusions: NGS results marked the end of a diagnostic odyssey in affected children, and provided a hope for prenatal diagnosis to families who approached after proband's death.

P-401

Mitofibrate CT1: a double-blind placebo controlled trial to evaluate efficacy and safety of bezafibrate for patients with mitochondrial myopathies

Lagler F B[1], Koch J[3], Freisinger P[2], Mayr J[3], Moder A[1], Sperl W[3]

[1]Inst Inborn Errors of Metab, PMU, Salzburg, Austria, [2]Child Hosp, Klinikum am Steinenberg, Reutlingen, Germany, [3]Dept Ped, Paracelsus Medical University, Salzburg, Austria

Background & Objectives: Findings in pre-clinical studies and observations in individual patients point to a beneficial role of bezafibrate for patients with mitochondrial myopathies. We

therefore, investigate the efficacy and safety of bezafibrate in a multinational trial.
Methods & patients: 16 patients with PDHc or Complex I defect are enrolled. The study is designed as responder-enrichment phase followed by randomized placebo-controlled withdrawal of bezafibrate. Primary endpoint is the time interval until pre-defined manifestation of disease progression measured by walking-distance in the 6-minutes-walking test. The subjective disease progression is continuously measured by patients or their parents via Newcastle Paediatric Mitochondrial Disease Scale. Once the score points to disease progression, the walking-test performance is evaluated in an unscheduled study visit. Elsewise, walking test performance is routinely evaluated at scheduled study visits. A decline of >20% in the 6-minutes-walking test leads to termination of study participation.
Preliminary Results & Conclusion: Of 12 patients at study site Salzburg, 1 patient dropped-out prior to randomization, 8 patients were assessed as responders and entered the randomized phase. So far, 7 patients have completed the trial. Among them, 3 showed disease progression and reached the primary endpoint at preterm. No serious adverse drug reactions have been reported.

P-402

5-Aminolevulinic acid and iron can bring a cure for mitochondrial respiratory chain disorders

Ohtake A[1], Murayama K[2], Harashima H[1], Yamazaki T[1], Tokuzawa Y[3], Kishita Y[3], Mizuno Y[3], Kohda M[4], Shimura M[2], Fushimi T[2], Taniguchi M[2], Ajima M[2], Takayanagi M[2], Okazaki Y[3 4]

[1]Dept Pediatr, Saitama Med Uni, Moroyama, Japan, [2]Dept Metab, Chiba Child Hosp, Chiba, Japan, [3]Div Funct Genom, RCGM, Saitama Med Uni, Hidaka, Japan, [4]Div Tr Res, RCGM, Saitama Med Uni, Hidaka, Japan

Background: Mitochondrial respiratory chain disorders (MRCD) are almost incurable and have the highest incidence among congenital metabolic diseases occurring at a rate of 1 in 5000 births. Many drugs are now in development for MRCD, but there is no drug for permanent cure. 5-aminolevulinic acid (5-ALA) is a heme precursor, and heme proteins play an important role at the catalytic site of subunits of mitochondrial respiratory chain enzyme complexes.
Methods: To study the effects of 5-ALA on respiratory chain, we have investigated the amount and function of the mitochondrial respiratory chain complexes II, III and IV in 5-ALA treated fibroblasts from patients with MRCD. Fibroblasts from patients with MRCD were cultured in the medium supplemented with 100 mM iron ion and 5-ALA. Using blue native-PAGE, the assembly of complexes II, III and IV were analyzed.
Results: 5-ALA and iron ion increased heme proteins, leading to induction and activation of respiratory chain complexes II to IV and resulting in increment of ATP production in patient-derived fibroblasts (mainly Complex I deficiency).
Conclusion: We expect that 5-ALA could be used to provide a symptomatic relief for patients with MRCD. The investigator initiated clinical trial for patients with Leigh disease are now undertaken in Japan.

P-403

Gracile like syndrome caused by BCS1L gene mutations

Rodrigues E[1], Eden Santos P[1], Teixeira A[2], Martins E[3], Nogueira C[4], Vilarinho L[4], Leão Teles E[1]

[1]Metab Unit, Ped Depart CHSJoao, Porto, Portugal, [2]Nephr Unit, Ped Depart CHSJoao, Porto, Portugal, [3]Metab Unit, Ped Depart CHP, Porto, Portugal, [4]Met and Genet Unit, H Gen Depart, INSA, Porto, Portugal

Background: GRACILE syndrome is an inherited severe mitochondrial disorder characterized by fetal growth restriction, aminoaciduria, cholestasis, iron overload, lactacidosis, and early death. It is caused by a mutation in the *BCS1L* gene, located on chromosome 2q35.
Case report: CGS, female, 18 months of age, second daughter of non-consanguineous parents. Normal newborn presenting at 36 hours of life with irritability and tachypnea, associated with severe metabolic acidosis, hyperlactacidemia, hyperammonemia and hepatic cytolysis. Multidisciplinary approach identified proximal tubulopathy, nephrocalcinosis and normal iron kinetics. Cardiac, opthalmological and ENT evaluations were normal. A peak of lactate in both lenticular nuclei was identified on cerebral spectroscopy without morphological changes. Under therapy with bicarbonate and Joulie solution patient had a good metabolic control and at discharge she was clinically well. During follow up the patient had a positive evolution of growth and psychomotor development. Hepatic enzymes values have been floating.
BCS1L gene analysis revealed heterozygous compound for two mutations (c. - 147 A > G and p. R56X). The same genotype was identified in the older sister, six years old, followed in another centre, with similar presentation.
Discussion The framework of data allows the diagnosis of GRACILE like syndrome, with a more favorable picture than previously described.

P-404

A homozygous p.Trp22Arg *NDUFB3* mutation identified in a cohort of patients with mitochondrial complex I deficiency presenting with persistent growth failure, subtle facial dysmorphism and a variable metabolic phenotype

Howard C[1], Alston C L[2], McCarthy P[1], Shield J P H[3], Murray P G[4], He L[2], McFarland R[2], Knerr I[1], Monavari A A[1], Clayton P E[4], Taylor R W[2], Crushell E[1], Hughes J[1]

[1]NCIMD, CUH, Temple Street, Dublin, Ireland, [2]Wellcome Trust Centre for Mito Research, Newcastle upon Tyne, United Kingdom, [3]Bristol Royal Hospital for Children, Bristol, United Kingdom, [4]Centre for Paediatrics and Child Health, Manchester, United Kingdom

Background: *NDUFB3* encodes an accessory subunit of complex I, and mutations have previously been associated with a severe, often lethal, phenotype.
Methods: We report a cohort with a homozygous p.Trp22Arg *NDUFB3* mutation, initially identified through targeted NGS which sequenced the genes encoding all structural subunits and ancillary proteins of patients with complex I deficiency. Subsequent patients with similar features were identified through targeted mutation screening. A review of clinical notes was undertaken to define the phenotype.
Results: Nine children, eight of Irish ethnicity, from seven families were identified. Age range 0.5 – 12 years. All presented with intrauterine growth restriction, persistent postnatal growth restriction and characteristic facial features. Lactic acidosis was identified in seven. Two had hypertrophic cardiomyopathy. Neurodevelopment is normal. One family had a sibling who died day two of life with lactic acidosis. Two patients were identified through genetic investigation of familial short stature and do not have lactic acidosis. The p.Trp22Arg *NDUFB3* mutation had previously been reported in association with a severe metabolic presentation and poor prognosis.
Conclusion: The combination of pre- and post-natal growth failure, lactic acidosis and the associated subtle facial dysmorphism should prompt consideration of this *NDUFB3* mutation, particularly in the Irish population.

P-405

FeS cluster biogenesis defect in a patient with mutations in ISCA2

Ahting U[1], Rolinski B[4], Haack T[3], Mayr J A[2], Alhaddad B[1], Prokisch H[3], Sperl W[2], Meitinger T[1 3], Freisinger P[5]

[1]Inst Human Genetics, Technische Univ, München, Germany, [2]Dep of Pediatrics, Paracelsus Med Univ, Salzburg, Austria, [3]Inst Human Genetics, Helmholtz-Zentrum, Neuherberg, Germany, [4]Laboratory Pathology, Elblandliniken, Meissen, Germany, [5]Dep of Ped, Kreisklinikum Reutlingen, Reutlingen, Germany

We report a case of neurodegenerative mitochondrial disease. The child of consanguineous Arabic parents showed normal development for the first 6 month of live. By 7 month developmental regression started. The patient developed tonic cramp seizures of the limbs. Respiratory insufficiency required tracheostomy. The girl died at age 2y.
MRI taken at the age of 7 months and 22 months showed progressive leukodystrophy and optic atrophy. Lactate in blood was normal as well as routine metabolic screening except of increased glycine in serum (1039 μmol/l; NR: 73-436). Glycine was also strongly increased in CSF (45 μmol; NR: 2-9). Most strikingly lactate was strongly elevated n CSF (8.3 mmol/l; NR: 2.1-2.1). Thus, mitochondrial disease was suspected. However, muscle biopsy showed no defect of respiratory chain complexes and pyruvate dehydrogenase activity.
Exome screening revealed the homozygous sequence variant c.[229G>A];[229G>A], p.[Gly77Ser];[Gly77Ser] in *ISCA2*, which is involved in mitochondrial FeS cluster biogenesis. Staining of fibroblasts showed reduced levels of lipoic acid in patient cells.
FeS cluster defects show a clinical and biochemical distinct pattern. Typically, glycine is elevated in most body fluids, whereas lactate is often elevated only in CSF. Muscle biopsy is often normal. These findings together with optic atrophy and leukodystrophy point to *ISCA2* defects.

P-406

RNase P complex formation is disrupted in HSD10 disease

Amberger A[1], Deutschmann A J[1], Oerum S[2], Yue W J[2], Zschocke J[1]

[1]Div Human Gen, Med Univ Innsbruck, Innsbruck, Austria, [2]Struc Gen Cons, Univ Oxford, Oxford, United Kingdom

Introduction: Missense mutations in 17β-hydroxysteroid dehydrogenase type 10 (HSD10) cause HSD10 disease, a disorder characterized by psychomotor regression and cardiomyopathy during childhood. HSD10 is part of the RNase P complex which processes 5`-ends of mitochondrial tRNAs. Here, we analyzed the impact of different HSD10 mutations on RNase P complex formation.
Methods: RNase P protein expression was analyzed by Western blot. QRT-PCR was used to quantify tRNA processing.

Pull-down assays and recombinant RNase P proteins were used to assess RNase P complex formation and function.
Results: Experiments in cell lines showed that HSD10 maintained MRPP1 expression. Cells with HSD10 mutations p.Q165H, p.R226Q, p.E249Q, p.L122V, and p.A154T expressed normal HSD10 levels. Interestingly, MRPP1 expression was reduced in p.R226Q and p.E249Q mutant cells. HSD10/MRPP1 complex formation using recombinant proteins revealed weak interaction of MRPP1 with $HSD10^{R226Q}$ or $HSD10^{E249Q}$ but normal interaction with other HSD10 mutants and $HSD10^{wt}$. Low HSD10/MRPP1 expression was associated with impaired tRNA processing and accumulation of pre-tRNAs.
Conclusion: Our results demonstrate that HSD10/MRPP1 interaction stabilizes MRPP1 protein and disruption of this interaction results in MRPP1 degradation. Failure of HSD10/MRPP1 complex formation result in impaired tRNA processing. The findings provide a novel model for mitochondrial dysfunction in HSD10 disease.

P-407

Molecular characterization of a mitochondrial tRNA processing complex implicated in the HSD10 disease

Oerum S[1], Kopec J[1], Fitzpatrick F[1], Amberger A[2], Deutschmann A J[2], Zschocke J[2], Yue W W[1]

[1]SGC, University of Oxford, Oxford, United Kingdom, [2]Div of Hum Gen, Innsbruck Medical Univ, Innsbruck, Austria

Background: Two proteins, MRPP1 and MRPP2, form a complex with methyltransferase activity towards mitochondrial tRNA. A third component, MRPP3, adds 5'-end ribonuclease processing activity to the complex. MRPP2 protein alone holds enzymatic oxidative/reductive functions in steroid metabolism and β-oxidation of fatty acids. *MRPP2* mutations cause the rare neurological HSD10 disease, where, due to the multifunctionality of MRPP2, no clear genotype-phenotype relationship has been established.
Methods & Results: Here we investigate the molecular properties of the individual proteins, and interactions within the MRPP1-MRPP2-(MRPP3) complexes. At the molecular level, the uncharacterized N-terminal region of MRPP1 is responsible for dimerization whilst the C-terminal region binds the methyl donor S-adenosyl-methionine. A low-resolution solution model of human MRPP3 structurally resembles a characterized homologue from plant, providing clues into the substrate-binding pattern of the overall complex. For the MRPP1-MRPP2 subcomplex, full-length proteins are required for both for correct complex assembly and neither of the co-factors NAD^+/NADH, utilized by MRPP2 in its oxidative/reductive function, cause disassembly. Furthermore, formation of the full MRPP1-MRPP2-MRPP3 complex requires the presence of a mitochondrial tRNA substrate.
Conclusions: Our structural data provides a starting point for understanding the molecular facet affected by each patient mutation, leading to a better understanding of the diverse disease severity observed in patients.

P-408

A novel presentation of *EARS2*-associated mitochondrial disease

Oliveira R[1], Sommerville E W[4], Thompson K[4], Nunes J[2], Pyle A[4], Grazina M[5], Chinnery P F[4], Diogo L[3], Taylor R W[4], Garcia P[3]

[1]Med Gen Unit, Hosp Ped, CHUC, Coimbra, Portugal, [2]Neurorad, Hosp Santos Silva, VNG, Coimbra, Portugal, [3]Metab Unit, Child Develop Ctre, CHUC, Coimbra, Portugal, [4]WTC Mitoc Res, Newcastle Univ, Newcastle upon Tyne, United Kingdom, [5]Bioch & Gen Lab, Fac Med Univ Coimbra, Coimbra, Portugal

Background: Defects of mitochondrial translation are important causes of early-onset mitochondrial disease. Although the biochemical signature and neuroimaging are usually distinctive, they are not diagnostic as the genetic origin is heterogeneous.
Clinical Report: A female child, born at term to unrelated parents, died at age 5 months with a suspected mitochondrial disorder. She presented after birth with severe feeding problems, hypotonia, failure to thrive, persistent and progressive hyperlactacidaemia with lactic acidosis, liver dysfunction and developed an encephalopathy. Brain MRI revealed hypogenesis of the corpus callosum and T2 signal abnormalities in the medulla, cerebellar nuclei, thalamic and subthalamic regions; MRS showed a significant lactate peak. Muscle histopathology revealed COX-deficient ragged-red fibers associated with decreased activities of respiratory chain enzyme complexes I and IV. Having excluded mtDNA mutations, we undertook whole exome sequencing identifying biallelic *EARS2* gene variants (p.Met1?) and p.(Ile62Phe), including one novel change.
Discussion: *EARS2* encodes the mitochondrial glutamyl-tRNA synthetase with defects reported to cause Leukoencephalopathy involving the Thalamus and Brainstem with high Lactate (LTBL) - a mitochondrial disorder characterized by adistinctive brain MRI pattern and a biphasic clinical course. We review the literature of reported patients with *EARS2* mutations, contrasting these with the atypical presentation in our case.

P-409

Unravelling a new mitochondrial disorder: PNPT1 mutations causing Leigh syndrome with a complex movement disorder

Lourenco C[1], Sobreira C[1], Marques Jr W[1]

[1]Univ of Sao Paulo, Ribeirao Preto, Brazil

Mitochondrial disorders are complex metabolic inborn errors of metabolism with a broad phenotypic range (such as Leigh syndrome, isolated sensorineural deafness).
Objective: To report a new hereditary metabolic cause of Leigh syndrome in a Brazilian patient caused by enzyme deficiency of a mitochondrial polynucleotide phosphorylase (PNPase, *PNPT1* gene).
Methodology: After excluding the main metabolic causes of Leigh syndrome, whole exome sequencing (WES) was performed.
Results: Mutations in *PNPT1* gene were identified in a female patient born to non-consanguineous parents. Patient showed a complex movement disorder with choreoathetosis, abnormal eye movements and hypotonia. Brain MRI was initially normal and later showed features of Leigh syndrome.
Conclusions: *PNPT1* gene was first identified in patients with sensorineural deafness, but later it was reported as cause of encephalopathy in two sibs born to a consanguineous couple. Our findings reinforce the broad clinical manifestation of mutations in such gene, to include complex movement disorder with abnormal eye movements as part of the clinical spectrum of the disease. PNPase is localized in the mitochondrial intermembrane space with an importante role in RNA targeting to human mitochondria. PNPase deficiency unravels another disease mechanism as cause of mitochondrial disorders: abnormal RNA import into mitochondria.

17. Mitochondrial disorders: mtDNA

P-410

Leigh syndrome presenting with Wolff-Parkinson-White syndrome due to a m.10254G>A mitochondrial DNA mutation

Ersoy M[1], Akyol M B[2], Ceylaner S[3]

[1]Child Metab Dis,Sadi Konuk Res&Tra Hosp, Istanbul, Turkey, [2]Div Pedi Cardio, Sadi Konuk Res&Tra Hosp, Istanbul, Turkey, [3]Intergen Genetics Center, Ankara, Turkey

Background and objectives: Leigh syndrome (LS) is a neurodegenerative disorder, characterized by bilaterally symmetric lesions, particularly in the basal ganglia, thalamus, and brainstem. Although LS presents with neurologic deficits, depending on the underlying mutation, cardiac symptoms can be observed. We describe a child who presented with LS and Wolf-Parkinson-White syndrome (WPW), caused by a recently identified mutation in *MT-ND3*.
Case report: A 13-month-old boy was brought to the hospital with the loss of acquired function and encephalopathy after immunization. He had strabismus and moderate left hemiparesia. He was hypotonic and deep tendon reflexes were increased. Serum lactate was 72 mg/dl (normal range: 4.5-19.8). Brain MRI revealed increased intensity in the dentate nucleus, globus pallidus, putamen and brainstem which was compatible with LS. WPW syndrome with short P-R interval and wide-QRS complexes were revealed by on electrocardiogram. Muscle biopsy showed lipid storage. The sequencing of mtDNA revealed a m.10254G>A heteroplasmic (77%) change in *MT-ND3* gene. He died with cardiac collapse due to ventricular fibrillation when he was 18-month-old.
Conclusion: Because it is an important cause of death, we want to emphasize the importance of the serial heart function monitoring in patients carrying mtDNA mutations.

P-411

Are we missing MtDNA depletion syndromes in infants with fulminant hepatic failure?

Joshi M M[1], Kudalkar K V[1], Jalan A B[1], Jalan R A[1], Shinde D H[1], Borugale M A[1], Mahakal J M[1], Sonalkar N D[1], Khubchandani S[2], Ramprasad V L[3]

[1]NIRMAN, Div of Biochemical Genetics, Navi Mumbai, India, [2]Histopath Dept, Jaslok Hosp, Mumbai, India, [3]MedGenome Labs, Bangalore, India

Background: MtDNA depletion syndrome is a quantitative abnormality of mitochondrial genome, affecting liver and brain in Hepatocerebral type.
Objective: To screen for MtDNA depletion syndrome in patients with fulminant hepatic failure (FHF).
Materials and method: We analysed data of 103 patients with liver disorders of which 8 patients presented with FHF, abnormal LFT, jaundice and high ferritin, where galactosemia, tyrosinemia, HLH, CDG, neonatal heomochromatosis were ruled out. Ferritin levels were 1232.2±499.1ng/ml. All 8 patients succumbed to death. Only one out of 8 agreed for genetic analysis and liver biopsy.
Result: One patient presented at 2 days of age with recurrent seizures, altered sensorium, hypotonia, hypoventilation, hepatomegaly, acute liver failure and high Ferritin (1,135 ngm/ml).

Metabolic studies did not reveal any specific disorder. Electron microscopy of Postmortem liver biopsy showed features consistent with MtDNA Depletion syndrome. Molecular genetic analysis revealed homozygous defect in *DGUOK* gene. An unreported homozygous splice variation which affects the 4th base from the exon-intron junction (c.142+4 A>C ; ENST00000264093.4) was detected.
Conclusion: MtDNA depletion syndrome should be considered in any critically ill infant with fulminant hepatic failure, high Ferritin, especially when other common disorders have been ruled out. EM of liver biopsy and molecular studies are essential for the diagnosis.

P-412

Body composition of adults with mitochondrial disease

Zweers H[1], Leij S[1], Wanten G[1], Janssen M C[1]

[1]Radboudumc, Nijmegen, Netherlands

Background: Recently, it was shown that, based on body mass index (BMI), malnutrition frequently occurs in patients with mitochondrial disease (MD). This study aimed at describing body composition (BC) in adults with MD.
Methods: After an overnight fast height, weight and BMI of adult MD patients were measured and compared with their fat mass index (FMI) and fat free mass index (FFMI) as determined using bio-impedance. BMI, FMI and FFMI were compared with their current standards for normal ranges.
Results: Patients (age: 44 yr ±13; male: n=11) with chronic progressive external ophthalmoplegia (CPEO, n=17) and m.3243A>G carriers (n=20) signed informed consent. 14% were underweight and 46% were overweight (according to BMI). In contrast, 51% of all patients had a decreased FFMI, while 51% had an increased FMI. 29% of the patients had sarcopenic obesity.
Conclusion: The majority of patients, even those with normal BMI, have an abnormal BC -i.e. decreased FFMI and/or increased FMI. They are at risk for sarcopenia which using BMI alone can remain undiscovered. These results are in line with data of patients with other neuromuscular diseases. It should be investigated whether and how nutrition and/or physical intervention may improve BC and quality of life in these patients.

P-413

Age-related decline in muscle mitochondrial function is different between men and women

Gaignard P[1], Lebigot E[1], Thérond P[1], Slama A[1]

[1]Biochemistry, Bicetre Hosp, APHP, Le Kremlin-Bicêtre, France

Background: Aging is associated with accumulative oxidative mitochondrial DNA damages leading to a progressive loss of mitochondrial respiratory chain (RC) function. The aim of our study was to determine whether the RC function evolution with age is similar for men and women.
Methods: A retrospective analysis of oxygen consumption and enzymatic activities of RC complexes was performed in 117 skeletal muscle and 96 cultured skin fibroblasts samples from individuals aged 15 to 80 years (mean=43±17 years, 43% of men for muscle; mean=41±18 years, 46% of men for fibroblasts).
Results: Analyses of covariance (age, sex and age x sex) revealed a main effect of sex in muscle RC function with a lower activity in women as compared to men. Linear regression analyses (age x RC function for men/women subgroups) showed that the oxygen consumption in presence of pyruvate or succinate and the enzymatic activities of the complexes I and III decreased with age in muscle, but only for the men subgroup. No sex or age effects were detected in RC function for fibroblasts.
Conclusion: Our study revealed that men are largely more affected by age-related decline in muscle mitochondrial function than women, suggesting a better protection against oxidative damages in women.

P-414

Respirometry in blood and fibroblasts of LHON patients

Krylova T[1], Tsygankova P[1], Itkis Y S[1], Sheremet N L[2], Nevinitsyna T A[2], Malakhova V A[1], Zakharova E Y[1]

[1]FSBI "Res Cent Med Gen", Moscow, Russian Federation, [2]FSBI "Res Inst Eye Dis", Moscow, Russian Federation

Background: Mitochondrial disorders are an heterogeneous group of diseases which associated with defects of mitochondrial respiratory chain. Leber's Hereditary Optic Neuropathy (LHON) is one of the most common mitochondrial syndrome. The most cases are caused by three mutations of mtDNA : m.11778G>A, m.3460G>A, m.14484T>C. And more than 15 other mutations are described nowadays.
Aim: To develop method for robust measurement of respiratory chain function in patients with LHON which could reveal biochemical defect in blood cells and fibroblasts of patients.
Material and methods: We used high-resolution respirometry (HRR) on the Oxygraph-2k (Oroboros corp., Austria) for complex analysis of mitochondrial respiratory function in intact human skin fibroblasts and platelets of LHON patients

and healthy controls. We analyzed fibroblasts from 5 LHON patients harboring mutations m.3460G>A (n=3), m.11778G>A(n=1), m.3635G>A(n=1) and platelets from 4 LHON patients with m.3460G>A (n=2), m.11778G>A(n=2).
Results: Oxygen consumption in the control's and the patient's samples was different. Flux control ratios (R/E, netR/E) were statistically ($p < 0.05$) different between groups.
Conclusion: These results show that changes in oxygen consumption could be seen in blood and fibroblasts of LHON patients. HRR in platelets is noninvasive, fast and cheap approach for biochemical diagnostic of LHON.

P-415

Mitochondrial DNA mutations associated with autism spectrum disorders

Avdjieva-Tzavella D[1], Mihailova S[2], Kathom H[1], Naumova E[2], Tincheva R[1]

[1]Dept Clin Genet, Univ Child Hosp, Sofia, Bulgaria, [2]Dept Clin Immunol, Univ Hosp Alexandovsk, Sofia, Bulgaria

Background: Autism spectrum disorders (ASDs) are a heterogeneous group of neurodevelopmental disorders characterized by impaired reciprocal social interaction, lack of communication, isolated interests and repetitive or stereotyped behaviors. Most cases are idiopathic, although there is increasing evidence that ASDs have an important genetic component with aetiological heterogeneity. Some cases of autism have been associated with several different organic conditions including mitochondrial dysfunction, however, very few individuals with mitochondrial DNA (mtDNA) mutations have been found.
Patients and Methods: In order to confirm these causative relationship we screened 88 individuals with idiopathic ASDs for a number of the most common mtDNA mutations. PCR - sequencing based technique (SBT) was used for detection of single nucleotide changes.
Results: We identified three patients with candidate mutations: m.3885C>T in the MT-ND2 gene, m.6852G>A that produce an aminoacid change Gly to Ser in the MT-CO1 gene and m.8033A>G (Ile→Val) in the MT-CO2 gene. Normal blood lactate levels were found in the three children.
Conclusion: Our study suggests that mtDNA mutations may be an additional pathogenetic factor for a subset of individuals with ASDs. Further studies are needed to prove the clinical significance of the present findings and to understand how mitochondrial defects may contribute to autism.

P-416

Unique presentation of LHON/MELAS overlap syndrome caused by m.13046T>C in *MTND5*

Kolarova H[2], Liskova P[2], Tesarova M[1], Kucerova Vidrova V[1], Ulmanova O[3], Forgac M[3], Hansikova H[1], Honzik T[1]

[1]Dpt Pediat,First Facult Med,Charles Univ, Prague, Czech Republic, [2]Dpt Ophtalmol, Gen Univ Hosp, Prague, Czech Republic, [3]Dpt Neurol, Gen Univ Hosp, Prague, Czech Republic

Background: LHON and MELAS syndromes are mitochondrially-inherited disorders characterized by acute visual failure and variable multisystemic presentation, respectively.
Materials and Methods: A 12-year-old girl presented with an abrupt, painless and simultaneous loss of vision. Ocular examination confirmed bilateral optic nerve atrophy. Over the next months, she developed a sensorineural hearing loss, vertigo, migrainous headache, anhedonia and thyroiditis. CSF lactate was elevated.
Results: Whole mtDNA sequencing revealed pathogenic heteroplasmic mutation m.13046T>C in *MTND5* encoding for ND5 subunit of complex I. This particular variant has been previously described in only one patient with MELAS/Leigh syndrome. Based on a combination of clinical symptoms in our patient, we concluded the condition as LHON/MELAS overlap syndrome.
Conclusions: We describe a unique presentation of m.13046T>C mutation in a 12-year-old girl with with LHON/MELAS overlap syndrome. In patients with sudden visual failure excluded to carry one of the three most prevalent mitochondrial mutations, mtDNA mutations in other complex I subunits must be investigated. Further, different clinical presentation must be expected even in previously well-described phenotype. Comprehensive systemic clinical and laboratory evaluation in patients suspected to suffer from mitochondrial disorders is of utmost importance considering the potential benefit of Idebenone therapy. *Supported by GAUK 38515, IGANT 14156/3A, 13114-4.*

P-417

Leigh-like syndrome due to homoplasmic m.8993T>G mutation with unusual biochemical features suggestive of multiple carboxylase deficiency (MCD) and hypocitrullinemia

Balasubramaniam S[1 2], Lewis B[3], Greed L[3], Mattman A[4], Fletcher J M[5 6], Van Karnebeek C[7], Christodoulou J[8 9], Rodenburg R J[10]

[1]Metab Unit, Princess Margaret Hosp, Perth, WA, Australia, [2]School Paeds, Child Health, UWA, Perth, WA, Australia, [3]PathWest Lab Med WA, PMH, Perth, WA, Australia, [4]Div Endo & Metab,Vancouver Gen Hosp, UBC, Vancouver, Canada, [5]Genet & Mol Path, SA Path, Adelaide, SA, Australia, [6]Univ of Adelaide,, Adelaide,SA, Australia, [7]Dept of Peds, UBC, Vancouver, Canada, [8]WSGP, Children's Hosp Westmead, Sydney, NSW, Australia, [9]Div Paeds & Genet Med, Uni Sydney, Sydney, NSW, Australia, [10]Nijmegen Center Mito Dis, Radboud,UMC, Nijmegen, Netherlands

Background: The *MT-ATP6* m.8993T>G mutation when homoplasmic is associated with Leigh or Leigh-like syndromes.
Case: 2-year old male had raised 3-hydroxyisovalerylcarnitine on newborn screening. Subsequent persistent plasma elevations of 3-hydroxyisovalerylcarnitine, propionylcarnitine and fluctuating urinary markers were suggestive of MCD. He developed psychomotor delay, central hypotonia, myopathy, failure to thrive, hypocitrullinemia, recurrent decompensations with lactic acidosis and an episode of hyperammonemia. Biotin treatment was associated with increased activity levels, alertness, and accruement of new developmental milestones, despite lack of significant correlation with biochemical improvements.
Results: MCD was excluded by normal enzymology and mutational analysis of *HLCS* and *BTD* genes. Biotin uptake studies were normal. Whole exome sequencing was uncontributary. Identification of *de novo* homoplasmic m.8993T>G mutations in muscle and blood led to the diagnosis of Leigh-like syndrome. Complex IV activity was mildly reduced in skeletal muscle. Apart from a small lactate doublet on spectroscopy, brain MRI was normal.
Discussion: Hypocitrullinemia has been reported in patients with m.8993T>G mutation and other mitochondrial disorders, possibly due to secondary impairment of pyrroline-5- carboxylate synthase and carbamoyl phosphate synthetase I activities. Similarly, biotin and ATP dependent carboxylases may be compromised, causing the unusual biochemistry noted here. Larger studies may elucidate the precise interactions between mitochondrial mutations and these abnormalities.

P-418

A very rare syndrome: Mitochondrial DNA depletion syndrome type 13

Onal Z[1], Ersen A[3], Mutlu N[1], Cansever M S[2], Onal H[1], Adal E[1]

[1]Div Metab Dis, KSS Edu Res Hosp, Istanbu, Istanbul, Turkey, [2]Cent Lab, Ist Univ Cer Med Fac, Istanbul, Istanbul, Turkey, [3]Pediatrics, Kasimpasa Military Hosp, Istanbul, Turkey

Background and objectives: Mitochondrial DNA depletion syndrome (MDS) refers to a group of autosomal recessive disorders with a significant drop in mitochondrial DNA which causes defects in mitochondrial oxidative phosphorylation and decreased mtDNA content. It manifests as myopathic, hepatopathic, and/or encephalomyopathic forms. MDS type 13 is an encephalomyopathic form and characterized by early infantile onset of encephalopathy, hypotonia, lactic acidosis, severe global developmental delay.
Methods: A 1.5 year-old girl whose birth weight was 3000 g, was suffering from feeding problems, poor weight gain. We noted developmental delay, hypotonia, persistent lactic acidosis, renal tubular acidosis. There weren't any craniofacial anomalies but MRI images identified mild cerebral atrophy and thin corpus callosum. The increased assay level of alanine but normal organic acid analysis was detected.
Results: Whole exome sequencing revealed homozygote c.1152C>G C384W mutation in FBXL4 gene confirmed diagnosis. Although no curative treatment is available, sodium bicarbonate, coenzyme q, carnitine and vitamin-B were given. She has been fed by gastrostomy, due to the difficulty swallowing and gastroesophageal reflux and followed-up for six months.
Conclusion: This case is presented herein since MDS type 13 is very rare with the total number of reported cases are not more than 10 in the literature so far.

P-419

Primary coenzyme Q10 deficiency- type 4: A case report

Kocak O[1], Kilic Yildirim G[2], Yarar C[1], Durmus Aydogdu S[2], Carman K B[1]

[1]Osmangazi Uni, Med Fac Dept of Ped Neuro, eskişehir, Turkey, [2]Osmangazi Uni, Med Fac Dept of Ped Metab, Eskişehir, Turkey

Introduction: Primary coenzyme Q10 (CoQ10) deficiency-type 4 is an autosomal recessive disorder due to mutations in the *ADCK3* gene. It is characterized by childhood-onset of exercise intolerance, cerebellar ataxia and seizures. We presented a patient with primary coenzyme Q10 deficiency-type 4 as the first one in Turkey.
Case Report: Sixteen-year-old male patient was admitted with seizures, growth retardation, and hepatomegaly. Cranial MRI was normal and EEG showed mid-amplitude spike-and-slow-wave complexes on bilateral temporal and parietal regions. Antiepileptic therapy was started. During the follow-up he had lactic acidosis, increased serum CPK and elevated liver function tests. Myopathic features on EMG and ragged fibers in the muscle biopsy were detected. He

developed uncontrolled seizures and stroke-like episodes. Repeated MRI was interpreted as MELAS. Genetic analysis showed compound heterozygote c.1042C>T (p.Arg348*)/ c.1015G>A (p.Ala339Thr) mutations on *ADCK3* gene and primary CoQ10 deficiency-type 4 was confirmed. Clinical status was improved with high dose of CoQ10, ketogenic diet and antioxidant therapy.
Conclusion: Primary CoQ10 deficiency is unique among mitochondrial disorders because early supplementation with CoQ10 can prevent the onset of clinical findings. It should be considered in the differential diagnosis of MERRF/MELAS because of the similar MRI and muscle biopsy findings.

P-420

Renal manifestations in DGUOK deficiency: an atypical presentation

Nascimento J[1], Silva E S[2], Rocha L[3], Garrido C[4], Nogueira C[5], Almeida L[5], Vilarinho L[5], Bandeira A[1], Martins E[1]

[1]Metab Dis Unit,Paed Serv,Cent Hosp Port, Porto, Portugal, [2]Paed Gastr Serv,Cent Hosp Port, Porto, Portugal, [3]Paed Nephr Serv, Cent Hosp Port, Porto, Portugal, [4]Paed Neur Serv, Cent Hosp Port, Porto, Portugal, [5]Newb Scren,Gen Metab Unit,N Inst Heal RJ, Porto, Portugal

Introduction: Mitochondrial DNA depletion syndromes (MDS) are a heterogeneous group of autosomal recessive disorders that are due to defects in nuclear genes responsible for mitochondrial nucleotide synthesis or mtDNA replication.
Case report: Female patient observed at day 24 after diagnosis of cholestatic jaundice and persistent failure to thrive. At this time blood creatinine was between 1.0-1.5mg/dl. She regained weight, but jaundice persisted (DB of 8.88mg/dl) and creatinine values got higher (3.9mg/dl) with metabolic acidosis. Renal Fanconi syndrome was diagnosed. At two months old, despite resolution of hepatic cholestasis, glomerular filtration rate reach 16ml/min/1.73m2 and it was decided to start peritoneal dialysis.The persistently elevated lactate leads us to consider the possibility of a MDS which was confirmed by sequencing analysis of *DGUOK*. The patient was found to be a heterozygous compound for p.A2S / p.Q170R mutations. At 8 months she had a good neurological development. MRI did not detect structural brain abnormalities and spectroscopy study was normal.
Conclusion: Approximately 100 individuals have been reported with *DGUOK* mutations associated to hepatocerebral form of MDS. Here we present a patient with absence of developmental delay and severe renal presentation making this case an atypical presentation of MDS with *DGUOK* mutations.

P-421

Blue and clear native electrophoresis in skin fibroblasts as a tool for detection of mitochondrial disease

Sosova I[1 8], Sinasac D[2 3], Snyder F[2 3], Hume S[4], Friederich M[6], Van Hove J[6], White C[5], Resch L[7], Khan A[2 3]

[1]Dept Lab Med and Path, Univ of Alberta, Edmonton, AB, Canada, [2]Alberta Children's Hospital, Calgary, AB, Canada, [3]Dept Medical Genet, Univ of Calgary, Calgary, AB, Canada, [4]Dept Medical Genet, Univ of Alberta, Edmonton, AB, Canada, [5]Dept Clin Neurosci, Univ of Calgary, Calgary, AB, Canada, [6]Dept Pediatr, Univ of Colorado, Aurora, CO, United States, [7]Dept Path and Lab Med, Univ of Calgary, Calgary, AB, Canada, [8]NMS and Biochem Genet Lab, U of A Hosp, Edmonton, AB, Canada

Case Report: A 47 year-old man was seen for muscle weakness, ophthalmoplegia, dysarthria, and an ischemic lesion the right thalamus. Muscle histopathology showed increased lipid content. No mitochondrial DNA (mtDNA) deletions were found on Southern blot. Muscle blue native polyacrylamide gel electrophoresis (BN-PAGE) revealed a band of incompletely assembled complex V. To investigate further for mitochondrial disease, we developed a method in skin fibroblasts using a hybrid blue/clear native PAGE (BN/CN-PAGE) and long range PCR to look for mtDNA rearrangements.
Results: Skin fibroblast BN/CN-PAGE confirmed incomplete assembly of complex V. Long range PCR showed a deletion (m.9090_16070del) at 20% heteroplasmy that disrupted the region coding for ATPase6. The deletion would be expected to lead to two different protein fragments leading to an abnormal band of complex V on BN/CN-PAGE.
Discussion: The finding of incomplete assembly of complex V on both skin and muscle PAGE suggests that the mtDNA deletion is more likely inherited rather than acquired by age. We suggest that skin fibroblast BN/CN-PAGE can be used as a complementary tool for detection of abnormal mitochondrial electron transport chain protein assembly. We also suggest that long-range PCR using muscle-extracted mtDNA is a more sensitive method of finding deletions than Southern blot analysis.

P-422

Biochemical and mutational spectrum of mitochondrial disorders: 1 Year prospective data from tertiary care centre of India

Kumar S[1], Lomash A[1], Khalil S[1], Polipalli S K[1], Kapoor S[1]

[1]MAULANA AZAD MEDICAL COLLEGE ,, New Delhi, India

Background: Mitochondrial disorders, though most common heterogeneous set of neurometabolic disorders, reported as isolated reports from our country. Considering the population referred to us has high rates of consanguinity and endogamus matings, the population with these disorders is likely to be high.
Materials and Methods: A total of 52 patients screened for lactate, pyruvate, alanine, glycine (LC-MSMS), lactic acid, Ethylmalonic acid, 3 Methylglutaconic acid (GCMS). Amplification of complete Mt. genome by LR-PCR, followed by library preparation for Illumina Next gen. Sequencer for Whole Mt.genome analysis.
Results: 52 patients suspected for mitochondriopathy were enrolled. 12 (23%) were >15years and >10 years age respectively. 10 children (19%)in between 5-10 years, 9 patients (18%) each were < 1 month and < 1 year of age. Seizures followed by encephalopathy, the most commonest causes for referrals. 10 children(19%) were diagnosed with mitochondriopathy.6 (60%) were product of 3° consanguineous marriage.3(30%) congenital lactic acidosis, 2 for m.G3460A(MT-ND1),m.G11778A(MT-ND5) and 1 each with m.3243A>G, m.8344A>G, m.8993T>C/G, m.A12361G(MT-ND5) for NAFL syndrome and 1 mutated mt.genome for missense m.4561T>C (MT-ND2)
Conclusions: Defined score may be helpful in targeting younger patients while older patients with suspected disease are easy to diagnose. Prospective data will helps in establishing 1st disease database of mitochondrial disorders in India.The mtWGS- NGS provides accurate, sensitive heteroplasmy measurement, one-step approach to detect/ map deletion and breakpoints, common and uncommon point mutations, deletions.

P-423

Comparison of biochemical and molecular diagnosis in children with Leigh syndrome

Lee Y M[1]

[1]Dept of Ped, Yonsei Univ College of Med, Seoul, Korea, Republic of

Objective: This study compared the aspects of biochemical and molecular diagnosis of mitochondrial respiratory chain complex (MRC) defects in Leigh syndrome, using methods of biochemical enzyme assays and molecular genetic analysis.
Methods: We included a total number of 82 patients who satisfied the clinical criteria of Leigh syndrome. All patients underwent muscle biopsy. We performed biochemical enzyme assays to analyze MRC enzymes and molecular genetic analysis of muscle tissues to search for mtDNA mutations of Leigh syndrome and *SURF1* mutation. Clinical aspects of cases without mutation were compared with patients carrying a mutation.
Results: MRC defect was found in 47 patients (57.3%). Complex I defect was seen in 23 (28.0%) cases and complex IV defect in 15 (18.3%). There were 12 with mutations including 9 with confirmed mtDNA mutations, 3 with *SURF1* mutations. Continuous ventilator care and perinatal asphyxia were reported significantly more often in the mutation(+) group. In brain MRI, the percentage of multiple lesions, brain stem and thalamus lesions were significantly higher in the mutation(+) group. Statistically higher proportion of mutation(+) patients had combined MRC defect.
Conclusions: Further gene analysis on an extended group of patients will enable us to improve diagnostic precision and reveal genotype/phenotype correlations.

P-424

Lipomas: an unexpected phenotype of mitochondrial DNA mutations

Smet J[1], Vanlander A V[1], Creytens D[5], Lengelé B[2], Revencu N[4], Seneca S[3], Van Coster R[1]

[1]Div Ped Neur, Univ Hosp Ghent, Ghent, Belgium, [2]Dep Pl Reconstr Surg, St Luc Univ Hosp, Brussels, Belgium, [3]Cent Med Genet, Brussels Univ Hosp, Brussels, Belgium, [4]Cent Hum Genet, St LucUniv Hosp, Brussels, Belgium, [5]Dept Path, Univ Hosp, Ghent, Belgium

Objective: To evaluate the possibility of mitochondrial involvement in patients with multiple symmetrical lipomatosis (MSL) by testing mitochondrial DNA (mtDNA) and by assaying OXPHOS complex activities.
Methods:Several tissues from one patient originating from a family affected by MSL on three generations has been assayed using biochemical and molecular tests to detect mtDNA alterations and OXPHOS deficiencies as potential cause of MSL.
Results:mtDNA sequencing in patient's lymphocytes revealed presence of the classic MERRF mutation (m.8344A>G) in heteroplasmic state (40%) and even higher percentages in skeletal muscle (68%) and adipose tissue (94%). Spectrophotometric analysis of OXPHOS activities in skeletal muscle revealed a mild decreased activity of complex IV. BN-PAGE followed by in–gel activity staining in skeletal muscle showed overall low activities of complexes I, III and IV and presence of subcomplexes of complex V. This latter is a hallmark of a defect in intramitochondrial translation.
Conclusion: MSL is characterized by presence of lipomas with axial symmetrical distribution. The pathophysiology of this disease is currently unknown. Assays of the OXPHOS complexes and mtDNA analysis are indicated in patients presenting MSL. As symmetrical lipomatosis may be the first

sign of a mitochondrial disease extensive evaluation and follow-up of these patients is mandatory.

P-425

HSD10 is an independent prognostic marker for overall survival in colorectal cancer (CRC) and regulates mitochondrial DNA (mtDNA) copy number

Deutschmann A J[1], Amberger A[1], Traunfellner P[1], Feichtinger R G[2], Kofler B[2], Zschocke J[1]

[1]Div Human Gen, Med Univ Innsbruck, Innsbruck, Austria, [2]Dep of Ped, Paracelsus Med Univ, Salzburg, Austria

Background: Mitochondrial dysfunction is an important feature of many cancers and may play a role in disease progression and prognosis. Mitochondrial RNase P consisting of three proteins MRPP1, HSD10, and MRPP3 cleaves polycistronic mtDNA transcripts to generate RNAs for translation. Here, we analyzed the role of RNase P proteins in colorectal cancer pathology and in regulating mitochondrial biogenesis.
Methods: MRPP1, HSD10 and porin expression was assessed by immunohistochemistry in 356 CRC samples. The effects of altered HSD10 expression on mtDNA quantity in cancer cell lines was studied by transfection and siRNA knockdown experiments. Mitochondrial DNA was quantified by real-time PCR.
Results: High expression of HSD10 and MRPP1 was found in 28% and 38% of CRC samples, respectively. In patient samples there was a highly significant association of high HSD10 expression with high mtDNA content and good overall survival ($p < 0.001$). Multivariate Cox analysis suggested high HSD10 expression as an independent prognostic marker. HSD10 knock-down or overexpression of HSD10 in cancer cells caused reduced or increased mtDNA levels, respectively.
Conclusion: There may be a functional link between HSD10 expression and mitochondrial biogenesis in CRC cells that affects cancer cell biology and patient prognosis.

P-426

Common mitochondrial syndromes but non typical mutations

Zakharova E Y[1], Itkis Y S[1], Tsygankova P[1], Rudenskaya G E[1], Krylova T[1]

[1]FSBI "Res Cent Med Gen", Moscow, Russian Federation

Background: Mitochondrial disorders (MD) represent a heterogeneous group of genetic diseases which caused by respiratory chain defects. They can onset at any age and mostly have multisystem involvement.
Methods: We have developed two MLPA panels for detection of the most common mutations in mtDNA (m.3697G>A, m.3460 G>A, m.8363 G>A, m.10197 G>A, m.11778 G>A, m.14484T>C, m. 13513 G>A, m.14459 G>A, m.3243 A>G, m.13094 T>C, m.8344 A>G, m.8993T>G/C) and in POLG gene (p.W748S, p.G848S, p.T914P, p.A467T, p.L304R, p.P587L, p.G737R).
Results: Using these panels as the first step of MD molecular diagnosis we confirmed the molecular defect in 62 patients among more than 1000 tested samples (6,2%). Some unusual findings were found in 7 families: no correspondence between suspected syndrome and DNA mutation. One patient with Leigh-like phenotype had two typical mtDNA LHON's homoplasmic mutations: m.11778G>A and m.14484T>C. Two patients had mutations in POLG gene (p.W748S / p.R597W and p.L304R/p.L304R), but they manifest classic clinical features of MELAS syndrome. 4 patients with KSS had common MELAS mutation m.3243A>G.
Conclusion: MLPA analysis as the first step of diagnostic of mitochondrial disorders helps to find new phenotypic manifestation of common mutations.

P-427

Atypical MEGDEL syndrome with hepatic presentation

Nobre S[1], Amaral R[2], Garcia P[3], Almeida L[4], Grazina M[5], Vilarinho L[4], Diogo L[3], Gonçalves I[1]

[1]Liver Unit, Ped Hosp Coimbra, CHUC, Coimbra, Portugal, [2]Hosp Divino Espírito Santo, Ponta Delgada, Portugal, [3]Metabolic Unit-CDC, Ped Hosp Coimb, CHUC, Coimbra, Portugal, [4]Human Genetics Depart, Dr Ricardo Jorge, Porto, Portugal, [5]Lab Biochemical Genet-CNC, Univ Coimbra, Coimbra, Portugal

Background: MEGDEL (methylglutaconic aciduria with deafness, encephalopathy, and Leigh-like syndrome) syndrome usually presents in early childhood, with deafness, progressive spasticity/dystonia, psychomotor retardation and 3-methylglutaconic aciduria.
Case report: We present a 3-y-old boy admitted for liver biopsy due to persistently increased transaminases and failure to thrive. He is the first child of nonconsaguineous parents, born from eutocic delivery at 35wks gestation: Apgar index 6/8/9, birth weight 2070g (P5). At 18 months, in the context of pneumonia, investigation revealed AST/ALT 125/155 UI/L. Liver ultrasound disclosed steatosis. Common causes of chronic liver disease were excluded. Psychomotor development and physical exam were normal, except for discrete abdominal collateral circulation. Cardiac evaluation and brain MRI/MRS were normal. Liver histology showed macro/microvesicular steatosis (25%),

portal tract inflammation and mild fibrosis. During an episode of intense prostration after anesthesia, he presented metabolic acidosis (pH-7.28), hyperlactacidemia (5.7; normal: 0.4-2) and 3-methylglutaconic aciduria (203 μmol/mmol creatinine; normal: 0-19), which persisted in a subsequent evaluation. Decreased hepatic activity of respiratory mitochondrial chain complexes (III,II+III,V) and marginal mtDNA depletion (28.1%) were found. Analysis of *SERAC1* showed homozygosity for c.777>G, exon 9 mutation (p.Y259*), not previously described. Conclusion: This is an atypical presentation of MEGDEL syndrome, with isolated hepatic involvement until now. 3-methylglutaconic aciduria was the clue to diagnosis.

18. Other disorders of energy metabolism, creatine disorders

P-428

Study of creatine uptake by cultured skin fibroblasts for functional diagnosis of SLC6A8 deficiency

Joncquel M[1 2], Bout M A[1], Parsy N[1], Lemaire V[1], Fontaine M[1 2], Dessein A F[1], Briand G[1 2], Mention-Mulliez K[2 3], Dobbelaere D[1 2], Vamecq J[1 2 4]

[1]Lab Bioch, Métabolisme, CBP, CHRU Lille, Lille, France, [2]RADEME, EA 7364, Univ Lille 2, Lille, France, [3]CR MHM Hôp Jeanne de Flandre, CHRU Lille, Lille, France, [4]Inserm, Lille, France

Creatine plays a key role in energy shuttling. It enters cells *via* SLC6A8, a specific Na^+-dependent plasma membrane transporter, deficiency of which was first described in 2001 as a X-linked disorder. Symptoms may include mental retardation, language delay, epilepsy and autistic behavior. Heterozygous females may be symptomatic. This phenotype variability hampers diagnosis and is attributed to random X inactivation.
Classically, diagnosis is established by a rise in creatine/creatinine ratio in the urine. Genetic study completes the diagnosis. MRI is normal or non-specific. ^{1}H-MRS shows a collapse of brain creatine. Functional study of the transporter is based on measurements of cell creatine uptake. We report a new approach to study this creatine uptake by cultured skin fibroblasts. It is adapted from the method of Salomons and coworkers (Am J Hum Genet. 2001, 68:1497-500) and based on the use of creatine-D3 as a substrate for the transporter. Its main strength lies in a reliable assay for control human fibroblasts and a good discrimination of control (0.40-1.22 nmol/mg proteins/h) from patients (0.00-0.15 nmol/mg proteins/h) activities, with potentially a predictive value for the patient response to oral creatine supplementation.

P-429

Guanidinoacetate specific effects of GAMT deficiency in developing brain cells

Hanna-El-Daher L[1], Loup M[1], Henry H[1], Tenenbaum L[2], Braissant O[1]

[1]Neurometab Unit, Biomedicine, Univ Hosp, Lausanne, Switzerland, [2]Clin Neurosci, Univ Hosp, Lausanne, Switzerland

Background: GAMT deficiency is the most severe of creatine deficiency syndromes, showing creatine deficiency and guanidinoacetate accumulation in CNS. So far, the brain guanidinoacetate-specific pathomechanisms occuring under GAMT deficieny were difficult to analyze due to concomitant effects of creatine deficiency.
Methods: A model of partial GAMT deficiency was developed in 3D organotypic rat brain cell cultures by AAV2-transduced GAMT knock-down, showing no creatine deficiency but guanidinoacetate accumulation. Guanidinoacetate-specific effects were confirmed by direct guanidinoacetate exposure. Cultures were transduced, respectively GAA-exposed, at DIV0 (AAV2/GAMT MOI:1000; GAA: 10;30μM), and followed during one month (DIV8;18;28).
Results: Mild guanidinoacetate exposure (GAMT knock-down: 9.0μM; controls: 0.9μM / direct guanidinoacetate exposure: 10 and 30μM) led to axonal hypersprouting and decrease in natural apoptosis (DIV8;18). This was paralleled by dysregulation of MAPK pathways (Erk1/2;SAPK/JNK;p38) and increased expression of GABA neurotransmission-related genes (GAD;$GABA_AR$). GAA exposure led to non-apoptotic cell death at later stages (DIV28). All guanidinoacetate-induced effects were prevented by creatine co-treatment.
Conclusions: Our findings demonstrate for the first time some of the specific effects of guanidinoacetate on brain cells under GAMT deficiency, and suggest new targets for its treatment.

P-430

Long-term outcome after creatine supplementation in the two italian AGAT deficient families

Battini1 R[1], Alessandrì1 M G[1], Casalini1 C[1], Casarano1 C[1], Tosetti1 M[1], Cioni1 2 G[1]

[1]IRCCS Fondaz Stella Maris, 2 Univ Pisa, Calambrone (Pisa), Italy

Background: AGAT deficiency is a rare inborn error of creatine (Cr) synthesis characterized by the absence or severe reduction of cerebral Cr. To date only 11 patients have been

reported worldwide, all of them presenting mainly cognitive impairment with language disorder. Cr supplementation improves clinical symptoms by restoring brain and peripheral Cr levels. However little is known about the optimum dosage, treatment duration and long term toxicity of Cr.
Patients: 4 patients have been reported: 3 affected patients started Cr supplementation at 400 mg/Kg bw/day and then gradually reduced the dosage at 100 mg/Kg bw/day; one patient, diagnosed at birth, was stably treated with 100 mg/Kg bw/day since the age of four months. All patients were periodically monitored toward Cr levels in brain (MRS) and body fluids, blood/urine routine tests and neuropsychological functions.
Results & Conclusion: Cr supplementation improves clinical symptoms in all patients, restoring the brain content up to 80% of normal levels. No neurological impairment was observed in the pre-symptomatic child. No important side effects were reported, except an increase of body weight, polyuria and renal stone in one patient. Long term Cr supplementation is safe and the early treatment prevents the neurological signs of the disease.

P-431

Plasma creatine levels in patients with different forms of homocystinurias

Boenzi S[1], Pastore A[1], Martinelli D[1], Goffredo B M[1], Dionisi-Vici C[1]

[1]Unit Metab Biochem, OPBG Child Hosp, Rome, Italy

Trans-methylation reactions are needed in crucial biological processes, essential for cell homeostasis, including control of gene expression. A significant proportion of the methyl groups generated by transsulfuration cycle (about 70%), is used for the biosynthesis of creatine by the action of the enzyme-guanidinoacetate methyltransferase utilizing as substrates guanidinoacetate and S-adenosylmethionine. Since the availability of methyl groups represents a rate limiting substrate for creatine biosynthesis, we investigate the potential presence of plasma creatine abnormalities in patients with different forms of homocystinurias, including CBS (2 pts), CblC (17 pts) and MTHFR (1 pt) deficiencies. Plasma creatine in samples obtained from CBS patients was significantly reduced (median 20.8 mmol/L) compared to healthy subjects (52.4 mmol/L; $p < 0.001$), CblC (median 40.8 mmol/L; $p < 0.001$) and MTHFR (median 54.9 mmol/L; $p < 0.001$). In addition, plasma creatine in both CblC and MTHFR were not significantly different from controls.Our study shows that among homocystinurias, CBS deficiency alone, a disorder of transsulfuration cycle, causes secondary creatine deficiency. In patients with other homocystinurias, unrelated to this pathway, plasma creatine levels are normal. This observation highlight the potential use of creatine supplementation in patients with CBS defect.

P-432

Disorders of the biosynthesis of lipoyl-proteins: a biochemical approach to improve diagnosis

Lebigot E[1], Gaignard P[1], Slama A[1], Rio M[2], Roche S[3], Ottolenghi C[2], Therond P[1], De Lonlay P[2], Boutron A[1]

[1]Biochemistry, Bicetre Hosp, APHP, Paris, France, [2]IEM Reference Center, Necker Hosp, APHP, Paris, France, [3]Ped Department, Children Hosp,, Toulouse, France

Background: Lipoic acid (LA), cofactor of mitochondrial ketoacid dehydrogenases and glycine cleavage system, plays a major role in oxidative decarboxylation. *De novo* LA biosynthesis is dependent on LIAS activity together with LIPT1, LIPT2 and sulfur donors. Genes such as *NFU1*, *BOlA3*, *IBA57* encoding proteins of the iron-sulfur cluster (ISC) biogenesis pathway, are involved in the multiple mitochondrial dysfunctions syndromes (MMDS). Disorders of the lipoyl-proteins biosynthesis are difficult to diagnose due to clinical, biochemical and genetic heterogeneity.
Methods: Retrospectively, fibroblasts from 8 patients carrying mutations in *BOlA3*, *NFU1*, *IBA57* or *LIPT1* were analyzed by protein-bound LA by immunoblotting. Activities of PDHc, Krebs cycle enzymes and respiratory chain were measured.
Results: In most cases, anti-LA antibody failed to detect the expected lipoyl-E2 proteins of PDHc, AKGDH and BCKDH, whereas bands were seen with anti-PDHE2 antibody. PDHc and AKGDH activities were decreased for all the patients. Aconitase, complex II and complex IV activities were decreased in patients with ISC defects.
Conclusion: These enzyme assays clearly discriminate between ISC biogenesis defects vs mutations leading to primary LA biosynthesis deficiency. Our study emphasizes the need for renewed efforts to develop efficient procedures for the diagnostic work-up of LA related defects.

P-433

Simultaneous measurement of creatine, creatinine and guanidinoacetate in dried urine spots by LC-MSMS : a pilot study

Holwerda U[1], Smith D E[1], Squires L[2], Salomons G S[1]

[1]Metabolic Unit, VU Univ Med Center, Amsterdam, Netherlands, [2]Lumos Pharma, Austin, Texas, United States

Background: Creatine deficiency syndromes (CDS : AGAT-, GAMT- and creatine transporter defects) are inborn errors of creatine metabolism and transport. Diagnosis is based on the combination of cerebral 1H-MRS, measurement of guanidinoacetate, creatine, and creatinine in body fluids and genetic testing. Traditionally, metabolite measurements are performed in urine samples stored frozen and transported on dry ice to specialized laboratories.We aimed to develop a method in dried urine spots (DUS) that allows transport and storage at room temperature.
Methods: We developed a LC-MS/MS method for measuring creatine, guanidinoacetate and creatinine simultaneously in DUS. Urine spots (representing 5 μL) were extracted (ultrasonic) with methanol. The supernatants were dried and butylated. The butylated compounds were separated by reversed-phase HPLC and detected by positive ion tandem mass spectrometry.
Results: DUS of proven AGAT-, GAMT- and SLC6A8- patients could be distinctly distinguished from control urine spots. One week of storage at room temperature did not significantly alter the metabolite concentrations in DUS samples in comparison to the original sample.
Conclusion: This method offers a faster and more cost effective approach for screening CDS allowing testing of a broader patient population (eg intellectual disability with unknown etiology).
Conflict of Interest declared.

P-434

A novel mouse model of creatine transporter deficiency

Baroncelli L[1], Alessandrì M G[2], Tola J[1], Putignano E[1], Migliore M[1], Amendola E[3], Zonfrillo F[3], Gross C[3], Leuzzi V[4], Cioni G[2 5], Pizzorusso T[1 6]

[1]Institute of Neuroscience, CNR, Pisa, Italy, [2]IRCCS Stella Maris Scientific Institute, Calambrone (Pisa), Italy, [3]European Molecular Biology Laboratory, Monterotondo (Roma), Italy, [4]Sapienza University of Rome, Roma, Italy, [5]University of Pisa, Pisa, Italy, [6]University of Florence, Florence, Italy

Background: Mutations in the creatine (Cr) transporter (CrT) gene lead to cerebral creatine deficiency syndrome-1 (CCDS1), an X linked metabolic disorder characterized by cerebral Cr deficiency causing intellectual disability, seizures, movement and behavioral disturbances, language and speech impairment (OMIM#300352). CCSD1 is still an untreatable pathology that can be very invalidating for patients and caregivers.
Methods & Results: Only two murine models of CCDS1, one of which is an ubiquitous knockout mouse, are currently available to study the possible mechanisms underlying the pathologic phenotype of CCSD1 and to develop therapeutic strategies. Given the importance of validating phenotypes and the efficacy of promising treatments in more than one mouse model we have generated a new murine model of CCSD1 obtained by ubiquitous deletion of 5-7 exons in the *Slc6a8* gene. We showed a remarkable Cr depletion in the murine brain tissues and cognitive defects, thus resembling the key features of human CCSD1.
Conclusion: These results confirm that CCDS1 can be well modeled in mice. This $CrT^{-/y}$ murine model will provide a new tool for increasing the relevance of preclinical studies to the human disease.

P-435

Guanidinoacetate methyltransferase deficient mice express electric seizure activity through systemic availability of guanidinoacetate affecting GABA(A) receptor function and seizure threshold

Schulze A[1], Tran C[1], Levandovskiy V[1], Patel V[1], Cortez M A[1]

[1]Hosp for Sick Children, Univ of Toronto, Toronto, Canada

Rationale: To study GABA(A) mediated mechanisms in electrical brain activity of Gamt-167 mice.
Methods: Electrocorticographic monitoring of pharmacological treatments with ornithine (5% in drinking water for 5 days) and picrotoxin (PTX) (a GABA(A) receptor antagonist) (1.5 mg/kg, I.P.) in Gamt-/- and Gamt+/+ groups (n=3 each, mean (SEM) age=6.9 (0.2) weeks). Mice were fitted with two frontal and two parietal epidural electrodes under ketamine/xylazine anesthesia. Baseline, spike-and-wave frequency, duration and the presence of electrodecremental epochs were determined from 1-2 hours continuous recordings.
Results: The EEG baseline of Gamt-/- exhibited a monotonous cortical rhythm (7-8 Hz) with little variability during awake and sleep states compared to wild type recordings. Ornithine treatment and also PTX administration led to a relative normalization of the Gamt-/- EEG phenotype. Gamt+/+ on PTX exhibited electro-behavioral seizures, whereas the Gamt-/- did not have PTX induced seizures at the same PTX dose. Gamt-/- treated with both ornithine and PTX did not show electro-behavioral seizures while ornithine elevated the PTX seizure threshold of Gamt-167 mice even further.
Conclusion: These preliminary data demonstrate (i) that there is expression of electrical seizure activity in this mouse strain, and (ii) that the systemic availability of guanidinoacetate affects GABA(A) receptor function and seizure thresholds.
Conflict of Interest declared.

P-436

Identification and re-purposing of drugs for the treatment of human guanidinoacetate methyltransferase deficiency (GAMT-D)

Tkachyova I[1], Tropak M[1], Datti A[3], Schulze A[2]

[1]The Hospital for Sick Children, Toronto, Canada, [2]Hosp for Sick Children Univ of Toronto, Toronto, Canada, [3]Mount Sinai Hospital, Toronto, Canada

Background: GAMT-D results in accumulation of its substrate guanidinoacetate (GAA) and lack of end-product creatine. Accumulation of GAA has been proven to be neurotoxic and epileptogenic in GAMT-D patients. Present treatments, such as ornithine supplementation and arginine deprivation, fail to normalize GAA.
Objectives: Our goal is to lower GAA accumulation by inhibiting GAA synthesizing enzyme, L-arginine:glycine amidinotransferase (AGAT).
Methods: We set up two high-throughput assays to screen for inhibitors of AGAT. Transcriptional inhibitors are found from drug library screening using permanent HeLa cell line that stably expressing AGAT promoter luciferase. Direct enzymatic inhibitors are validated from the thermal shift denaturation profiles of the enzyme in the presence of the ligand.
Results: Inhibition assays of *AGAT* transcription and AGAT enzyme activity were established and validated. We confirmed that our stably expressed *AGAT* promoter responded to the creatine treatment in a dose-dependent manner. From the screening of a small subset of libraries we found several compounds as potential *AGAT* transcription inhibitors. For one of these hits effectiveness was validated in selected cell lines by RT-PCR.
Conclusion: We demonstrated that our established assays could be used as a powerful tool towards finding new possibilities to treat metabolic disorders.
Conflict of Interest declared.

19. Disorders of purines, pyrimidines and nucleic acids

P-437

New biomarkers for early diagnosis of Lesch-Nyhan disease revealed by metabolic analysis on a large cohort of patients

Ceballos-Picot I[1 2 6], Le Dantec A[3], Brassier A[6], Jaïs J P[4], Ledroit M[1], Petitgas C[1], Ea H K[5], Daignan-Fornier B[3], Pinson B[3]

[1]Metab Biochem, Univ Necker Hosp, Paris, France, [2]Univ Paris Descartes, Paris, France, [3]Univ Bordeaux, IBGC UMR 5095, Bordeaux, France, [4]Biostat Univ Necker Hosp, Paris, France, [5]Center Viggo Petersen, Inserm UMR 1132, Paris, France, [6]Metab Dis Ref Center, Paris, France

Background: Lesch-Nyhan disease (LND) is a rare X-linked metabolic disorder caused by mutations in the *HPRT1* gene leading to a deficiency of the purine recycling enzyme HGPRT. The prevalence is underestimated due to the difficulty of diagnosing the less severe forms. We searched for metabolic changes that would facilitate an early diagnosis and give potential clues on the disease pathogenesis.
Methods: Diagnosis using HGPRT enzymatic assay in erythrocytes and identification of *HPRT1* gene mutations. Patients classificatied into three phenotypic subgroups. Metabolites quantified by high performance ionic chromatography.
Results: 139 patients from 112 families were studied. 98 displayed LND full phenotype (86 families) and 41 (26 families) attenuated clinical phenotypes. Genotype/phenotype correlations showed that attenuated phenotypes are associated with mutations allowing some residual HGPRT activity. Analysis of metabolites revealed strong variations specific to HGPRT deficiency for six metabolites (AICAR mono- and tri-phosphate, nicotinamide, nicotinic acid, ATP and succinyl-AMP) as compared to controls including hyperuricemic patients without HGPRT deficiency.
Conclusions: Highly significant correlation between six metabolites and the HGPRT deficiency was established providing an easily measurable marker of LND. Their combination strongly increases the probability of an early and reliable diagnosis for HGPRT deficiency.

P-438

Intrastriatal hypoxanthine administration alters inflammatory profile in striatum of Wistar rats

Biasibetti H[1], Pierozan P[1], Rodrigues A F[1], Sebotaio M[1], Wyse A T S[1]

[1]UFRGS, Porto Alegre, RS, Brazil

Lesch–Nyhan disease is characterized by deficiency of the enzyme hypoxanthine–guanine phosphoribosyl-transferase, resulting in accumulation of hypoxanthine. Although the underlying mechanisms of brain dysfunction in Lesch–Nyhan disease are poorly understood, hypoxanthine accumulation seems to contribute to neurological damage. We analyzed the effect of hypoxanthine on inflammatory parameters such as cytokine levels, immunocontent of NF-κB/p65 subunit and

iNOS, nitrite levels, acetylcholinesterase (AChE) activity and immunocontent of infant and young adult rats subjected to stereotaxic surgery. 21 and 60 days old Wistar rats underwent stereotactic surgery and were divided into two groups: control (saline) and hypoxanthine (10μM). Animals were decapitated 30 minutes after the injection. Results showed that intrastriatal administration of hypoxanthine increased IL-6 levels in striatum of both ages of rats analyzed, while TNF-α was increased only in 21-day-old rats. Results show an augmented nuclear immunocontent of NF-κB/p65 subunit in striatum of both ages of rats. Hypoxanthine administration decreased nitrite levels in the striatum of 21-day-old rats, but iNOS immunocontent was increased in striatum of hypoxanthine groups, as well as AChE immunocontent. According to our results, hypoxanthine increases inflammatory parameters, suggesting that this process may be involved in neurological disorders found in patients with Lesch-Nyhan disease. Supported by CNPq.

P-439

Adenine Phosphoribosyltransferase deficiency: an under-recognized cause of urolithiasis and renal failure

Morgan, Ledroit M[1], Lionel, Mockel L[1], Véronique, Droin V[1], Céline, Petitgas C[1], Michel, Daudon M[3], Guillaume, Bollée G[4], Ceballos-Picot I[1 2]

[1]Metab Biochem, Univ Necker Hosp, Paris, France, [2]Univ Paris Descartes, Paris, France, [3]Funct Explor Unit, Tenon Hosp, Paris, France, [4]Nephrol. CHU Montréal, Montréal, Canada

Background: Adenine phosphoribosyltransferase (APRT) deficiency is an under-recognized autosomal recessive disorder causing 2,8 dihydroxyadenine (2,8-DHA) urolithiasis and crystalline nephropathy. The disease can be efficiently treated by inhibitors of xanthine dehydrogenease, which makes early diagnosis and treatment essential to prevent recurrence of urolithiasis and nephropathy.
Methods: Diagnosis of complete APRT deficiency was determined by null erythrocyte APRT enzymatic activity in a large cohort of pediatric and adult patients (67 patients from 56 families) identified at Necker Hospital between 1978 and 2014. We analyzed *APRT* gene mutations in 54 patients (46 families).
Results: Twenty seven different mutations were identified. A single T insertion at the intron 4 splice donor site (c.400+2dup) leading to a truncated protein, accounted for 36 % of mutated alleles. A striking finding was a diagnosis delay in 70 % of patients ranging from 0 to 43 years from first episode of urolithiasis to diagnosis.
Conclusions: The c.400+2dup mutation, previously identified in several patients from Europe, is the most common cause of APRT deficiency in this population. APRT deficiency remains poorly recognized despite the availability of diagnostic tools.

P-440

Diagnosis of xanthinuria type II using untargeted mass spectrometry-based next generation metabolic ccreening (NGMS)

Engelke U F H[1], Heeft van der E[1], Schreuder M[1], Boer de S[1], Engel J[2], Huigen M[1], Wevers R A[1], Kluijtmans L A J[1]

[1]Radboud University Medical Centre, Nijmegen, Netherlands, [2]Radboud University, Nijmegen, Netherlands

Xanthinuria typeII (XAII) is an IEM caused by a combined deficiency of xanthine dehydrogenase and aldehyde oxidase. The diagnosis usually relies on purine/pyrimidine-analysis combined with an allopurinol loading test. Here we describe the ability to unambiguously establish this diagnosis by an untargeted metabolomics assay without performing an allopurinol loading test.
Our patient, a 2-month-old boy, presented with kidney stones. Plasma of controls (n=20) and of our patient were analyzed by UHPLC-QTOF-MS. Accurate masses, retention times and signal intensities were processed using alignment software (XCMS) and were statistically analyzed in MATLAB with an in-house-developed script. The Human Metabolome Database was subsequently queried to identify the diagnostic metabolites.
Several biomarkers, common to XAI and XAII were identified in abnormal intensity in plasma of the patient relative to controls (xanthine, xanthosine and uric acid). Additionally, four biomarkers related to adehyde oxidase activity were found to be present in abnormal intensity relative to controls, i.e.N-methylnicotinamide, hydantoin propionate, N-(3-acetamidopropyl) pyrrolidin-2-one and pyridoxal), and were consistent with a deficiency of aldehyde oxidsase.
This result demonstrates that NGMS as a newly established technique involving untargeted metabolomics approach is superior to classical metabolic screening in diagnosing XAII, i.e. establishing the diagnosis without the need of an allopurinol test.

P-441

A new case of AICA-ribosiduria

Marie S[1], Ceballos-Picot I[2], Delorière E[2], Imbard A[3], Benoist J F[3], Vincent M F[1], Dewulf J[1], Rio M[4]

[1]Biochem Genetics, Clin St-Luc, UCLouvain, Brussels, Belgium, [2]Metab Biochem, Univ Necker Hosp, Paris, France, [3]Biochem Horm, Robert Debré Hosp, APHP, Paris, France, [4]Medical Genetics, Univ Necker Hosp, Paris, France

Background: ATIC (AICAR transformylase-IMP Cyclohydrolase) is a bifunctional enzyme catalysing the two final steps in the purine *de novo* synthesis pathway. We described the first ATIC-deficient patient in 2004 and we report here the second family with AICAribosiduria.
Case report: The proband was referred at 14 months due to hypotonia and developmental delay. Hypotonia with poor eye contact was observed from the first weeks of life. She developed with growth retardation, microcephaly, nystagmus, hypotonia and severe psychomotor delay. Epilepsy occurred at the age 3. Her older sister was born prematurely with intrauterine growth retardation and presented with West syndrome in the first year of life. At 10 years, she presented with motor impairment and was unable to walk and had no language. A positive urinary Bratton-Marshall test in both sisters suggested accumulation of SAICA-riboside and/or AICA-riboside. HPLC analysis of urinary purines showed the same profile as the first ATIC-deficient patient, with high AICA-riboside concentration, and lower level of SAICA-riboside and succinyladenosine (S-Ado). Molecular analysis showed the presence, in heterozygous state, of one mutation in the AICAR-transformylase domain, also found in the first ATIC-deficient patient. The second mutation was not identified, but the mutation found was shown to lead to an unstable mRNA.

P-442

Preparation and characterization of the model cells system to study to date unknown genetically determined defects of *de novo* purine synthesis

Zikanova M[1], Skopova V[1], Krijt M[1], Souckova O[1], Baresova V[1]

[1]Inst of Inh Metab Dis, First Fac of Med, Prague, Czech Republic

Background: The diagnosis of patients with neurological impairment is set down only in about 2% of studied cases. The reason might be the inability to capture new, to date unknown, diseases. One of the metabolic pathways whose malfunctions could lead to neurological impairment is *de novo* purine synthesis (DNPS). Currently two genetically determined defects of DNPS enzymes are described. Therefore, the existence of other defects manifested by neurological symptoms and by accumulating DNPS intermediates in body fluids, is highly presumable.
Methods: We developed methods for the enzymatic preparation of the DNPS intermediates and demonstrated its use in quantitative LC-MS/MS analysis. To describe the defects of DNPS enzymes we prepared model HeLa cells deficient for particular steps of DNPS using CRISPR method and analyzed DNPS metabolites in cell lysates and growth medium.
Results: We have prepared five cells lines, deficient for particular DNPS enzyme, which have accumulated substrates of the knocked-out enzymes.
Conclusion: Analysis of the model cells provided us with the information about metabolites secreted and accumulated in particular defects of DNPS. Using the knowledge obtained from the model cell lines, the population of patients with neurological impairment can be examined and the new diagnosis determined.

P-443

New biochemical markers in adenosine kinase deficiency

Wiame E[1], Balthausen D[2], Dewulf J[3], Marie S[3], Vincent M F[3]

[1]de Duve Institute, UCLouvain, Brussels, Belgium, [2]Center for Molecular diseases, CHUV, Lausanne, Switzerland, [3]Biochem Genetics, Clin univ Saint-Luc, UCLouvain, Brussels, Belgium

Here we present not yet previously described biochemical parameters found in the urine of a patient with adenosine kinase deficiency (ADKD).
The patient is the first child of first-degree cousins of Turkish origin. In the newborn period he presented repetitive hypoglycaemia and liver dysfunction. At one month a mild lactic acidosis and moderate hyperammonemia were recognized. He is now 2 ½ years old and shows psychomotor retardation.
Amino acid analysis showed mild hypermethioninemia. There was a raised urinary excretion of adenosine. The diagnosis of ADKD was confirmed by molecular analysis showing homozygozity for the mutation p.Phe302Ser in the *ADK* gene. Furthermore, we found a marked hyperexcretion of 5-amino-4-imidazolecarboxamide (AICA) riboside, also found in urine of patients with 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase (ATIC) deficiency. This finding can be explained by substrate cycling between AICAriboside and its monophosphate derivative, AICAR, as previously described in isolated hepatocytes. Succinyl-AICAriboside was also detected in the patient urine.
The accumulation of AICAriboside, and most probably of intracellular AICAR, may contribute to the pathophysiology in ADKD, as numerous effects are known due to the resemblance to respectively adenosine and AMP, and the effects on AMPK.

Urinary AICAriboside might be considered as a new diagnostic marker of ADKD.

20. Lipid and lipoprotein disorders, porphyrias

P-444

Characterization of plasma lipoprotein particles in Spanish patients with lysosomal acid lipase deficiency (LAL-D)

Yahyaoui R[1 11], Rodríguez-García E[1], Heras M[5 6 7 8 10], García-Jiménez M C[2], González-Diéguez M L[3], Mallol R[4], Amigó N[4 5 6 7 9]

[1]Clin Lab. Málaga Regional Hospital, Málaga, Spain, [2]Pediatrics. Miguel Servet Hospital, Zaragoza, Spain, [3]Hepatology. Univ Hospital of Asturias, Oviedo, Spain, [4]Biosfer Teslab, Reus, Spain, [5]CIBERDEM, Reus, Spain, [6]IISPV, Reus, Spain, [7]Universitat Rovira i Virgili, Reus, Spain, [8]Res Unit of Lipids and Atherosclerosis, Reus, Spain, [9]Metabolomic Platform, Reus, Spain, [10]University Hospital Sant Joan de Reus, Reus, Spain, [11]IBIMA, Málaga, Spain

Background: LAL-D patients typically present with dyslipidaemia (elevated plasma concentrations of total cholesterol and triglycerides) due to accumulation of ApoB-containing lipoproteins such as VLDL and LDL. The objective of this study was to characterize plasma lipoprotein particles in LAL-D.
Patients/Methods: Five patients with LAL-D were included: two 9-year-old children treated solely with statins and three young adults with liver transplants without lipid-lowering treatment. Plasma samples were analyzed by a novel advanced lipoprotein test based on 2D diffusion-ordered ^{1}H-NMR spectroscopy (Liposcale®). The lipoprotein particle concentrations, lipid load and sizes were determined. Lipoprotein fractions were obtained by sequential ultra-centrifugation and apolipoproteins levels were measured.
Results: Children's lipoprotein profiles presented a high concentration of VLDL-C, VLDL-TG, IDL-C and LDL-C (22.1, 117.7, 19.2, 131.5 mg/dL), and a low concentration of HDL-C and apoA-I levels. Small subclasses of VLDL, LDL and HDL particles represented the 81%, 51% and 67% of the total. The adults´lipoprotein profiles were shifted to a healthy state.
Discussion: LAL-D patients present with a very atherogenic lipoprotein pattern in spite of lipid-lowering therapy, characterized by high levels of small ApoB-containing lipoproteins including IDL. These are capable of easily entering the arterial wall and reduced mature HDL particle levels. Liver transplantation seems to cure dyslipidaemia.

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Familial hypercholesterolemia due to *LDLR* gene mutations: about six new Moroccan families

Touzani A[3], Di-Filippo M[2], Sassolas A[2], Rousson R[2], Gaouzi A[1], Chabraoui L[3]

[1]CHU-Unit Endocrinology-Diabetology-Pedia, Rabat, Morocco, [2]Centre de Biologie et de Pathologie Est, Lyon, France, [3]Biochem Lab, FMPR, University Med V, Rabat, Morocco

Familial hypercholesterolemia (FH) is an autosomal dominant disorder mainly due to LDLR mutations. The rare homozygous or compound heterozygous forms result in major hypercholesterolemia, xanthomas and cardiovascular risk. We identified six unrelated Moroccan probands with major hypercholesterolemia, belonging to six families. We also analysed seventeen of their family's members. We sequenced the 18 LDLR gene exons in the six probands. Patients have high LDL-C level (7 ± 1.4 g / L). They present with xanthomas on elbows, knees and buttocks. Hypercholesterolemia was found in parents and some other relatives, indicating their heterozygous status. Three siblings had high cholesterol levels consistent with a homozygous status. The molecular studies showed the presence of two new mutations in exon 4 at homozygous state and in exon 9 at compound heterozygous state. We also identified three other point mutations, two at the homozygous state in exons 4 and 6 and the third in the exon 12 at the compound heterozygous state. Two of these mutations were found for the first time in Moroccan patients. In this study, we reported two new mutations in the LDLR gene while emphasizing the genetic heterogeneity in the Moroccan population. The molecular studies allowed us also to give an adequate genetic counseling to the families studied.

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Treatment experience in a patient with serious mevalonic aciduria

Erdöl S[1], Çekiç S[2], Dorum S[1], Cakı S[3], Saglam H[1], Kılıç S S[2]

[1]Uludag Univ, Div Metab Dis, Bursa, Turkey, [2]Div İmmunol Dis, Uludag Univ, Bursa, Turkey, [3]Div Hemat Dis, Medical Park Hosp, Istanbul, Turkey

Background: Mevalonic aciduria (MVA) is an autosomal recessive disorder caused by a deficiency of the enzyme mevalonate kinase involved in cholesterol biosynthesis.
Case Report: A ten-day-old boy presented with mild coarse facies and massive hepatosplenomegaly and was diagnosed as

having mevalonic aciduria. Ascites was detected at the age of 44 days and recurrent paracentesis was required. The patient did not benefit from furosemide and spironolactone treatment. At the age of 70 days, coenzyme Q10 (5 mg/kg/day) and prednisolone (2 mg/kg/day) were started and prednisolone was tapered and stopped at 25 days. At 80 days, ibuprofen and at 124 days, canakinumab were started. Physical examination findings and laboratory values did not improve with prednisolone, ibuprofen and canakinumab though there was a decrease in the frequency of paracentesis with ibuprofen. Thereafter, a bone marrow transplantation (BMT) was performed at 138 days from a sibling donor. Despite regression of ascites and normalisation of urinary mevalonolactone excretion after transplantation, organomegaly did not improve. The patient died from septicemia 3.5 months after BMT.
Conclusion: In this severe case of MVA, ibuprofen, prednisolone, and canakinumab were ineffective, and bone marrow transplantation did not correct the clinical outcome.

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Plasma lipids and proprotein convertase subtilisin/kexin type 9 (PCSK9) in patients with Smith-Lemli-Opitz syndrome (SLOS)

Tavori H[1], Roullet J - B[1 2], Minnier J[1], Pappu A[1], Steiner R D[3], Fazio S[1]

[1]Oregon Health & Science University, Portland, OR, United States, [2]Washington State University, Spokane, WA, United States, [3]Marshfield Clinic Research Foundation, Marshfield, WI, United States

Background & Objectives: SLOS is an inborn error of metabolism caused by mutations in *DHCR7* resulting in cholesterol (C) deficiency and 7-dehydrocholesterol (7DHC) excess. We aimed to study the effect of dietary cholesterol and statins on plasma lipid and PCSK9 levels in SLOS patients.
Methods: A retrospective study of 30 SLOS patients on high cholesterol diet (HC) was carried out. For some participants the HC diet was replaced with a 4-week cholesterol-free diet (CF). For others, simvastatin was added to the HC diet (HC+S). Plasma lipid and PCSK9 levels were measured and results analyzed using linear mixed model adjusting for age.
Results: CF diet did not significantly affect cholesterol, LDL, or 7DHC compared with HC diet. Addition of simvastatin to dietary cholesterol (HC+S) reduced 7DHC levels (-25.4%, p=0.011), and increased total (+17.6%, p=0.048) and LDL cholesterol (+21.6%, p=0.064) levels. PCSK9 levels increased in the HC+S group (+21.5%), but differences were not statistically significant (p=0.108).
Conclusions: This report emphasizes the benefits of dietary cholesterol supplementation with statins as a means to reduce 7DHC and increase total and LDL cholesterol levels, and suggests a role for PCSK9 in cholesterol regulation in SLOS patients on HC diet treated with statins.

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Cases of Acute Intermittent Porphyria and Congenital Erythropoietic Porphyria

Canda E[1], Köse M[1], Kağnıcı M[1], Karakoyun M[3], Kurt I[4], Aksoylar S[2], Kalkan Ucar S[1], Aydınok Y[2], Kansoy S[2], Çoker M[1]

[1]Div Metab Dis, Ege Univ Med Faculty, Izmir, Turkey, [2]Div Ped Hemat Oncol, Ege Univ Med Facult, Izmir, Turkey, [3]Div Ped Gastro, Ege Univ Med Faculty, İzmir, Turkey, [4]Div Biochemistry, GATA, İzmir, Turkey

Background: Acute Intermittent Porphyria (AIP) is caused by constitutive Hydroxymethylbilane Synthase (HMBS) deficiency due to heterozygous mutations of *HMBS* gene, is associated with neurological symptoms, especially intense and recurrent abdominal pain attacks, and exhibits typical urine darkening. Congenital erythropoietic porphyria (CEP) is due to constitutive deficiency of Uroporphyrinogen 3 Synthase due to recessive mutations of *UROS* gene. Our cases of AIP and CEP are evaluated with their follow-up.
Case Report:
Case 1: A 16-year old female patient presented with recurrent abdominal pain attacks and pins and needles in hands and feet. Previous diagnosis was ulcerative colitis and FMF. A p.R173W(c.517C>T) mutation was found in the *HMBS* gene.
Case 2: A 15-year old female patient presented with recurrent abdominal pain attacks. The levels of ALA and porphobilinogen in urine were high during attack. Specific heme arginate was administered to both patients during attack.
Case 3. A four year five month old female patient presented with a cholestatic hepatitis in the neonatal period, hemolysis and bullous lesions after phototherapy. Uroporphyrin and coproporphyrin isomers I and III were elevated. *UROS* gene analysis revealed a homozygous mutation. The patient had Bone marrow transplantation (BMT) at the age of 13 months.
Conclusions: AIP must be considered in differential diagnosis in the presence of recurrent abdominal pain attacks. BMT is main treatment option for CEP patients early in life to avoid irreversible lesions.

P-449

Splicing mutation in aminophospholipid transporter protein ATP8A2 in a Turkish family

Dursun A[1], Yalnizoglu D[3], Yucel-Yilmaz D[1 2], Serdaroglu E[3], Unal O[1], Gormez Z[5], Demirci H[5], Sagiroglu M[5], Topcu M[3], Ozgul R K[1 4]

[1]Div Metab Dis, Hacettepe Univ Child Hosp, Ankara, Turkey, [2]Inst of Child Health, Hacettepe Univ, Ankara, Turkey, [3]Div Pediatric Neurol, Hacettepe Univ, Ankara, Turkey, [4]Institute of Child Health, Hacettepe Uni, Ankara, Turkey, [5]TÜBİTAK, BİLGEM, İGBAM, Kocaeli, Turkey

Background and objectives: Aminophospholipid transporter protein ATP8A2 had been associated with cerebellar ataxia, mental retardation and dysequilibrium syndrome. These patients had quadrupedal locomotion. We report a consanguineous Turkish family with two siblings who presented with severe cognitive impairment and extrapyramidal involvement.
Case report: The first patient developed hypotonia after a fairly normal development in the first 3 months. After 12 months she displayed progressive involuntary movements sensitive to tactile stimulus. She had axial hypotonia, poor light perception and severe choreathetoid movements. Her brother lost acquired developmental milestones after 4 months. His clinical findings were similar. They both were unable to hold their heads correctly. They had a characteristic supine crawl with good mobility.
Methods and Results: Whole exome sequencing revealed a homozygous splicing mutation (c.3075+2T>G) in the *ATP8A2* gene.
Discussion: ATP8A2 is expressed in brain tissues, especially in cerebellum, retina and spinal cord. It is required for normal visual and auditory function. Quadrupedal locomotion due to *ATP8A2* mutations was attributed to its role in the development of the cerebro-cerebellar structures required for posture and gait. Two patients presented herein, displayed crawling on their back, severe intellectual disability and choreathetoid movements of limbs.
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P-450

Neutral lipid storage disease (Chanarin-Dorfman syndrome): a report of three cases

Elkhateeb N[1], Selim L A[1], Sobhi A[1]

[1]Div ped neuro, Cairo univ child hosp, Cairo, Egypt

Background: Neutral lipid storage disease (NLSD) is a rare non-lysosomal, autosomal recessive lipid storage disorder characterized by systemic triacylglycerol deposition in multiple tissues, including skin, muscle, liver, central nervous system, and blood leukocytes. Patients affected present with myopathy, hepatomegaly, cardiomyopathy, and a specific form of ichthyosis in the form of nonbullous congenital ichthyosiform erythroderma. Peripheral blood smear shows Jordans' anomaly; lipid-containing vacuoles in leukocytes.
Case report: We report 3 patients with NLSD, a 2 years old female, and two 9 months old twin females. All three presented with motor delay and hypotonia, hepatomegaly and icthyosis. EMG for the 3 patients showed a myopathic picture. Liver biopsy for the older child showed hepatic steatosis. Peripheral blood smears showed Jordans' anomaly, while results of muscle biopsy and molecular diagnosis for the 3 patients are pending.
Conclusion: Neutral lipid storage disease is a rare autosomal recessive lipid storage disease which is characterized by the deposition of triacylglycerol in multiple tissues including liver, skin, muscle, central nervous system and blood leukocytes. Emollients and dietary modification remain the mainstay of treatment. Reduced long-chain fatty acids, increased medium-chain fatty acids are beneficial. Ursodeoxycholic and vitamin E are given in steatohepatitis because of their cytoprotective effects.

21. Peroxisomal, sterol and bile acid disorders

P-451

A novel mutation of the *PEX16* gene in a patient with slowly progressive atypical presentation of Zellweger sydrome

Ersoy M[1], Tatli B[3], Ceylaner S[2]

[1]Child Metab Dis,Sadi Konuk Res&Tra Hosp, Istanbul, Turkey, [2]Intergen Genetics Center, Ankara, Turkey, [3]Div Pediat Neur, Istanbul Medical Fac, Istanbul, Turkey

Background and objectives: Zellweger Syndrome Spectrums (ZSSs) are a group of inherited metabolic disease caused by one of the *PEX* genes requried for normal peroxisome assembly. Clinical presentation varries due to the affected *PEX* gene and the related mutations. Most of the patients die before their second-year bithday. However, late-onset-mild phenotypes are diagnosed resently. We report a case diagnosed with ZSS who shows a late-onset, slowly progressive clinic with a novel mutation of *PEX16* gene.
Case report: Two-year-old girl with mild neuromotor retardation and walking difficulty admitted to hospital. She showed normal

development in the first year of life, but then stopped acquiring new skills, and lost her ability to walk independently due to spasticity and ataxia. Her speech and cognitive functions deteriorated slowly over time. At the age of 4 years, she had nystagmus and involuntary movements. Bilateral symetric axonal sensorimotor polyneuropathy was determined at electromyography. Brain MRI showed widespread white matter changes on a background pattern of global delay in myelin maturation.Very long chain fatty acids were mildly elevated. We found (c.9531974del23ins5) homozygous mutation in *PEX16* gene.
Conclusion: *PEX16* gene mutations cause late-onset slowly progressive phenotype and we can speculate the existence of the genotype-phenotype correlation.

P-452

Protective effect of antioxidants on DNA damage in leukocytes from X-linked adrenoleukodystrophy patients

Marchetti D P[1 2], Donida B[1 2], Da Rosa H T[4], Manini P R[4], Moura D J[4], Saffi J[4], Deon M[1], Mescka C P[1 2], Coelho D M[1], Jardim L B[1], Wajner M[1], Giugliani R[1 3], Vargas C R[1 2]

[1]HCPA/Medical Genetics Service, Porto Alegre, Brazil, [2]UFRGS/PPGBioq, Porto Alegre, Brazil, [3]UFRGS/PPGBM, Porto Alegre, Brazil, [4]UFCSPA/LabGentox, Porto Alegre, Brazil

Background: Oxidative stress in X-linked adrenoleukodystrophy (X-ALD), an inborn error of peroxisome metabolism, was already described in literature; however, DNA injuries were not studied yet. The aims were to investigate DNA damage in heterozygotes and symptomatic X-ALD patients, to search for associations between DNA damage and lipid peroxidation and to evaluate the *in vitro* effect of N-acetyl-L-cysteine and trolox on DNA damage in leukocytes from symptomatic patients.
Methods: Whole blood cells from 6 symptomatic patients, 5 heterozygotes and 8 healthy controls were incubated with NAC (1 and 2.5 mM), TRO (25 and 75 μM) and/or phosphate buffer saline for 6 hours at 37°C, before DNA damage analysis (comet assay). Plasmatic TBA-RS was analyzed.
Results: Symptomatic patients presented higher DNA damage levels than those found in heterozygotes and controls; heterozygotes and controls showed similar results. N-acetyl-L-cysteine and trolox were capable to reduce, *in vitro*, DNA damage in symptomatic patients until control levels. Finally, DNA damage correlated with plasmatic TBA-RS levels, allowing to hypothesize that DNA damage might be induced by lipid peroxidation.
Conclusion: Administration of N-acetyl-L-cysteine and trolox in X-ALD patients might be of relevance as an adjuvant treatment, since there is still not any satisfactory therapy for X-ALD.

P-453

Primary hyperoxaluria in infancy: a rare cause of early-onset renal insufficiency

Eyskens F[1], Trouet D[2]

[1]Div Metab Dis, Univ Hosp Antwerp, Antwerp, Belgium, [2]Div Nephrol, Univ Hosp Antwerp, Antwerp, Belgium

Background: Primary hyperoxaluria (PH1) is a rare inborn autosomal recessive metabolic disorder due to the defiency of hepatic alanine-glyoxylate-aminotransferase, resulting in excessive synthesis and urinary excretion of oxalate inducing renal stone formation and deposition of calcium oxalate in the kidney and other organs (systemic oxalosis) in the most severely affected individuals.
Case report: We report a 5 month-old girl, first child of non-consanguineous parents of caucasian origin, presenting with a nephrotic syndrome (tot. prot./creat: 16,850 mg/mg) and rapidly progressive renal insufficiency. The abdominal ultrasonography revealed normal sized kidneys with intense cortico-medullary hyperechogenicity. Organic acid analysis in urine showed extremely elevated excretion of oxalic acid (548-922 μmol/mmol creat) and glycolic acid (310-2235 μmol/mmol creat). Plasma oxalic acid (GC/MS) was also highly elevated:121-150 μmol/L. The diagnosis of PH1 was confirmed by mutation analysis of *AGXT* gene: she is compound heterozygous for the mutations c.358+1G>T (splice site mutation) and c.454T>A (p.Phe152Ile). The latter mutation is prevalent in the Dutch population and associated with a pyridoxine responsive form of PH1.
Conclusion: A trial with high dose of pyridoxine gave insufficient lowering of the plasma oxalate. The patient is treated by peritoneal dialysis and hemodialysis until combined liver-kidney transplantation.

P-454

Leukoencephalopathy associated with mutation in the gene encoding peroxisomal sterol carrier protein X and hepatocellular carcinoma

Jovanovic A[1], Wilcox G[1], Donaldson E[1]

[1]Salford Royal NHS Foundation Trust, Salford, United Kingdom

Background: Liver disease has been a feature of many peroxisomal disorders with neonatal onset. However, liver complications have not previously been reported with presentation in adulthood.

Case report: Here we present a 60 year old male who developed walking and balance difficulties in adult life. His symptoms were initially mild. Progressively over years he developed increasing gait instability with ataxia and mild spasticity. Subsequently, he was diagnosed with leukoencephalopathy associated with mutation in the gene encoding peroxisomal sterol carrier protein X, a very rare peroxisomal disorder associated with increased phytanic acid, pristanic acid and very-long-chain fatty acids. Further testing confirmed sensorineural hearing loss, mild retinopathy and mild optic pathway demyelination. Sensory nerve conduction studies were consistent with myelopathy. In one adult case described in literature neurological changes had responded well to normalisation of phytanic and pristanic acid levels. Therefore he was commenced on a low-phytanate diet. Extensive workup revealed elevated a-fetoprotein leading to diagnosis of hepatocellular carcinoma. He had a successful resection and has remained under regular review.
Conclusions: Based on this case, we propose that adult patients with peroxisomal disorders associated with elevated phytanic acid, pristanic acid and very-long-chain fatty acids undergo regular liver surveillance for hepatic neoplasia.

P-455

Pigmentary retinopathy and neuropathy: clinical, radiological and pathological features of α-methylacyl-CoA racemase (AMACR) deficiency

Merwick A[1], King R[4], Manji H[2], Graham E[2], Reilly M M[2], Wanders R J[3], Lachmann R[1]

[1]Charles Dent Metabolic Unit, NHNN, London, United Kingdom, [2]Ntnl Hsptl for Neurology & Neurosurgery, London, United Kingdom, [3]University of Amsterdam, Amsterdam, Netherlands, [4]Royal Free & UCL Medical School, London, United Kingdom

Background: α-methylacyl-CoA racemase (AMACR) deficiency is rare autosomal recessive peroxisomal disorder, first described in two patients suffering from adult-onset sensorimotor neuropathy with or without associated pigmentary retinopathy.
Case report: A now 67 year old woman presented at age 27 with difficulty reading small print and deteriorating night vision. She had a pigmentary retinopathy, absent ankle reflexes and impaired vibration sensation to mid shin. The patient was assessed by electrophysiological methods, sural nerve biopsy, serum lipid profile, phytanic and pristanic acids, as well as by MRI brain.
Results: Involvement of sensory fibres was demonstrated by nerve conduction studies. Pristanic acid levels were raised disproportionate to phytanic acid levels. Biochemical investigation of cultured fibroblasts revealed normal levels of *de novo* plasmalogen biosynthesis, dihydroxyacetonephosphate-acyltransferase activity, ß-oxidation of palmitate (C16:0) and cerotate (C26:0), and phytanate but partially deficient ß -oxidation of pristanic acid. A diagnosis of AMACR deficiency was made based on cultured fibroblasts where no detectable AMACR activity could be demonstrated. MRI brain showed symmetrical abnormalities in the thalami, cerebral peduncles and pons.
Conclusion: We report a patient in whom a deficiency of the peroxisomal enzyme α-methylacyl-CoA racemase has been demonstrated, with a progressive deterioration in vision as well as central nervous system involvement.

P-456

Rhizomelic chondrodysplasia punctata type II: a case diagnosed by whole exome sequencing

Dursun A[1], Pektas E[1], Yucel-Yilmaz D[2], Ozgul R K[2]

[1]Div Metab Dis, Hacettepe Univ Child Hosp, Ankara, Turkey, [2]Division of Metabolism, Hacettepe Univer, Ankara, Turkey

Background: Rhizomelic chondrodysplasia punctata (RCDP) is an autosomal recessive disorder due to the deficiency in ether lipid synthesis. The peroxisomal disorders (PDs) are usually subdivided into two groups: the peroxisome biogenesis disorders and the single peroxisomal enzyme (transporter) deficiencies. Dihydroxyacetone-phosphate acyltransferase (DHAPAT) is one of the two enzymes involved in ether phospholipid biosynthesis, whose deficiency causes rhizomelic chondrodysplasia punctata type II. DHAPAT is a peroxisomal enzyme catalysing the first step in ether-phospholipid biosynthesis.
Case report: We present a 14-month-old female from consanguineous parents with short stature, rhizomelic and asymmetric limb shortening, bilateral cataracts, facial dysmorphism, punctate calcifications located in the epiphyses, severe growth and mental retardation. Urine & blood aminoacid chromatography, blood tandem mass spectrometry, urine organic acid analysis, blood cholesterol levels, very long chain fatty acid and phytanic acid levels were normal. Whole exome sequencing analyses revealed a homozygous c.743G>T; p.Arg248Leu mutation in *GNPAT* gene.
Conclusions: Children with developmental abnormalities and various systemic findings are commonly referred to metabolic clinics. Conventional laboratory and metabolic tests are sometimes inadequate to reach a diagnosis. This case illustrates the usefulness of the exome sequencing in patients who cannot be provided a specific diagnosis by routine metabolic investigations.

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P-457

Adrenoleukodystrophy and metachromatic leukodystrophy cases who underwent bone marrow transplantation

Kalkan Ucar S[1], Canda E[1], Köse M[1], Kağnıcı M[1], Aksoylar S[2], Kitiş Ö[3], Tosun A[4], Kansoy S[2], Çoker M[1]

[1]Div Metab Dis, Ege Univ Med Faculty, İzmir, Turkey, [2]Div Ped Hemato Onco,Ege Univ Med Faculty, İzmir, Turkey, [3]Div Radiology, Ege Univ Med Faculty, İzmir, Turkey, [4]Div Ped Neurol, Adnan Menderes Univ, Aydın, Turkey

Background: Bone marrow transplantation (BMT) is an important treatment option for some congenital metabolic diseases. Cases of adrenoleukodystrophy (ALD) and metachromatic leukodystrophy (MLD), in which BMT was performed, are presented.
Cases reports: Case 1. An 8-year 9-month old male patient presented with behavioral change and hyperactivity over the last two years. VLCFA releaved ALD. Cranial MRI Loes Score was 8. BMT was performed at the age of 8 years.
Case 2. An 11-year 6-month old male patient, was diagnosed with adrenal insufficiency when 5-year 2-month old,. VLCFA analysis revealed ALD. Cranial MRI Loes Score was 11. BMT was performed at the age of 7 years four months. Case 3. A twenty-year old male patient received a diagnosis of adrenal insufficiency at the age of 10. The results of the VLCFA analysis revealed ALD. BMT was performed at the age of 12 years Case 4. A 14-year old female patient presented with seizures at the age of 6 years. Arylsulfatase enzyme was 2 nmol/h/mg of protein (50-990). Cranial MRI revealed bilateral cerebral hemispheral hyperintentities.
BMT was performed at the age of 8 years.
Conclusions: The chances of a successful BMT are especially high in asymptomatic ALD patients and juvenile adult form of MLD.

P-458

Avoid rapid weight loss in Refsum disease

Douillard C[1], Le Poulennec T[3], Fontaine M[2], Vanmalleghem A[1], Parent A S[3], Dobbelaere D[1], Mention K[1]

[1]Div Metab Dis, Ref Cent Metab, Lille, LILLE, France, [2]Div Bioch Metab, CBP Hosp Lille, Lille, France, [3]Div Endocrinol Dis, Huriez Hosp, Lille, Lille, France

Background: Adult Refsum disease is a peroxisome disorder with high level of neurotoxic phytanic acid. Diagnosis is usually made during the first or second decade, with symptoms such as nyctalopia, anosmia, polyneuropathy, deafness and cerebellar ataxia.
Case report: A woman in whom the diagnosis was made at the age of 16, in a context of family genetic investigation. At this moment, she was considered asymptomatic. Twenty-six years later, she was seen at the age of 42, before a bariatric surgery (BMI 37.8 Kg/m2). She reported disturbances of smell and a loss of sensation in the lower limbs during a protein diet with a weight loss of 12 kg.
Results: Plasma phytanic acid 183 μmol/l (0.08 – 4.7 μmol/l), sensorineuronal hearing loss, hyposmia, retinitis pigmentosa, no polyneuropathy, mild vermian atrophy but no white matter signal abnormality on brain MRI.
Conclusion: Neurological worsening occurs during rapid weight loss, possibly related to phytanic acid mobilization in adipose tissue and/or protein diet with food rich in phytanic acid (ruminant meat). So bariatric surgery was not recommended and we proposed a medical management for Refsum disease and obesity with weight loss no faster than 1 to 2 kg a month.

P-459

Stem cell transplantation for symptomatic patients with childhood onset adrenoleukodystrophy

Kato K[1], Sakaguchi H[1], Yoshida N[1], Onodera M[2]

[1]Dept Ped Hem/Onc, JRC Nagoya First Hosp, Nagoya, Japan, [2]Brain Research Inst, Niigata Univ, Niigata, Japan

Background: Adrenoleukodystrophy (ALD) is an X-linked recessive disorder with progressive neurodegeneration and stem cell transplantation (SCT) at its early stage is the only treatment modality. But the indication and transplant outcomes of SCT for symptomatic ALD patients are not well understood. Here we report the transplant outcomes of symptomatic ALD patients in our institution.
Methods: From 2010 to 2014, nine patients with symptomatic ALD underwent allogeneic SCT in our institution. The conditioning regimen given to them were fludarabine, melphalan, and 4Gy of total body irradiation and they received either bone marrow (n=3) or cord blood (CB, n=6). The median Loes score before SCT was 14 (10 to 18).
Results: Engraftment was observed in all patients but one who got engraftment after second SCT and all of

them are alive for 1-5 years. Patients without involvement of internal capsule (IC) (n=6) before SCT showed lower median Loes score after SCT than those with involvement of IC (n=3), (13.5 versus 21, respectively, P=0.066) and stabilization of neurological symptoms was more evident in CB transplantation patients.
Conclusion: Even when patients with ALD are symptomatic, CB transplantation could stabilize their neurological status if their IC was not affected before SCT.

P-460

A novel *ABCD1* gene mutation in a Turkish patient with X-linked adrenoleukodystrophy who had atypically normal plasma levels of very long chain fatty acids

Zubarioglu T[1], Kiykim E[1], Aktuglu Zeybek A C[1], Cansever M S[2], Aydin A[1]

[1]Div Nutr Metab Dis, Ist Univ Cer Med Fac, Istanbul, Turkey, [2]Cent Lab, Ist Univ Cer Med Fac, Istanbul, Turkey

Background: X-linked adrenoleukodystrophy(X-ALD) is a rapidly progressive neurodegenerative disorder resulting from dysfunction of peroxisomal adenosinetriphosphate - binding fatty-acid transporter due to mutations in the *ABCD1* gene. It is characterized by progressive demyelination of central nervous system, adrenocortical insufficiency and elevated levels of very long chain fatty acids (VLCFAs) in plasma and tissues. Diagnosis is usually based on clinical, radiological and serological examinations and should be confirmed with molecular analysis of *ABCD1* gene.
Case report: A seven years old patient who had atypically normal plasma levels of VLCFAs and whose diagnosis of X-ALD is confirmed by a novel mutation of *ABCD1* gene is described. He was admitted with complaints of weakness of lower extremities, difficulty in spatial orientation, aggressive behavior and inarticulate speech. His physical examination revealed reduced muscle strength as 3/5 in lower extremities, hyperreflexia and pathological Babinski sign. His brain MRI was highly predictive of X-ALD. Fasting plasma levels of VLCFAs were normal. Molecular genetic analysis of *ABCD1* gene showed the homozygous c.713_730del18 mutation.
Conclusion: atypical pattern of normal plasma levels of VLCFAs despite existence of neurological manifestations and MRI findings compatible with X-ALD led the necessity of molecular analysis of *ABCD1* gene to lead the proper diagnosis.

P-461

Serum VLCFA levels as a biomarker of the severity of peroxisomal beta-oxidation impairement in peroxisomal biogenesis disorders and single enzyme deficiency

Stradomska T J[5], Syczewska M[1], Jamroz E[2], Pleskaczyńska A[4], Kruczek P[6], Tylki-Szymanska A[3]

[1]Dept Ped Rehab, Child Mem Health Inst, Warsaw, Poland, [2]Dept Neuroped, Med Univ Sil, Katowice, Poland, [3]Dept Metab Dis, Child Mem Health Inst, Warsaw, Poland, [4]Dept Neo Path, Child Mem Health Inst, Warsaw, Poland, [5]Dept Bio Exp Med, Child Mem Health Inst, Warsaw, Poland, [6]Dept Neo Path, Child Univ Hosp, Cracow, Poland

Background: Peroxisomal disorders are a heterogeneous group in the family of inborn errors of metabolism. There are two categories of the diseases resulting from peroxisomal biogenesis and single–enzyme deficiency. Peroxisomes, contain more than 50 enzymes that catalyze numerous specific biochemical process. There are 14 *PEX* genes responsible for peroxisomal biogenesis. Mutations in one of them make impossible the fulfillment of their biochemical functions similar to mutations in the genes of enzymes involved into peroxisomal metabolic pathways. The main biochemical marker for peroxisomal diseases are very long-chain fatty acids (VLCFA).
Methods: Samples from fifteen patients with peroxisomal disorders were investigated: 7 with classical Zellweger syndrome (ZS), 2 with mild outcome of ZS and 6 with D-Bifunctional Protein deficiency (DBP). VLCFA levels were analyzed by GC method.
Results: Accumulation of serum VLCFA (mean±SD) in severe ZS were higher than in patients with mild form of ZS and in patients with DBP, for *C26:0/C22:0* 0.68±0.20; 0.13±0.12; 0.23±0.05 ($p< 0.001$) and for *C26:0*[*mg/mL*] 5.51±2.22; 0.80±0.61; 2.40±0.59($p< 0.001$) respectively.
Conclusion: VLCFA levels correlate with the severity of the clinical course; ZS, DBP and mild ZS. The best predictive value for predicting of the disease severity is a concentration of *C26:0*.

P-462

Is hyperoxaluria phenotypes a second contribution to determine the disease?

Ozer I[1], Candan C[2], Turhan P[2]

[1]Ped Metab Dis, Goztepe Edu Res Hosp, Istanbul, Turkey, [2]Ped Neph, Goztepe Edu Res Hosp, Istanbul, Turkey

Background: Type I hyperoxaluria (PH1) (OMIM259900) is a deficiency of pyridoxal 5 ' phosphate dependent Alanine glyoxylate Aminotransferase (AGT) enzyme, an autosomal recessive disease. Frequency is defined as 1-3 per million. It is characterized by progressive kidney failure due to renal deposition of calcium oxalate.
Methods: Patients with PH1 were evaluated by urine oxalate, glomerular filtration rate and *AGXT* molecular analysis. Genotype, phenotype were reviewed in terms of the relationship
Results: Age range of the 20 patients was 65.3 ± 69. 3 months (7-123 months). First clinic finding age was 37.7 ± 40.8 months (1-123months). Ile340Met compound heterozygote mutation was most common.
Discussion: By the family screening, 5 asymptomatic patients were identified. Except the earliest symptomatic one, all of them were pyridoxine-responsive. Patients with PH1 and methylenetetrahydrofolate reductase (MTHFR) mutations coincidence was found in half of the group. All of them have bilateral seriously affected kidneys.
Conclusion: Genotype-phenotype relationship of hyperoxaluria could not be found.It may contribute to the second disease in patients in determining the phenotype. The *MTHFR* mutation causing structural and functional abnormalities to the urinary system has to be recognized since early treatment can be lifesaving.

P-463

Lorenzo's oil therapy. Follow up of three patients

Espinosa E[4], Ortiz T[3], Pulido N F[2], Ardila Y A[2], Guevara J M[1], Echeverri O Y[1], Barrera L A[1]

[1]I.E.I.M. Pont. Univ. Javeriana, Bogota, Colombia, [2]Hosp. Univ. San Ignacio, Bogota, Colombia, [3]Lab. Invest. Hormonal, Bogota, Colombia, [4]Univ. Mil. Nueva Granada - IOIR, Bogota, Colombia

Background: X-Linked Adrenoleukodistrophy is a peroxisomal disorder affecting VLCFA oxidation, involving adrenal function and CNS with high clinical heterogeneity. The classical form usually presents during childhood, as a rapidly progressive neurodegenerative disorder. Lorenzo's-oil (LO) therapy has proven to reduce plasma VLCFA levels and can benefit patients before any CNS compromise.
Methods: We present the follow-up of three patients from two families, who were biochemically diagnosed in early childhood due to family history of affected sibblings.
Results: Currently patients ages are 11, 8 and 7 years old, and have been under LO treatment for 6 years respectively. Up to date none of them have manifested any CNS involvement either clinically or by images, while the onset of neurological disease of their affected male siblings was around 7 years old. All patients present adrenal function alterations and are receiving replacement therapy.
Conclusion: Although, there is controversy in the literature regarding LO effectiveness, it is still the least invasive and most accesible treatment available. In our experience, there has been a good clinical response with delay in the onset of the neurological manifestations, however longer follow-up is needed to determine the treatment‘s effect.

P-464

Incidental finding of X-linked adrenoleukodystrophy in a male patient and gonosomal mosaicism in his mother

Beysen D[2], Castermans E[3], Sacré N[3], Roelens F[4], Verloo P[1]

[1]Div Metab Dis, Univ Child Hosp, Ghent, Belgium, [2]Div Ped Neur, Univ Child Hosp, Ghent, Belgium, [3]Dep Hum Gen, CHU, Liege, Belgium, [4]Dep Ped, AZ Delta, Roeselare, Belgium

Case report: In a 5-year old boy, demyelination of the splenium of the corpus callosum was observed on brain MRI after prolonged headache complaints after minor head trauma. Elevated plasma VLCFA levels and adrenal insufficiency were consistent with the diagnosis of X-linked adrenoleukodystrophy (X-ALD). Sequencing analysis identified a novel intronic *ABCD1* mutation (c.1866-11C>A), creating a novel splice acceptor site. Carrier testing in the mother showed a low level of heterozygosity of the mutation, suggestive of gonosomal mosaicism and was confirmed by pyrosequencing, restriction enzyme assay and subsequent sequencing of the restriction fragments.
Conclusion: This is the second report on gonosomal mosaicism in X-ALD. Although the level of mosaicism is low (estimated 10%), it is not possible to predict the clinical outcome in the mother, as it is currently unknown to what extent X-chromosome inactivation and modifier genes play a role in the development of the AMN-like phenotype in female carriers.

P-465

Nutrient intake in children with Smith-Lemli-Opitz syndrome

Myrie S[4], Haas K[1], Porter F D[5], Heubi J[6], Steiner R D[3], Jones P H[4], Roullet J - B[1 2]

[1]Oregon Health & Science University, Portland, OR, United States, [2]Washington State University, Spokane, WA, United States, [3]Marshfield Clinic Research Foundation, Marshfield, WI, United States, [4]University of Manitoba, Winnipeg, MB, Canada, [5]Eunice Kennedy Shriver NICHD, Bethesda, MD, United States, [6]Cincinnati Children's Hosp. Med. Center, Cincinnati, OH, United States

Background: Smith-Lemli-Opitz syndrome (SLOS) is an inborn error of metabolism caused by mutations in *DHCR7*. Growth failure is common. However, systematic studies of nutrition in SLOS are lacking.
Methods: Three 24-hr dietary recalls were performed over 1 year in 26 SLOS patients (13/13 M/F; 2-21 years old). Seventeen patients were oral feeders (O), 4 were gastrostomy-fed (G), and 5 used both feeding methods (OG). Daily nutrient intakes were calculated using Nutrition-Data-System Research software and analyzed adjusting for age, body weight or caloric intake when appropriate.
Results: Average caloric intake was 75 cal/day/kg (40%/45%/15% fat/carbohydrates/proteins), similar in all 3 groups and ~16% higher than the recommended intake for healthy children. The intake of minerals, B vitamins, and antioxidants (selenium, vitamins A, C and E) was lower in O than in G or OG patients but overall exceeded daily recommended intake (from 114% to 416%). Cholesterol intake (mg/kg/day) was significantly higher in OG patients (36±3) than in O (29±2) or G (24±4) patients.
Conclusions: This is the first controlled systematic evaluation of nutrition in SLOS. The data provide a basis for examining nutrition-clinical outcomes relationships and suggest that in SLOS growth failure is not the consequence of nutritional deficiency.

22. Lysosomal disorders: mucopolysaccharidoses, oligosaccharidoses

P-466

The first screening results of six lysosomal storage disorders using a HPLC-MS/MS multiplex assay in Turkey

Akbas H S[1], Soyucen E[2], Yucel G[1]

[1]Dep Biochemistry, Univ Akdeniz Hosp, Antalya, Turkey, [2]Dep Metab Dis, Univ Akdeniz Hosp, Antalya, Turkey

Background: Mass spectrometry has been used for the diagnosis of lysosomal storage disorders (LSD) such as Pompe, Fabry, Gaucher, Krabbe, Niemann-Pick A/B and mucopolysaccharidosis I in dried blood spots (DBS). In this study we aim to investigate a HPLC-MS/MS method for multiplex screening of LSDs in dried blood spots in Turkey.
Methods: Dried blood spots were incubated for 20 h with cocktails containing substrates and internal standards. We determined the resulting product and internal standard using LC-MS/MS (Shimadzu 8030 Triple Quadrupole Liquid Chromatograph Mass Spectrometer, Shimadzu Scientific Instruments, Japan). The method did not require offline sample preparation such as liquid-liquid and solid-phase extraction.
Results: A total of 450 dried blood samples were analyzed for the lysosomal α-glucosidase, β-glucocerebrosidase, α-galactosidase, acid sphingomyelinase, galactocerebrosidase, and α-L-iduronidase activities. Affected patient's enzyme activities were found as significantly lower. Carryover were not observed, whereas between and within-run imprecisions were < 10%.
Conclusions: Our data shows that the mass spectrometric techniques can be easily used for the screening of lysosomal storage diseases which presents remarkable technical advantages compared with traditional methods. The screening for several LSDs simultaneously is appropriate for use in high-throughput screening laboratories.

P-467

Effect of high doses of enzyme replacement therapy by systemic infusion on the central nervous system defects in a mouse model of mucopolysaccharidosis type 2

Kim J[1], Yang A[1], Huh R[1], Cho S Y[1], Lee J[1], Ko A R[2], Kim C[3], Chung Y K[4 5], Kim S J[6], Sohn Y B[7], Park S W[8], Jin D K[1]

[1]Samsung Medical Center, Seoul, Korea, Republic of, [2]CRC, Samsung Biomedical Research Ins, Seoul, Korea, Republic of, [3]Rare Disease Team, MOGAM Biotech Ins, Yongin, Korea, Republic of, [4]Biopress Eng, CRC, Green Cross Cor, Yongin, Korea, Republic of, [5]Dep of molec sci and tech, Ajou univ, Suwon, Korea, Republic of, [6]Dep of ped, Myungji hos, Goyang, Korea, Republic of, [7]Dep of Medical Genetics, Ajou Univ, Suwon, Korea, Republic of, [8]Dep of Ped, Kwandong Univ, Cheil hosp, Seoul, Korea, Republic of

Background and objective: Despite the availability of intravenous enzyme replacement therapy (ERT) in Mucopolysaccharidosis type II, the improvement of central nervous system defects is limited. This study was performed to investigate brain responses to the systemic infusion of high-dose IDS in KO mice.
Method: ERT was performed using three doses (1, 5, and 10 mg/kg weekly) of IDS for different durations (1, 3 and 6 months) in KO mice of two age groups (2, 8 months). GAG

measurement in tissues, brain pathology, and behavioral assessment were analyzed.
Result: The GAG level and histopathology of brains improved in a dose-dependent manner, particularly with a high-dose, prolonged infusion of IDS in young mice only. The spontaneous alternation behavior was recovered in young mice treated with 5 mg/kg or higher IDS; however, no significant improvement was observed in old mice
Conclusion: These results suggest that high-dose ERT given to mice of earlier ages may play a role in preventing GAG accumulation and preventing CNS damage in IDS KO mice. Therefore, ERT above the standard dose, starting in early childhood, could be a promising treatment regimen for reducing neurological impairment in Hunter syndrome

P-468

MPS II – patient's profile - objective evaluation of the body stature in patient who started idursulfase treatment presymptomatically at the age of 3 months

Różdżyńska-Świątkowska A[2], Tylki-Szymanska A[1 3]

[1]The Children's Memorial Health Institute, Warsaw, Poland, [2]Ant Lab, Child Mem Health Inst, Warsaw, Poland, [3]DeptPedNutr&MetabDis,ChildMemHealth Inst, Warsaw, Poland

Background: Mucopolysaccharidosis type II (Hunter syndrome) is an X-linked, recessive, lysosomal storage disorder caused by a deficiency of iduronate-2-sulfatase (I2S). Deficient enzyme activity leads to widespread accumulation of the glycosoaminoglycans (GAG) dermatan and heparan sulfate.
Objective: To show change in proportion of body stature and head shape in patient with MPS II who started enzyme replacement therapy (ERT) presymptomatically at the age of 3 months.
Material and methods: The analysis of somatometric and craniofacial features was performed regularly for the first 8 years of treatment. Individual anthropometric data were standardized in order to show the degree and direction of deviations. The patient grew faster than normal during eight years of life. At the 3 months of age head circumference was between 75 and 97 centiles. This tendency held up during the time of observation. From 18 months until 7 years of age larger values of chest depth was observed. In analysis performed at the age of 8 the patient showed no body disproportion characteristic for MPS II. Disproportion remained only in the shape of head.
Conclusions: Normal body proportions may suggest a beneficial effect of early introduction of ERT.

P-469

Molecular analysis of 22 patients with mucopolysaccharidosis IVA from Poland, Belarus and Kazakhstan identifies 6 novel *GALNS* mutations

Tylki-Szymanska A[5], Różdżyńska-Świątkowska A[1], Marucha J[5], Kulpanowicz A[2], Tulebayeva A[3], Jurecka A[4]

[1]Ant Lab, Child Mem Health Inst, Warsaw, Poland, [2]Genet Unit, Nat Med Cent "Mother...", Minsk, Belarus, [3]ScCentPedChildSurg,SD.Asf.Kazakh Nat Med, Almaty, Kazakhstan, [4]Dept Gen, Univ Gdansk, Gdansk, Poland, [5]DeptPedNutr&MetabDis,ChildMemHealth Inst, Warsaw, Poland

Background and objectives: Mucopolysaccharidosis type IVA (MPS IVA, Morquio syndrome) is an autosomal recessive lysosomal disorder caused by severe deficiency of galactosamine-6-sulfate sulfatase (GALNS), encoded by the *GALNS* gene. Patients who carry two mutated alleles of the *GALNS* gene have a decreased ability to catabolise keratan and chondroitin 6-sulfates, which results in their accumulation and leads to connective tissue impairment and consequently to multiorgan clinical outcome. We aimed to analyse the spectrum of mutations in the *GALNS* gene responsible for the disorder in Poland, Belarus and Kazakhstan.
Patients and methods: Twenty two patients with MPS IVA, in whom diagnosis was confirmed biochemically and enzymatically, were studied.
Results: In total, fifteen different disease-causing mutations were identified. Six novel mutations included c.680delT, p.D183Y, p.A321G, IVS13+5G>A, c.121-9T>G, and p.Q476P. No mutations were identified on 9/44 alleles. Eight patients were homozygous and had either related parents or parents from neighbouring villages. All patients, except one (homozygous for p.R529Q) presented with a severe phenotype.
Conclusion: Genotypic data in our multi-ethnic study group, did not allow for prediction of disease severity (phenotype). The analysis failed to identify mutations on 21% of alleles suggesting that the only reliable diagnostic method for Morquio A remains enzymatic testing.

P-470

Severe tracheal collapse in mucopolysaccharidosis type 2

Langeveld M[1], Rutten M[2], Ciet P[3], Van den Biggelaar R[4], Oussoren E[1], Langendonk J G[1], Van der Ploeg A T[1]

[1]Center for Lysosomal and Metab Diseases, Rotterdam, Netherlands, [2]Depart Pulmonology, Erasmus MC, Rotterdam, Netherlands, [3]Depart Radiol, Erasmus MC, Rotterdam, Netherlands, [4]Depart Int Care Med, Erasmus MC, Rotterdam, Netherlands

Purpose: In mucopolysaccharidosis type 2 patients dyspnoea is a frequent symptom of incompletely understood aetiology. In this study we describe the occurrence of changes in tracheal diameter between in- and expiration.
Methods: Five adult MPS2 patients (mean age 40 years) were included. Repeated pulmonary function tests were obtained as well as inspiratory and expiratory chest CT scans. The cross-sectional area of trachea and main bronchi was measured at end-inspiration and -expiration and % collapse was calculated.
Results: There was a diffuse narrowing of the entire intra-thoracic trachea and the main bronchi and severe expiratory collapse of the trachea in all patients. At 1 cm above the aortic arch the median % collapse of the trachea was 68% (range 60 to 77%) and at the level of the aortic arch 64% (21-93%). The collapse of the main bronchi was 58% (26-66%) on the left and 44% (9-76%) on the right side. Pulmonary function tests showed obstructive airway disease in all but one patient (FEV1 ranged from 18-62%).
Conclusion: In adult MPS 2 patients central airway calibre is strikingly reduced and upon expiration there is a severe collapse of the trachea and main bronchi explaining the severe respiratory symptoms in MPS2 patients.

P-471

Impact of mucopolysaccharidosis (MPS) on daily living, employment, general health and parenthood of adult patients

Lavery C[1], Wedehase B[2], Harmatz P[3], Hendriksz C[4]

[1]Society for Mucopolysaccharide Diseases, Amersham, United Kingdom, [2]National MPS Society, Durham, NC, United States, [3]UCSF Benioff Child Hosp, Oakland, CA, United States, [4]Salford Royal Hospital NHS Trust, Salford, United Kingdom

Background & Objectives: The impact of MPS on adult patients in the UK, Canada, and USA was evaluated in an online survey developed by MPS societies, with support from expert physicians, BioMarin Pharmaceutical Inc., and Ismar Healthcare.
Methods: 41 survey questions covered topics including living arrangements, employment, education, general health/well-being, and health-related quality of life (derived from the EQ-5D-5L questionnaire).
Results: 81 patients completed the survey; most (55.6%) lived with their parents. 31 patients (38.3%) were employed, mostly outside the home (87.1%); 39.5% were unemployed, mostly due to MPS (87.5%); and 22.2% were studying. 42.5% of patients perceived it as more difficult for them than for peers to maintain friendships/personal relationships. Mobility issues (76.0%) and pain (59.5%) were the most important aspects of MPS impacting ability to socialize; 48.7% indicated that anger or frustration affected them emotionally and psychologically more than peers, but independence was perceived as less important for them than for peers (39.7%). The EQ-5D-5L responses showed moderate-severe problems with mobility in 69.3% and moderate-severe pain/discomfort and anxiety/depression in 66.7% and 30.7%, respectively.
Conclusion: MPS has a great impact on daily living, employment, general health and well-being, parenthood, and (in particular mobility- and pain-related) HRQoL of adult patients.
Conflict of Interest declared.

P-472

Correlation between phenotype and genotype in 81 Japanese patients with mucopolysaccaridosis type II

Kosuga M[1], Kumagai T[2], Fuji N[2], Hirakiyama A[2], Igarashi H[2], Mashima R[2], Nikaido M[3], Okuyama T[2]

[1]Div Clin Gene, Nat Cent Child Health Dev, Tokyo, Japan, [2]Dept Clin Lab Med,NCCHD, Tokyo, Japan, [3]BioBank,Nat Cent Child Health Dev, Tokyo, Japan

Background: Mucopolysaccaridosis type II is an X linked recessive lysosomal storage disorder caused by deficiency of iduronate-2-sulfatase encoded by the *IDS* gene.
Methods: We analyzed the *IDS* gene mutations of 81 individuals with MPS II patients and carriers.
Results:The type of identified mutations were as follows: 37 missense mutations, 8 nonsense mutations, 11 frame shift mutations, 4 intronic changes affecting splicing, 10 recombinations and 11 others. We classified these mutations into three clinical phenotypes, attenuated type, severe type and unknown type according to intellectual and cognitive status. 83% of individuals with attenuated type had missense mutations. On the other

hand, 28% with severe type had missense mutations. Other individuals with severe type had various mutations including splicing variants, nonsense mutations, frame shift mutations and *IDS-IDS2* recombination. There were a few common mutations of *IDS* gene in Japanese MPSII families. Each family had unique individual mutations. 23 novel mutations were identified in this study. Many of them in severe phenotype had frame shift, recombination, deletion and nonsense mutations. More than 80% of individuals with attenuated type had missense mutations.
Conclusion: These results suggest that *IDS* gene analysis may be useful for predicting clinical phenotypes in Japanese MPS II patients.

P-473

Disorder in the house: actin level decrease in leukocytes of patients with Hunter syndrome

Magner M[1], Kulhanek J[1], Ujcikova H[2], Poupetova H[3], Zeman J[1], Svandova I[2]

[1]Dep Pediatr, 1st Facult Med, Charles Uni, Prague, Czech Republic, [2]Dep Physiol, Facult Science, Charles Uni, Prague, Czech Republic, [3]Inst IMD, 1st Facult Med, Charles Uni, Prague, Czech Republic

Background and objectives: Mucopolysaccharidosis II (MPS II) is a rare X-linked lysosomal storage disorder, characterised with an excessive storage of glycosaminoglycans and alterations in composition of extracellular matrix (ECM) due to a deficiency of iduronate-2-sulfatase. The physical cross-linking of ECM molecules affects the condition of the actin cytoskeleton. Actin level changes in some diseases linked to the loss of the ECM proper structure. Thus, we focused on actin level in MPS II.
Patients and Methods: Whole blood samples of three male patients with MPS II and their age- and sex-related controls were collected, primary leukocytes were separated and postnuclear supernatant was prepared. Proteins of supernatant were fractionated by 10% SDS-PAGE and actin level measured by Western blot using goat polyclonal IgG actin antibody (I-19, sc-1616).
Results: Actin was detected on 43 kDa. The Patient 1 actin level reached 63.80% of his pair-matched control ($p< 0.001$), Patient 2 actin level 78.50% ($p=0.008$), and Patient 3 actin level 84.95% ($p=0.04$), respectively.
Conclusion: This study represents the first work investigating actin levels in leukocytes of MPS II patients. We showed significant decrease of actin level in three MPS II patients. This work was supported by grants RVO-VFN 64165/2012 and IGA MZ NT14015-3/2013.

P-474

Oxidative DNA damage in mucopolysaccharidosis type IVA patients treated with enzyme replacement therapy

Donida B[1 2], Marchetti D P[1 2], Manini P R[4], Da Rosa H T[4], Moura D J[4], Saffi J[4], Deon M[1], Giugliani R[1 3], Vargas C R[1 2]

[1]HCPA/Medical Genetics Service, Porto Alegre, Brazil, [2]UFRGS/PPGBioq, Porto Alegre, Brazil, [3]UFRGS/PPGBM, Porto Alegre, Brazil, [4]UFCSPA/LabGentox, Porto Alegre, Brazil

Background: Mucopolysaccharidosis type IVA (MPS IVA) is an inborn error of glycosaminoglycans catabolism due to deficient activity of N-acetylgalactosamine-6-sulfatase that leads to keratan sulfate and chondroitin-6-sulfate accumulation in body fluids and lysosomes. The pathophysiology of this lysosomal storage disorder is not completely understood. The aim of this study was to investigate oxidative DNA damage in MPS IVA patients on enzyme replacement therapy (ERT).
Methods: We analyzed urine and blood samples of MPS IVA patients on ERT (n=17) and healthy age-matched controls (n=10-15). Basal DNA damage was investigated by comet assay and we assessed DNA repair using endonuclease III enzyme which recognizes oxidized pyrimidines bases and converts them into breaks reflected in comet tail. We also determined the urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels, a product of DNA oxidative damage due to the hydroxyl radical attack at the C8 position of deoxyguanosine.
Results: Our results showed higher basal DNA damage levels in MPS IVA patients when compared to control group and this damage has an oxidative origin in purines and pyrimidines bases.
Conclusion: The data presented showed experimental evidence that oxidative DNA damage occur in cells from MPS IVA patients even on ERT.
Financial support: CNPq, CAPES, FIPE/HCPA.

P-475

Natural history of Mucopolysaccharidosis type III (Sanfilippo disease) in United Arab Emirates

Al-Jasmi F[1]

[1]United Arab Emirates University, Abu Dhabi, United Arab Emirates

Background: Mucopolysaccharidosis type III (MPS III) is caused by deficiency in enzymes involved in the lysosomal degradation of heparan sulphate.
Methods: A retrospective analysis of medical records of 9 patients with MPS III (4 males, 5 females; age range, 3-17 years) seen at Tawam hospital from 2010-2015.
Results A total of 9 children, MPS IIIB (67%) and MPS IIIC (33%). Mean ages of onset of symptoms and confirmed diagnosis were 2.5 and 10.6 years, respectively. The most prevalent clinical manifestations were mental retardation, hyperactive and aggressiveness, coarse facies, and hepatosplenomegaly (100%), recurrent URTI and recurrent otitis media (90%), sleep problem (78%), heart disease (77%), abnormal gait (55%), hearing loss and diarrhea (44%), feeding difficulties (33%), seizure (11%), and Eight patients (88%) experienced at least one surgical procedure with the most common being ear tube insertion and adenoidectomy(44%), dental rehabilitation(33%), inguinal hernia repair (22%). Molecular analysis of our cohort for MPS IIIB for *NAGLU* gene reveals (4 Emirati patients had (c.1694 G>T/ c.1694 G>T) and 2 Syrian siblings had c.889C>T/ c.889C>T). MPS IIIC for *HGSNAT* gene (2 Emirati siblings had c.1348delG/ c.1348delG and 1 Emirati with c.1600 A>G/ c.1600 A>G)
Conclusion: MPS III in UAE present with CNS and systemic manifestations.
Conflict of Interest declared.

P-476

AAV-GNPTAB gene delivery attenuates bone loss in the GNPTAB knock out mouse model of mucolipidosis II

Sohn Y B[1], Ko A R[2], Kim J S[3], Jin D K[4]

[1]Dept Med Genet, Ajou Univ Hosp, Suwon, Korea, Republic of, [2]Samsung Biomedical Research Center, Seoul, Korea, Republic of, [3]Dept Pathol, Samsung Medical Center, Seoul, Korea, Republic of, [4]Dept Pediatrics, Samsung Medical Center, Seoul, Korea, Republic of

Background: Mucolipidosis-II (ML-II, I-cell disease) is an autosomal recessive lysosomal storage disease characterized by a deficiency of UDP-GlcNAc-1-phosphotransferase. To date, *GNPTAB* is the only gene in which mutations are known to cause ML II. Here, we envisaged that a gene therapy approach would provide a new therapeutic method and that this could provide an effective treatment for ML-II.
Methods: We have generated *GNPTAB* knock out mouse and analyzed clinical and biochemical manifestations. The gene therapy using associated virus-*GNPTAB* plasmid (AAV-*GNPTAB*) was performed via tail vein injection. At 16 and 32 weeks after gene transfer, the bone mineral density and pathology were analyzed. And IL-6 expression was also analyzed.
Results: The KO mice resembled the human pathology of ML-II including dwarfism and facial dysmorphism. We observed that the gene transfer inhibited the bone loss and lean mass in KO mice by decrease of IL-6 production.
Conclusion: Our study demonstrated that AAV-mediated GNPTAB gene transfer can delay bone loss in ML-II. And gene therapy will highlight the hope for a novel treatment approach to ML II in which no therapeutic modality is available.

P-477

Age-dependent mitochondrial dysfunction in brain of mucopolysaccharidosis type III C mouse model

Hansikova H[1], Hulkova H[2], Martins C[3], Tesarova M[1], Svobodova E[2], Langer J[1], Hrebicek M[2], Pshezhetsky A V[3], Zeman J[1]

[1]Dep Pediatr, Charles Univ, Gen Univ Hosp, Prague, Czech Republic, [2]Inst Inher Metab Dis, Charles Univ, Prague, Czech Republic, [3]CHU Ste-Justine, Univ of Montreal, Montreal, Canada

Mucopolysaccharidosis type III C (MPS IIIC) is a progressive neuro-metabolic disease caused by mutations in the *HGSNAT* gene leading to deficiency of acetyl-CoA: a-glucosaminide N-acetyltransferase involved in the lysosomal catabolism of heparan sulphate. To better understand the pathophysiology of the disease, we analysed mitochondrial function and ultrastructure in brain of MPS IIIC mouse model (Hgsnat-Geo) at the age between 4 and 12 months. Age-dependent changes of activity and amount of respiratory chain complexes I,II,III and IV, pyruvate dehydrogenase, citrate synthase (CS) and total coenzyme Q10 were analysed in isolated brain mitochondria. Activities of complex IV and complex II were significantly lower in brains of Hgsnat-Geo mice than in the corresponding wild-type controls at the ages of 8 and 12 months and 8 months, respectively. Complex II, II+III, CS activity and coenzyme Q10 content in brain of Hgsnat-Geo mice decreased significantly between the ages of 8 and 12 months in comparison to age-related wild-type animals. Electron microscopy showed enlarged, structurally abnormal mitochondria in brain neurons since the age of 5-months in Hgsnat-Geo mice in comparison to aged matched controls.

Conclusion: Age-dependent mitochondrial dysfunction in neurons contribute to the explanation why MPS IIIC manifests primarily as a neurodegenerative disease. *Supported*: *RVO-VFN64165*, *PRVOUK P24/LF1/3*

P-478

Efficacy of early enzyme replacement therapy in severe Morquio A disease: a case report

Do Cao J[1], Quintaux T[1], Mainard L[3], Froissart R[2], Journeau P[4], Feillet F[1]

[1]Ref Cent for IEM, Univ Child Hosp, Nancy, France, [2]Bioch and Molec Biol, Univ Hosp, Lyon, France, [3]Radiol Dpt, Child Univ Hosp, Nancy, France, [4]Orthop surg, Child Univ Hosp, Nancy, France

Background: Patients under 5 years where not evaluated in a phase-3 study for enzyme replacement therapy (ERT) in Morquio A and there is no data about the efficacy of ERT in patients less than two years old.
Methods: We report the case of a patient with Morquio A diagnosed at 19 months of age because of thoraco-lumbar kyphosis and pectum carinatum. Patient showed growth retardation and dysostosis, increase of urinary glycosaminoglycans, and null activity of NAGS. He is homozygous for the severe mutation: c.871G>A. Elosulfase alfa treatment was initiated at 21 months.
Results: At 16 months follow-up, urinary GAGs normalized. We observed a stop in growth retardation (which remains at -1SD). Inferior and superior limbs did not worsen, but x-rays showed a progressive flattening of the femoral heads. Kyphosis remained. Medullar compression requiring surgery developed at 34 months. Neuropsychological evaluation showed normal cognitive development. We observed adverse events of ERT such as vomiting, chills or fever which required corticotherapy before infusions for 8 months.
Conclusion: ERT seems to improve the height prognosis of this early treated patient with Morquio A. Further follow-up is required to evaluate the long-term effect of early ERT in Morquio A.
Conflict of Interest declared.

P-479

Two Cases with Mucopolysaccharidosis Type VII (Sly's Syndrome)

Sivri S[1], Pektas E[1], Yildiz Y[1], Dursun A[1], Tokatli A[1], Coskun T[1]

[1]Div Metab Dis, Hacettepe Univ Child Hosp, Ankara, Turkey

Background: Mucopolysaccharidosis type-VII (MPS-VII) is caused by deficiency of β-glucuronidase. We present two female patients with this rare disorder.
Patient A was hospitalized postnatally due to edema and pericardial effusion detected by prenatal ultrasonography. She had coarse facies and a short neck. Peripheral smear showed granulated leukocytes. Urinary glycosaminoglycans (GAG) were high and leukocyte β-glucuronidase activity was low, establishing the diagnosis of MPS-VII. She was lost to follow-up until referral to an orthopedics clinic with walking difficulties 10 years later. She had tonsillar hypertrophy, hearing loss, shallow acetabulae, valvular insufficiencies, and mild intellectual disability. She was recently enrolled in an enzyme replacement therapy trial.
Patient B presented to the gastroenterology department at 2 months of age with jaundice and hepatosplenomegaly. Bone marrow examination revealed granulated cells. Liver biopsy showed intracellular cholestasis. She was lost to follow-up until she was 8 years old, complaining of limited neck movements. She now had coarse facies. GAG electrophoresis suggested MPS-VII, confirmed by profoundly low β-glucuronidase activity. She is now 22 years old, has kyphoscoliosis, hepatomegaly, valvular insufficiencies and mild intellectual disability.
Conclusion: MPS-VII patients usually exhibit milder phenotypes than other types of MPS. These cases underline the possibility of MPS-VII in the differential diagnosis of various systemic findings.

P-480

Characteristics of patients with mucopolysaccharidosis type II identified at a very young age: data from the Hunter Outcome Survey (HOS)

Burton B K[1], Ficicioglu C[2], Bosch J[3], Morin I[4], Jurecka A[5], Tylki-Szymanska A[6]

[1]Div Genet, Lurie Child Hosp, Chicago, United States, [2]Child Hosp Philadelphia, Philadelphia, United States, [3]Iowa Child Hosp, Iowa City, United States, [4]Cytel, Inc, Geneva, Switzerland, [5]Shire, Lexington, United States, [6]Child Mem Health Inst, Warsaw, Poland

Background and objectives: Despite the fact that mucopolysaccharidosis II (MPS II, Hunter syndrome) starts prenatally, affected individuals usually appear normal at birth and therefore early diagnosis remains

challenging. This analysis aimed to determine characteristics of patients identified and treated with idursulfase at a very young age.
Patients and methods: Patients (n=31), who had started idursulfase therapy ≤18 months of age, were analyzed (09/2014) in HOS (a Shire-sponsored, global, observational registry).
Results: 4.5% of treated patients enrolled in HOS started idursulfase treatment before 18 months of age. Family history was negative in 27.6% of patients. The median (10-90th percentiles)age at onset of symptoms was 0.6 years (0.0-1.1) and main presentations included hernia, respiratory infections, coarse facial features, and hepatosplenomegaly.The median age of diagnosis was 0.6 years (0.0-1.3) and the maximum delay of diagnosis was 9.6 months. Although the disease was suspected primarily by geneticists, paediatricians, and metabolic specialists, some patients were diagnosed by pulmonologists, ENT and orthopaedic surgeons. The median age at treatment initiation was 0.8 years (0.1–1.4).
Conclusion: Although in majority of cases, a positive family history played a critical role in early diagnosis, almost 30% of patients were diagnosed based on a clinical suspicion.
Conflict of Interest declared.

P-481 – Moved to A-099

22. Lysosomal disorders: mucopolysaccharidoses, oligosaccharidoses

P-482

Quantitative analysis by UPLC-MS/MS of dermatan and heparan sulfate in urine of mucopolysaccharidosis patients

Ruijter G J[1], Harquouli B[1], Agirbas E[1], Oussoren E[2], Langereis E J[3], Van Vlies N[3], Wijburg F A[3], Van der Ploeg A T[2], Van den Bosch J C[1]

[1]Dep Clin Genet, Erasmus Med Center, Rotterdam, Netherlands, [2]Dep Pediatr, Erasmus Med Center, Rotterdam, Netherlands, [3]Dep Pediatr Met Dis, Acad Med Center, Amsterdam, Netherlands

Background: Common methods to screen for mucopolysaccharidoses (MPS) in urine are quantitative GAG tests (e.g. dimethylmethyleneblue) and GAG electrophoresis. These methods are hampered by limited specificity and demanding interpretation. Novel methods to analyse GAG have been proposed, using enzymatic or chemical hydrolysis of GAG and subsequent quantification of resulting disaccharides by LC-MS/MS. We propose a modified LC-MS/MS approach with excellent sensitivity and specificity.
Methods: GAG was precipitated from 125 μL urine using ethanol to remove interfering compounds. GAG was then degraded by methanolysis and, following addition of deuterated internal standards, analysed by UPLC-MS/MS.
Results: Extracted ion chromatograms improved substantially by a simple ethanol precipitation step. Response curves were linear up to 1000 mg/L dermatan sulfate and 4000 mg/L heparin sulfate. Recoveries were 101-112% and variation coefficients 8-18%. All MPS patients tested (n=49; types I, II, III and VI) were easily differentiated from normal controls (n=78). Six out of 11 samples, with a false-positive DMB test and doubtful electrophoresis results, had normal DS and HS levels in the LC-MS/MS test.
Conclusion: This modified method for GAG analysis is robust, requires only 125 μL urine and is suitable to diagnose MPS patients and to monitor GAG as a biomarker during therapies.

P-483

Ten years of the Hunter Outcome Survey (HOS): a decade of improving our understanding of Hunter syndrome

Muenzer J[1], Giugliani R[2], Scarpa M[3 4], Tanaka A[5], Tylki-Szymanska A[6], Beck M[7], Paabøl Larsen M[8], Morin I[9], Whiteman D[8]

[1]Dept Ped, Univ NC, Chapel Hill, United States, [2]Med Genet Service/HCPA, Porto Alegra, Brazil, [3]Rare Dis Centre, HSK, Wiesbaden, Germany, [4]Dept Ped, Univ Padova, Padova, Italy, [5]Dept Ped, Osaka City Univ Grad Schl Med, Osaka, Japan, [6]Child Mem Health Inst, Warsaw, Poland, [7]Univ Med Center, Johannes Gutenberg Univ, Mainz, Germany, [8]Shire, Lexington, United States, [9]Cytel, Inc., Geneva, Switzerland

Background and Objectives: Hunter syndrome (mucopolysaccharidosis type II [MPS II]) is a rare, X-linked disorder caused by deficiency of iduronate-2-sulfatase. The Hunter Outcome Survey (HOS; a Shire-sponsored, global, observational registry initiated in 2005) collects real-world, long-term data on the natural history of this disease and the safety of idursulfase treatment.

Patients and Methods: Individuals with a confirmed diagnosis of Hunter syndrome are eligible for enrolment. Prospective clinical data are collected during routine visits, according to local standard practice; historical data may also be recorded.
Results: More than 1000 patients have been enrolled at 118 clinics in 26 countries, with 81% (715/886) of prospective patients having received idursulfase treatment (January 2015 data; median age at treatment start, 7.4 years). The data collected have contributed to understanding the onset and prevalence of clinical manifestations (including cardiac involvement and hearing loss), growth patterns, and identification of characteristic surgical histories (including hernia repair and ENT procedures). Important insights have also been gained about the tolerability of idursulfase treatment in different age groups.
Conclusions: As the only registry for Hunter syndrome, HOS is a unique source of valuable data that facilitates wider goals of informing physicians, improving clinical outcomes and quality of life for patients.
Conflict of Interest declared.

P-484

Preserving hand function in mucopolysaccaridosis type 1: a systematic review

Schrijver W A M[2], Van der Beek E S J[2], Mink van der Molen A B[2], Van Hasselt P M[1]

[1]Dept Met Dis, Wilhelmina Child Hosp,UMCU, Utrecht, Netherlands, [2]Dept Plast Surg, UMCU, Utrecht, Netherlands

Background: Mucopolysaccharidosis type I (MPS-I) is a lysosomal storage disorder with accumulation of glycosaminoglycans (GAGs) in various tissues. The accompanying hand problems are not yet fully elucidated.
Methods: Embase, Cochrane and Pubmed databases were searched to identify all studies describing hand abnormalities in MPS-I.
Results: Accumulation of GAGs in fibroblasts, collagen fibers, chondrocytes, osteocytes and periosteal cells can induce joint stiffness and contractures, carpal tunnel syndrome (CTS) and trigger fingers (TVS). Together with the skeletal deformities this leads to reduced hand function. Neither ERT nor HSCT appears to be able to fully prevent GAG accumulation and its consequences. The clinical presentation of both CTS and TVS differs strikingly from other causes, reflecting the unique underlying pathophysiology. Importantly, CTS cannot be reliably recognized based on clinical symptoms. Delayed recognition may cause irreversible loss of hand function, stressing the need for reliable diagnostic tools.
Conclusions: Standard evaluation of hand function in MPS-I is important to preserve hand function and should focus on early symptoms of CTS and TVS. As CTS may be symptom free, evaluation should include EMG analysis or ultrasonography. Timely surgery is well tolerated and could lead to improved hand use, range of motion and function.

P-485

Beta-mannosidosis is a rare cause of hypomyelination

Renaud D L[1]

[1]Mayo Clinic, Rochester, MN, United States

Background: Beta-mannosidosis is a rare autosomal recessive disorder due to a deficiency of lysosomal beta-mannosidase associated with mutations in MANBA. Significant clinical variability has been noted in the small number of described cases. Naturally occurring goats with beta-mannosidosis demonstrate a paucity of myelin. The MRI findings in patients with beta-mannosidosis have not been well-described.
Case report: A 4 year 3 month old boy presented to the Neurometabolic Clinic for evaluation of global developmental delay and hypotonia. His hearing and eye examination were normal. His general examination was significant for growth parameters at the 95th percentile, epicanthal folds and absence of hepatosplenomegaly. On neurological examination, he had mild hypotonia with hyperreflexia and clonus in the lower extremities. His gait was stiff and immature and his speech was difficult to understand. MRI of the brain revealed near absence of myelination in the deep and subcortical white matter of the brain with no interval myelination since an MRI performed three years earlier. These findings are suggestive of hypomyelination. Metabolic evaluation revealed significantly decreased but not absent beta-mannosidase activity. Sequence analysis of *MANBA* revealed one previously reported mutation (c.563_572 dup) and one novel mutation (c.1449 G>A).
Conclusion: Beta-mannosidosis is a rare cause of hypomyelination.

P-486

Every other week enzyme replacement therapy in mucopolysaccharidosis type I: efficacy of alternative double-dose regimen in 17 patients

Horovitz D D G[1], Acosta A X[5], Martins A M[2], Souza C F M[3], Esposito A C[1], Barth A L[1], Kyosen S O[2], Giugliani R[3], Araujo E K E[5], Cardoso Jr L[5], Burin M G[3], D'Almeida V[2], Scalco F[4], Oliveira M L[4]

[1]Instituto Fernandes Figueira - Fiocruz, Rio de Janeiro, Brazil, [2]CREIM - Univ Federal de São Paulo, São Paulo, Brazil, [3]Serviço de Genetica Medica, HCPA, Porto Alegre, Brazil, [4]LABEIM - UFRJ, Rio de Janeiro, Brazil, [5]Serviço de Genetica Medica, UnivFedBahia, Salvador, Brazil

Objectives: To evaluate safety and efficacy of an alternative regimen for enzyme replacement therapy (ERT) in mucopolysaccharidosis-I (MPS-I) of 1.2 mg/kg laronidase every 2 weeks versus the 0.58 mg/kg weekly dose in patients followed in four Brazilian centers.
Methods: Seventeen patients [10F/7M; Hurler (4), Hurler-Scheie (9), Scheie (4)], ages 4-25 years, had their dosing regimen changed to 1.2 mg/kg every 2 weeks. All had been on weekly ERT for over one year, responding well and without adverse events. The recommended follow-up protocol for MPS-I was performed, and urinary glycosaminoglycan (GAG) levels were monitored.
Results: Patients have been on every other week regimen for 1-7 years (mean 3.6 yrs). Urinary GAG excretion is following the same pattern as prior to regimen change in all. No significant infusion-related events were reported; no acceleration of disease progression was documented.
Conclusions: The every other week double-dose regimen did not change the disease progression or the urinary GAG excretion. All families were satisfied with such alternative, especially due to less school/work days missed and fewer venepunctures. Considering lifelong therapy and that interval between doses may change patients' quality of life, we believe this alternative regimen must be considered, with strict clinical and laboratory follow-up. More dose optimization studies should be encouraged.
Conflict of Interest declared.

P-487

Histological and mechanical characterisation of growth plates in a MPS VI rat model

Guevara J M[1], Frohbergh M[2], Garzon-Alvarado D A[3], Barrera L A[1], Schuchman E[2], Simonaro C[2]

[1]Inst. Inborn Errors Metab - PUJ, Bogota, Colombia, [2]Genet Genom Dept - Mount Sinai Sch Med, New York, United States, [3]Org & Tiss Mech Group - Univ Nac Col, Bogota, Colombia

Background/Objectives: In the mucopolysaccharidoses (MPS), skeletal dysplasia is characterized by changes in bone length and shape resulting from alterations in growth plate (GP) function. We hypothesize that such alterations are related to an abnormal mechanical response within GP due to glycosaminoglycan accumulation within the GP extracellular matrix. Therefore, our aim is to characterize the GP in the MPS-VI rat model histologically and its mechanical properties.
Materials and Methods: Distal femurs from MPS-VI rats at three different developmental stages (birth, 1 and 3 months of age) were examined both histologically and using scanning electron microscopy. Young modulus was determined for newborn samples by compressive testing.
Results: At birth, MPS-VI rat GPs displayed normal histological characteristics. At 1 month changes in cell size and zonal distribution became evident, and progressed to complete loss of columnar arrangement by 3 months of age. Mechanical evaluation of young animals showed a decreasing trend when compared to normal.
Discussion/Conclusion: Although the structural alterations within MPS-VI GPs develop post-natally, the mechanical properties of the tissue are abnormal at birth. These studies will help to understand the pathophysiology of the MPS GP and the biomechanical alterations that may contribute to the development of bone abnormalities in these disorders.

P-488

Cardiac features and effects of enzyme replacement therapy for 28 patients with mucopolysaccharidosis I, II, IVA and VI

Lin H Y[1 2 3 4 6], Lin S P[1 2 3 4], Chuang C K[2], Chen M R[1 3 4], Lin S M[1 3 4], Hung C L[2 3 6 7], Chiu P C[8], Tsai W H[9], Niu D M[5 6]

[1]Dep Pediatrics, Mackay Memorial Hospital, Taipei, Taiwan, [2]Dep Med Res, Mackay Memorial Hospital, Taipei, Taiwan, [3]Dep Medicine, Mackay Medical College, New Taipei City, Taiwan, [4]Mackay Jun Col of Med, Nurs Management, Taipei, Taiwan, [5]Dep Ped, Taipei Veterans General Hosp, Taipei, Taiwan, [6]Inst Clin Med, National Yang-Ming Univ, Taipei, Taiwan, [7]Div of Cardiol, Mackay Memorial Hospital, Taipei, Taiwan, [8]Dep Ped, Kaohsiung Veterans General Hosp, Kaohsiung, Taiwan, [9]Dep Ped, Chi Mei Medical Center, Tainan, Taiwan

Background: Cardiac abnormalities have been observed in patients with mucopolysaccharidoses (MPS) of any type.
Methods: We reviewed medical records, echocardiograms, and electrocardiograms of 28 Taiwanese MPS patients (19 males and 9 females; age range, 0.7 to 34 years; 9 with MPS I, 7 with MPS II, 7 with MPS IVA, and 5 with MPS VI) treated with enzyme replacement therapy (ERT) for 0.4 to 10.8 years.
Results: At start of ERT, z scores > 2 were identified in 46%, 75%, and 29% for left ventricular mass index (LVMI), interventricular septum diameter in diastole (IVSd), and left ventricular posterior wall diameter in diastole (LVPWd) in these patients, respectively. Twenty-four patients (86%) had valvular heart disease. After ERT, the IVSd z score of all patients decreased significantly from 3.87 to 2.57 (p=0.016). Interestingly, for 11 patients starting ERT before 12 years of age, the z scores for both LVMI and IVSd decreased significantly (p< 0.01) after ERT. However, the condition of valve regurgitation or stenosis didn't show improvement despite ERT.
Conclusions: Although ERT apparently had little impact on valvular heart disease, it appears to be effective in reducing intraventricular septal hypertrophy, and may have better results when started before 12 years of age.

P-489

Sulfated disaccharide from heparin are chaperone candidate for treatment of mucopolysaccharidosis type II

Hoshina H[1 2], Shimada Y[2], Higuchi T[2], Kobayashi H[1 2], Eto Y[3], Ida H[1 2], Ohashi T[1 2]

[1]Dep Ped, Jikei Univ, tokyo, Japan, [2]Div Gen Ther, Jikei Univ, tokyo, Japan, [3]Adv Clin Res Cent, Ins Neuro Dis, kanagawa, Japan

Background: Mucopolysaccharidosis type II (MPS II) is an inherited metabolic lysosomal storage disorder caused by a deficiency of iduronate-2-sulfatase (IDS). In recent years, pharmacological chaperone therapy has been investigated as a potential treatment for lysosomal storage disorders such as Fabry disease and Pompe disease. However, candidate chemical of chaperone for treatment of MPS II has not been developed yet.
Methods: In this study, we focused on the degradation products of heparin, and analyzed the utility of the oligosaccharides as chemical chaperone for MPS II. When sulfated disaccharides from heparin, digested with heparin lyases, were incubated with recombinant human IDS (rhIDS), sulfate groups were cleaved from a part of the oligosaccharides. Addition of sulfated disaccharides to rhIDS solution significantly prevented the thermal degeneration of IDS. Stabilization of rhIDS by treatment of sulfated disaccharides was dependent on the concentration of the saccharides. Furthermore, sulfated disaccharides increased the IDS activity in the fibroblasts from patient with MPS II.
Conclusion: These results suggests that disaccharides from heparin is candidate chemical for pharmacological chaperone therapy to MPS II.

P-490

Screening mucopolysaccharidosis type IX in patients with juvenile idiopathic arthritis

Kiykim E[1], Cansever M S[3], Barut K[2], Aktuglu Zeybek A C[1], Zubarioglu T[1], Kasapcopur O[2], Aydin A[1]

[1]Div Nutr Metab Dis, Ist Univ Cer Med Fac, Istanbul, Turkey, [2]Div Rheum, Ist Univ Cer Med Fac, Istanbul, Turkey, [3]Cent Lab, Ist Univ Cer Med Fac, Istanbul, Turkey

Background: Mucopolysaccharidosis type IX is the rarest form of mucopolysaccharidosis and only four patients have been reported to date. The first reported patient had mild short stature and periarticular soft tissue masses; the other reported patients are clinically indistinguishable from juvenile idiopathic arthritis. In the present study we aimed to screen mucopolysaccharidosis type IX, among patients with juvenile idiopathic arthritis with hyaluronidase enzyme assay.
Materials and Methods: This was a cross-sectional study of 108 juvenile idiopathic arthritis patients attending the outpatient Pediatric Rheumatology clinic of Cerrahpasa Medical Faculty Children's Hospital. Healthy age matched 50 control subjects were enrolled in the study. Serum hyaluronidase activity was assessed for both patient and control group.
Results: Among all patients, none had deficient hyaluronidase activity. Despite, serum hyaluronidase activity was significantly increased in juvenile idiopathic arthritis patients, compared with control subjects (P < 0,000), no correlation was found between acute phase reactants and Hyal-1 activity (P= 0,187).
Conclusion: As conclusion the data reported in our study indicates that systemic metabolic investigation for hyaluronidase activity is not recommended in all patients with juvenile idiopathic arthritis.

P-491

Prevalence of Mucopolysaccharidosis (MPS) type I, II and VI in the paediatric and adult population with carpal tunnel syndrome (CTS). Retrospective and prospective analysis of patients who have been treated for CTS

Nørmark M B[1], Lund A M[2]

[1]Dept of Neurology, Hillerød Hosp, Hillerød, Denmark, [2]Cent for inherited Metab Dis, Copenhagen, Denmark

Background: We wanted to investigate whether the prevalences of mucopolysaccharidoses (MPS) I, II and VI were higher than expected in a selected cohort of patients with carpal tunnel syndrome (CTS). CTS is a common finding in patients with MPS and therefore it was decided to screen patients who had undergone surgery for CTS for undiagnosed MPS.
Patients and Methods: Patients who had been operated for CTS were found in databases from two hospitals. Furthermore patients who had undergone surgery for CTS when under the age of 18 were retrieved from the National Patient Registry. All included patients had a filter paper blood spot sample taken that was subsequently analyzed enzymatically for MPS I, II and VI.
Results: 427 patients were included. 408 patients were tested negative in the first test. Five patients had two inconclusive tests each and were referred for further examination at the Center for metabolic diseases where the diagnosis was excluded. Thus, all included patients were negative for both MPS I, II and VI.
Discussion/Conclusion: Though our sample size is relatively small, results indicate that MPS is not prevalent in a cohort of patients with CTS and that screening is not indicated in this setting.
Conflict of Interest declared.

P-492

The frequency and structure of mucopolysaccharidosis in Kazakhstan Republic

Sharipova M N[1], Boranbayeva R Z[1], Adamova G S[1], Tulebayeva A K[2]

[1]Scient Cent of Pediatr and Child Surgery, Almaty, Kazakhstan, [2]Asfendiyarov Kazakh Nation Med Uni, Almaty, Kazakhstan

MPS are rare diseases in Kazakhstan, and prevalence is unknown. In 2009 was launched a program for identifying children with MPS, by determining the activity of lysosomal enzymes. MPS VI was diagnosed in 2 children, and MPS I in 1 child. In 2011 Scientific Center of Pediatrics and Children's Surgery was defined as coordinator for diagnosis and treatment of MPS among children in Kazakhstan. In the same year, the first 3 children received enzyme replacement therapy (ERT) in Kazakhstan. Currently, in Kazakhstan 21 patients with MPS I, II and VI, are treated by ERT, which is funded by government budget. HSCT was performed for one child with MPS I. Now the number of diagnosed cases in Kazakhstan is 33, mainly boys – 22 and 11 girls: 10 children with MPS I (30.3% of the total number of children with MPS), 12 boys with MPS II (36.4%). 2 cases of MPS III (6.1%), 3 cases of MPS IV (9.1%) and 6 children with MPS VI (18.2%). The frequency is 0.63 cases per 100 000 child population. There are siblings among children with MPS: 2 families with MPS I and one with MPS VI.
Thus, the most common type in Kazakhstan is MPS II.

P-493

The therapeutic efficacy of bone marrow transplantation from heterozygous donor in mucopolysaccharidosis type II mice

Akiyama K[1 2], Shimada Y[2], Higuchi T[2], Yokoi T[2 3], Yokoi K[2 3], Kobayashi H[2 3], Ida H[2 3], Ishii M[1], Ohashi T[2 3]

[1]Dept Ped, kitasato Univ, Kanagawa, Japan, [2]Div Gen Ther, Jikei Univ, Tokyo, Japan, [3]Dept Ped, Jikei Univ, Tokyo, Japan

Background: Mucopolysaccharidosis type II (MPS II), is characterized by deficient activity of iduronate-2-sulfatase (IDS), resulting in accumulation of glycosaminoglycans (GAGs) in various tissues. Although there is not yet unified consciousness whether bone marrow transplantation (BMT) has an indication for MPS II treatment, our previous report demonstrated the therapeutic efficacy of BMT to several mouse tissues. Now then, efficacious BMT are based on appropriate donor selection. HLA matched sibling donor has distinctive advantage over other donors in GVHD morbidity and time to engraftment.
Methods and Results: In this study, we compared the efficacy of wild type and heterozygous donor cell transplantation to murine model of MPS II. There is no significant difference in donor cell engraftments between wild type BMT (WBMT) and heterozygous BMT (HBMT). MPS II mice treated by WBMT showed a rise in IDS activity which reached 15% of WT levels, and mice treated by HBMT were limited two-thirds rising of WBMT. Any treatment reduced tissue accumulated GAGs, and WBMT reduced GAGs more profoundly than HBMT. This observation was consistent with the result of pathological analysis.
Conclusion: WBMT has apparent advantage over HBMT in terms of reduction of GAGs in tissues.

P-494

A prospective study on early diagnosis of MPS diseases in young patients with particular bone and joints manifestation of disease

Eyskens F[1 2], Devos S[2]

[1]Div Metab Dis, Univ Hosp Antwerp, Antwerp, Belgium, [2]PCMA vzw, Antwerp, Belgium

Background: The diagnosis of mucopolysaccharidoses, MPS diseases, is significantly delayed in most patients, especially the more attenuated subtypes, and misdiagnosis is common. Since effective disease-specific treatments have become available and timely initiation of these treatments is necessary to prevent the development of severe, disabling and irreversible manifestations, early diagnosis has become essential.
Methods: As musculoskeletal manifestations are common, raising awareness for better recognition of these metabolic diseases based on the presenting signs and symptoms by all doctors who may encounter these patients is mandatory: pediatricians, orthopedic surgeons, rheumatologists, child neurologists and neurosurgeons (48 investigators of 8 different clinics). The screening is performed in a multiplex manner using UPLC-MSMS so that different MPS can be screened for on the same blood spot in the same time keeping costs to a minimum.
Results: in a pilot study over a period of 1 year 50 patients were screened. We detected 1 MPS type 2 (Hunter), confirmed by DNA analysis of having a deletion of the *IDS* gene.
Conclusion: Aim of this study is to implement the ESI-MSMS technique in the screening for MPS I, II, IVA and VI in dried blood spots with the focus on to simplify and optimize the pre-analytical phase.
Conflict of Interest declared.

P-495

Development and reliability assessment of the MPS II Disease Severity Score (DSS)

Amartino H[1], Burton B K[2], Giugliani R[3], Harmatz P[4], Jones S[5], Scarpa M[6], Solano M[7], Zafeiriou D[8], Vernon M[9], Raluy-Callado M[9], Trundell D[9], Wiklund I[9], Pulles T[10], Whiteman D A H[11], Muenzer J[12]

[1]Child Neurol Dept, Hosp Univ Austral, Pilar, Argentina, [2]Div Genet, Lurie Child Hosp, Northwester, Chicago, IL, United States, [3]Med Genet Service/HCPA, Porto Alegre, Brazil, [4]Child Hosp & Res Center, Oakland, CA, United States, [5]St Mary's Hosp, Univ Manchester, Manchester, United Kingdom, [6]Rare Dis Centre, HSK, Wiesbaden, Germany, [7]Dept Neuroped, Fund Cardioinf, Bogotá, Colombia, [8]Aristotle Univ Thessaloniki, Thessaloniki, Greece, [9]Evidera, London, United Kingdom, [10]Shire, Zug, Switzerland, [11]Shire, Lexington, MA, United States, [12]Dept Ped, Univ NC, Chapel Hill, NC, United States

Background and objectives: The current classification for MPS II categorises patients with cognitive involvement as severe and the remainder as attenuated, but no disease-specific measure captures the actual range of severity. The objective of this activity was to develop a Clinician-reported Outcome (ClinRO) measure designed to quantify disease severity in MPS II patients, and assess its reliability.
Materials and Methods: Literature review and input from recognised clinical experts were used to develop the MPS II DSS. Clinical experts completed the DSS for 7 hypothetical patient vignettes (4 attenuated, 3 severe) at two timepoints (N=11 at T1; N=10 at T2). Inter-rater item reliability at both timepoints, and intra-rater reliability for the unchanged items were assessed using the kappa statistic with $\kappa \geq 0.7$ considered acceptable.
Results: The DSS contains two components: Somatic (11 items) and CNS (6 items). Agreement between clinicians was high ($\kappa \geq 0.7$) for all but two CNS items. After revision, higher kappa values at T2 was achieved (language: $\kappa=0.90$; activity: $\kappa=0.70$). High intra-rater reliability was demonstrated for all items ($\kappa > 0.80$).
Discussion: The MPS II DSS seems a reliable and useful measure for quantifying disease severity in both attenuated and severe MPS II patients, but needs to be confirmed through clinical use.
Conflict of Interest declared.

P-496

Analyzes of biomarkers of oxidative stress in Mucopolysaccharidosis I and Mucopolysaccharidosis VI

Cé J[1], Mello A S[1 2], Antunes C[2], Bortolato G[2], Marinho J[2], Machado F[2], Funchal C[2], Dani C[2], Coelho J C[1]

[1]Lab EIM-DLD, Dep Bioq ICBS-UFRGS, Porto Alegre, Brazil, [2]Lab Bioq, Centro Pesq, Centr Univ Metod, Porto Alegre, Brazil

Background: High levels of reactive oxygen species have been associated with oxidative stress and inflammation being related to cellular changes characteristic, caused by changes in metabolic pathways of individuals with Inborn Errors of Metabolism. Mucopolysaccharidoses (MPS) are characterized by biomolecular and tissue damage that results in the accumulation of glycosaminoglycans not degraded in cells of various organs and systems. The aim of this study was to evaluate biomarkers of oxidative stress in plasma of MPS I and VI individuals.
Patients and Methods: The groups were MPSI, MPSVI (n=7, each) and healthy controls (HC n=14). The antioxidant

capacity was measured by superoxide dismutase (SOD) and catalase (CAT), since the damage to lipids was measured by the presence of thiobarbituric acid reactive substances (TBARS).
Results: The results found a significant increase ($p< 0.02$) in lipid peroxidation in MPSI, but in MPSVI no change in any of the parameters studied was observed. Analyzing SOD and CAT, there was no significant differences ($p>0.05$) among the groups.
Conclusion: there was an increase in damage to lipids in MPSI suggesting that this disease is more vulnerable to oxidative damage than MPSVI.

P-497

N-acetylgalactosamine-6-sulfate in leukocytes: kinetic parameters in diagnosis of Mucopolysaccharidosis type IV A

Moraes V C[1], Cé J[1], Coelho J C[1]

[1]Lab EIM-DLD, Dep Bioq ICBS-UFRGS, Porto Alegre, Brazil

Background: Mucopolysaccharidosis IVA is characterized biochemically by the accumulation of keratan sulfate and chondroitin-6-sulfate in lysosomes, caused by the deficiency of the N-acetylgalactosamine-6-sulfatase (GALNs) enzyme. The aim of this study was to determine the kinetic parameters, Km and Vmax of GALNs in leukocytes of healthy individuals in order to improve the diagnosis of Mucopolysaccharidosis IV A.
Methods: Leukocytes from six healthy individuals were isolated from 10mL of heparinized blood. GALNs activity was measured according to van Diggelen et al. (1990) with 4-methylumbelliferyl-β-D-galactoside-6-sulfate (MU-βGal-6S) as artificial substrate. The curve of Michaelis-Menten (substrate curve) was obtained with MU-βGal-6S solutions at concentrations of 1 to 20mM. The linearity was observed and new points of substrate concentration were established for the calculation of the Km and Maximum Velocity (Vmax) parameters using the Lineweaver and Burk plot.
Results: The Km and Vmax of GALNs in leukocytes of healthy subjects was 7.16 mM and 77.3 nmol/17h/mg protein, respectively.
Conclusion: The determination of the kinetic parameters of the enzyme N-acetylgalactosamine-6-sulfatase is of great importance for the improvement of fluorometric techniques in the diagnosis and the development of enzyme replacement therapy for individuals affected with Mucopolysaccharidosis IVA and to aid in distinguishing healthy individuals affected and heterozygous.

P-498

Impact of elosulfase alfa on exercise capacity in patients with Morquio A syndrome in a randomised, double-blind, pilot study

Burton B[2], Berger K I[3], Lewis G D[4], Tarnopolsky M[5], Harmatz P[1], Mitchell J J[6], Muschol N[7], Jones S A[8], Sutton V R[9], Pastores G M[3 10], Lau H[3], Sparkes R[11], Genter F[12], Shaywitz A[12]

[1]UCSF Benioff Child Hosp, Oakland, CA, United States, [2]Lurie Child Hosp & NWU Feinberg, Chicago, United States, [3]NY Univ School of Med, New York, United States, [4]Mass Gen Hosp, Boston, United States, [5]McMaster Univ Med Ctr, Hamilton, Canada, [6]Montreal Child Hosp, Montreal, Canada, [7]Univ Med Ctr Hamburg-Eppendorf, Hamburg, Germany, [8]Centre for Genomic Med, St Mary's Hosp, Manchester, United Kingdom, [9]Baylor Coll of Med and TX Child Hosp, Houston, United States, [10]Mater Misericordiae Univ Hosp, Dublin, Ireland, [11]Alberta Child Hosp, Calgary, Canada, [12]BioMarin Pharmaceutical Inc, Novato, United States

Background and objectives: To assess baseline performance and impact of elosulfase alfa enzyme replacement therapy on maximal exercise capacity in patients with Morquio A syndrome (mucopolysaccharidosis IVA).
Patients & Methods: After a 3-week screening period, 25 patients aged ≥7 years and able to walk >200 meters in the 6-minute walk test were randomised in a double-blind fashion to elosulfase alfa 2.0 mg/kg/week (n=15) or 4.0 mg/kg/week (n=10) for 27 weeks. The primary endpoint was safety; the secondary endpoint was change from baseline to week 25 in peak exercise capacity on the cardiopulmonary exercise test (n=15; dosage groups combined).
Results: At baseline, weight-adjusted peak oxygen uptake was mildly/moderately impaired. There was no evidence of dynamic cardiac impairment. At 25 weeks, median percent change in exercise capacity was positive: 16.9% exercise duration, 26.5% peak workload, 10.7% O_2 pulse. Median percent VO_2/work ratio declined 7.6%, indicating that patients were performing work at reduced oxygen cost. Limited patient numbers precluded dose comparisons, and the small sample size and lack of a control group should be considered when interpreting these results.
Conclusion: Maximal exercise capacity improved during 25 weeks of elosulfase alfa treatment in this Morquio A patient group with mildly/moderately impaired baseline endurance/exercise capacity.
Conflict of Interest declared.

P-499

Long-term outcomes of treatment with elosulfase alfa for Morquio A Syndrome (mucopolysaccharidosis IVA)

Hendriksz C[1], Jones S[2], Geberhiwot T[4], Van Tuyl A[3], Schweighardt B[3], Slasor P[3], Decker C[3]

[1]Salford Royal Hospital NHS Trust, Salford, United Kingdom, [2]Centre for Genomic Med, St Mary's Hosp, Manchester, United Kingdom, [3]BioMarin Pharmaceutical Inc, Novato, United States, [4]Birmingham Child Hosp, Birmingham, United Kingdom

Background & Objectives: Elosulfase alfa (EA) is the only approved drug for mucopolysaccharidosis MPS IVA, a lysosomal storage disorder caused by deficiency of N-acetylgalactosamine-6-sulfatase. This Phase 1/2 extension study evaluated the long-term safety and efficacy of weekly EA infusions in MPS IVA patients.
Patients & Methods: Seventeen patients previously treated with varying doses of EA for 72–84 weeks received an additional 178–190 weeks of treatment at 2.0 mg/kg/wk during a multi-center, open-label extension study.
Results: Phase 1/2 study improvements in the 6-minute walk and 3-minute stair climb tests were generally sustained during the extension study, as were decreases in the glycosaminoglycan urine keratan sulfate and improvements in forced vital capacity measures. Most adverse events (AEs) were grade 1 or 2, with no AE-related drug discontinuations or on-study deaths. All evaluable patients developed antidrug and neutralizing antibodies to elosulfase alfa, but with no association between antibody titers or neutralizing antibody presence/absence and efficacy or safety. Transient drug-specific IgE positivity in a small number of patients was not associated with hypersensitivity AEs.
Conclusions: EA maintained a favorable safety and sustained efficacy profile in patients for over 4 years, with further improvement in functional measures in some cases.
Conflict of Interest declared.

P-500

Safety and pharmacodynamic activity of elosulfase alfa in pediatric patients less than 5 years of age with Morquio A Syndrome (Mucopolysaccharidosis IVA)

Harmatz P[1], Jones S A[2], Bialer M[4], Parini R[5], Martin K[3], Wang H[3], Shaywitz A[3]

[1]UCSF Benioff Child Hosp, Oakland, CA, United States, [2]Centre for Genomic Med, St Mary's Hosp, Manchester, United Kingdom, [3]BioMarin Pharmaceutical Inc, Novato, United States, [4]North Shore LIJ Health System, Manhasset, United States, [5]Az. Ospedaliera S. Gerardo, Monza, Italy

Objective: The 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. Standing heights were measured in >2 yo, and one 18-month-old (n=13) and lengths for all (n=15).
Results: The mean (range) age was 3.1 (0.8-4.9) years at study entry. The 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) μg/mg creatinine and decreased by 43.1 %(±22.15) at Week 52. Standing heights (centimeters) increased by mean (±SD) 6.7 (±3.76) from baseline. Baseline Z-scores for height/length (n=15) were −1.6 (±1.61) and −1.9 (±1.62) at week 52(mean (±SD)).
Conclusion: In children< 5 yo, 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-501

Impact of long-term elosulfase alfa treatment on six-minute walk test distance in patients with Morquio A syndrome

Harmatz P[1], Burton B K[2], Giugliani R[5], Hughes D[6], Mitchell J J[3], Raiman J[7], Solano Villarreal M L[8], Stewart F[9], Slasor P[4], Shaywitz A[4]

[1]UCSF Benioff Child Hosp, Oakland, CA, United States, [2]Lurie Child Hosp & NWU Feinberg, Chicago, United States, [3]Montreal Child Hosp, Montreal, Canada, [4]BioMarin Pharmaceutical Inc, Novato, United States, [5]HCPA, UFRGS & INAGEMP, Porto Alegre, Brazil, [6]Royal Free London NHS FT & UC, London, United Kingdom, [7]Hosp for Sick Child, Toronto, Canada, [8]Assoc Colombiana de Neurol Infant, Bogata, Colombia, [9]Belfast City Hosp, Belfast, Ireland

Background: Long-term safety and efficacy of elosulfase alfa enzyme replacement therapy (ERT) was evaluated in patients with Morquio A in an open label, multi-center, phase 3 extension study.
Methods: In part 1, patients initially randomized to ERT in the original placebo-controlled 24 week study remained on their assigned regimen (2.0 mg/kg/week or every other week); placebo patients were re randomized to one of the two regimens. At part 2, all patients received weekly infusions of ERT 2.0 mg/kg. The primary efficacy endpoint, six-minute walk test distance (6MWD), was evaluated in 173 patients for up to 120 weeks and compared to baseline for intent-to-treat (ITT), and pre-specified per-protocol (PP) populations.
Results: The 6MWD further increased at 36 weeks in those on weekly doses in the ITT and PP populations. At 48 weeks, the 6MWD did not further improve compared to week 24 in the ITT; in the PP the improvement achieved at week 36 was maintained. At 120 weeks, 6MWD was sustained at the 24-week level, with generally greater effects in the PP. There were no new safety signals and the safety profile was consistent with that observed the first 24 weeks.
Conclusion: Long-term ERT stabilizes or improves endurance/mobility of Morquio A patients.
Conflict of Interest declared.

P-502

Impact of long-term elosulfase alfa treatment on three-minute stair climb test, pulmonary function tests and normalized urine keratan sulfate in patients with Morquio A syndrome

Giugliani R[5], Burton B K[2], Harmatz P[1], Hughes D[6], Mitchell J J[3], Raiman J[7], Solano Villarreal M L[8], Stewart F[9], Slasor P[4], Shaywitz A[4]

[1]UCSF Benioff Child Hosp, Oakland, CA, United States, [2]Lurie Child Hosp & NWU Feinberg, Chicago, United States, [3]Montreal Child Hosp, Montreal, Canada, [4]BioMarin Pharmaceutical Inc, Novato, United States, [5]HCPA, UFRGS & INAGEMP, Porto Alegre, Brazil, [6]Royal Free London NHS FT & UC, London, United Kingdom, [7]Hosp for Sick Child, Toronto, Canada, [8]Assoc Colombiana de Neurol Infant, Bogata, Colombia, [9]Belfast City Hosp, Belfast, Ireland

Background: Long-term safety and efficacy of elosulfase alfa enzyme replacement therapy (ERT) was evaluated in Morquio A patients in an open label, multi-center, phase 3 extension study.
Methods: Part 1: Patients initially randomized to ERT in the original placebo-controlled 24-week study remained on their regimen (2.0 mg/kg/week or every other week); placebo patients were re randomized to one of the two regimens. Part 2: All patients received weekly infusions of ERT 2.0 mg/kg/week. Secondary efficacy endpoints (n=173) were the three-minute stair climb test (3MSCT), pulmonary function tests (PFTs), and normalized urine keratan sulfate (uKS) in the intention to treat (ITT) and pre-specified per protocol (PP) populations. Change from baseline to 120 weeks was calculated.
Results: In the 24-week Phase 3 study, non-statistical increases were seen for 3MSCT and PFTs (FVC, FEV1, and MVV) versus placebo. Increases from baseline were sustained (3MSCT and MVV) or improved (FVC and FEV1) up to 120 weeks during extension. The reduction in uKS compared to baseline achieved at 24 weeks was sustained on weekly doses: 47.1% at 48 weeks, ~60% at 120 weeks. Results were similar for the ITT and PP.
Conclusion: Long-term ERT for Morquio A patients results in sustained improvements in 3MSCT, PFTs, and uKS.
Conflict of Interest declared.

P-503

Management of fertility and pregnancy in individuals with mucopolysaccharidosis (MPS)

Stewart F[5], Harmatz P[1], Braunlin E[6], Bentley A[7], Burton B[2], Guffon N[8], Hale S[9], Johnston T[10], Kircher S[11], Kochhar P[12], Mitchell J J[3], Plöckinger U[13], Semotok J[14], Sisic Z[4]

[1]UCSF Benioff Child Hosp, Oakland, CA, United States, [2]Lurie Child Hosp & NWU Feinberg, Chicago, United States, [3]Montreal Child Hosp, Montreal, Canada, [4]BioMarin Pharmaceutical Inc, Novato, United States, [5]Belfast City Hosp, Belfast, Ireland, [6]University of Minnesota, Minneapolis, United States, [7]Univ Hosp S Manchester NH Fdn Trust, Manchester, United Kingdom, [8]Hôpital Femme Mère Enfants, Lyon, France, [9]Seattle Child Hosp, Seattle, United States, [10]Birmingham Women's NHS Fdn Trust, Birmingham, United Kingdom, [11]Inst Med Chem Med Gen Med Univ Vienna, Vienna, Austria, [12]Central Manchester Univ Hosp, Manchester, United Kingdom, [13]Charité - Universitätsmedizin Berlin, Berlin, Germany, [14]Adult Metab Gen Clinic Univ Hlth Ntwk, Toronto, Canada

Background and objectives: Due to the rarity of mucopolysaccharidoses (MPS) there is limited documented experience in managing fertility and pregnancy in these disorders. As individuals with MPS are living longer, clinicians are more likely to encounter family planning issues.
Methods: This work is based on discussion of existing literature and the experience of the authors with issues of fertility,

pregnancy, delivery and post-partum sequelae in individuals with MPS.
Results: MPS individuals should receive counselling from early adolescence onward to address their expectations and needs with respect to fertility, contraception and pregnancy, including genetic counselling, and planning/managing for the pregnancy and delivery. Clinicians caring for such individuals need to be aware of unique medical aspects and existing guidelines related to including cardiac, pulmonary and skeletal manifestations and anaesthetic considerations. A multidisciplinary individualized approach for females before becoming pregnant is required, centralized around the patient's needs and desires and to address the potential complications. The risks and benefits addressing the use of enzyme replacement therapy during pregnancy and breastfeeding also need to be determined.
Conclusions: It is essential to establish early ongoing dialogue, provide accurate information and an appropriate multi-disciplinary plan for fertility, contraception and pregnancies in individuals with MPS.
Conflict of Interest declared.

P-504

Medical issues and other challenges in adult patients with mucopolysaccharidosis (MPS)

Mitchell J J[2], Berger K I[1], Quartel A[4], Braunlin E[5], Wang R[7], Pastores G M[1 3], White K[6], Jurecki E R[4]

[1]NY Univ School of Med, New York, United States, [2]Montreal Child Hosp, Montreal, Canada, [3]Mater Misericordiae Univ Hosp, Dublin, Ireland, [4]BioMarin Pharmaceutical Inc, Novato, United States, [5]University of Minnesota, Minneapolis, United States, [6]Seattle Child Hosp, Seattle, United States, [7]CHOC Child Spec, Orange County, United States

Background and objectives: The number of adult patients being treated for mucopolysaccharidosis (MPS) has increased because of better diagnosis and management. Nevertheless, multidisciplinary evaluation and management of these patients remains largely unexplored. We present an overview of medical issues of adults with MPS and challenges associated with disease management.
Methods: This work is based on presentations and discussions among paediatricians and adult specialists at a meeting about MPS and adulthood and on additional literature searches.
Results: Internists should be aware of the importance of establishing "medical homes" providing MPS-centred multidisciplinary outpatient, inpatient, and surgical care throughout adulthood. Because surgical procedures in adult MPS patients are complicated by short stature, bone and other tissue abnormalities, and anaesthetic considerations, an individualised approach balancing risks and benefits is required that respects patient autonomy and self-determination. To improve management of MPS in adults, registries should capture the related natural history of these disorders, and clinical studies should include and focus on adult patients, including reproductive considerations.
Conclusions: Programmes are needed that guide transition from paediatric to adult health care for MPS patients and coordinating physicians who can monitor disease progression while organising a team of expert local specialists properly trained to treat these patients.
Conflict of Interest declared.

P-505

Novel therapeutic options for mucopolysaccharidosis (MPS) type IIIA based on the crystal structure of human sulfamidase

Steinfeld R[1], Sidhu N S[2], Kraetzner R[1], Usón I[3], Gärtner J[1], Sheldrick G M[2]

[1]University Medical Center Goettingen, Goettingen, Germany, [2]Inst of Inorg Chemistry, Univ Goettingen, Goettingen, Germany, [3]IBMB–CSIC, Barcelona Science Park, Barcelona, Spain

Background: Mucopolysaccharidosis (MPS) type IIIA is an autosomal recessively inherited lysosomal storage disease that is caused by mutations in the *SGSH* gene. Affected children suffer from developmental delay, abnormal behavior, progressive loss of cognitive and motor skills, cerebral convulsions and spasticity. More than 100 different pathogenic *SGSH* mutations have been described and about 80% of them represent missense mutations that cause conformational changes of sulfamidase.
Methods: We crystallized human sulfamidase to elucidate the structural effects of *SGSH* mutations. The X-ray crystal structure at 2.0 Å resolution revealed a butterfly-like shape of two sulfamidase molecules. The catalytical center comprises a Ca2+ion that is bound to Asp31, Asp32, Asp273, Asn274 and the phosphorylated formyl Gly70. The formyl glycine is a characteristic feature of sulfatases and crucial for the enzymatic cleavage of the sulfate group. On the basis of the sulfamidase structure we have investigated 79 missense mutations and described their functional effects on the activity and stability of sulfamidase. We tested several substances for their potential to rescue sulfamidase activity in selected mutants.
Conclusion: Our results provide new insight into the molecular mechanisms leading to the loss of enzymatic sulfamidase activity and offer novel therapeutic options for the fatal neurodegenerative disease MPS IIIA.

P-506

Mucopolysaccharidosis type I is the most common lysosomal storage disorder in Morocco and the P533R is the founder mutation

Talbaoui H[1], Puech J P[3], Dahri S[1], Dini N[2], Kriouile Y[2], Caillaud C[3], Chabraoui L[1]

[1]Biochem Univ Hosp Lab of Rabat, Rabat, Morocco, [2]Neuropediatr Univ Hosp of Rabat, Rabat, Morocco, [3]Biochem Genet Lab Hosp Necker, Paris, France

In 1991 we developed a selected screening protocol of mucopolysaccharidoses which comprises a colorimetric quantification of urinary glycosaminoglycans and their separation and identification by electrophoresis. The diagnosis is confirmed by the study of leukocyte enzymes. During 24 years we have diagnosed 119 patients with a deficiency of α-L-iduronidase. Aged 1 to 16 years at diagnosis, all patients have typical symptoms (facial dysmorphia, dysostosis multiplex, hernia, hepatosplemegaly). Consanguinity was observed in 68% of the families. The search for the P533R mutation by sequencing of exon 11 of the *IDUA* gene was positive in the homozygous state in 91% of the studied patients. This suggests a founder effect of this mutation in the Moroccan population. Only 12 patients were treated by enzyme replacement therapy with Aldurazyme using a dose of 100 I/U every week. The biological monitoring showed a decreased urinary GAGs level in all treated patient.

P-507

Mucopolysaccharidosis Type I in two Mexican siblings treated with enzyme replacement therapy at different ages

Flores L L[1], Ruiz E D[1], Marquez M A[1], Vega M E[3], Franco S J[2]

[1]Clin Gen Dept Gen Hosp Soc Ins Mex Inst, Mexico City, Mexico, [2]Hem Ped Dept, Gen Hosp, Soc Ins Mex Inst, Mexico City, Mexico, [3]Med Int Ped, Gen Hosp, Soc Ins Mex Inst, Mexico City, Mexico

Background and objectives: Mucopolysaccharidosis type 1 patients receiving enzyme replacement therapy (ERT, laronidase) have different clinical improvement, this variation in the expected response to ERT is due to several factors among them: type of mutation, age and pathological complications, individual response, lack of discontinuity in ERT and several other environmental factors. MPS I siblings have a better prognosis due to earlier diagnosis. We present data of two siblings treated with enzyme replacement therapy at different ages and their clinical evolution.
Patients and Methods: A female and male brotherhood from non-consanguineous parents, were diagnosed and received ERT at 7 and 2 years, respectively. In this report we compared the clinical outcome of two siblings after three years of therapy.
Results: Clinical response was not as expected since infiltrated face and dysostosis multiplex remained unchanged in the female, the male sibling had no face, nor osteo-articular involvement since first evaluation. Both had hepatosplenomegaly improvement, yet without complete regression in the female. Favorable outcomes were reduction in respiratory airway infections.
Conclusion: ERT for MPS 1 is being evaluated in siblings and different settings offers an important point of comparison in clinical evolution. It can be observed that if started earlier, ERT has a better prognosis.

P-508

A new case of an adolescent with alpha-mannosidosis

Spodenkiewicz M[1], Garnotel R[2], Venot P[3], Bednarek N[3], Gaillard D[1]

[1]Genetics Unit, CHU Reims, Reims, France, [2]Pediatrics Lab, CHU Reims, Reims, France, [3]Pediatrics Unit, CHU Reims, Reims, France

Background: Alpha-mannosidosis is a rare autosomal recessive lysosomal storage disorder. Its clinical manifestations are progressive and diverse, making it a possibly under- or misdiagnosed disease. We report a case of alpha-mannosidosis with epilepsy in a 14-year-old Chadian girl.
Case report: The patient shows coarse facial features, daily epilepsy seizures, global developmental delay and progressive psychomotor regression since 3 years of age. Many paroxysmal slow waves transient disturbances, spikes-waves and slow notched-waves patterns in the bi-frontal derivations were found in electroencephalography (EEG) record. Magnetic resonance imaging (MRI) of the brain reveals cerebellar vermis atrophy and anteroposterior lengthening of the skull with significant thickening of the cranial inner table and the orbital bones. Enzymatic activity test revealed a significant decrease alpha-mannosidase activity (32 U/mg proteins - reference value: 200-1000), which is a hallmark of alpha-mannosidosis. The gene *MAN2B1* sequencing found a homozygous missense mutation c.2248C>T (p.Arg750Trp) in exon 18.
Conclusion: Epileptic seizures associated with psychomotor regression could be an important indication of alpha-mannosidosis. However, further research of their relation is needed.

P-509

Developing substrate reduction therapy for six mucopolysaccharidoses by targeting NDST1

Tkachyova I[1], Fan X[1], Schulze A[1], Mahuran D[1]

[1]The Hospital for Sick Children, Toronto, Canada

Background: Accumulation of undegraded heparan sulfate (HS) results from deficiencies in any of eight HS degrading lysosomal enzymes. Stored HS eventually affects the function of the lysosomes, which directly impacts all body-functions including the central nervous system (CNS). Presently used bone marrow transplantation and enzyme replacement therapies cannot be applied for lysosomal storage disorders (LSDs) with neurological complications since access to the CNS is precluded by the blood brain barrier. We plan to identify small molecules that can be used in substrate reduction therapy (SRT) to lower the O-sulfation and/or epimerization of the HS sugar moieties. Our aim is to down-regulate the transcription of the first modifying enzyme in HS biosynthesis, N-deacetylase/N-sulfotransferase 1 (NDST1).
Methods: HeLa cells stably expressing the human NDST1 promoter-firefly luciferase were used in a high-throughput based assay to screen compounds in the Prestwick library.
Results: Screening of the Prestwick library resulted in 35 potential NDST1 transcription inhibitors. Further evaluation of dose response curves resulted in 22 bona-fide hits. Also, one of these compounds significantly reduced the activity and protein levels of endogenous NDST1.
Conclusion: We have identified several potential compounds that target NDST1 transcription and could be applied as SRT to treat a group of HS LSDs.

P-510

Fertility in patients with Mucopolysaccharidosis type VI

Teresa Cardoso M[1], Castro Chaves P[1], Rodrigues E[2], Martins E[3], Lacerda L[4], Leão Teles E[2]

[1]Metab Dis Unit, Int Med Depart, CHS João, Porto, Portugal, [2]Metab Dis Unit, Paed Depart, CH S João, Porto, Portugal, [3]Metab Dis Unit, CHP, Porto, Portugal, [4]CGMedica, CHP, Porto, Portugal

Background: Mucopolysaccharidosis type VI (MPS VI) is a rare lysosomal storage disorder with a wide spectrum of symptoms from rapidly progressing forms to slowly advancing disease. To date, little is described about the fertility of MPS VI patients and about the influence of enzyme replacement therapy (ERT) on it.
Patients: We report two brothers of non consanguineous parents with MPS VI (hererozygous compound mutation c.11438T>G and c.1213+4A>C). The younger, 25 years old was observed at 10 years-old due to articular pain. He has a previous history of frequent otitis in infancy, hearing loss, compromised vision, inguinal hernia, dysostosis multiplex, carpal tunnel syndrome (CTS) and severe aortic regurgitation. At 13 years he required cervical medular decompression. He is on ERT since 18 yearsold with significant functional improvement. He has two sons with 6 and 3 years-old. His sibling, 35 years old, with later clinical presentation (hearing loss, inguinal hernia, CTS, dysostosis multiplex), has an offspring of 3 children and is now waiting for ERT.
Conclusion: To our knowledge, fertility of patients with MPS VI is a topic rarely described. The purpose of this presentation is to contribute to a better understanding of the natural history of the disease particularly in late presentations.

P-511

Efficacy on brain of hematopoietic stem cell transplantation and enzyme replacement therapy for patients with mucopolysaccharidosis type II severe form

Tanaka A[1], Hamazaki T[1], Okuyama T[2], Sakai N[3], Kosuga M[2], Shinpo M[3], Kato K[4], Yaba H[5], Ishige M[6], Suzuki Y[7], Sawada T[1], Kudo S[1], Kadono C[1], Kobayashi R[8], Mugishima H[6], Tabuchi K[9], Kato S[5]

[1]Osaka City University, Osaka, Japan, [2]National Center for Child Health and Dev, Tokyo, Japan, [3]Osaka University, Osaka, Japan, [4]Japanese Red Cross Nagoya Daiichi Hosp, Nagoya, Japan, [5]Tokai University, Isehara, Japan, [6]Nippon University, Tokyo, Japan, [7]Gifu University, Gifu, Japan, [8]Sapporo Kitanire Hospital, Sapporo, Japan, [9]Tokyo Komagome Hospital, Tokyo, Japan

Background & Objectives:Mucopolysaccharidosis type II (MPS II) is an X-linked disorder caused by deficiency of iduronate-2-sulfatase, associated with a broad spectrum of chronic and progressive symptoms. We performed a retrospective study of the efficacy of enzyme replacement therapy (ERT) and hematopoietic stem cell transplantation (HSCT) on brain for MPS II patients by analyzing developmental quotient (DQ).
Patients & Methods: MPS II patients with severe form were divided into two groups according to the gene mutation, missense mutations as Type C and null mutations as Type D.

Effects of HSCT and ERT on brain function were analyzed by scatter diagram of developmental age against chronological age historically in each patient.
Results: When HSCT was performed before the age 2.5 years without significant delay (DQ>70), linear development was achieved until at least eleven years of age in Type C. On the other hand, none of the ERT patients showed development after 40 months old even though ERT started in young ages.
Conclusions: HSCT could diminish the brain involvement of MPS II when it was performed at early disease stage. Early treatment of HSCT for MPS II is recommendable when the early diagnosis was confirmed as a severe form by molecular analysis.

P-512

A case of fucosidosis with a new mutation in *FUCA1* gene

Pektas E[1], Yucel-Yilmaz D[2], Ozgul R K[2], Dursun A[1]

[1]Div Metab Dis, Hacettepe Univ Child Hosp, Ankara, Turkey, [2]Division of Metabolism, Hacettepe Univer, Ankara, Turkey

Fucosidosis is an autosomal recessive disorder caused by a deficiency of alpha-L-fucosidase. Accumulation of fucosylated glycoproteins occurs in various tissues such as the central nervous system, endothelial cells, and skeletal tissues. A 4-year-old boy presented with global developmental delay with no speech, followed by regression of acquired milestones from 2 years of age onwards. He was the second child of a consanguineous marriage (first-cousins), born normally after an uneventful pregnancy. He was referred to our pediatric neurology clinic for the evaluation of cognitive delays to determine whether there was an underlying metabolic disease. He had coarse facies, thickened lips, gingival hypertrophy and sacral mongolian spot. There was no visceromegaly on physical examination. His blood counts, liver and renal functions were normal. Abdominal ultrasonography revealed no organomegaly. Metabolic workup and urine mucopolysaccharide levels were normal. Bone X-rays showed dysostosis multiplex. Characteristic MRI findings were detected. All these findings led us to the diagnosis for fucosidosis which was confirmed by the presence of a homozygous c.1160G>A (p.Trp387Stop) mutation on *FUCA1* gene, identified by whole exome sequencing. We present a case of fucosidosis, a rare lysosomal storage disease, for which we describe a novel mutation.
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P-513

Vitamin D deficiency – a preventable co-morbidity in mucopolysaccharidosis

Faria A[1], Garcia P[1], Rodrigues E[4], Macário M C[5], Martins E[3], Janeiro P[2], Diogo L[1]

[1]Metab Dis Unit, Ped Hosp, CHUC, Coimbra, Portugal, [2]Metab Unit, Pediatric Department, CHLN, Hospital de Santa Maria, Lisboa, Portugal, [3]Metab Unit, Pediatric Department, Centro Hospitalar do Porto, Porto, Portugal, [4]Metab Unit, Centro Hosp São João, Porto, Portugal, [5]Neurology Department, HUC, CHUC, Coimbra, Portugal

Background: Hypovitaminosis D may lead to accelerated bone loss and compromise general health and quality of life.
Objective: Evaluate vitamin D status in Portuguese MPS patients.
Methods: Plasma 25-OH-cholecalciferol, calcium, phosphorus, alkaline phosphatase and parathormone were evaluated in 31 patients (54,8% male) aged 20 months to 32 years: 5 MPSI, 4 MPSII, 9 MPSIII, 4 MPSIV, 9 MPSVI, 1 MPSVII.
Results: Mean 25-OH-vitamin D was 47.4nmol/L (8.0-86.6). Fifteen patients (48.4%) presented hypovitaminosis D (< 50nmol/L) and insufficient levels (50-74.9nmol/L) were found in 12. Adequate values (≥75nmol/L) were detected in the youngest patient and in three other under supplementation. No significant difference was found between MPS types.
Hypocalcemia and hypercalcemia were recognized in two patients each. Five patients presented hyperphosphatemia.
Hyperparathyroidism was disclosed in two cases, one with vitamin D insufficiency (74.9nmol/L) and the other with hypovitaminosis (24.5nmol/L).
Hyperphosphatasemia was identified in two patients with vitamin D deficiency.
Discussion and Conclusion: Hypovitaminosis D is rather prevalent. However, it seems even more frequent among Portuguese MPS patients, as only 12.9% showed adequate levels. Low mobility or severe behavioural problems may predispose them to limited sun exposure and consequent vitamin D deficiency. This problem should be anticipated to prevent additional damage.
Conflict of Interest declared.

P-514

Expansion of mutation spectrum in *IDS* and *IDUA* genes: Report of eight and one novel mutations in Indian patients with Hunter syndrome and Hurler syndrome

Verma, G[1], Kabra, M[1], Gupta, N[1], Shastri S[1], Mishra P[1], Roy Chowdhury M[1], Sapra S[1], Gulati S[1], Kaur P[2]

[1]Dep Peads, All India Inst of Med Sciences, New Delhi, India, [2]Dep Biophy,All India Inst of MedSciences, New Delhi, India

Background: Deficiency of lysosomal enzymes α-L-iduronidase and iduronate-2-sulfatase caused by mutations in *IDUA* and *IDS* genes result in MPS-I and MPS-II.
Objective: To identify mutation spectrum of Indian patients with MPS-I and MPS-II.
Patients and Methods: Thirty MPS-I and 37 MPS-II patients with deficient enzyme levels in leukocytes were recruited. Bidirectional Sanger-sequencing of the entire coding region and flanking intronic-splice region was done for *IDUA* gene in 8 MPS-I patients and for *IDS* gene in 31 MPS-II patients. Sequences were analyzed using NCBI/BLAST and UCSC/BLAT.
Results: Four pathogenic variations were identified in four of eight MPS-I patients, each being homozygous for a different nonsense mutation, and 17 pathogenic variations (8 missense, 2 nonsense, 2 splice site variants and 5 frame-shift variants) were identified in 17 of 31 MPS-II patients, all hemizygous for a different mutation. Of these, 1 *IDUA* (nonsense) and 8 *IDS* mutations (2 missense, 1 nonsense and 5 frame-shift) were novel.
Discussion/Conclusion: The mutation spectrum of Indian MPS-I and II patients seem to be different from reported literature. *IDS*/*IDS2* recombination was not ruled out, which could be the main reason for not finding mutations in some patients. Exon-9 appears to be mutational hot spot in MPS-II in Indian patients.

P-515

Ventriculo-peritoneal shunt and hematopoietic stem cell transplantation in Hurler patients

Galimberti C[1], Motta S[1], Gasperini S[1], Parini R[1], Rovelli A[2]

[1]Rare Metab Dis, Ped. Dept, MBBM Foundation, Monza, Italy, [2]Bone Marrow Transplantation Unit, MBBM F, Monza, Italy

Background: Combined enzyme replacement therapy and hematopoietic stem cell transplantation (HSCT) is the standard of care for Hurler syndrome (MPS I-H) patients. They often have hydrocephalus and need ventriculo-peritoneal shunt (VPS) that has been suggested to be placed before HSCT, in order to avoid the risk of infection in immunosuppressed patients.
Case report: Three of 15 children with MPS I-H transplanted at our centre underwent shunting before HSCT because of hydrocephalus at 6, 12 and 16 months of age. They underwent HSCT at the age of 8, 16 and 20 months of age. The first one had 5 febrile episodes considered sepsis after HSCT treated with antibiotics. The VPS was removed and there was no need of reimplantation. The second one had fever before HSCT, a Staphylococcus epidermidis was found in the CSF. He was treated with antibiotics and VPS exteriorization for one month; after that a new VPS was positioned. The post-VPS evolution of the other patient was uneventful. They are now 5, 10 and 11 years old and never showed any other complication.
Conclusion: Although the rate of VPS infections is higher in transplanted patients, in our cohort VPS shunt did not determine untreatable complications and did not affect patients' survival

P-516

First assessment of elosulfase (Vimizin) early access program for a group of 7 Spanish pediatric patients with Morquio A disease

Pintos-Morell G[1], Del Toro-Riera M[2], Blasco-Alonso J[3], Couce-Pico M L[4], Guillén-Navarro E[5], González-Gutiérrez-Solana L[6], O'Callaghan M[7]

[1]Dept Ped, Germans Trias i Pujol Uni Hosp, Badalona, Spain, [2]Neuropediatrics, Univ Hosp Vall d'Hebron, Barcelona, Spain, [3]Div Metab Dis, Univ Hosp Carlos Haya, Málaga, Spain, [4]Div Metab Dis, Univ Hosp Sant Compostela, Santiago de Compostela, Spain, [5]Med Genet Sect, Univ Hosp V Arrixaca, Murcia, Spain, [6]Neuropediatrics, Univ Hosp Niño Jesús, Madrid, Spain, [7]Neuropediatrics, Univ Hosp St Joan deDéu, Barcelona, Spain

Objectives: To analyze the response to elosulfase (Vimizim) treatment in 7 Spanish pediatric patients with Morquio A disease under an individualized early access program (MOR-EAP) sponsored by BioMarin, for a duration of 8 months.
Methods: The selection of the patients to be included in the MOR-EAP was determined by each of the authors who are responsible for different expert centers for lysosomal disorders in Spain. A controlled study design with objective variables, similar to those of the Morquio clinical trial, was established in order to obtain safety and efficacy results.
Results: Age of patients ranged from 8 to 17 years. 7/7 patients showed an improvement in the endurance, as assessed by 6MWT and/or 3MSC. The EQ-5D-5L score at 8 months was better than baseline. Generally, urine GAGs decreased. No severe adverse events related to elosulfase were reported. No patient required surgical intervention during the study period.
Conclusions: Overall, the opportunity of this short experience was considered very positive for patients and physicians. We hope that the report of these results will aid to the availability of Vimizin for the rest of Morquio patients in Spain.
Conflict of Interest declared.

P-517

Neuroimaging findings and cerebrospinal fluid flow study using MR imaging in patients with Mucopolysaccharisosis (MPS)

Mendes C S C[1], Rand M H[1], Curiati M A[1], Feliciano P[1], Aranda C S[1], Martins A M[1]

[1]Universidade Federal de São Paulo, São Paulo, Brazil

Background and objectives: The frequent brain MRI findings in MPSs are dilated perivascular spaces, white matter abnormalities and communicating hydrocephalus (CH). Cerebrospinal fluid (CSF) flow study is a non-invasive imaging technique that is useful for evaluation of patients with hydrocephalus. The aims of this report are to describe the neuroimaging findings and CSF flow study, in MPS patients.
Patients and methods: Retrospective analysis of clinical neurological assessment and CSF flow study by phase-contrast magnetic resonance imaging (PC-MRI), in 11 MPS patients (types I, II, VI).
Results: The findings in neurologic exam were pyramidal signs (9/11, 81,8 %), cognitive imparment (6/11, 54, 5 %), macrocephaly (10/11, 90,9 %) and behavior disturbances (3/11, 27,2 %). Neuroimaging abnormalities: dilated perivascular spaces (7/11, 63,6 %), white matter abnormalities (8/11, 72,7 %), ventriculomegaly (8/11, 72,7%), cortical atrophy (5/11, 45,4 %), craniocervical stenosis (9/11, 81,82%) and abnormalites in CSF flow study (7/11, 63,6%).
Discussion and conclusion: No clinical signs of high intracranial pressure were found in our patients, but CSF flow study showed abnormal flow in a significant number of patients. The brain CSF flow study is an important tool to plan therapy for abnormalities like hydrocephalus and high intracranial pressure, and survey additional information to conventional MRI.
Conflict of Interest declared.

P-518

Aortic tortuosity: A new finding in patients with Mucopolysaccharidosis type IVA (MPS IVA)

Goekce S[1], Tanyildizi Y[2], Mengel K E[1], Kampmann C[3], Mueller-Forell W[2], Hennermann J B[1]

[1]Villa Metabolica, Div Metab Dis, Uni Med, Mainz, Germany, [2]Inst Neurorad Uni Med Center, Mainz, Germany, [3]Div Ped Card, Child Hosp, Uni Med Center, Mainz, Germany

Background: MPS IVA is a lysosomal storage disorder characterized by skeletal dysplasia. Affection of the vascular system has not been described yet. Goal of this study was the examination of the vascular system on the basis of the aorta.
Methods: We analyzed retrospectively the aortic course in 56 craniospinal MRs and 6 CTs in 33 patients with MPS IVA aged 9-49 years (μ 22,5; m 21). Aortal kinking was defined as high buckled arteries in relation to the length of the affected aortal part, aortal coiling as a moderate twist in relation to the length of the affected aortal part.
Results: From a total of 38 patients, 5 could not be analyzed. 14/33 patients had an aortal kinking, 10/33 an aortal coiling, 9/33 a normal aortal course.
Conclusion: This study reveals for the first time the occurrence of aortic tortuosity in patients with MPS IVA. Although the etiology is still unknown, we hypothesize that this may be caused by glycosaminoglycan depositions in the aortic wall, resulting in an increased vulnerability and a rupture of elastic fibers. Examination of the vascular system should be included in regular follow-up protocols of patients with MPS IVA.

P-519

Proteoglycan expression in patients with MPS II and III

Batzios S[1 2], Papakonstantinou E[2], Klagas I[2], Kontopoulos E[1], Vargiami E[1], Zafeiriou D[1]

[1]1st Dep of Paeds, A.U.T.H, Thessaloniki, Greece, [2]Dep of Pharmacology, A.U.T.H, Thessaloniki, Greece

Background: The aim of the present study was the assessment of the expression of proteoglycans (PGs) in patients with mucopolysaccharidoses (MPS) type II and III, with the goal to use those molecules as potential biomarkers for patients' diagnosis and follow-up.
Patients and Methods: The sample included 12 patients with MPS (9 patients with MPS III, 3 with MPS II), and an equal number of sex and age matched healthy subjects. The concentration of circulating PGs was measured by ELISA, while their expression at the genetic level was assessed with Real-Time RT-PCR.
Results: We have detected an increase in the concentration of aggrecan, biglycan, decorin and syndecan -2, -3 and -4, while a reduction in syndecan-1 and versican was noted. Especially in the MPS III patients, syndecan-2 and decorin were significantly increased (p=0.021 and 0.006 respectively). None of the PGs was found to be significantly altered at a genetic level, while also no alterations have been found during the aministration of ERT.

Conclusion: The index study revealed the potential use of multiple PGs as biomarkers for MPS. Yet, confirmation of these findings in a larger population of MPS patients is needed before they can be used in clinical decision making.

P-520

Cardioembolic stroke as a manifestation of Scheie syndrome

Duque C[1], Gouveia A[1], Tomás J[1], Lacerda L[2], Cordeiro G[1], Macário M C[1]

[1]Neurology Dep, Coimbra Univ Hosp, Coimbra, Portugal, [2]Bio Gen Unit, CGMJM, Porto, Portugal

Background: Mucopolysaccharidosis type I (MPS I) is a lysosomal storage disorder caused by a deficiency of the enzyme alpha-L-iduronidase (IDUA). There are three variants, differing widely in their severity, with Scheie syndrome (SS) being the mildest. Manifestations include coarse facial features as well as cardiac valve thickening.
Case report: A 45-year-old male presented to the emergency department due to sudden left-sided weakness. Past medical history included mechanical aortic and mitral valve replacement, corneal distrophy and inguinal and umbilical hernias. He was under acenocumarol (INR 2.93). Neurologic examination revealed mild weakness affecting his left arm and leg and a left superior quadrantanopia. His physical examination showed coarse facial features, clouding of the cornea and flexion contracture of the fingers with the appearance of claw hands. Computed tomography of the brain revealed a lesion consistent with a recent stroke in the right middle cerebral artery. Due to its peculiar characteristics we considered mucopolyssacharidoses and further testing confirmed deficient activity of IDUA – MPS I.
Discussion/Conclusion: Cerebral infarction was previously reported in one Sheie syndrome patient, due to cardiac embolism probably related with GAG accumulation. We report the first case of cardioembolic stroke complicating Scheie syndrome due to subtherapeutic anticoagulation after valve replacement.

P-521

Evaluation of the disease advancement in patients with mucopolysaccharidosis

Opoka-Winiarska V[1], Żuber Z[2]

[1]Medical University of Lublin, Lublin, Poland, [2]St. Louis Children's Hospital, Cracow, Poland

Background: One of the most important manifestations of mucopolysaccharidoses (MPS) are progressive osteoarticular disorders. The evaluation of the disease advancement is difficult due to the complexity of symptoms. A uniform scale has not been developed for these patients.
Objectives: The aim of this study was to use the experience in the evaluation of rheumatic diseases for patients with MPS.
Methods: 17 patients with MPS (6 MPS II, 5 MPS IV, 6 MPS VI) were evaluated. The following parameters were selected: Physician global assessment of disease advancement (PGA), Patient/parent global assessment of well-being (PGE), functional ability (CHAQ), nr of joints with limited movement (LJC) and visual analogue scale for pain (VAS). The disease advancement score (range 0–104) was the linear sum of PGA(1-10)+PGE (1-10)+CHAQ(1-3)+LJC(0-71)+VAS(1-10).
Results: The values of disease advancement scores ranged from 4,4 to 48,6 (MPS II: 7,9-45; MPS IV: 13,7-40,3; MPS VI: 4,4-48,6), including PGA: 1-8, PGE: 0-6, CHAQ: 0-3, LJC: 2-37 and VAS: 0-6.
Conclusion: The described parameters may be applied for assessment of the MPS severity and add valuable information to the physician. With their implementation, the progression of the disease and the effect of the treatment can be assessed and compared.

P-522

Hyaluronic acid metabolism in patients with MPS II and III

Batzios S[1 2], Klagas I[2], Papakonstantinou E[2], Vargiami E[1], Kontopoulos E[1], Zafeiriou D[1]

[1]1st Dep of Paeds, A.U.T.H, Thessaloniki, Greece, [2]Dep of Pharmacology, A.U.T.H, Thessaloniki, Greece

Background: The aim of the present study was the assessment of hyaluronic acid (HA) metabolism in patients with Mucopolysaccharidoses (MPS) type II and III, with the goal to unravel possible aspects of disease aetiopathogenesis.
Patients and Methods: The sample included 12 patients with MPS (9 patients with MPS III, 3 with MPS II), and an equal number of sex and age matched healthy subjects. The concentration of circulating molecules was measured by ELISA, while their expression at the genetic level was assessed with Real-Time RT-PCR. The enzyme activity of hyaluronidases was measured with the use of zymography.

Results: HA levels were significantly increased in all MPS types while also increased were the circulating levels of hyaluronan synthases, and of the receptors CD44 and RHAMM. Hyaluronidase-1 activity was decreased in patients when compared to the control group. No alteration in the concentration of the studied molecules has been noted during ERT. No alterations have been found at a genetic level.
Conclusion: Our study revealed that HA acid metabolism is altered in patients with MPS and the molecules which are involved in it could represent potential biomarkers for the diagnosis of these disorders.

P-523

Pregnancy in a patient with mucopolysaccharidosis type I (MPS I) treated with enzyme replacement therapy: A case report

De Souza C F M[1], SIlva A A[1], Sanseverino M T V[1 2], Magalhaes J A[2], Fagondes S[1], Manica D[1], Vairo F P[1], Barrios P[1], Giugliani R[1]

[1]Medical Genetic Service - HCPA, Porto Alegre, Brazil, [2]Fetal Medical Group, Porto Alegre, Brazil

Introduction: Mucopolysaccharidosis type I (MPS I) is a progressive lysosomal storage disease that results from the deficiency of α-l-iduronidase (IDUA). Enzyme replacement therapy (ERT) with laronidase is the only specific treatment for the disease.
Objectives: To describe the case of a MPS I patient in her first pregnancy, treated with laronidase, every other week, in double dose.
Methods: Chart review and description of gestational and postnatal follow-up data.
Results: The mother is a 28-year-old with Sheie MPS I (genotype R89Q/W402X), no consanguineous couple. The patient remained in ERT every other week and monitored by a multidisciplinary team throughout pregnancy. On physical examination, the patient had height = 136cm. Echocardiogram shows mild to moderate valve insufficiency and espirometry suggest moderate restrictive respiratory disorder. A healthy 1,7kg female was delivered by cesarean section at 31 weeks. There are no complications or congenital malformations. No adverse event related to ERT was reported. The IDUA enzymatic dosage of the baby was normal.
Conclusions: There are still few reports of pregnancies in patients with MPS. No teratogenic effects are expected of ERT. The strict monitoring of patients for possible complications is important and multidisciplinary management was critical to the successful outcome of this case.

P-524

Evaluation of inflammatory markers in mucopolysaccharidosis I and mucopolysaccharidosis VI

Mello A S[1 2], Dorneles G P[2], Breier A C[1], Cé J[1], Peres A[2 3], Coelho J C[1]

[1]Department of Biochemistry, UFRGS, Porto Alegre, Brazil, [2]Methodist University Centre – IPA, Porto Alegre, Brazil, [3]UFCSPA, Porto Alegre, Brazil

Background: Mucopolysaccharidoses (MPS) are a group of lysosomal storage diseases caused by deficiency or lack of a-L-iduronidase, for the catabolism of glycosaminoglycans (GAGs). Changes in inflammatory processes are connected in the development of several rare diseases, however their relationship with the MPS still remain uncertain.
Methods & Results: Plasma analyzes were performed on 14 individuals (MPSI = 7; healthy controls = 7) and 14 individuals leukocyte (MPS VI = 7; healthy controls = 7). Measurement of the cytokines IL-6, IL-10, IL-17, TNF-α, and INF-? (pg/ml) was performed by ELISA. The TNF-α values ??were lower in MPS VI patients than in controls ($p = 0.05$), whereas they were significantly increased in patients with MPS I compared to controls ($p = 0.04$). For IL17, a significant difference was found ($p = 0.05$) only in the patient samples from MPS VI, showing increased levels compared to healthy controls, showing a pattern inversely proportional to TNF-α in this disease. The other analyzed parameters showed no significant differences in both analyzed diseases.
Conclusion: The results obtained in TNF-α levels in individuals with MPS I and MPS VI compared to controls, suggest that this cytokine may be a potential inflammatory marker for monitoring disease.

P-737

Three-plex MS/MS method to measure MPS II, MPS IVA and MPS VI enzyme activities in dried blood spots

Potier A[1], Cournoyer J[1], Trometer J[1], Rehnberg J[2], Kuracina M[1], Schermer M[1], Gelb M [3]

[1]PerkinElmer, Waltham, United States, [2]PerkinElmer, Turku, Finland, [3]University of Washington, Seattle, United States

The mucopolysaccharidoses (MPS) family of lysosomal storage disorders (LSDs) is caused by defects in the metabolic breakdown of glycosaminoglycans (GAGs). With enzyme

replacement therapies available for MPS I (IDUA), MPS II (I2S), MPS IVA (GALNS) and MPS VI (ARSB) there is an increasing interest for reliable methods that can effectively distinguish samples with low enzyme activities. Previous work demonstrated a new six-plex FIA-MS/MS assay that includes the measurement of IDUA. Additionally, the enzyme activities of ID2S, GALNS and ARSB can now be measured with a single 3.2 mm dried blood spot (DBS) punch and one cocktail. This three-plex is incubated overnight at 37 °C followed by a post-incubation workup that is less than 30 minutes per plate. Sample-to-sample time using MS/MS analysis is only two minutes, which allows the possibility to obtain more than 2000 results per day if desired. Method performance studies show good linearity for each enzyme in their respective activity range. Furthermore, a study consisting of presumed healthy neonates (n=1000), confirmed low I2S/GALNS/ARSB activity and CDC control DBS showed excellent resolution and a clear distinction between the different enzyme activity levels.

P-738

New MS/MS method to measure MPS II enzyme activity in dried blood spots

Trometer J[1], Potier A[1], Cournoyer J[1], Kuracina M[1], Rehnberg J[2], Schermer M[1], Gelb M[3]

[1]PerkinElmer, Waltham, United States, [2]PerkinElmer, Turku, Finland, [3]University of Washington, Seattle, United States

The mucopolysaccharidoses (MPS) family of lysosomal storage disorders (LSDs) is caused by defects in the metabolic breakdown of glycosaminoglycans (GAGs). More specifically, MPS II, or Hunter Syndrome, is an X-linked disorder caused by a deficiency in the activity of the enzyme iduronate-2-sulfatase (I2S). Deficient I2S activity results in reduced degradation of heparan sulfate and dermatan sulfate. A new tandem mass spectroscopy (MS/MS) method has been developed to measure the enzyme activity of I2S in dried blood spots (DBS). This approach uses a substrate that is similar in structure to the natural substrate and an isotope labeled internal standard designed to match the resulting product from the assay. A recent 2-day study using presumed healthy neonates (n=875) showed an average blood-to-filter paper ratio of 135. Confirmed low I2S activity DBS (n=14) presented enzyme activities below 10% of the average presumed healthy neonate activity. Good precision in the assay was demonstrated using three levels of control DBS from the Center of Disease Control (CDC) (n=20 per level). The %CV for CDC Low/Mid/High control DBS were 5%, 6% and 6%, respectively.

23. Lysosomal disorders: sphingolipidoses

P-525

Changes in antioxidant enzymes and DNA damage in peripheral blood of patients with Gaucher disease type I treated with enzyme replacement therapy

Turcatel E[1], Schweinberger B M[1], Rodrigues A F[1], Trindade C[1], Henriques J A P[1], Coelho J C[1], Wyse A T S[1]

[1]UFRGS, Porto Alegre, RS, Brazil

Gaucher disease (GD) is the main lysosomal storage disease, caused by a mutation in the gene encoding β-glucosidase, which leads to glucosylceramide accumulation in the lysosomes of the reticulo endothelial system (macrophages and monocytes), affecting organs such as liver, spleen and bone marrow. The enzyme replacement therapy (ERT) has greatly improved the clinical outcome, especially in GD type I patients. In this sense our objective was to evaluate the activities of antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) in plasma and erythrocytes, as well as DNA damage by comet assay in whole blood. Patients were divided into two groups: patients with negative GD diagnosis (controls, N=10) and patients diagnosed GD (patients, N=10). Peripheral blood samples were collected, before ERT infusion, when the β-glucosidase levels were low. We observed a reduction of superoxide dismutase and an increase in glutathione peroxidase activities in erythrocytes and plasma, respectively. Catalase activity was not altered. We also observed DNA damage in whole blood. Our findings suggest that the increase in superoxide caused by the reduction in superoxide dismutase activity may be, at least in part, responsible for the DNA damage, which could be associated with pathophysiology of GD. Supported by CNPQ

P-526

Patient with Niemann-Pick type C presenting with lymphatic involvement with Niemann-Pick cells in the left jaw

Inci A[1], Okur I[1], Esendaglı G[2], Okur A[3], Ezgu F S[1], Tumer L[1]

[1]Div Pediatr Metab Dis, Gazi Univ Hosp, Ankara, Turkey, [2]Dept Pathol, Gazi Univ Hosp, Ankara, Turkey, [3]Div Pediatr Haematol Dis, Gazi Univ Hosp, Ankara, Turkey

Background: Niemann-Pick C disease (NPC) is an autosomal recessive lipidosis resulting in lysosomal accumulation of non-esterified cholesterol and complex lipids especially in liver, spleen, lung and central nervous system.

Methods: An 8 year old girl with NPC disease was admitted to our department with swelling of her left jaw for 2 weeks. She had 3x3 cm painless, fixed and rigid swelling on the left angular mandibular region. There were no supraclavicular, cervical, occipital, axillar or inguinal lymphadenopathy. She had hepatosplenomegaly with liver 2 cm and spleen 4 cm palpable. Other systems were normal. Haemogram, peripheral blood smear, biochemical and microbiological evaluation revealed no abnormality.
Results: Superficial ultrasonography revealed heterogeneous hypoechogenic hard consistency lymphatic nodules in parotid tail with 8x11 mm dimension. Lymph node biopsy showed lipid-laden phagocytic cells consistent with NPC.
Conclusion: Our case is important as it has been the first case of mandibular lymph node involvement with NPC cells.

P-527

Clinical characterization of Korean Gaucher patients

Ko J M[2], Yoo H W[1]

[1]Ped & Med Genet, Asan Med Ctr, Ulsan Uni, Seoul, Korea, Republic of, [2]Ped, Seoul Natl Univ Child Hosp, Seoul, Korea, Republic of

Background & Objectives: Gaucher disease (GD) is quite rare in Korea and only about 80 patients have been diagnosed to date. Their clinical and molecular characteristics including bone manifestations were analyzed.
Methods: Nation-wide survey was conducted by sending questionnaire to experts of GD. Clinical data, genotype and bone imaging study results were obtained and critically reviewed.
Results: Seventy-one Korean GD patients from 66 families were included in this study. About only 40% of patients were non-neuronopathic, while the others were neuronopathic (among them, 1/3 acute, 2/3 chronic). The mean age at diagnosis was 10.6 years. Most patients presented with hepatosplenomegaly, thrombocytopenia, and variable neurological features including seizures, tremor, and gaze palsy in the neuronopathic group. The B cell lymphoma, protein-losing enteropathy, and hydrops fetalis were uncommon manifestations. Abnormal bone remodeling, osteonecrosis and bony infarcts, osteopenia with fractures, and osteomyelitis were demonstrated in most patients. p.L444P (21.9%) was the most common mutation. Thirty-nine patients have been on ERT. Chitotriosidase is not a good biomarker for Korean patients due to high null allele frequency (40%) of *CHITI*.
Conclusion: While the clinical outcome for GD has improved remarkably following ERT, there still remain unmet needs with co-morbidities due to the preponderance of neuronopathic type.

P-528

A multicenter, open-label phase III study to evaluate the efficacy of biosimilar product of imiglucerase in patients with type 1 Gaucher disease

Beshlawy A[2], FathyAbdalla A[3], Yoo H W[1]

[1]Ped & Med Genet, Asan Med Ctr, Ulsan Uni, Seoul, Korea, Republic of, [2]Abou EI Reesh Child Hosp, Cairo Univ, Cairo, Egypt, [3]Ped Masoura Univ Child Hosp, Mansoura, Egypt

Background and objective: Gaucher disease (GD) is an autosomal recessive inborn error of metabolism due to a deficiency of the lysosomal enzyme β-glucocerebrosidase. This study was aimed to evaluate the efficacy and safety of a product biosimilar to imiglucerase in type I GD patients.
Methods: The study was designed to administer a biosimilar product of imiglucerase (which was produced by CHO cell-based expression system and its structural, immunological and biological properties were well characterized by pre-clinical and phase I, II clinical studies) at a dose of 60 U/kg/eow for 6 months in treatment-naïve GD patients. The primary endpoint was the increment of hemoglobin concentration. The secondary endpoints were change of platelet counts, visceral volumes, biomarker levels, skeletal changes compared to the baseline.
Results: Eight patients completed the study. The mean change of hemoglobin level was 1.96±0.35 g/dL (p-value=0.0013). Also, statistically significant increase in platelet count, decrease in spleen volume, biomarker levels also indicated the efficacy. No drug related adverse event was observed. One patient had anti-imiglucerase antibodies without neutralizing activity.
Conclusions: The above results demonstrated the efficacy and safety of the biosimilar product to imiglucerase in patients with Type 1 GD. Accordingly it can be used as ERT in Type 1 GD patients.
Conflict of Interest declared.

P-529

Long-term efficacy of enzyme replacement therapy (ERT) for Fabry disease: Experience of single institution

Kim J H[2], Cho J H[1], Lee B H[1], Choi J H[1], Yoo H W[1]

[1]Ped & Med Genet, Asan Med Ctr, Ulsan Uni, Seoul, Korea, Republic of, [2]Ped, Hanyang Univ Hosp, Guri, Korea, Republic of

Background: ERT in Fabry disease is efficacious in alleviating neuropathic pain and sweating problems as well as improving

or stabilizing the renal and cardiac functions. This study aimed at evaluating the long-term ERT efficacy.
Methods: This study included 18 patients with Fabry disease (10 male adults, 4 male pediatric and 4 symptomatic female patients), who had been on Fabrazyme for longer than 5 years. Their medical records were reviewed retrospectively.
Results: Their median age at ERT and duration of treatment were 31.2 and 8.7 years. Mean annual eGFR reduction rate was -1.6 ± 1.7 (n = 4), -5.9 ± 7.7 (n = 2), and -6.6 ± 6.3 mL/min/1.73m^2/yr (n = 4) in adult males with 24hrs urine protein < 0.1, 0.1–1, and >1 gm, respectively. Left ventricular mass index decreased throughout the ERT in male patients with LVH (-2.0 ± 1.6 g/m$^{2.7}$/yr, n = 4) and remained stable in patients without LVH. The renal and cardiac functions remained stable in female and pediatric patients.
Conclusion: Progressive deterioration of eGFR was observed in adult male patients with overt proteinuria, not in pediatric or female patients. These results indicate that earlier diagnosis is critical for the prevention of irreversible damage to vital organs.

P-530

The need for disease-specific patient-reported outcome measures for lysosomal storage disorders

Elstein D[2], Weinreb N[7], Belmatoug N[6], Schwartz I[3], Deegan P[4], Renault L[1], Goker-Alpan O[5]

[1]Shire, Zug, Switzerland, [2]Shaare Zedek Med Cent & Hadassah Med Sch, Jerusalem, Israel, [3]Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil, [4]Addenbrooke's Hospital, Cambridge, United Kingdom, [5]LSD Unit & Cent Clin Trials, O&O Alpan, Fairfax, United States, [6]Hôpitaux Univ Paris Nord Val de Seine, Clichy, France, [7]Univ Research Foundation for LSDs, Coral Springs, United States

Background: Patient-reported outcome (PRO) measures enable direct health-status reporting by patients without third-party interpretation. They are useful and important in rare disease research, in clinical trials and surveillance registries, but generic PRO and health-related quality of life instruments have several limitations when applied to lysosomal storage disorders (LSDs). Each of the LSDs is in itself and all LSDs collectively are a phenotypically heterogeneous group of diseases. Disease-specific treatment options are not available for some LSDs or uniformly effective for all types of LSDs; generic instruments may miss disease-specific aspects. The solution may be to develop a basic PRO template that is customizable for each LSD. We are validating a Gaucher disease-specific PRO questionnaire.
Methods: We suggest combining some salient questions from generic instruments (e.g., mortality) with disease-specific features (e.g., risk of disease progression) but also impact of disease-specific and non-disease-specific interventions, which would be customized for each disease, and finally concerns of psycho-social functioning.
Conclusions: There is a need for disease-specific PROs that reflect the individual characteristics of each LSD and the phenotypic disease variance. The development and validation of a series of LSD-specific PROs may enable us to capture the patient experience better than using generic instruments.
Conflict of Interest declared.

P-531

Insight into the pre-diagnosis period of patients with Gaucher disease: results of a physician survey

Mehta A[9], Belmatoug N[8], Bembi B[7], Deegan P[4], Elstein D[2], Goker-Alpan O[5], Lukina E[6], Mengel E[11], Nakamura K[12], Pastores G M[10], Pérez López J[13], Schwartz I[3], Serratrice C[14], Szer J[15], Zimran A[2], Panahloo Z[1], Hughes D[9]

[1]Shire, Zug, Switzerland, [2]Shaare Zedek Med Cent & Hadassah Med Sch, Jerusalem, Israel, [3]Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil, [4]Addenbrooke's Hospital, Cambridge, United Kingdom, [5]LSD Unit & Cent Clin Trials, O&O Alpan, Fairfax, United States, [6]Hematology Research Center, Moscow, Russian Federation, [7]Regional Coord Cent Rare Dis, Univ Hosp, Udine, Italy, [8]Hôpitaux Univ Paris Nord Val de Seine, Clichy, France, [9]Royal Free Hospital, London, United Kingdom, [10]Mater Misericordiae University Hospital, Dublin, Ireland, [11]University Medical Center, Mainz, Germany, [12]Kumamoto University Hospital, Kumamoto, Japan, [13]Vall d'Hebron Hospital, Barcelona, Spain, [14]Foundation Hospital Saint Joseph, Marseille, France, [15]Royal Melbourne Hospital, Parkville, Australia

Objective: To examine the pre-diagnosis period of patients with Gaucher disease (GD).
Methods: Experts in lysosomal storage disorders (LSDs) completed a survey to assess earlier diagnostic trends in GD in preparation for a scientific meeting (supported by Shire pharmaceuticals). Experts provided estimated responses to 9 questions on the pre-diagnosis journeys of their GD patients.
Results: 17 experts from 12 countries participated. Pooled responses represent 798 GD patients, 89% with GD1. Haematology/haem-oncology, paediatrics, and primary care were the most cited specialties to which patients first presented with GD-related symptoms. These were also the main specialties that referred patients to the LSD experts. Internal/general medicine, gastroenterology and hepatology were specialties less frequently involved in referring patients. Splenomegaly was the most frequent clinical feature present at

diagnosis, followed by thrombocytopenia and anaemia. As the main cause of diagnostic delay, 8/12 experts stated lack of awareness of GD or misdiagnosis; 4/12 stated phenotypic heterogeneity, non-specific clinical presentation or mild symptomology; and 1/12 stated outsourced testing.
Conclusion: Trends from a physician survey suggest that haematology/haem-oncology is the main specialty to which GD patients first present, and splenomegaly is the main presenting feature at GD diagnosis. There is a need to increase awareness of LSDs across a range of specialties.
Conflict of Interest declared.

P-532

Insight into the pre-diagnosis period of patients with Gaucher disease: results of the OnePath® US patient survey

Pastores G M[3], Panahloo Z[1], Mehta A[2]

[1]Shire, Zug, Switzerland, [2]Royal Free Hospital, London, United Kingdom, [3]Mater Misericordiae University Hospital, Dublin, Ireland

Objective: To examine the pre-diagnosis period of patients with Type 1 Gaucher disease (GD1).
Methods: Consenting GD1 patients registered in the US OnePath® patient support system completed a survey with 13 questions related to their pre-diagnosis journey.
Results: 212/576 invited patients responded. An enlarged stomach/abdomen was cited most as the first symptom or sign of GD that patients recall experiencing, although an abnormal blood test result was the most cited final GD-related problem that prompted patients to seek further medical advice. Paediatrics, haematology/haem-oncology and primary care were the most cited specialties to which patients first presented regarding their health problems, with haematology/haem-oncology being the main specialty to make the final GD diagnosis (70/116 patients, 60%). 78/208 patients (38%) were diagnosed before the age of 10 years. Most patients (112/154, 73%) were diagnosed ≤1 year after first seeing a doctor, but for others (22/154, 14%), diagnosis took ≥7 years. Commenting on their path to diagnosis, many patients described experiences with physicians lacking awareness of GD and previous misdiagnoses.
Conclusion: An enlarged stomach/abdomen was the most common first GD-related problem. Haematology/haem-oncology was the main specialty involved in making a GD diagnosis, although it could take more than 7 years from earliest symptoms to eventual diagnosis.
Conflict of Interest declared.

P-533

Plasma and urinary levels of glycosphingolipids in cardiac variant (*N215S*) Fabry patients

Alharbi F J H[1], Ward D G[1], GeberHiwot T[2]

[1]School Cancer Scien- Univ of Birmingham, Birmingham, United Kingdom, [2]Dep Endocrinology, Queen Elizabeth Hosp, Birmingham, United Kingdom

Introduction: Fabry disease is an X-linked lysosomal storage disease caused by defective α-galactosidase A. This defect leads to accumulation of glycosphingolipids in various organs and body fluids. Globotriaosylceramide (Gb3), globotriaosylsphingosine (lyso-Gb3) and related isoforms/analogues have been investigated as biomarkers for Fabry disease severity and treatment efficacy. We now report the levels of glycosphingolipids in the plasma and urine of cardiac variant patients with the *N215S* mutation.
Methods: Plasma and urine were collected from 40 cardiac variant Fabry patients, 73 classical Fabry patients and 34 age/gender matched healthy controls. Lyso-Gb3 and 8 related analogues were enriched by solid phase extraction and analysed by LC-MS/MS. Urinary Gb3 was measured using a novel rapid method utilising liquid-liquid extraction and MALDI-TOF-MS.
Results: Plasma and urine levels of lyso-Gb3 and several of its analogues were significantly higher in *N215S* male patients than in healthy male controls but significantly lower than in classical male Fabry patients. These glycosphingolipids were also significantly elevated in the plasma of *N215S* female patients but did not reach statistical significance in urine. Urinary Gb3 was not significantly elevated in *N215S* patients.
Discussion: This study shows that plasma levels of lyso-Gb3 and its analogues may prove useful in monitoring cardiac variant Fabry disease.

P-534

Prenatal diagnosis of Gaucher disease using next generation sequencing technology

Yoshida S[1 3], Nakamura K[1], Matsumoto S[1], Mitsubuchi H[1], Shimazu T[2], Sugawara K[3], Endo F[1]

[1]Dep Ped, Grad Schl of Med,Univ Kumamoto, Kumamoto, Japan, [2]Kumamoto Saishunso National Hospital, Kumamoto, Japan, [3]Kaketsuken, Kumamoto, Japan

Background and purpose: Gaucher disease (GD) shows severe progressive neurological manifestations. To analyze genetic

information of the family for prenatal diagnosis quickly, the next-generation sequencer (NGS) was used.
Methods: Long-range PCR was performed to amplify the 13.6kb region which contains glucocerebrosidase gene *GBA* and upstream and downstream regions of *GBA*. Using these PCR products NGS gene analysis was performed.
Results: The father has one mutation (IVS2+1G>A), the mother has two mutations (I(-20)V), M85T) in the *GBA* gene. The GD patient (Child 1) has all of these mutations, the fetus (child 3) has none of these mutations. IVS2+1G>A was reported as a disease-related mutation which causes exon skipping. I(-20)V was a novel mutation. M85T was reported, but there was not a detailed clinical report.
Discussion: Child 3 was able to be diagnosed as not having the GD-related mutations found in Child1. The combination of IVS2+1G>A and I(-20)V or M85T probably cause disease as compound heterozygosity.
Conclusions: Using NGS we could provide genetic analysis data of four members in about 1 week (DNA amplification - genetic analysis) without prior genetic information of the family. In comparison with conventional methods, this method has usefulness in time and amount of information.
Conflict of Interest declared.

P-535

Pharmacological chaperone-mediated reduction of glucosylsphingosine in a Gaucher mouse model

Clark S W[1], Pellegrino L[1], Dungan L[1], Hamler R[1], Sanders A[2], Kaspieva O[2], Clarke L[2]

[1]Amicus Therapeutics, Cranbury, United States, [2]Child and Fam Res Inst, U Brit Columbia, Vancouver, Canada

Pharmacological chaperones (PCs) are highly specific small molecules that bind and stabilize their target protein, leading to increased enzyme activity in the proper cellular compartment. For diseases such as Gaucher, reduction of enzyme activity and concomitant elevation of the enzyme substrate is often due to a change in the protein arising from a single missense mutation. The enzyme affected in Gaucher disease, glucocerebrosidase (GCase), may be misfolded and/or unstable, resulting in degradation by the ER quality control system and reduced trafficking to its lysosomal site of action. Decreased GCase activity leads to accumulation of its substrates, glucosylceramide and glucosylsphingosine (GlcSph). Mouse models expressing the human L444P or N370S variants of GCase have been shown to accumulate GlcSph. We orally administered PCs specific for GCase to this model for up to two months, substantially elevating the tissue activity of both L444P and N370S GCase. A range of administration regimens and doses were explored that decreased the accumulation of GlcSph in several disease-relevant tissues, with reductions of more than 50%, 40%, and 30% seen in spleen, liver, and bone marrow, respectively. Taken together, these data suggest that orally-administered PCs may provide an alternative to ERT to reduce accumulated substrate in Gaucher disease.
Conflict of Interest declared.

P-536

Miglustat treatment in an early infantile form of GM1 gangliosidosis

Erdöl S[1], Dorum S[1], Saglam H[1]

[1]Uludag Univ, Div Metab Dis, Bursa, Turkey

Background: GM1 gangliosidosis is a sphingolipid metabolism disorder due to lysosomal acid β-galactosidase deficiency. In the early infantile forms, rapid neurological regression, seizures and difficulty in swallowing are seen after age one year and patients often die before age two years.
Case Report: Seizures started at six months of age, with an increased seizure frequency over time, and upon the development of progressive hypotonia and neurological regression, miglustat 100 mg/day was started in a 15-month-old patient with early infantile form GM1 gangliosidosis following consent from the family. One month after treatment, a reduction of 3 and 3.5 cm in liver and spleen size was observed on palpation, respectively. Urinary oligosaccharide chromatography revealed a slight decrease in the intensity of the GM1 oligosaccharide pattern. Four months after treatment, MRI showed an increase in hypomyelination compared to the pre-treatment examination. Despite miglustat treatment, neurological deterioration, seizures, edema, and proteinuria did not improve, and the patient died of pneumonia and sepsis at age of 2.5-years.
Conclusion: Although improvements in hepatosplenomegaly and the reduced excretion of urinary oligosaccharides were observed following miglustat treatment, neurological deterioration continued and the patient died of pneumonia and sepsis.

P-537

A rapidly progressive neurodegeneration case: Gaucher type 2 disease

Kilic M[1], Eski A[2], Aydin H I[3]

[1]Div Metab Dis, Dr Sami Ulus Child Hosp, Ankara, Turkey, [2]Dr. Sami Ulus Child Hosp, Ankara, Turkey, [3]Div Met Dis, Turgut Ozal Univ Hosp, Ankara, Turkey

Background and objectives: Gaucher disease, the inherited deficiency of the enzyme glucocerebrosidase, is the most common lysosomal storage disorder. Type 2 Gaucher disease, the most severe and progressive form, manifests either prenatally or in the first months of life, followed by death within the first years of life.
Case Report: A15 months old male patient was referred to our hospital with complaints of throwing back his head and developmental delay. His symptoms began five months ago. Parents had first degree cousin marriage and there was a history of sibling death with jaundice and hepatosplenomegaly at three months old. Physical examination revealed head retroflexion, hepatosplenomegaly, spasticity of hands and feet, and bilateral hip flexion contractures. Acute neuronopathic Gaucher (Gaucher type 2) disease was suspected and ß-glucocerebrosidase activity: 0.3 mmol/L/h (> 3.2) was significantly low. Molecular genetic analysis showed a mutation p.N370S in one allele and deletion of 55 bases (including D409H, L444P, A456P, V460V) in the other allele of exon 9.
Conclusion: Gaucher type 2 disease should be considered in differential diagnosis of patients with retroflexion, hepatosplenomegaly and neurodegeneration.

P-538

Awareness study of Gaucher disease from southeast part of Turkey

Bulut D[1], Kor D[1], Seker Yilmaz B[1], Ozbek M N[2], Onenli Mungan N[1]

[1]Div Ped Metab, Cukurova University, Adana, Turkey, [2]Div Metab Dis, Diyarbakir Child Hosp, Diyarbakir, Turkey

Background: Gaucher Disease type-1(GD1) is a frequent lysosomal storage disease, presenting with multiorgan involvement.
Methods: Our clinic is the only metabolic center in Southeastern Turkey. Analysis of awareness is evaluated by reviewing the patients' records retrospectively. Both pediatric and adult patients are included in the study.
Results: Mean age of complaints, admission, diagnosis and initiation of ERT was 93,8±3,8; 116,4±4,7; 122,4±2,8 and 132,4±4,99 months, respectively. Mean time between complaints and diagnosis, diagnosis and ERT was 29,6±1,7 and 12,8±1,1 months, respectively. 66% patients had family history. First admission of patients was to pediatricians (59,1%), hematologists (14,2%), pediatric metabolism specialists (12, 2%), internists (4%), family physicians (2%), pediatric endocrinologists (2%), and others (6,1%). Patients were referred with hepatomegaly (26%), abdominal distension (17,4%), growth retardation(17,4%), hematologic abnormalities (13%), splenomegaly (8,7%), hepatosplenomegaly (8,7%) and epistaxis (4,3%). 29 patients had visceral-hematologic, 10 had visceral-hematologic and skeletal, 9 had visceral, one had visceral-skeletal and one had hematologic findings at diagnosis. 22 patients had undergone bone marrow aspiration before diagnosis.
Discussion: This study is one of the largest cohort of GD1 from Turkey. As early diagnosis and ERT administration decreases the severe-irreversible complications, also enzyme assay and mutation analysis are reliable and non-invasive; increasing the awareness is important.

P-539

Cornea verticillata in the first reported Cypriot female with Anderson-Fabry disease

Georgiou T[1], Mavrikiou G[1], Spanou-Aristidou E[2], Krasia M[3], Christophidou-Anastasiadou V[2], Tanteles G A[2]

[1]Biochem Genet Dept, Cyprus Inst Neur Gen, Nicosia, Cyprus, [2]Clinical Genet Dep, Cyprus Inst Neur Gen, Nicosia, Cyprus, [3]Ophthalmology Clinic, Nicosia, Cyprus

Anderson-Fabry disease is an X-linked lysosomal storage disorder resulting from a deficiency of the hydrolytic enzyme α-galactosidase A (α-Gal A). It is characterized by progressive lysosomal accumulation of globotriaosylceramide (Gb3) and multisystem pathology, affecting the skin, nervous and cerebrovascular systems, kidneys, and heart.Heterozygous females typically exhibit milder symptoms at a later age of onset than males. Rarely, they may be relatively asymptomatic throughout a normal life span or may have symptoms as severe as those observed in males with the classic phenotype.
We report on a 17 year old female with a history of cornea verticillata and no other clinical symptoms. The α-galactosidase activity in her leucocytes was in the overlap range between Fabry heterozygotes and normal controls. Sanger sequencing of the *GLA* gene failed to reveal any pathogenic variants. Multiplex Ligation-dependent Probe Amplification (MLPA) analysis revealed a deletion of exon 7. Using a long-range PCR walking approach we managed to identify the deletion breakpoints. The deletion spans 1182bp, with its 5' end located within exon 6 of the *GLA* gene and its 3' end located 612bp downstream of exon 7. This finding represents a novel deletion identified in the first reported female with Anderson-Fabry disease of Cypriot descent.

P-540

A new HPLC/MS-MS assay for quantification of total plasma glucosylsphingosine in Gaucher disease

Polo G[1], Furlan F[1], Del Rizzo M[1], Celato A[1], Giordano L[1], Burlina A P[2], Burlina A B[1]

[1]Div Metab Dis, Dep Ped, Padua Univ Hosp, Padua, Italy, [2]Neurol Unit, St. Bassiano Hospital, Bassano del Grappa, Italy

Background: Glucosylsphingosine (GlcSph), a deacylated form of glucosylceramide, has been found markedly elevated in patients with Gaucher disease (GD). The available methods are not able to resolve GlcSph from psychosine, the biomarker of Krabbe disease.
Objective: To set up a rapid plasma HPLC/MS-MS assay in order to quantify the total GlcSph from the interference of psychosine.
Methods: Plasma processed samples were injected into a normal phase UPLC column and monitored by ESI/MS-MS (5 minutes run time).
Results: The method was fully validated: precision across 3 levels QC samples for intra and inter day batch was < 10%; linearity r2≥0.998, recovery ≥87 % and LLOQ 0.3 nmol/l. The plasma GlcSph reference value in normal subjects were 1.12 and 3.00 nmol/L (2.5th-97.5th percentile). In confirmed GD patients (n=8) GlcSph was significantly increased (median 360, range 73-733 nmol/L). We analyzed 12 patients in whom GD was suspected and we found high level of GlcSph (range 360-734 nmol/L) in 3 patients, confirmed as GD by molecular analysis. No psychosine was detected.
Conclusion: GlcSph is a reliable biomarker for diagnosis of GD patients. Our method allowing to separate GlcSph from psychosine is a new and rapid assay for definitive diagnosis.

P-541

Optimizing molecular detection of large *GLA* gene deletions in Fabry patients

Ferri L[1 2], Cavicchi C[1], Fiumara A[3], Parini R[4], Pantaleo M[5], Giglio S[5], Guerrini R[1 2], Morrone A[1 2]

[1]Clin Paed Neurol & Lab, Meyer Child Hosp, Florence, Italy, [2]Dept NEUROFARBA, Univ of Florence, Florence, Italy, [3]Dept of Paed, Univ Catania, Catania, Italy, [4]Metab Unit, San Gerardo Hosp, Monza, Italy, [5]Med Genet Unit, Meyer Child Hosp, Florence, Italy

Background: X-linked Fabry disease (FD) is caused by enzyme deficiency of lysosomal alpha-galactosidase A (α-GAL A) due to mutations in the *GLA* gene. The heterozygosity of the X-chromosome in females makes it important to combine routine sequence analysis with an allelic dosage assay. MLPA is the most commonly used technique in FD for this purpose.
Results: We identified two new large deletions involving *GLA* gene not properly detected by MLPA. CASE 1: a male in whom PCR analysis indicated a deletion of *GLA* exon 7. In contrast, MLPA did not confirm such result. We applied a Quantitative-Fluorescent PCR assay which definitively confirmed exon 7 deletion in the proband and excluded it in his mother (*de novo* mutation). CASE 2: a male proband in whom PCR amplification detected a deletion of *GLA* exons 5 and 6, while MLPA indicated deletion of exon 5 only. By Long-Range-PCR (LR-PCR) we characterized the breakpoint confirming a large deletion involving both exons 5 and 6. LR-PCR allowed also to detect this *GLA* mutation in proband's mother and in his brother.
Conclusion: A combination of different allele dosage assays is mandatory to properly detect large *GLA* gene rearrangements in order to optimize molecular confirmation of FD.

P-542

Clinical spectrum of Farber disease with 4 novel acid ceramidase mutations in the Iranian population

Hadipour F[1 2], Shafaghati Y[1 2], Levade T[3], Rolfs A[4], Tavasoli A[5], Hadipour Z[1 2]

[1]Sarem Fertility & Infertility Research C, Tehran, Iran, Islamic Republic of, [2]Med Gen Dep, Sarem Cell research Center, Tehran, Iran, Islamic Republic of, [3]Laboratoire de BiochimieMétabolique,, Toulouse, France, [4]Albrecht-Kossel-Institute for Neurore, Rostock, Germany, [5]Children Medical Center, Tehran Universi, Tehran, Iran, Islamic Republic of

Background: Farber disease (FD, OMIM 228000) is a rare lysosomal disorder caused by deficient activity of acid ceramidase. Less than 100 cases have been reported. FD is classically characterized by a triad of painful and swollen joints, subcutaneous nodules, and laryngeal involvement.
Methods: Clinical data on 10 Iranian patients (4 males, 6 females, age range: 1 month to 5 years) with diagnosis of FD were reviewed. Diagnosis was confirmed by molecular analysis of the *ASAH1* gene.
Results: Manifestations in our patients with FD ranged from the most severe form with neonatal onset to the early infantile onset variant. Consanguinity of parents were documented in all cases. The main clinical findings were painful swelling of the joints,

palpable subcutaneous nodules, and a hoarse cry. Abnormalities were first noted at 2 weeks to 4 months of age. Progressive impairment of psychomotor development was seen in half of those cases. Macular cherry red spot may be present. Four novel mutations were found.
Conclusion: Most of our patients had overlapping features of joint manifestations, evidence of myopathy and significant hepatosplenomegaly and neonatal onset. It indicates the severity of disease in our cases. For Farber disease there is no efficient treatment, so, with precise diagnosis in hand, we can prevent recurrence of the disease.

P-543

Clinical spectrum of 13 Iranian families with GM1-gangliosidosis with 4 novel mutations in *GLB1* gene

Hadipour F[1 2], Shafaghati Y[1 2], Sarkhail P[1 2], Vertinjien F W[3], Hadipour Z[1 2]

[1]Sarem Fertility & Infertility Research C, Tehran, Iran, Islamic Republic of, [2]Med Gen Dep, Sarem Cell research Center, Tehran, Iran, Islamic Republic of, [3]Universitair Medisch Center Rotterdam,Er, Rotterdam, Netherlands

Background: GM1-gangliosidosis is a very rare autosomal recessive disorder. It is caused by deficiency of the lysosomal enzyme ganglioside-β-galactosidase that results in accumulation of glycosaminoglycans, oligosaccharides, and specially GM1-ganglioside.
Materials & Methods: Herein, we report the clinical and laboratory findings of 13 Iranian consanguineous families with 35 affected cases. In the probands, enzyme assay showed that β-galactosidase activity was deficient in all of them.
Results: Measurements of the activity of the lysosomal enzyme β-galactosidase was deficient in all of the probands. Sphingomyelinase and other lysosomal enzyme activities were within normal limits. In seven families mutation analysis has been done and six homozygous mutations have been detected. Three of these mutations have not been reported in the relevant literature.
Conclusion: According to enzyme assay and molecular analysis, diagnosis in these families was GM1-gangliosidosis. Because most of the lysosomal storage disorders present with similar symptoms and course, enzymatic assay and mutation analysis are essential for confirmation of clinical diagnosis. For GM1-gangliosidosis there is no efficient treatment. With precise diagnosis in hand, we can provide informative genetic counseling, prenatal diagnosis, and prevent recurrence of the disease.

P-544

Alterations of the *GBA* gene in 120 Russian patients with Gaucher disease

Savostyanov K V[1], Pushkov A A[1], Gevorkyan A K[1], Gundobina O S[1], Lukina E A[2], Lukina K A[2], Movsisyan G B[1], Namazova-Baranova L S[1], Baranov A A[1]

[1]Scientific Centre of Children Health, Moscow, Russian Federation, [2]Div Orph Dis, Hematology Research Center, Moscow, Russian Federation

Background: Gaucher disease (GD) is the most common autosomal recessive glycolipid storage disorder resulting from glucocerebrosidase deficiency due to mutations in the gene *GBA*,coding for this enzyme.
Methods: Study was performed in 120 unrelated patients with low β-glucocerebrosidase activity in leukocytes. Among the patients studied was 89 children aged from 7 months to 18 years, representing more than 95% of sick children from the Federal Gaucher's Register. The exons and exon-intron boundaries of the *GBA* gene were bidirectionally sequenced using an automated sequencer.
Results: We identified twelve novel missense mutations, two novel nonsense mutations: *p.Tyr61X*, *p.Tyr244X* and two novel deletions: *p.Ile158_Pro161del*, *p.Glu112Valfs*32*. *p.Asn409Ser* was the most common mutant allele identified in 116/240 (48,3%) allelic variants of the *GBA* gene non-neuronopathic form based on clinical presentation. Other common mutant allele was *p.Leu483Pro* identified in 56/240 (23,30%) allelic variants of the *GBA* gene non-neuronopathic form and in two sick children this mutation in the homozygous state has led to the development of sub-acute neuronopathic form.
Conclusion: We identified total 42 different alterations of the gene *GBA*. This may indicate the diversity of populations inhabiting the territory of the Russian Federation in terms of geography, as well as on a national basis.

P-545

Nine novel mutations in the alpha-galactosidase A gene in Russian patients with Fabry disease

Savostyanov K V[1], Pushkov A A[1], Kuzenkova L M[1], Podkletnova T V[1], Pakhomov A V[1], Namazova-Baranova L S[1], Baranov A A[1]

[1]Scientific Centre of Children Health, Moscow, Russian Federation

Background: Anderson-Fabry disease is a rare X-linked inborn error of glycosphingolipid catabolism that leads to excessive deposition of neutral glycosphingolipids in various body's cells. Fabry disease is caused by mutations in the GLA gene, encoding alpha-galactosidase A.
Methods: We investigated more than 1000 patients aged 4 to 67 years with clinical suspicion of Fabry disease. Alpha-galactosidase A activity is measured in dried blood spots using tandem MS-MS. For mutation detection, each exon of GLA gene with flanking intronic regions was sequenced.
Results: We found 20 patients from 17 families with reduced alpha-galactosidase A activity and *GLA* gene mutations, while nine mutations was novel. It was seven novel missense mutations: *p.Cys174Tyr*, *p.Trp204Cys, p.Ser297Pro*, *p.Arg301Leu*, *p.Gln327His*, *p.Leu388His*, *p.Arg404Lys*, and two novel small deletions: *p.Pro362Hisfs*8, p.Lys374Gly375delinsArg*. Molecular analysis extended to other family members led to the identification of two additional heterozygous females and one hemizygous male with phenotype of Fabry disease, who subsequently underwent ERT. In total we identified 17 different alterations of the gene *GLA*,while none mutation was found in other families.
Conclusion: High frequency of novel mutations (52,9%) among undocumented earlier unrelated patients may indicate insufficient examination patients inhabiting the territory of Russia.

P-546

Treatment with miglustat reverses the progression of the disease in juvenile/adult GM1-gangliosidosis

Deodato F[1], Procopio E[2], Rampazzo A[3], Taurisano R[1], Donati M A[2], Dionisi-Vici C[1], Caciotti A[4], Morrone A[4], Scarpa M[5]

[1]Div Metab Dis, Bambino Gesù Child Hosp, Rome, Italy, [2]Metab and Musc Unit Meyer Child Hosp, Florence, Italy, [3]Dep of Women's and Children's Health, Padua, Italy, [4]Mol and Cell Biol Lab, Meyer Child Hosp, Florence, Italy, [5]Center for rare Dis, Horst Schmidt Klini, Wiesbaden, Germany

Background: The juvenile and adult GM1-gangliosidosis are invariably characterized by progressive neurological deterioration. To date only symptomatic therapies are available.
Patients and Methods. We report the results of miglustat treatment on 3 Italian GM1-gangliosidosis patients (follow-up 2-6 years). Pt1 and 2 had a juvenile form (enzyme activity ≤5%, *GLB1* genotype *p.R201H*/*c.1068+1G>T*; *p.R201H*/*p.I51N*), whereas pt3 had an adult form (enzyme activity 7%, *p.T329A*/*p.R442Q*). Treatment (600 mg/day) was started at age 10, 17 and 28 years, respectively. Treatment response was evaluated by neurological examination in all patients every 4-6 months, by assessment of Movement Disorder-Childhood Rating Scale (MD-CRS) in pt2, and by 6 Minute Walking Test (6MWT) in pt3.
Results: The baseline neurological status was severely impaired, with loss of autonomous ambulation and speech in pt1 and 2, and gait and language difficulties in pt3. While on treatment, all patients showed gradual improvement: both juvenile patients regained walking ability, along with increased alertness and vocalization. In pt2 MD-CRS class score decreased from 4 to 2. Pt3 improved in movement and speech control, the walking distance at 6MWT increased from 338 to 475 meters.
Discussion: These results suggest that miglustat may be of help in slowing and reversing the disease progression in juvenile/adult GM1-gangliosidosis.

P-547

Biomarkers for Fabry disease: How to screen patients with late-onset cardiac variant mutations

Abaoui M[1]

[1]Div. Med Genet, Univ de Sherbrooke, Sherbrooke, QC, Canada

Background: Fabry disease is an X-linked lysosomal storage disorder due to α-galactosidase A deficiency. It leads to accumulation of glycosphingolipids, including globotriaosylceramide (Gb_3) and globotriaosylsphingosine (lyso-Gb_3) in biological fluids and various organs. Recent metabolomic studies highlighted novel biomarkers for patients having the cardiac variant mutation p.*N215S*. Most of these patients had normal Gb_3 and lyso-Gb_3 urinary levels jeopardizing the screening/diagnosis. The incidence of late onset cardiac mutation can be frequent (1:1600 in Taipei).
Materials/Methods: Time of flight mass spectrometry (UPLC QTof Synapt G1, Waters Corp.) was used to perform a semi-targeted metabolomic approach for biomarker discovery. After appropriate extraction, biomarkers of interest were structurally identified, followed by development and validation of multiplex methods on the UPLC Xevo TQ-S (Waters) tandem mass spectrometer.
Results: Abnormal excretion of urinary lyso-Gb_3 analog(s) was observed in Fabry patients having the p.*N215S* mutation (n=4), as well as in 67% of untreated patients with the Chinese *IVS4+919G>A* hotspot mutation (n=176). Similarly, other novel biomarkers such as urinary methylated Gb_3 isoforms were detected in patients with the p.*N215S*mutation.
Conclusion: These mass spectrometry results confirm the importance of analyzing various biomarkers for high-risk

screening of Fabry patients, considering the marked phenotypic and genotypic heterogeneity observed.
Conflict of Interest declared.

P-548

The Gaucher Disease Outcome Survey: first description of the population in an ongoing international observational disease registry

Elstein D[3], Schwartz I[4], Deegan P[5], Lau H[6], Goker-Alpan O[7], Lukina E A[8], Bembi B[9], Belmatoug N[10], Fernandez-Sasso D[11], Giraldo P[12 13], Fabien V[2], Dinh Q[1], Zimran A[3]

[1]Shire, Lexington, United States, [2]Shire, Zug, Switzerland, [3]Shaare Zedek Med Cent & Hadassah Med Sch, Jerusalem, Israel, [4]Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil, [5]Addenbrooke's Hospital, Cambridge, United Kingdom, [6]NYU Langone Medical Center, New York, United States, [7]LSD Unit & Cent Clin Trials, O&O Alpan, Fairfax, United States, [8]Hematology Research Center, Moscow, Russian Federation, [9]Regional Coord Cent Rare Dis, Univ Hosp, Udine, Italy, [10]Hôpitaux Univ Paris Nord Val de Seine, Clichy, France, [11]Criocenter, Buenos Aires, Argentina, [12]CIBER de Enfermedades Raras, Zaragoza, Spain, [13]Miguel Servet University Hospital, Zaragoza, Spain

Background: The Gaucher Disease (GD) Outcome Survey (GOS) was designed to monitor GD's natural history and the long-term safety and effectiveness of treatment.
Methods: GOS is an open-ended, international, observational disease registry initiated in 2010 by Shire. Participation is voluntary and open to any centre managing GD patients. Patient data from routine follow-up visits are entered into GOS through a secure online application.
Results: As of 30-01-2015, 30 sites in 9 countries had enrolled 905 patients (48% from Israel, 34% USA). Population characteristics: 46% male; 12% aged < 18 years (0.1-17.9, mean 11.1 years) at GOS entry; 88% ≥18 years (18.0-93.1, mean 46.4 years); 16% previously splenectomized; 97% for whom clinical subtype was reported as type 1 GD (3% type 3); 65% for whom ethnicity was reported are Ashkenazi Jewish. 466 patients were reported to be receiving treatment at GOS entry, with a bias towards velaglucerase alfa (n=280/466) because the first centres to enrol patients participated in Shire velaglucerase alfa trials (GOS is a post-approval commitment to regulatory agencies) or used velaglucerase alfa during the global imiglucerase shortage. 145 patients were untreated (n=98/145 asymptomatic).
Conclusion: GOS is a disease-specific registry designed to assess longer-term, real-life outcomes in untreated patients and patients exposed to various treatments.
Conflict of Interest declared.

P-549

First assessment of Fabry-specific Paediatric Health and Pain Questionnaire (FPHPQ) scoring in children in the Fabry Outcome Survey

Ramaswami U[1], Pintos-Morell G[2], Karabul N[3], Parini R[7], Rohrbach M[4], Bizjajeva S[6], Kalkum G[5]

[1]Royal Free NHS Found. Trust, Univ Coll, London, United Kingdom, [2]Dept Ped, German Trias i Pujol Univ Hosp, Badalona, Spain, [3]Ctr Ped Med, Johannes Gutenberg Univ, Mainz, Germany, [4]Child Res Ctr, Univ Child Hosp, Zurich, Switzerland, [5]Dept Ped, Dr-Horst-Schmidt Kliniken HSK, Wiesbaden, Germany, [6]Shire, Zug, Switzerland, [7]San Gerardo Hosp, Monza, Italy

Objectives: To analyse Fabry-specific Paediatric Health and Pain Questionnaire (FPHPQ) scores among children in the Fabry Outcome Survey (FOS; sponsored by Shire) registry treated with agalsidase alfa (agalα) or treatment-naive.
Methods: Retrospective analysis of baseline data from 25 treated (9 girls, 16 boys) and 68 untreated (47 girls, 21 boys) children < 18 years of age in FOS to examine FPHPQ scores in 3 subscales: general pain associated with heat or exertion (A), general pain associated with cold (B), and physical pain and fatigue symptoms (C). Possible score ranges: (A) and (C) 0-36, (B) 0-20.
Results: Baseline median (range) FPHPQ subscale scores were: untreated (A) girls 2.0 (0-31), boys 5.0 (0-30); (B) girls 1.0 (0-11), boys 2.0 (0-16); (C) girls 6.0 (0-20), boys 9.0 (0-17); and treated (A) girls 15.5 (0-29), boys 18.0 (0-29); (B) girls 0.0 (0-2), boys 4.0 (0-10); (C) girls 12.0 (1-17), boys 11.0 (1-22). Baseline treated FPHPQ scores for (A) were generally >10 and higher than untreated.
Conclusions: Overall, baseline FPHPQ scores were higher in boys than girls and lower in untreated than treated patients, suggesting that more severely affected children were more likely to receive treatment. FPHPQ scoring may help assess disease severity in children.
Conflict of Interest declared.

P-550

Evolutionary studies of Arylsulphatase-A and Beta-galactocerebrosidase

Echeverri O Y[1], Barrera L A[1], Montaño A M[2 3]

[1]I.E.I.M. Pont. Univ. Javeriana, Bogota, Colombia, [2]Doisy Res. Cent. Dep. Ped. St. Louis U., St Louis, United States, [3]Dep.Bioche. Molec. Biol. S. Med. SLU, St Louis, United States

Background and objectives: Myelin sheath is composed by over 70% of lipids; it is responsible for providing stability and improvement of the transmission speed in the nerve cells. Throughout evolution the concentration of sulfatide plays a major role in the biophysical properties of the myelin. To gain insights of the evolutionary role of these galactosylceramides we have studied arylsulfatase-A (ARSA) and galactocerebrosidase (GALC), which are essential enzymes for myelin degradation.
Methods: We conducted phylogenetic analyses of *ARSA* and *GALC* genes in 28 and 25 species, respectively, ranging from bony fishes to human, including over 600-mya. Evolutionary history, nucleotide site divergence, ancestral reconstruction and 3D-structure predictions of both genes were studied.
Results and Discussion: The *ARSA* and *GALC* ratio of nonsynonymous to synonymous nucleotide-substitution rates were 0.28 and 0.26, respectively. Selective pressure analyses did not reveal any site under positive selection and the 3D-reconstruction of ARSA and GALC for many species did not show any change in the structure. These results suggest that the protein function is preserved along the evolution, and it has not allowed the occurrence of a high rate of changes that could affect the structure or catalytic function of these enzymes to preserve myelin metabolism.

P-551

Characteristics of 12 Turkish Adult Patients with Gaucher Disease Type 1

Sivri B[1], Kav T[1], Altan E[1]

[1]Div Gastroenterol, Hacettepe Uni Hosp, Ankara, Turkey

Gaucher disease type 1 is an autosomal recessive lysosomal storage disease caused by deficiency of beta-glucosidase, resulting in deposition of glucosylcerebroside in the monocyte-macrophage system. Here, we present the characteristics of 12 (4 male, 8 female) adult Turkish patients with Gaucher disease type 1.
The patients are aged 20-46 with a mean age of 18.6 (range: 1-45) at diagnosis. Seven of the 12 patients were diagnosed before 18 years of age by their pediatricians and were referred to us as they matured into adulthood. Pain and abdominal distension were the most common presenting symptoms, followed by failure to thrive and fatigue. All patients had hepatosplenomegaly at presentation and splenectomy was performed in 6 patients. 5 patients had osteoporotic compression fractures and 3 patients had to undergo multiple orthopedic surgical procedures. Aside from low beta-glucosidase activity, anemia was the most common laboratory finding, present in all but one patient. Of the 11 anemic patients, 8 also had thrombocytopenia. All 12 patients are currently receiving enzyme replacement therapy.
Increasingly more patients with Gaucher disease type 1 survive into adulthood. The characteristics of our patients may help provide better understanding of long-term follow-up of adult patients with Gaucher disease type 1.

P-552

Clinical and molecular characteristics of a large cohort of patients with Fabry disease

Dasouki M[1 2], Kimber C[1], Tuffaha A[1]

[1]University of Kansas Medical Center, Kansas City, United States, [2]King Faisal Specialist Hospital & Resear, Riyadh, Saudi Arabia

Background and objectives: Fabry disease is a progressive, life threatening multisystemic disease caused by mutations in the X-linked *GLA* gene and is characterized by renal insufficiency, cardiac dysfunction, peripheral neuropathy and brain parenchymal microvascular disease. We aimed to examine the clinical and molecular characteristics in a large cohort of patients with Fabry disease.
Materials/Patients and Methods: We reviewed the medical records, renal biopsies, and molecular studies in this cohort.
Results: In 20 unrelated families, 30 patients (11 M/19 F) were confirmed to have Fabry disease. Symptoms included: peripheral neuropathy (18/30), stroke (4/30), deafness (3/30), tinnitus (6/30), GI disturbances (18/30), palpitations (5/30) and cardiomyopathy (6/30) requiring heart transplantation (1 male) and myomectomy (1 female). Renal biopsies (13/30) including one which showed crescentic glomerulopathy were performed. Three patients (2 males, 1 female) underwent renal transplantation. ERT (26/30) was well tolerated. Twelve pathogenic *GLA* mutations including a novel one (W245G associated with crescentic glomerulopathy) were identified. Two patients with recurrent strokes died.
Discussion/Conclusion: Fabry disease is associated with significant morbidity in males as well as females. Renal biopsies are still being done frequently in such patients and crescentic glomerulopathy is an unusual renal histologic phenotype associated with a novel *GLA* mutation.
Conflict of Interest declared.

P-553

Pilot selective screening for Niemann-Pick type C disease in Slovakia

Hlavatá K[1], Jungová P[2], Dvoráková L[3], Hlavatá A[1]

[1]2nd Dep of Pediat, Univ Child Hospital, Bratislava, Slovakia, [2]Center of Medical Genetics, Univ Hosp, Bratislava, Slovakia, [3]Inst of Inherit Metab Dis, Charles Univ, Prague, Czech Republic

Background: Niemann-Pick type C disease (NP-C) is a rare neurovisceral disease characterized by progressive neurodegeneration and premature death. The incidence has been estimated as 1: 125 000 live births. NP-C is caused by autosomal recessive mutations in the *NPC1* (in 95% of cases) or in the *NPC2* gene. Clinical presentations of NP-C feature a range of systemic and neurological signs.
Methods: Since June of 2014 to April 2015 we have realized selective screening for NP-C disease. In collaboration with clinical genetics we have selected adult patients with unknown diagnosis presenting with neurological impairment (e.g. dementia, saccadic eye movements and tremor). Children patients selected from our clinic presented with prolonged jaundice, splenomegaly and neurological impairment. Blood samples from selected patients were analyzed for level of oxysterols in Münster, Germany. Diagnostic process of positive samples was completed by molecular genetic analyses in Prague, Czech Republic.
Results: From 12 selected adult patients, the disease causing mutation in *NPC1* gene was found in homozygous state in 1 case. From 10 selected children patients, two mutations in *NPC1* gene were found in heterozygous state in 1 patient.
Conclusion: New screening methods are very useful for effective diagnostic process and early start of the treatment.

P-554

Clinical profile of children with Fabry disease in a brazilian reference centre

Caneloi T P[1], Kyosen S O[1], Mendes C S C[1], Aranda C S[1], Curiati M A[1], Rand M H[1], Feliciano P[1], D´Almeida V[1], Pesquero J B[1], Martins A M[1]

[1]Univ Federal de São Paulo, Unifesp, São Paulo, Brazil

Background: Fabry Disease (FD) diagnosis is often delayed in children. Although there is an expressive morbidity in childhood, available information about FD symptoms are limited in this age group. We aim to describe FD clinical profile in pediatric patients.
Methods: Retrospective, single-center, observational study in 16 FD patients under 18 years old.
Results: Most patients (14/16) were symptomatic before diagnostic confirmation through familiar screening. Male were majority (10/16). Median age 13.8y, age at onset of first symptom 5.0y, age of diagnosis 10.8y and time between onset of symptoms and diagnosis 2.9y. Most common complaints: acroparesthesia (9/14); hypohidrosis (7/14); headache (6/14); fatigue (5/14); fever of unknown origin (5/14). Three children showed bilateral cornea verticillata; six presented microalbuminuria, two with arrhythmia. Enzyme replacement therapy eligible patients: female, 14y, by renal manifestation and two male, 15 and 16y, both by renal manifestation and limitation of daily activities.
Conclusion: Even the majority of patients with typical FD symptoms, diagnosis was only made by familiar screening. Recent studies show that female patients also have major organ dysfunction during childhood, which corroborates our findings. To improve prognosis, it is important to disclose FD among pediatricians so that diagnosis can be made before onset of irreversible damage.
Conflict of Interest declared.

P-555

Niemann-Pick type C disease in adults: the multiple faces of a complex lysosomal disorder

Lourenco C[1], Almeida M[1], Nobre L[1], Leprevost C[1], Timm F[2], Saraiva M[2], Giugliani R[2], Marques Jr W[1]

[1]Univ of Sao Paulo, Ribeirao Preto, Brazil, [2]Federal Univ Rio Grande do Sul, Porto Alegre, Brazil

Introduction: Niemann-Pick type C disease (NP-C) is a rare inborn error of metabolism caused by defective intracellular transport of cholesterol. Symptoms can be present since early childhood until adulthood.
Objective: To present the investigation of 45 adult patients with suspicion of Niemann-Pick type C disease.
Material and Methods: Retrospective study and review of neuroimaging, neurophysiological, biochemical and molecular studies carried out in the course of 45 adult patients with NPC suspicion.
Results: Eight out of 45 patients were confirmed to have NPC by filipin staining (2 patients required molecular analysis because of "variant filipin pattern"). Regarding clinical presentation, all positive NPC cases had VSGP with typically downward gaze palsy. Two patients had generalized dystonia with dementia; one patient had cerebellar ataxia with typical VGSP but no cognitive decline; one patient had laryngeal dystonia as prominent feature without cerebellar signs or pyramidal features. Psychiatric disorder was observed in two of the patients with generalized dystonia with dementia.
Conclusions: Neurological manifestations in NPC patients is extremely variable, even in the same sibship. Better understanding of the natural history of the disease is crucial for evaluation of potential therapeutic approaches in such a devastating disorder.

24. Lysosomal disorders: others

P-556

Childhood Pompe disease: clinical spectrum and genotype in 31 children

Van Capelle C I[1 13], Van der Meijden J C[1 13], Van den Hout J M P[1 2 13], Jaeken J[3], Baethmann M[4], Voit T[5], Kroos M A[1 6], Van Spronsen F J[8], Rubio-Gazalbo M E[7], Willemsen M[9], Lachmann R H[11], Mengel E[10], Michelakakis H[12], Reuser A J J[1 6], Van der Ploeg A T[1 13]

[1]Center for Lys and Metab Dis, Erasmus MC, Rotterdam, Netherlands, [2]Dept of Neurology, Erasmus MC, Rotterdam, Netherlands, [3]Cent for Metab Dis, Univ Hosp Gasthberg, Leuven, Belgium, [4]Dept of Pediatrics, Hosp Dritter Orden, Munich, Germany, [5]Inst de Myologie, Univ Piere et Marie Cu, Paris, France, [6]Dept of Clin Gen, Erasmus MC, Rotterdam, Netherlands, [7]Dept of Ped and Lab Gen Met Dis, MUMC, Maastricht, Netherlands, [8]Div of Metab Dis, Beatr Child Hosp, UMCG, Groningen, Netherlands, [9]Dept of Ped Neurology, Radboud UMC, Nijmegen, Netherlands, [10]Villa Metab, Centre for Ped and Adol Med, Mainz, Germany, [11]Charles Dent Metab Unit, Nat Hosp Neur, London, United Kingdom, [12]Dept of Enzym & Cel func, Aghia Sop Hosp, Athens, Greece, [13]Dept of Pediatrics, Erasmus MC, Rotterdam, Netherlands

Background: As little information is available on children with non-classic presentations of Pompe disease, we wished to gain knowledge of clinical characteristics and genotypes.
Methods: This cross-sectional observational study included all patients younger than 18 years who had been referred to our center between 1975 and 2012. Patients with the classic-infantile phenotype were excluded. We collected information on first symptoms, diagnosis, wheelchair and/or respirator use, and enzyme and mutation analysis. We assessed muscle strength, pulmonary function, and cardiac parameters.
Results: Thirty-one patients participated (median age at symptom onset 2.3 years, and at diagnosis 4 years). Children typically presented with limb-girdle muscle weakness and/or delayed motor development, with disproportional involvement of neck flexors. Respiratory problems never preceded proximal muscle weakness, but had led to ventilator support or death during childhood in eight patients (26%). Two patients had cardiac hypertrophy; 68% of patients – 86% of whom male – carried the c.-32-13T>G mutation.
Conclusion: Our study shows that patients with non-classic Pompe disease may already have severe mobility and respiratory problems during childhood. As in adults, the commonest genotype was c.-32-13T>G mutation/null. Interestingly, most symptomatic children with this genotype were male. While children with other mutations were generally affected more severely, age at symptom onset did not differ.
Conflict of Interest declared.

P-557

Novel mutations in Tay-Sachs Egyptian patients

Fateen E M[1], Ali Ibrahim D M[2], Abdel Aleem A K[2], Mohamed Ali O S[4], Zaki M S[3]

[1]Biochem.gen dep, National Research Cent, Cairo, Egypt, [2]Med Mol Dep,National Research Centre, Cairo, Egypt, [3]Clin Gen Dep,National research Centre, Cairo, Egypt, [4]Bioch Dep Fac Phar, AlAzhar Uni, Cairo, Egypt

Background: Tay-Sachs disease (TSD) is a fatal autosomal recessive disorder caused by reduced hexosaminidase A (Hex A) enzyme activity.
Objective: to detect mutations on the HEXA gene in Egyptian TSD patients.
Patients and Methods: 14 exons of HEXA gene encoding the hexosaminidase alpha chain were sequenced in 10 infantile TSD patients and 10 controls.
Results: 40% of TSD alleles carried the previously reported mutations p.R504C, p.R504H and p.R499C, but 35% exhibited four novel mutations p.H318D (Ex 8), p.E307A (Ex 8),p.S3T (Ex 1) and c.484delG (162). In 25% of alleles, mutations were not detected. Silent polymorphisms p.1436V and p.E506E were detected in 100% of controls and 84.6% of TSD patients. In 15.4% of TSD patients, a novel silent mutation 6.A≥G (T2T) was detected.
Conclusions: Four novel TSD pathogenic mutations were identified for the first time in our study.

P-558

Spectrum of lysosomal storage disorders in India and Pakistan

Jalan R A[1], Jalan A B[1], Kudalkar K V[1], Borugale M A[1], Joshi M M[1], Sonalkar N D[1], Shinde D H[1], Eichler S[2], Zielke S[4], Zielke S[4], Giese A[4], Rolfs A[2 4], Rao H A[3], Alam M A[5]

[1]NIRMAN, Div of Biochemical Genetics, Navi Mumbai, India, [2]Centogene AG, Rostock, Germany, [3]MedGen, Clin Gen Div, Medicare Hosp, Rawalpindi, Pakistan, [4]Albrecht Kossel Institute, Univ Rostock, Rostock, Germany, [5]BioCare Lab, Islamabad, Pakistan

Introduction: Inherited defects or deficiencies of lysosomal enzymes can result in accumulation of undegraded metabolites.
Objective: To find out the spectrum of lysosomal storage disorders in patients from India and Pakistan.
Method: This work is a prospective study over a period of 4 years (2011-2014) of patients from India and Pakistan. Biochemical investigations included urine GAG analysis, urine MPS electrophoresis, enzyme studies, urine oligosaccharides, chitotriosidase, CCL18 and molecular studies.
Results: A total of 87 cases (India-38, Pakistan- 49) were confirmed by DNA studies out of 151 biochemically proven. Commonest presentations were coarse facies, hepatosplenomegaly, short stature, bone deformities, delayed milestones and mental retardation. Incidence in our cohort was - Gaucher's disease (20.7%), MPS I (13.8%) and MPS IVA (12.6%). The most common mutation in cases of Gaucher's disease was c.1448T>C (homo) or p.L483P in exon 10 and in MPS I was c.1469T>C (homo) or p.L490P in exon 10.
Discussion: In India, not much data are available on LSDs. Agarwal S et al. and Jayesh Sheth et al. found high incidence of Gaucher's disease and MPS in India. This is in accordance with our findings, confirmed by mutation analysis.
Conclusion: Gaucher's disease, MPS I and MPS IVA were found to be the commonest LSDs in India and Pakistan.

P-559

Secondary hemophagocytosis in a patient with Wolman disease

Küçükçongar Yavas A[1], Genç P[2], Kılıç N[2], Erdoğan H[2], Özdemir Ö[2], Ekici A[2], Orhaner B[2]

[1]Div Metab Dis, Dışkapı Child Hosp, Ankara, Turkey, [2]Div Child Health, Ş. Yılmaz Child Hosp, Bursa, Turkey

Background: Hemophagocytic lymphohistiocytosis (HLH) is hyperinflammatory disease caused by an uncontrolled immune response, which develops in patients with underlying genetic diseases, or which can occur secondary to infection, malignity, autoimmune or metabolic disorders. Complete loss of lysosomal acid lipase (LAL) activity in infancy results in Wolman disease (WD). Here, we reported a patient with a diagnosis of WD who presented with HLH.
Case report: A two months old female patient applied with diarrhea, fever, hepatosplenomegaly. The most conspicuous features were very high levels of lactic dehydrogenase, ferritin and bicytopenia on laboratory examinations. Bone marrow aspiration showing numerous hemophagocytoses diagnosed HLH. Although under chemotherapy, her clinical condition got worse. Metabolic screening tests were normal. Enzymatic analyses were performed for lipid storage disorders. Only LAL activity was low < 0.02 nmol/punch/hr (0.07-2.3). She was diagnosed Wolman disease and died in short time. Sequence analysis of all coding regions of the LIPA gene (NM_000235, LIPA_vENST00000336233) showed the c.260G>T (p.Gly87Val) variation in both parents (heterozygote). This variation was reported as a disease-causing variant; it was given the number CM960943 in HGMD database.
Conclusion: Except classical manifestations like progressive hepatosplenomegaly, steatosis, cholestasis, adrenal calcification of WD, HLH can be added as the feature of WD. On the other hand, WD can cause secondary HLH.

P-560

Glucocerebrosidase (GBA1) deficiency and Parkinson's disease. Potential modifying effects of glucocerebrosidase 2 (GBA2) and oxidative stress

Burke D G[1 2], Heales S J R[1 2]

[1]Great Ormond Street Hospital, London, United Kingdom, [2]UCL Institute of Child Health, London, United Kingdom

Background: Mutations affecting lysosomal glucocerebrosidase (GBA1) are associated with increased risk of developing Parkinson's Disease (PD). Previously, we suggested that non-lysosomal glucocerebrosidase (GBA2) could have a moderating effect with regards to disease severity for Gaucher /PD. Furthermore, we have also reported that brain GBA1 activity is decreased in PD patients without GBA1 mutations. Building on our observations, we have evaluated the effects of oxidative stress, a condition associated with PD, on neuronal viability in the presence of GBA1 and/or GBA2 inhibition.
Methods: SHSY5Y cells were used and exposed to increasing concentrations (0-0.4 mM) of hydrogen peroxide (H_2O_2) for 7 days. Cell viability and the activities of GBA1, GBA2, β-hexosaminidase (β-Hex) and acid alpha-glucosidase (GAA) were then evaluated.
Results: H_20_2 caused a dose dependent loss of cell viability, the magnitude of which was not altered by GBA1 and/or GBA2 inhibition. Oxidative stress alone did not affect GBA1 or GBA2 activities. However, β-Hex and GAA activities were significantly increased.

Conclusions: Loss of GBA1 activity in idiopathic PD may not be explained by oxidative stress. However, the oxidative stress mediated increase in β-Hex and GAA may provide a mechanism for the reported increased activity of these enzymes in neurodegeneration and neuronal stress models.

P-561

Evaluation of blood-brain barrier (BBB) integrity and structural abnormalities in mucopolysaccharidosis (MPS) IIIB patients using cerebrospinal fluid/serum albumin index (CSF-AI) and multimodal MRI

Vijay S[2], Parker G[3], Roberts C[3], Riches V[3], Leavitt M C[1], Rossomando A[1], Kemp T[1], Quinn A G[1], Patki K C[1], Rojas-Caro S[1]

[1]Synageva BioPharma Corp., Lexington, United States, [2]Birmingham Children's Hospital, Birmingham, United Kingdom, [3]Bioxydyn Limited, Manchester, United Kingdom

Background: MPS IIIB (Sanfilippo B syndrome) is a lysosomal storage disorder that increases heparan sulfate (HS) levels in the brain and other organs, for which no treatment exists. Studies indicate possible BBB defects in mice with MPS IIIB and MPS IIIA/IIID patients.
Methods: We evaluated BBB integrity and structural brain abnormalities in 5 MPS IIIB patients (4 males, 1 female; Caucasian; 6–8 years old) using CSF-AI and multimodal MRI. Dynamic contrast-enhanced MRI determined blood plasma volume, BBB transfer coefficient (K^{trans}) and tissue MRI relaxation time (T_1). Exploratory biomarkers such as HS and hepatocyte growth factor (HGF) levels were evaluated.
Results: CSF-AI was above ULN in 4 patients (5.5–11.3; ULN 4.9). K^{trans} values in grey and white matter were very low, similar to those in adult healthy brains; T_1 values in white matter were comparable to those in healthy volunteers of similar age. Other MRI findings: cerebral atrophy, increased skull thickness, and abnormal areas of white matter hyperintensity. Substantially higher HS levels (CSF, serum, and urine) and higher-than-normal HGF levels (CSF) were found.
Conclusion: Although CSF-AI provided evidence of mild BBB leakage, MRI did not. MRI volumetric measurements showed global and tissue-specific atrophy. These findings may have implications for MPS IIIB disease pathogenesis.
Conflict of Interest declared.

P-562

Neuronal ceroid lipofuscinosis-2 (CLN2) disorder, a type of Batten disease caused by TPP1 enzyme deficiency: Current knowledge of the natural history from international experts

Schulz A[1], Cohen-Pfeffer J L[2], Crystal R[3], De los Reyes E[4], Eto Y[5], Guelbert N[6], Héron B[7], Mikhailova S[8], Miller N[2], Mink J W[9], Perez-Poyato M S[10], Simonati A[11], Sims K[12], Williams R E[13]

[1]Univ Medical Center Hamburg-Eppendorf, Hamburg, Germany, [2]BioMarin Pharmaceutical Inc., Novato, United States, [3]Weill Cornell Medical College, New York, United States, [4]Nationwide Children's Hospital, Columbus, United States, [5]Southern Tohoku Brain Research Center, Kawasaki, Japan, [6]Hospital de Niños de Cordoba, Corboda, Argentina, [7]Hôpital d'Enfants Armand-Trousseau, Paris, France, [8]Russian Pediatric Regional Hospital, Moscow, Russian Federation, [9]University of Rochester Medical Center, Rochester, United States, [10]Hosp Universitario Marqués de Valdecilla, Santander, Spain, [11]University of Verona, Verona, Italy, [12]Massachusetts General Hospital, Boston, United States, [13]Guy's and St Thomas' NHS Fdn Trust, London, United Kingdom

Background and objectives: The neuronal ceroid lipofuscinoses (NCLs) are the most common group of neurodegenerative disorders in children and adolescents. CLN2, a type of NCL caused by TPP1 enzyme deficiency, is characterized by seizures, rapid deterioration of language, cognition, motor skills and vision, and premature death. Our aim is to describe expert knowledge of CLN2 disease.
Methods: 18 international NCL experts answered a survey on CLN2 natural history.
Results: Clinical suspicion for CLN2 is low due to its rarity and non-specific presenting symptoms. A 1-4 year delay was reported between first onset of symptoms and diagnosis. Speech delay/decline, developmental delay/regression and seizures/epilepsy were identified as initial presenting symptoms. Symptom onset typically occurs between 1.5-5 years of age, but may occur later (9-12 years). Myoclonic epilepsy was the most commonly reported seizure type. Notably, seizures are refractory oftentimes requiring polytherapy. Cardiac rhythm anomalies, not previously associated with CLN2, were also identified.
Conclusions: CLN2 is a severe, progressive, pediatric-onset neurodegenerative disorder. Disease awareness is low, causing delays in diagnosis. Seizures in concert with a regression of language and/or motor milestones should raise suspicion for CLN2. Knowledge of CLN2 is paramount to ensure timely diagnosis and to enable early initiation of future therapies.
Conflict of Interest declared.

P-563

Pulmonary function predictors (VC, FVC, MIP, MEP) of ventilator use in late-onset Pompe disease

Quartel A[1], Mozaffar T[2], Roberts M[3], Young P[4], Johnson E M[1], Berger K I[5]

[1]BioMarin Pharmaceutical Inc., Novato, CA, United States, [2]University of California, Irvine, CA, United States, [3]Salford Royal NHS Foundation Trust, Salford, United Kingdom, [4]University Hospital Munster, Munster, Germany, [5]New York University, New York, NY, United States

Late-onset Pompe disease (LOPD) is characterized by a deficiency of the enzyme acid alpha-glucosidase, resulting in progressive myopathies. Respiratory muscle involvement can lead to respiratory failure, the most common cause of mortality. Studies on sleep-disordered breathing in primary myopathies have suggested that the onset of night-time ventilation can be predicted with non-invasive pulmonary function tests (PFTs). To determine if PFTs can predict both night-time and daytime ventilator usage in LOPD, a systematic literature review was performed to identify relevant clinical data. Patient data were divided into three cohorts (no-ventilation; night-time; daytime) that were defined by either a ventilation description or the amount of ventilator hours (no ventilation; night-time or <=12 hours; daytime or >12 hours, respectively). Data for six relevant PFTs were analyzed, including maximum inspiratory pressure (MIP) and maximum expiratory pressure (MEP), as well as vital capacity (VC) and forced vital capacity (FVC) in the upright (-U) and supine (-S) positions. These PFTs were able to differentiate the three groups (no ventilation; night-time; daytime), with VC-U and MIP having both high AUC values and consistency in the two transitions. These analyses suggest that PFTs may be useful in predicting the need for both night-time and daytime ventilator assistance. Conflict of Interest declared.

P-564

Measurement of lysosomal acid lipase activity on dried blood spot: a French national screening program

Brasse-Lagnel C[2], Bekri S[1]

[1]Metab Biochem Rouen University Hospital, Rouen, France, [2]Metab Biochem Lyon University Hospital, Lyon, France

Background and objectives: The lack of the lysosomal acid lipase (LAL) enzyme leads to lysosomal storage disorders, Wolman disease and cholesterol ester storage disease. A new method for the measurement of lysosomal acid lipase on dried blood spots (DBS) have recently been described and a clinical trial evaluating the first enzyme replacement therapy is ongoing. Therefore, the aim of this study was to implement the measurement of LAL in dried blood spots in our lab and to screen for LAL deficiency.
Method: LAL was measured from DBS using a fluorimetric method. To support the quality of this assay, we used quality control materials provided by the Center for Disease Control (Atlanta, USA). At-risk populations screening may allow to identify patients presenting with LAL deficiency and subsequently to propose ERT, if appropriate. Thus, 1000 DBS were assessed.
Results: The assay showed a robust analytical performance with good intra and inter-day precisions ranging from 4 to 20 %. Analysis of DBS from healthy patients and LAL deficiency patients showed that the assay readily distinguished affected from non-affected individuals.
Conclusion: This assay made diagnosis of LAL deficiency more accessible with a short turnover compatible with screening laboratories workflow.

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Real-world experience in the diagnosis of neuronal ceroid lipofuscinosis type 2 (CLN2): report from an international collaboration of experts

Miller N[2], Mole S E[9], Cohen-Pfeffer J L[2], Crystal R[3], De los Reyes E[4], Eto Y[5], Fietz M[10], Héron B[6], Izzo E[2], Kohlschütter A[1], Lourenço C M[11], Noher de Halac I[12], Pearce D A[13], Perez-Poyato M S[7], Simonati A[8], Schulz A[1]

[1]Univ Medical Center Hamburg-Eppendorf, Hamburg, Germany, [2]BioMarin Pharmaceutical Inc., Novato, United States, [3]Weill Cornell Medical College, New York, United States, [4]Nationwide Children's Hospital, Columbus, United States, [5]Southern Tohoku Brain Research Center, Kawasaki, Japan, [6]Hôpital d'Enfants Armand-Trousseau, Paris, France, [7]Hosp Universitario Marqués de Valdecilla, Santander, Spain, [8]University of Verona, Verona, Italy, [9]University College London, London, United Kingdom, [10]SA Pathology, North Adelaide, Australia, [11]University of São Paulo, São Paulo, Brazil, [12]Universidad Nacional de Cordoba, Cordoba, Argentina, [13]Sanford Child Health Research Ctr, Sioux Falls, United States

Background: CLN2 disorder is a lysosomal storage disorder resulting from TPP1 enzyme deficiency that causes progressive neurological degeneration and early mortality. CLN2

disorder is rare and often unsuspected, leading to delays in diagnosis.
Methods: In late 2014, 18 international CLN2 experts (clinicians, academic researchers, and laboratory directors) answered a comprehensive survey on CLN2 disorder and a subset met to discuss experiences, current practices and shortcomings in diagnosis of CLN2.
Results: 70% of laboratory experts considered the standard for CLN2 diagnosis to be a demonstrated decrease in TPP1 enzyme activity, with the remaining experts favoring molecular detection of pathogenic *CLN2*/*TPP1* mutations. Delays in the diagnosis of CLN2 were identified as a crucial concern: 82% of the group responded that patient referral to a specialist can typically take longer than one year. Laboratory experts identified the challenge in reaching a suspicion of CLN2 (50%) and lack of awareness of available tests (83%) as common reasons for delays.
Discussion: Experts agreed that reliable techniques exist for CLN2 diagnosis and identified timely referral as a key challenge. An upcoming CLN2 expert meeting will define laboratory-based screening and diagnostic guidelines in order to establish best practices for use of biochemical genetics testing in CLN2 diagnosis.
Conflict of Interest declared.

P-566

Immune irregularities in lysosomal storage disease patients

Pashali V[1], Dimitriou E[2], Moraitou M[2], Kanariou M[1], Michelakakis H[2]

[1]Dept Immun-Histocom Ag Sophia Child Hosp, Athens, Greece, [2]Dept Enzym Cellular Fun, In Child Health, Athens, Greece

Background: Immune irregularities have been described in several lysosomal storage diseases (LSD) indicating a role for the lysosomal system in many immune system functions. In particular, autoantibodies have been described in patients and animal models of these disorders.
Methods: In the present study, we investigated the presence of autoantibodies in plasma of patients with Gaucher (GD, n=6), Niemann Pick type C (NPC, n=5) and Sanfilippo type B disease (SFB, n=8). All patients were examined for ANA, anti-DNA, autoantibodies to extractable nuclear antigens-ENA [PM-Scl-70, Scl-70,Ku, CENP A-C, AMA-M2, RNP, SS-A (60KDa), SS-A (52KDa), SS-B, JO-1, rib.P-Protein, Sm, CENP-B], ASMA and anti-ganglioside antibodies using IIF, ELISA and WB methods. In addition, immunoglobulin (IgG, IgA, IgM) levels were measured by nephelometry.
Results: Anti-ganglioside IgM Abs were detected in 4/5 NPC and in 4/8 SB patients. Anti-ganglioside IgG Abs were detected in 2/5 NPC and 4/8 SFB patients. Anti-Sm E/F Abs were detected in 1NPC and 5/8 SFB patients. ASMA Abs were detected in 1/6 GD and 3/8 SFB patients. 2/6 GD, 1/5 NPC and 2/8
SFB patients showed increased immunoglobulin levels.
Conclusion: Our findings suggest that independently of the development of an autoimmune disease in LSD patients, there seems to be an autoimmune activation towards specific antigens that differs in different disorders.

P-567

Farber disease: a case report with a novel mutation

Onenli Mungan N[1], Seker Yilmaz B[1], Bulut D[1], Kor D[1], Ceylaner S[2]

[1]Div Metab Dis, Univ Cukurova, Adana, Turkey, [2]Intergen Genetic Lab, Ankara, Turkey

Background and objectives: Farber disease is a very rare lipogranulomatosis with heterogeneous clinical manifestations due to the deficiency of acid ceramidase. Subcutaneous nodules, joint swelling, contractures, hoarseness, and neurological involvement are the frequent signs and symptoms. Mutations in *ASAH1* gene lead to enzyme deficiency. Currently there is no specific therapy.
Case Report: 3 months old female infant admitted to the hospital with complaints of restlessness, hoarseness, hypotonia, and inadequate nutrition. Parents are first degree cousins and an 8 months old daughter died with same complaints. Although she had microcephaly, severe hypotonia, laryngomalacia, thickened skin, and contractures on physical examination, any subcutaneous nodules were detected. Mutation analysis revealed a novel homozygous mutation (c.92G>T or p.C31F) on the *ASAH1* gene.
Conclusion: Here, we report a case of Farber lipogranulomatosis, a very rare storage disease accompanied with immune deficiency, and a novel mutation was detected.

P-568

Onset of cognitive decline in CLN3 disease: a systematic review and meta-analysis

Kuper W F E[1], Van Hasselt P M[1]

[1]Dept Met Dis, Wilhelmina Child Hosp, UMCU, Utrecht, Netherlands

Background: Neuronal ceroid lipofuscinosis involving the *CLN3* gene is an important cause of childhood neurodegeneration. Onset with visual failure around 6 years of age is thought to precede cognitive deterioration by several years. However, casuistic reports imply that cognitive deficits may already manifest around presentation.
Objective: To delineate the onset of cognitive decline in CLN3 disease.
Methods We performed a systematic review, searching PubMed and Embase for patients with confirmed CLN3 disease. Patients displaying classical and protracted forms were analyzed separately. Survival analysis was used to delineate age at onset of visual and cognitive deterioration. Additionally, time course of IQ scores was determined.
Results: 130 patients were retrieved. Information on cognitive function (IQ and/or onset of cognitive decline) was provided in 58 cases. Mean onset of visual decline was slightly earlier in classical vs protracted CLN3 (6.1 years (n=47) versus 7.2 years (n=9), p=0.02).
Reported onset of cognitive decline was at 6.75 years in the classical phenotype (n=20), and a clear decrease in IQ scores was observed from 4-5 years of age ($p< 0.0001$). No cognitive dysfunction was reported in protracted forms until adulthood (n=10), supported by stable IQ scores.
Conclusion: Cognitive dysfunction is an early symptom of classical CLN3 disease.

P-569

Large epidemiological study on selected lysosomal storage diseases

Oprea G E[1], Eichler S[1], Schmidt K[1], Kramp G[1], Wittmann G[1], Cozma C[1], Nahavandi N[1], Rolfs A[1 2]

[1]CENTOGENE AG, Rostock, Germany, [2]Albrecht-Kossel-Inst, Univ Rostock, Rostock, Germany

Background: Lysosomal storage disorders (LSDs) comprise inherited genetic diseases, resulting from a disturbed lysosomal function.
Patients & Results: In the last 4 years, Centogene AG/Germany has genetically screened worldwide about 20,000 individuals for a broad spectrum of different LSDs, and confirmed for 3,071 patients the clinical suspicion. Fabry disease was the most common (45.6%), followed by mucopolysaccharidosis type II (MPS2; 15.2%), Gaucher disease (10.7%) and Pompe disease (5.3%); the rest comprising other LSD types. The median age at diagnosis was calculated for each LSD type, and this showed that average was 24 years, and the range from 1 month to 86 years. The geographical distribution of the LSD cases revealed that a greater part of diagnosed LSD samples were identified in Latin America (42.4%), and Europe (34.2%). 19% of identified LSD cases are originating from Middle East. Molecular diagnostics resulted in detection of 1,130 unique sequence variants. 50.3% were missense mutations, followed by frameshift mutations (16.1), nonsense (10.4%) and splicing mutations (7.7%), the most of them located within the coding region (91.6%). 1.6% were represented by gross gene rearrangements. From the total of 1,130 unique variants, 55.5% were previously described in the literature, and 44.6% represent new, previously unpublished genetic variants.

P-570

Voice quality in patients with late-onset Pompe disease

Szklanny K[1], Gubrynowicz R[1], Marasek K[1], Iwanicka-Pronicka K[2], Ratyńska J[2], Tylki-Szymanska A[2]

[1]Polish-Japanese Academy of IT, Warsaw, Poland, [2]The Children's Memorial Health Institute, Warsaw, Poland

Background: Pompe disease (PD) is an autosomal recessive lysosomal myopathy caused by deficiency of acid a-glucosidase (GAA). In Pompe disease speech is impaired by reduced movement and/or weakness of the lips and/or tongue. The articulation is disordered featuring consonant substitutions, consonant omissions and cluster reductions, hypernasal resonance, and significantly impaired speech intelligibility.The aim of this study was to assess voice quality of patients with late-onset PD.
Patients and methods: Analysis of nasalence and vocal folds vibration was performed for 10 patients with late-onset PD. All patients have confirmed diagnosis of Pompe disease. For that Glottal Enterprises electroglottograph EG2-PCX2 and nasalance separator handle have been used. Additional information on structure of vocal folds and glottis was obtained by laryngoscopy. Soft nasalization of vowels, unstable pitch, non-synchronous movement of vocal folds, limitation in soft palate movement, irregularity in vocal folds functioning and glottal insufficiency in the middle part of vocal folds has been observed.
Conclusion. Careful analysis of signals gathered by sensitive devices such as electroglottograph and nasalance separator handle allows for recognition of pathological changes in voice which are not possible to be noted in routine phoniatric voice evaluation.

P-571

Molecular mechanism of autophagic degradation pathway in Gaucher's disease

Dokmeci Emre S[1]

[1]Hacettepe Univ Dept.of Medical Biology, Ankara, Turkey

Background and objectives: Lysosomes are involved in the degradation of macromolecules through the activity of lysosomal hydrolases and lysosomal membrane-bound proteins. Gaucher disease is a lysosomal storage disease resulting from the mutation of a lysosomal membrane-associated glycoprotein glucocerebrosidase and its cofactor saposin C. The disease leads to the intracellular accumulation of glucosylceramide and other glycolipids. Autophagy is a lysosome-dependent degradation pathway, therefore disorders in lysosomal action may directly affect autophagy.
Materials and Methods: We analyzed the expression of autophagy and/or lysosome-related genes and proteins in fibroblast cells isolated from patients with different mutations. Then we carried out active lysosome staining and confocal microscopy analyses of autophagy and/or lysosome-related proteins. Finally, we tested autophagic flux by utilizing the differential pH sensitivities of RFP and GFP in mRFP-GFP-LC3 probe.
Results: We showed significant attenuation in autophagy/lysosome-related gene and accumulation of some of the key autophagy proteins in patient cells. Moreover, there was a clear lysosome and lysosomal protein accumulation and an increase in lysosome numbers in starved-patient cells. Microscopy analyses of autophagy revealed some abnormalities in mutant cells.
Conclusion: Thus, autophagic degradation pathway is directly affected in Gaucher's patient cells. Acknowledgement: This Project is supported by TUBITAK-3501 National Young Researchers Career Development Program, Project No: 112T130

P-572

Cholesteryl ester storage disease (CESD): successful outcome of five pregnancies in Greece

Drogari E[1], Pappas G[2], Iliadis E[3], Manolaki N[4]

[1]1st Dept Ped,Univ Athens,Agia Sofia Hosp, Athens, Greece, [2]Dept Gynecol,Univ Athens, Alexandra Hosp, Athens, Greece, [3]Dept Obst/Gynecol,Mitera Gen Hosp, Athens, Greece, [4]2nd Dept Ped, NHS, Agia Sofia Hosp, Athens, Greece

CESD is a rare inborn error of metabolism. The mode of inheritance is autosomal recessive. It is caused by lysosomal acid lipase (LAL) deficiency. In the adult type, children and adults present mainly with liver disease and early atherosclerosis.
Four women (clinical and molecular criteria) have been followed up monthly (biochemistry,etc) during their pregnancies (n=5) for 9 months and 6 months before (planned pregnancies). All stopped the hypolipidemic drug treatment and followed low fat and low carbohydrate diet, high protein diet with high doses of fish oils (2000 mg/dl).
We present the successful outcome of these 5 pregnancies (3 baby girls and 2 baby boys). All mothers raised their lipid levels (cholesterol 249-360 mg/dl, LDL 199-298 mg/dl) with low levels of HDL (27-33 mg/dl) and upper normal triglycerides (160-190 mg/dl). Glucose tolerance test, thyroid hormones, blood pressure remained under good control. Liver enzymes were also raised, but they were reduced compared to the one's at diagnosis of the disorder. The body weight increased between 11-13 Kg. All babies were born full term and healthy. Two out of five were heterozygous.
Pregnancies of women with CESD can have a successful outcome for the mothers and babies. Enzyme replacement therapy could contribute more to them.

P-573

Successful implementation of plasma oxysterol for screening of Niemann Pick disease type C in Manchester UK

Wu H Y[2], Cooper J A[2], Church H J[2], Tylee K L[2], Heptinstall L[3], Hartley C L[3], Philippo S[2], Jameson E[2], Broomfield A[2], Hendriksz C[1], Jones S A[2]

[1]Metab Unit, Salford Royal NHS Trust, Manchester, United Kingdom, [2]Willink Biochem Genet Unit, Univ Hosp, Manchester, United Kingdom, [3]Centre of Genomic Medicine, Manchester, United Kingdom

Background: Plasma oxysterol has enabled timely diagnosis of 7 new cases of neonatal and infantile NPC1 since its implementation in Manchester. Its diagnostic specificity for NPC was greatly enhanced by parallel requesting of lysosomal enzyme screen. Its clinical sensitivity was further characterised as more experience was gained by analysing NPC positive and obligate heterozygotes samples.
Method: Over 300 samples were analysed for cholestane-3β, 5α,6β-triol by LC-MS/MS, from known NPC1 (n=52), carriers (n=23), variants (n=4) and non-NPC (n=220). The data was reviewed against age, disease status by mutation+/-Filipin staining, and types of clinical signs: jaundice, hepatosplenomegaly, atxia, gaze, psychosis, cognitive decline etc. A cutoff of 37.0ng/ml was applied.
Results: All genetically confirmed NPC had elevated oxysterol. Compared to the neurological concerns, the frequency of abnormal results amongst patients investigated due to liver diseases were relatively high (55%), of which 5 were diagnosed as NPC1 by mutation. Of note were the fluctuating levels observed in two adult NPC variants giving rise to false negative results.

Conclusion: An upper normal level in a patient with suspected attenuated NPC should be interpreted with caution, mutation+/-filipin should be pursued. The ability of plasma oxysterol in enabling early diagnosis and treatment of NPC remains indisputable.

P-574

Chitotriosidase activity for the screening of Niemann-Pick disease type C

Proshlyakova T Y[1], Baydakova G V[1], Mikhaylova S V[2], Zakharova E Y[1]

[1]FSBI Res Cent Med Gen, Moscow, Russian Federation, [2]Dep Med Gen, Rus Child Clin Hosp, Moscow, Russian Federation

Background: Niemann–Pick disease type C (NPC) is an inherited disorder belonging to the group of lysosomal storage diseases (LSD). Sequencing of the *NPC1* and/or *NPC2* genes can prove presence of the disease. Some studies described the possibility of using various biochemical markers for selective screening of NPC. We analyzed chitotriosidase activity in a cohort of patients suspected for NPC.
Materials: 210 patients were selected: 160 patients with high Suspicion Index (scores>70) and 50 patients with isolated splenomegaly and cholestasis. Genetic analyses focused on *NPC1* and *NPC2* genes direct sequencing and analysis of lysosomal enzymes (for differential diagnosis of other LSD) were performed for all patients.
Results: Diagnosis of NPC was confirmed in 14 patients. Diagnosis of other LSD was established in 16 patients. The increased chitotriosidase activity (>100 nmol/h/ml) was found in 93% (13/14 patients) with NPC (range 150-2088 nmol/h/ml), in 11% (20/180) of patients without mutations in *NPC1* or *NPC2* genes and 44% (7/16) with other LSD. Thereby, the sensitivity of the test was 93% (CI 95% 66-99%) and specificity 85% (CI 95% 83-86%).
Conclusion: Test of chitotriosidase activity has high sensitivity and can be used in combination with other biochemical markers as a first step in selective screening for NPC.

P-575

An unusual neurodegeneration in late infantile neuronal ceroid lipofuscinosis

Gonzalez M J[1 7], Fernandez-Marmiesse A[2], Gort L[3 6], Armstrong J[4], Montero R[5 6], Pineda M[1 7], De Diego V[7], García Cazorla A[1 6 7], O´Callaghan Gordo M M[1 6 7]

[1]Neurometab, Sant Joan de Deu Hospital, Barcelona, Spain, [2]Treatment and diagnosis IEM Unit, Santiago de Compostela, Spain, [3]Biomed Diag Center, Clínic Barc Hosp, Barcelona, Spain, [4]Genetic Dep, Sant Joan de Deu Hospital, Barcelona, Spain, [5]Biochemical Dep, Sant Joan de Deu Hosp, Barcelona, Spain, [6]C Biomed Netw Research Rare Dis, CIBERER, BCN, Spain, [7]Neuroped Dep, Sant Joan de Deu Hosp, Barcelona, Spain

Background: Neuronal ceroid lipofuscinosis type 2 is caused by a lack of tripeptidyl-peptidase I (TPP1) enzyme activity. Fast neurodegeneration, beginning by 2 - 4 years of age, is due to progressive lysosomal storage in CNS. Death occurs in early adolescence.
Case report: We present a male who started at 8 years with progressive learning disabilities lasted 2 years, bradypsychia without dysartria and isolated generalized tonic-clonic seizures. Eight months later, he developed clumsiness, progressive ataxia, isolated myoclonias, saccadic and slow ocular movements with limitation of ocular supraversion. He was on wheelchair 6 months later. IQ: 66 at 10 years, mild intellectual disability. MRI revealed severe cerebellar atrophy without white matter demyelination. EMG showed mild motor distal axonal neuropathy. VEP and ERG were normal up to 5 years after the onset. Genetic test based on lysosomal storage disorder sequencing panel found two heterozygous mutations (c.622C>T and IVS7as-10T>C) on the TPP1 gene and confirm CLN2 disease. Current Weill Cornell scale: 5 (Feeding:3, Gait:0, Motor:0, Language:2). Nowadays, he has seizures controlled by valproate and phenobarbital. He has special educational needs; he receives physiotherapy and equine therapy. He remains stable during last 12 months.
Conclusion: This case is a mild phenotype with atypical slow progression of CLN2 disease with no retinopathy and well-controlled epilepsy

P-576

Atypical pediatric presentation in two siblings with Gaucher disease type 3

Gonzalez M J[1 7], Lozano R[7], Díaz-Conradi A[6], Armstrong J[3], Gort L[2 5], Ortez C I[1 7], Montero R[4 5], García Cazorla A[1 5 7], O´Callaghan Gordo M M[1 5 7]

[1]Neurometab, Sant Joan de Deu Hospital, Barcelona, Spain, [2]Biomed Diag Center, Clínic Barc Hosp, Barcelona, Spain, [3]Genetic Dep, Sant Joan de Deu Hospital, Barcelona, Spain, [4]Biochemical Dep, Sant Joan de Deu Hosp, Barcelona, Spain, [5]C Biomed Netw Research Rare Dis, CIBERER, BCN, Spain, [6]Hematology Dep, Nens Hospital, Barcelona, Spain, [7]Neuroped Dep, Sant Joan de Deu Hosp, Barcelona, Spain

Background: Gaucher disease (GD) is an autosomal recessive lysosomal disease caused by glucocerebrosidase deficiency and subsequent accumulation of glucocerebroside. There are 3 types: type 1 with visceral involvement and types 2 and 3 with neurological involvement mainly related to D409H and L444P mutations.
Clinical cases: A 12-month-old boy was admitted with neonatal hepatomegaly and mild psychomotor retardation. Biochemical analysis revealed thrombocytopenia, increased AST, ALT and LDH. TORCH serology was negative. MRI was normal. VEP: delayed conduction optical pathways. SAEP: central and bilateral pathways involvement. GD was suspected but glucocerebrosidase activity in white blood cells was in the normal range. NPC1-NPC2 gene sequencing was negative. Two months later, twin sister consulted for mild psychomotor retardation and in exam, they showed bilateral oculomotor apraxia. Faced with clinical suspicion of GD, genetic testing is done and repeated glucocerebrosidase activity.
Results: Chitotriosidase activity 2976 and 3126 nmol/h/ml (17-211) Glucocerebrosidase activity: 1 nmol/h/mg protein (3.5-14 nmol/h/prot) and 26% (>30%). GBA gene: D409H mutation and deletion
Rec [Del55pb+D409H+L444P+A456P+V460V] in two brothers, confirming Gaucher disease type 3.
Conclusion: Clinical suspicion of GD and early diagnosis is important, because it can offer combined enzyme replacement therapy (imiglucerase) and inhibitor substrates (miglustat).

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Oxidative DNA damage is risen in Fabry patients

Biancini G B[1 2], Moura D J[3], Manini P R[3], Faverzani J L[2], Netto C B O[2], Deon M[2], Giugliani R[1 2], Saffi J[3], Vargas C R[1 2 4]

[1]PPG CB: Bioquímica, UFRGS, Porto Alegre, Brazil, [2]Medical Genetics Service, HCPA, Porto Alegre, Brazil, [3]Lab Gen Tox, UFSCPA, Porto Alegre, Brazil, [4]PPG Ciências Farmacêuticas, UFRGS, Porto Alegre, Brazil

Background: Beyond α-galactosidase A substrates accumulation, other mechanisms have been demonstrated to be involved in Fabry disease (FD) pathophysiology, such as inflammation and oxidative stress. Oxidative damage to proteins and lipids in Fabry patients were previously reported. However, DNA attack by oxidative species in FD has not been studied yet. The aim of this study was to investigate, in Fabry patients, the basal DNA damage, the nature of its damage (oxidative or not) and associate it with oxidative species levels.
Methods: Heparinized whole blood samples of ten adult patients with FD classic form and six healthy controls matched by age and sex were collected and used in the alkaline version of the comet assay to verify DNA damage. To investigate oxidative damage to purines and pyrimidines, the same assay was performed with two endonucleases (formamidopyrimidine DNA-glicosylase, FPG, and endonuclease III, EndoIII). Plasma aliquots were used to determine oxidative species levels by the dichlorofluorescein assay.
Results: Patients presented significantly higher basal DNA damage, oxidative damage to purines and oxidative species levels than controls.
Conclusions: DNA damage occurs in Fabry patients and has an oxidative origin in purines. Probably, the consequences of this damage are involved in FD pathophysiology.

P-578

Non-invasive biochemical diagnostic procedures for Niemann-Pick C (NPC): Filipin staining in blood smear and oxysterol determination by TOF MS

Eto Y[1], Takamura A[1], Fujisaki M[1], Umeda T[1], Iwamoto T[2], Ohashi T[2], Ida H[2], Eto K[3], Sakai N[4]

[1]Adv. Clin. Res. Centr, Inst. of Neurol, Tokyo, Japan, [2]Tokyo Jikei Uiv., Tokyo, Japan, [3]Dept. Ped., Tokyo Womens Med.Sch., Tokyo, Japan, [4]Dep. Ped. Osaka Univ., Osaka, Japan

Background: The diagnosis of Niemann-Pick C (NPC) patients are generally determined by invasive procedure by filipin staining on cultured skin fibroblasts. Here, we demonstrated two procedures for a diagnosis of NPC; these are Filipin staining of blood smear and also determination of 7-ketocholesterol (7-KC) in plasma by Q-TOF MS.
Materials and Methods: The blood smears from patients with late infantile, juvenile and adult type were staining by filipin staining. This procedure is simple and non-invasive for the diagnosis of NPC patients. Furthermore, we determined plasma oxysterol, 7-ketocholesterol(7KC) by Q-TOF MS.
Results: All types of NPC patients showed more or less positive staining in blood smears by filipin staining. Patients with NPC demonstrated more than 5-10 times higher concentration of 7-KC in plasma. The dried blood spot (DBS) from patients with NPC indicated that data from DBS was not consistent. The amount of plasma 7-KC was declined after Miglustat treatment in a patient with infantile NPC. These two non-invasive procedures can be useful for the diagnosis of NPC.
Conclusion: These two biochemical markers, filipin staining in blood smear and also plasma 7-KC values, can be used for non-invasive procedures to give a diagnosis of NPC.
Conflict of Interest declared.

P-579

Rare case of a liver Gaucheroma in a young child on ERT

Owens P[1], Korula S[1], Bhattacharya K[2]

[1]Metab services, Childrens hosp Westmead, Sydney, Australia, [2]Child hosp Westmead, clin sch, Univ Syd, Sydney, Australia

Background
A 5 year old boy treated from 16 months with type 1 Gaucher developing focal Gaucheroma in the liver at 3.5 years is presented.
Case: The subject presented at 15 months of age with anaemia, thrombocytopenia and hepatosplenomegaly, liver palpable 7 cm below the right costal margin and spleen 15 cm below left costal margin. Gaucher disease was confirmed by leucocyte enzyme assay. A homozygous change: c.1193G>A(p.Arg398Gln) in the *GBA* gene was identified. He had normal neurology with normal saccades. Imiglucerase was administered at 60 IU/Kg/fortnight from 16 months as per Australian regulations with good clinical response. At 3.5 years, hepatic ultrasound demonstrated a nodular cystic lesion measuring 5 x 3 x 4 cm in the right lobe of liver, confirmed on MRI. Biopsy demonstrated portal-portal bridging fibrosis with nodules of Gaucher cells. Cystic fluid comprised necrotic debris and Gaucher cells. Further evaluations over 18 months including repeated MRI, biopsy, alpha-fetoprotein monitoring and whole-body FDG-Pet scan demonstrate no malignancy.
Conclusion: GD is the most common lysosomal storage disorder. The aetiology, natural history and optimal management strategy of rare Gaucheroma in paediatric cases has not been defined particularly in regards to malignancy risk.

P-580

Biochemical study and molecular analysis identifying novel alleles in children affected with Sandhoff disease from India

Mistri M[1], Tamhankar P[2], Kodurkar P[2], Sheth F[1], Sheth J[1]

[1]FRIGE's Institute of Human Genetics, Ahmedabad, India, [2]Genetic Research centre, NIRRH, ICMR, Mumbai, India

Background and objectives: Sandhoff disease is a rare glycolipid storage disorder caused due to deficiency of β-Hexosaminidase activity. About 87 mutations in *HEXB* gene are known, but none from India. Present study will biochemically identify these children, with further confirmative diagnosis using molecular analysis.
Methods: Enzyme study using 4-MU synthetic substrate for β-Hexosaminidase and molecular analysis were performed by sequencing the *HEXB* gene.
Results: Fifteen children showed deficient activity of hexosaminidase A and B in leucocytes. Molecular analysis of the *HEXB* gene using genomic DNA identified 10 disease-causing alleles in 15 patients. These include three previously known missense mutations (p.T212S, p.C309Y, p.R533C in one patient each), the p.R284X nonsense mutation in 4 children, 1 splice site mutation (c.1417+1 G-A) and 1 insertion (c.1591_1592insC; p.R531TfsX22) in one patient each. Four mutations were novel, including 2 nonsense mutation (p.W111X in 2 children and p.R100X in 1 child) and 2 small in-frame deletions (c.534_541delAGTTTATC; p.V179RfsX10 and c.1563_1573delTATGGATGACG; p.M522LfsX2) in one patient each.
Conclusions: Our study demonstrate that 33.3% (5/15) of affected children have novel mutations while 4/15 (26.6%) patients have the p.R284X mutation which can be used as mass screening. Present study provides new insights into the molecular basis of Sandhoff disease, with possible founder effect in quarter of affected children.

P-581

Evaluation of CCL18 and chitotriosidase as biomarkers for Gaucher's and Niemann-Pick disease in patients from India and Pakistan

Sonalkar N D[1], Jalan A B[1], Mahakal J M[1], Kudalkar K V[1], Jalan R A[1], Shinde D H[1], Borugale M A[1], Joshi M M[1], Rao H A[2], Alam M A[3]

[1]NIRMAN, Div of Biochemical Genetics, Navi Mumbai, India, [2]MedGen, Clin Gen Div, Medicare Hosp, Rawalpindi, Pakistan, [3]BioCare Lab, Islamabad, Pakistan

Background: Chitotriosidase and CCL18 are common biomarkers for Gaucher's disease (GD) and Niemann pick disease (NPD). These are the two commonest LSDs in India and Pakistan.
Objective: To evaluate suitability of CCL18 or chitotriosidase as primary biomarkers for screening of GD / NPD patients.
Material and methods: Chitotriosidase and CCL18 levels were analysed by fluorometry and ELISA respectively, in 7 patients with GD (5 males, 2 females; 4 Indian/ 3 Pakistanis) and 10 patients with NPD (6 males, 4 females; 4 Indian/ 6 Pakistanis). All these cases were genetically confirmed.
Result: We found grossly elevated levels of chitotriosidase in our cohort of GD (5,488.46±7,954.93 umol/hr/ml, NR= 9-46) and NPD (NPC1, 1 NPC2) and 6 NPA/B (208.83 ±263.65umol/hr/ml). However, 2/7 with GD and 5/10 NPD

patients had undetectable activity. CCL 18 was elevated in all these patients : GD (2,450.68±5071.27 ng/ml, NR=1-72) and NPD (957.26±867.77 ng/ml).
Conclusion: Although chitotriosidase is generally considered as a primary marker for GD and NPD, its use as a biomarker is hampered by the fact that 5-6% population is deficient in activity due to a genetic defect (4-bp duplication). CCL18 appears to be more reliable with 100% sensitivity and therefore may be used as a primary biomarker.

P-582

Pompe diagnostic criteria - analysis of determining factors for correct and timely diagnosis of Pompe disease: a hypothese-generating cross-sectional study

Lagler F B[1], Rohrbach M[4], Hennermann J[3], Mengel E[3], Karabul N[3], Moder A[1], Hundsberger T[5], Rösler K[6], Huemer M[2]

[1]Inst Inborn Errors of Metab, PMU, Salzburg, Austria, [2]Kinderabteilung, LKH Bregenz, Bregenz, Austria, [3]Villa Metabolica, Mainz, Germany, [4]Children´s hospital, Zürich, Switzerland, [5]Klinik f Neuro, Kantonsspital St. Gallen, St. Gallen, Switzerland, [6]Uniklinik f Neuro, Inselspital Bern, Bern, Switzerland

Background: Pompe disease is a rare inherited lysosomal storage disorder caused by deficiency of the enzyme alpha-Glucosidase (EC 3.2.1.20). Clinical presentation and course of disease are highly heterogeneous. Therefore, delay to diagnosis is significant. To date, Pompe disease is regarded an incurable but treatable disease. Initiation of treatment at an early stage is associated with higher efficacy of treatment. The aim of this multinational study performed in Austria, Germany and Switzerland is to investigate patients' paths to diagnosis and to delineate improvement strategies for diagnostic performance.
Methods and Patients:
- Assessment of qualitative and quantitative aspects of disease-associated symptoms at onset and during the course of the disease by using patient and physician specific questionnaires
- Identification of information sources (internet and other media) proactively used by patients or parents by patient /parent-specific questionnaires.
- Identification of symptoms or symptom patterns, which constitute diagnostic pitfalls by physician-specific questionnaires
The target populations are : 30 patients with infantile or adult-onset Pompe disease and/or their parents and their attending physicians.
Results and Conclusion: Preliminary results show that prominent symptoms in infantile-onset include generalized muscle hypotonia, failure to thrive, macroglossia, weak crying, poor sucking and a hypomimic face. Most frequent symptoms in adult-onset are fatigue and motor retardation.
Conflict of Interest declared.

P-583

Characterization of gait in late-onset Pompe disease

Austin S[1], McIntosh P[1], Case L[1], Kishnani P S[1]

[1]Duke University Medical Center, Durham, North Carolina, United States

Background: The skeletal muscle manifestations of late-onset Pompe disease (LOPD) cause significant gait impairment. However, the specific temporal and spatial characteristics of abnormal gait in LOPD have not been described. The aim of this study was to characterize the gait of individuals with LOPD using several temporospatial gait parameters.
Methods: The gait characteristics of 22 individuals with LOPD were evaluated using the GAITRite® gait analysis system. The gait parameters were compared to normal reference values and correlations were made between gait abnormalities and other measures of disease progression including the six minute walk test, a 10 meter fast walk velocity measurement, and the gross motor function measure.
Results: Overall, the LOPD population demonstrated significant abnormalities in temporospatial parameters of gait when compared to norms. The LOPD population exhibited temporal gait discrepancies including decreased velocity and cadence, a prolonged stance phase and shorter swing phase, and a prolonged double limb support and shorter single limb support period.
Conclusion: Describing these gait characteristics has potential in increasing the understanding of the disease natural history, especially in regard to the impact of ERT and could serve as an important clinical endpoint as it is quick and easy to use.

P-584

Clinical features and outcomes in multiple sulfatase deficiency: a single centre experience

Sreekantam S[1], Simmons L[1], Hutchins T[1], Wassmer E[2], Vijay S[1], Santra S[1]

[1]Dept Of IMD, Birmingham Children's Hosp, Birmingham, United Kingdom, [2]Neurology, Birmingham Children's Hosp, Birmingham, United Kingdom

Background: Multiple sulfatase deficiency (MSD) is a rare lysosomal storage disorder due to mutations in the *SUMF1* gene resulting in inactive sulfatases. To date, there are very few cases reported in the literature. We report a series of 6 patients with MSD in our centre.
Methods: Retrospective case note review of all patients diagnosed with MSD in our centre focusing on clinical phenotype, mutation analysis and disease outcomes.
Results: 6 patients (4 males, 2 females) were diagnosed with MSD between 1998-2015. Mean age at presentation was 2.8 yrs with hypotonia, ataxia, developmental regression, ichtyosis and visual impairment. 2/6 had congenital talipes. There was a mean 4.9 years delay from presentation to diagnosis in index cases. MSD was confirmed by enzymatic assay of two sulfatases and *SUMF1* sequencing in 5/6 patients. Subsequent clinical problems included epilepsy (4/6), recurrent respiratory infections (3/6) and dysphagia requiring enteral feeding (5/6). One child died at age 10 y from respiratory failure. Contrary to published cases, no patient developed cardiomyopathy.
Conclusion: Our study noted variable clinical course and outcomes in MSD. It is a lethal neurodegenerative condition with no cure. Early recognition is important to facilitate genetic counselling and prevention in future pregnancies.

P-585

Is an increased Tau-protein in cerebrospinal fluid a marker for lysosomal storage diseases with neurodegeneration?

Scholl-Bürgi S[1], Michel M[1], Humpel C[3], Pichler K[1], Haberlandt E[2], Albrecht U[1 2], Baumann M[2], Karall D[1]

[1]Med Univ of Innsbruck, Pediatr, Met Dis, Innsbruck, Austria, [2]Med Univ of Innsbruck, Pediatr, Neuroped, Innsbruck, Austria, [3]Med Univ of Innsbruck, Psychiat, Exp Res, Innsbruck, Austria

Introduction: About half of the known lysosomal storage diseases (LSD) present with a neurodegenerative course. Diagnosis might be difficult as initial symptoms are unspecific. Routine diagnostic includes evaluation of clinical symptoms, examination of the urine (oligosaccharides and mucopolysaccharides), leucocytes and fibroblasts (vacuoles, enzyme activity) and genetic testing.
Methods: In 8 patients with an initially unclear neurodegenerative disease and later stated LSD, the concentration of Tau-protein in cerebrospinal fluid (CSF) was determined.
Results: Concentrations of Tau-protein in the CSF were increased in 7/8 patients with LSD (= Tau-protein > 450 pg/L) (P1, neuronal ceroid lipofuscinosis (NCL): 1920 pg/L; P2, Krabbe disease: 1837; P3, NCL: 606; P4, Niemann Pick disease type C: 1112; P5, GM1 gangliosidosis: 2800; P6, metachromatic leukodystrophy (MLD): 458; P7, MLD: 652; P8, NCL: 232.
Conclusion: Tau-protein in the CSF reflects neurodegeneration. It is elevated in Alzheimer disease and other neurodegenerative diseases. We could show that Tau-protein was elevated in 7/8 patients with diverse LSD. Therefore, elevated Tau-protein in CSF might be a new biomarker for diagnosing LSD with neurodegeneration in the oligosymptomatic state.

P-586

Pycnodysostosis: three patients with cathepsin K analysis

Kilic E[2], Elmaogullari S[4], Ucakturk S A[4], Demirel F[4], Karaer K[3], Ceylaner S[3], Kilic M[1]

[1]Div Metab Dis, Dr Sami Ulus Child Hosp, Ankara, Turkey, [2]Div Genet Dis, Hemato-Onco Child Hosp, Ankara, Turkey, [3]Intergen, Ankara, Turkey, [4]Ped End Dis, Hemato-Onco Child Hosp, Ankara, Turkey

Background and objectives: Pycnodysostosis is a rare autosomal recessive skeletal dysplasia characterized by short limb type dwarfism, dysmorphic findings and increased bone fragility. Pycnodysostosis is caused by mutations of the Cathepsin K gene (*CTSK*) located on chromosome 1q21. Cathepsin K is a lysosomal cysteine protease involved in the intralysosomal degradation of bone matrix proteins. To date, less than 200 cases have been reported. Here, we describe three new patients from two consanguineous families.
Case report: Patient 1 was a 12 year-old boy who have typical clinical and radiologic findings of pycnodysostosis. *CTSK* analysis revealed homozygosity for the c.746T>C (p.I249T) mutation. Patient 2, 12 year-old girl and patient 3, 10 year-old boy were siblings and diagnosis was made by clinical and radiologic findings. Molecular analysis of *CTSK* gene revealed the presence of the c.3G>T (p.M1I) mutation. Major clinical and radiological signs were dolicocephaly, open anterior fontanel, small face, bulding eyes, prominent nose, irregular teeth, partial anodontia, osteosclerosis, clavicular dysplasia, wormian bones, lack of frontal sinus and acro-osteolitic dysplasia of phalanges. Except for increased bone fragility, associated fractures prognosis is good and life span is normal. Growth hormone treatment had good responses in some patients.
Conclusion: Pycnodysostosis is a rare bone dysplasia-lysosomal disorder which must be noticed in pediatric practice.

P-587

Development of missense murine model of Pompe disease

Shimada Y[1], Fukuda T[2], Nishimura E[1], Hoshina H[1 3], Kobayashi H[1 3], Higuchi T[1], Ida H[1 3], Ohashi T[1 3]

[1]Div Gene Thera, Jikei Univ Sch of Med, Tokyo, Japan, [2]Div NeuroPath, Jikei Univ Sch of Med, Tokyo, Japan, [3]Dept Pedi, Jikei Univ Sch of Med, Tokyo, Japan

Background: Pompe disease is an autosomal recessive lysosomal storage disorder caused by a deficiency of acid α-glucosidase (GAA). We previously demonstrated that function of some missense GAA mutants in patient fibroblasts and transiently expressed cell lines was rescued by treatment with proteasome inhibitor bortezomib. However, efficacy of bortezomib treatment on animal model is still unclear.
Methods: To achieve the study, we generated a missense mouse model (M519V mice) by cross breeding murine GAA-deficient mice with transgenic mice expressing human GAA mutant M519V.
Results: Cross-reactive immunologic material (CRIM) was observed in major tissues including skeletal muscle and heart from M519V mice. Accumulation of glycogen was also observed in those tissues from M519V mice. In addition, autophagic buildup was observed in skeletal muscles from M519V mice as well as GAA-deficient mice. These results indicate that M519V mice are useful model for analyzing the effect of bortezomib treatment on CRIM-positive Pompe disease. We will discuss the impact of bortezomib on mutant GAA in M519V mice.

P-588

Persistent transaminitis in a patient with galactosaemia and I-cell disease despite treatment

Stepien K M[1], Broomfield A[3], Hutchesson A[1], Settle P[2], Seshadri D[2]

[1]Clin Biochem, Bolton Royal Hospital, Bolton, United Kingdom, [2]Neonatal Unit, Bolton Royal Hospital, Bolton, United Kingdom, [3]Willink Biochemical Genetics Unit, CMFT, Manchester, United Kingdom

Case Report:
A boy, born at term from Irish consanguineous parents, presented with prolonged jaundice. His total bilirubin was high (336 μmol/L) with predominantly conjugated bilirubin (70%). His liver enzymes were persistently deranged (ALT 185 IU/L, ALP 828 IU/L) despite treatment with ursodeoxycholic acid, and vitamins E and K. A family history of galactosaemia and Hurler syndrome led to detailed metabolic investigations and subsequently confirmed galactosaemia and mucolipidosis type II (I-cell disease). The GAL-1-P level was 1465 μg/ml (< 20 μg/ml). A large amount of sialic acid and a non-specific pattern in an oligosaccharides screen were seen. A plasma enzyme analysis showed high beta hexosaminidase A+B and alpha mannosidase. Subsequently, GNPTAB sequencing confirmed mucolipidosis type II.
Both conditions are well recognised in Irish travellers, but the combination of galactosaemia and I-cell disease is not documented in the literature. Despite a galactose-free diet, mild transaminitis persisted. A disproportional rise in ALP (but normal GGT) was most likely to have reflected overt hyperparathyroidism (PTH 30.6 pmol/L; reference ranges 1.5-9.2), as described in I-cell disease. Thus, careful dietetic input is required to balance the dietary lactose restrictions required to treat galactosaemia and calcium abnormalities seen in patients with I-cell disease to maintain bone mineralisation and help prevent pathological fractures.

P-589

A case of Gaucher Disease with negative bone marrow aspirate and normal initial beta-glucocerebrosidase assay

Yuliarti K[1], Masnadi N R[1], Sjarif D R[1]

[1]Div Metab Dis, Univ of Indonesia, Jakarta Selatan, Indonesia

Background: Gaucher disease (GD) is a lysosomal storage disease characterized by a deficiency of enzyme beta-glucocerebrosidase. Bone marrow examination is the hallmark for the diagnosis of GD.
Case: A 17-month-old boy presented with hepatosplenomegaly and severe wasting since age one year. There was no history of fever, ictere, bleeding, or bone pain. Peripheral blood smears showed hemolytic anemia and thrombocytopenia. Lipid profile showed low cholesterol (85 mg/dL), LDL (12 mg/dL), HDL (3 mg/dL), and Apo A1 (28 mg/dL). Working diagnosis was storage disease with differential diagnosis Tangier disease. Bone marrow aspirate examined by three pediatric hematologists did not reveal any Gaucher cell. Screening for storage disease using blood spot demonstrated normal acid sphingomyelinase and beta-glucocerebrosidase. Gene sequencing for Tangier found no mutation. Follow-up showed progressing hepatosplenomegaly. An investigation was performed on leukocytes and revealed low beta-glucocerebrosidase (0.18 nmol/mg prot/h) and high chitotriosidase (6155.65 nmol/mL/h) which were consistent with Gaucher disease. As no neurologic involvement was present, this patient was classified as GD type 1.
Conclusion: Negative bone marrow aspirate and normal glucocerebrosidase on screening test did not exclude GD. A possibility of GD should be considered in patients with progressive hepatosplenomegaly.
Keywords: Hepatosplenomegaly, bone marrow aspirate, Gaucher Disease

P-590

Cystinosis in an adult metabolic clinic - A truly multi-systemic disease requiring multi-professional and multi-disciplinary management

Sharma R[1]

[1]The Mark Holland Metabolic Unit, SRFT, Salford, United Kingdom

Introduction: Cystinosis is a lysosomal storage disease caused by *CTNS* gene mutations causing cystine-specific lysosomal membrane transporter cystinosin defect. Accumulated cystine leads to cellular dysfunction. We present the clinical details of our adult patients illustrating the multi-systemic nature of the disease as well as demonstrating that ESRD is not inevitable.
Method: In Manchester, there is a transition process of cystinosis patients from paediatric (nephrology) care into the adult IMD service. We report the current clinical condition of 7 patients (age 19-25y; 3M, 4F). All had presented with the classical infantile form of the disease.
Results: Average height was 157 (range 140-176) cms with BMIs average 22.6 (range 18.1-27.9) and all had ocular involvement. Three patients had functioning renal transplants, 2 on dialysis (PD and HD) and two patients had stable eGFRs of 86 and 27 mls/min. Four patients had hypothyroidism, three abnormal echocardiogram, one gonadal dysfunction, one osteopenia and one with PEG and feeding issues. Adherence to cysteamine was inadequate.
Discussion: Cystinosis in the long term is a multi-systemic disease. Adult patients require ongoing renal support, but at the same time will benefit from a formal transition into appropriate adult services where a multi-professional and MDT can ensure a high standard of care of the wide range of problems.

P-591

Fabry / non-Fabry: is *GLA* p.S126G a pathogenic mutation?

Arrizza C[1], Nowak A[2], Hahn D[1], Gautschi M[1], Rohrbach M[3], Ballhausen D[4], Nuoffer J M[1]

[1]Univ Inst Clin Chem, Inselspital, Bern, Switzerland, [2]Univ Child Hosp, Inselspital, Bern, Switzerland, [3]Univ Child Hosp, Bern, Switzerland, [4]Univ Inst Pathol, Bern, Switzerland

Background: Fabry disease is often screened or diagnosed genetically and biochemical investigations are not always performed. Pathogenicity of some mutations may be unclear.
Case report: 45 year old man with a patent foramen ovale and a positive family history for stroke, had a stroke. This led to a familial genetic investigation for Fabry disease. Neither the index patient nor the family members were otherwise symptomatic and renal, cardiac and neurological investigations were normal in positively screened relatives.
Results: The index patient and two cousins, brothers of 52 and 55 years had *GLA* gene mutation (p.S126G), reported as pathological (Branton, Medicine, 2002). The index case had a 66% residual enzymatic activity and both cousins showed normal activities in lymphocytes. The 52 year old patient further had normal enzymatic activity in fibroblasts and normal plasmatic and urinary ceramide trihexoside.
Conclusion: In the literature, this mutation is associated with a stroke-like Fabry phenotype (Rolfs, Stroke, 2013), but enzymatic and biochemical analyses are not mentioned. In our patients, both enzymatic activity and biomarkers are completely normal. In our opinion, this raises the question of the pathogenicity of this mutation and certainly the need of further investigation in order to adequately advice genetically screened patients.

P-592

Isolated elevated transaminases as an early sign of late-onset asymptomatic Pompe disease

Garnotel R[1]

[1]Pediatrics Lab, CHU Reims, Reims, France

Pompe disease (acid maltase deficiency) is an autosomal recessive metabolic disease, in which the enzyme acid alpha glucosidase (GAA) is deficient.
A 7-year-old boy was seen by the general paediatrician because of viral infection. Routine blood analysis showed persistently elevated plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations [176 IU/L (reference range : 10-30 IU/L and 132 IU/L (reference range : 10-40 IU/L), respectively). Liver pathology was excluded by Doppler ultrasound and a muscular rather than a hepatic problem was suspected, with creatine kinase (CK) concentration of 839 IU/L (reference range < 200 IU/L). Cardiac ultrasound was also normal. A muscular metabolic assessment was carried out with the assay for alpha glucosidase activity from whole blood using dried blood spot filter paper. The ratio alpha-glucosidase/hexosaminidase was 0.8 (reference range > 3), suggestive of Pompe's disease. Glc4 concentration in urine was 7.1 mmol/mol of creatinine (reference range < 2.5). DNA analysis revealed two mutations on the GAA gene encoding acid alpha-glucosidase : c.-32-13T>G and p.G643R.
The question thus remains : what is wise in cases like this, where no symptoms of the disease in terms of myopathy are present, but with evident and persistent elevations of AST, ALT and CK ?

P-593

Think outside the box: how genetic findings can be misleading

Arrizza C[2], Pavlovic M[2], Rieubland C[2], Hewer E[4], Perret Hoignè E[2], Hahn D[1], Casaulta C[2], Nuoffer J M[2], Gautschi M[3]

[1]Univ Inst Clin Chem, Inselspital, Bern, Switzerland, [2]Univ Child Hosp, Inselspital, Bern, Switzerland, [3]Univ Child Hosp, Bern, Switzerland, [4]Univ Inst Pathol, Bern, Switzerland

Background: Genetic panels are increasingly used as a routine investigation, but mutations may not always confirm the causality.
Case report: Hypertrophic interventricular septum was found in an asymptomatic newborn. A genetic panel for hypertrophic cardiomyopathy (HCM) revealed a heterozygous mutation p.Thr1929Met of *MYH7* (Michels, Eur Heart J, 2009) also found in the asymptomatic father. After 2 years, he developed progressive truncal-proximal myopathy, growth and developmental delay. Transaminases and CK were 5-10 x increased. MYH7 mutations have been associated with myopathies with variable expressivity, p.Thr1929Met however classified as benign variant by some prediction tools. At 6 years, an infection caused respiratory insufficiency and acute regression, requiring invasive ventilation.
Results: At 5 8/12 years a muscle biopsy showed glycogen storage. Pathological urinary oligosaccharides, low α-glucosidase activity and known pathogenic homozygous *GAA* mutation (p.Leu355Pro) confirmed the diagnosis of Pompe disease, with associated CRIM-positive status. Four months after starting ERT and enteral nutrition, he has made some progress, but still needs BiPAP-mask at night. Alglucosidase alpha IgG-antibodies were found.
Conclusion: Genetic panels should be carefully interpreted in the light of clinical, neurological, metabolic and genetic findings. In this patient, asymptomatic HCM and the possibly causative MYH7 mutation, have delayed the recognition of Pompe disease.

P-594

Niemann-Pick C Brazil network: five-year report of diagnostic results

Giugliani R[1 2], Souza F T S[1 2], Kessler R G[1 2], Burin M G[1 2], Michelin-Tirelli K[1 2], Civallero G E[1 2], Polese-Bonatto M[1 2], Trapp F B[1 2], Rafaelli C L[1 2], Souza C F M[1 2], Vairo F P[1 2], Ribas G S[1 2], Vargas C R[1 2], Saraiva-Pereira M L[1 2]

[1]Medical Genetics Service, HCPA/UFRGS, Porto Alegre, Brazil, [2]NPC Brazil Network, Headquarters, Porto Alegre, Brazil

Background: Niemann-Pick C (NP-C) is a progressive and severe lysosomal disease of the cholesterol trafficking, caused by mutations in any of the two genes involved in this route (*NPC1* and *NPC2*). The disease is raising more interest in the last years, as a treatment (miglustat), which modifies its natural history, became available. The number of identified cases is growing, but until now diagnosis has been difficult, usually invasive, always expensive and time-consuming. It has been based on a test (Filipin) performed on living fibroblasts obtained from a skin biopsy, with the positive and inconclusive results usually further investigated by molecular analysis of the *NPC1*/*NPC2* genes (30 exons in total).
Methods & Results: Our group coordinates a large network of Brazilian services which investigates patients at risk for presenting NPC and diagnosed 80 cases from 2010 to 2014. The data emerging from this program provides valuable information about the diagnostic challenges faced by a reference laboratory in a large developing country, and also helps to delineate the profile of this disease in Brazil, including the most prevalent mutations in each region. The recent introduction in this program of new biomarkers (as oxysterols) and of new genotyping technology (massive parallel sequencing) may significantly change the present diagnostic protocol.
Conflict of Interest declared.

P-595

Acid α-glucosidase protein interactions in control and Pompe disease fibroblasts

Coletta M[1 2], Porto C[1 2], Canetti D[3 4], Carrella A[2], Acampora E[1], Gatto F[2], Tarallo A[1 2], Monti M[3 4], Pucci P[3 4], Parenti G[1 2]

[1]Dep Transl Med Sc, Univ Federico II, Naples, Italy, [2]TIGEM, Pozzuoli, Italy, [3]CEINGE, Naples, Italy, [4]Dep Chem Sc, Univ Federico II, Naples, Italy

Background: Pompe disease (PD) is a metabolic myopathy caused by the deficiency of acid α-glucosidase (GAA). Extensive glycogen storage, massive accumulation of autophagic vesicles and debris in muscles are the disease pathological hallmarks. In order to improve our understanding of PD pathophysiology, we carried out functional proteomic experiments aimed at the identification of interactors of both wild type (wt) and mutant (p.L552P) GAA.
Methods: Control and p.L552P fibroblasts total lysates were immunoprecipitated with an anti-GAA polyclonal antibody.

Immunoprecipitated proteins were fractionated by SDS-PAGE. Protein bands were analyzed by nanoliquid chromatography-tandem mass spectrometry (nanoLC-MS/MS). To confirm GAA interactions with selected proteins, co-immunoprecipitation and western blot analysis were performed.
Results: LC-MS/MS analysis generated a list of interactors, many of them are cytoskeletal proteins. We selected two proteins, gelsolin and myosin VI. These studies showed that, while gelsolin interacts only with the wt GAA, mainly with the precursor GAA isoform, myosin VI shows interactions with all GAA isoforms (including the mature forms) both in wt and mutant fibroblasts. In fibroblasts from PD patients, gelsolin levels appeared to be increased by western blot analysis.
Conclusion: These results suggest that GAA interacts with different proteins at different stages of its trafficking and maturation.

P-596

Infantile onset Pompe disease : the French experience

Tardieu M[1], Chabrol B[2], Feillet F[3], Valayannopoulos V[6], Cano A[2], Carneiro M[8], Masurel A[9], Rivier F[8], Barth M[14], Eyer D[10], Kuster A[5], Mention K[4], Peralta M[11], Pitelet G[12], Vanhulle C[13], Ogier de Baulny H[7], Labarthe F[1]

[1]Pediatrics, CHRU, Tours, France, [2]Pediatrics, CHU, Marseille, France, [3]Pediatrics, CHRU, Nancy, France, [4]Pediatrics, CHU, Lille, France, [5]Pediatics, CHRU, Nantes, France, [6]Pediatrics, APHP Necker, Paris, France, [7]Pediatrics, APHP Debré, Paris, France, [8]Pediatrics, CHRU, Montpellier, France, [9]Genetics, CHRU, Dijon, France, [10]Pediatrics, CHRU, Strasbourg, France, [11]Pediatrics, CHRU, Mulhouse, France, [12]Pediatrics, CHRU, Nice, France, [13]Pediatrics, CHRU, Rouen, France, [14]Genetics, CHRU, Angers, France

Background: The aim was to report the French experience of infantile-onset Pompe disease (IOPD) since enzyme replacement therapy (ERT) was available.
Patients & Method: Outcomes of IOPD French patients diagnosed between 2004 and 2012 were retrospectively collected.
Results: Thirty-one IOPD patients have been diagnosed, 25 classical and 6 atypical forms. At diagnosis, patients with a classical form had hypertrophic cardiomyopathy (100%), hypotonia (92%) and assisted ventilation (29%). Seven patients died without ERT at the age of 6.8 [5.9-9.5] months. Among the 18 patients treated with ERT (median duration: 5.0 [0.5-7.6] years), a significant improvement was reported in 61% of patients for cardiac symptoms, 50% for respiratory symptoms and solely 31% for muscular symptoms. Seven children died after an ERT duration of 9.9 [2.0-22.6] months. Similar results were obtained for atypical forms. The risk factors related to a good response to ERT were the precocity of diagnosis and of treatment with ERT, which were both correlated to respiratory outcome.
Conclusion: Treatment with ERT prolonged survival and improved most of the clinical symptoms of children with IPD, but prognosis at long-term remained poor.

P-597

Diagnosis of cystinosis in the practice of the Russian diversified medical center

Pushkov A A[1], Sukhozhenko A V[2], Savostyanov K V[1], Mayansky N A[1], Tsygin A N[1]

[1]Scientific Centre of Children Health, Moscow, Russian Federation, [2]GosNIIGenetika, Moscow, Russian Federation

Background and objectives: Cystinosis is a metabolic disease characterized by an accumulation of cystine inside the lysosomes, causing damage in different organs and tissues, particularly in the kidneys and eyes.
Materials and Methods: In our center for the first time in Russia we developped and validated a method for measuring the concentration of cystine in the peripheral blood leukocytes by HPLC-MS. Cystine was measured in leukocytes obtained from the whole venous blood. Leukocyte protein extracts were analyzed by means of HPLC-MS, and cystine content was expressed in nmol 1/2 cystine per mg protein.
Results: We have analyzed about 80 patients with suspected cystinosis. As a result, 11 of them were identified with increased concentration of cystine in white blood cells, and then the diagnosis was confirmed by molecular genetic analysis. As a result we have found two previously undescribed mutation (c.100delA and c.450G>A), which according to in silico analyzes are pathogenic.
Discussion/Conclusion: Such a diagnostic algorithm will allow for selective screening of cystinosis in the territory of Russia, which will greatly improve the early detection of the disease. Early diagnosis and early treatment of cystinosis have significant impact on the long-term prognosis.

25. Lysosomal disorders: treatment, enzyme replacement therapy

P-598

Efficacy of enzyme replacement therapy with agalsidase alfa for 32 naïve Fabry disease patiets

Tsuboi K[1], Yamamoto H[1], Goto H[1]

[1]LSD Center, Nagoya Central Hospital, Nagoya, Japan

Background and objectives: Fabry disease is one of lysosomal diseases caused by deficiency of alpha-galactosidase activity and the substrate, mainly globotriaosylceramide (Gb3) progressively accumulates in lysosome. In this study, we investigated the efficacy and safety of the enzyme replacement therapy (ERT) in 32 naïve patients (14 males and 18 females) with Fabry disease who were treated with agalsidase alfa at our institution.
Material and Methods: We regularly measured plasma Gb3 and globotriaosylsphingosine (lyso-Gb3) at baseline and through ERT, as well as LVMI (left ventricular mass index), BNP, and high-sensitivity troponin I (hsTnI) and eGFR for renal functions. We also investigated the incidence of adverse events and appearance of infusion reactions related to anti-agalsidase alfa IgG antibody.
Results: Parameters related to cardiac function were maintained stably in most of the patients. eGFR was stable within the normal range in the patients in G1 and G2 showed a trend to improve categories according to CKD guideline. Plasma Gb3 and lyso-Gb3 markedly decreased after the initiation of treatment and maintained at low levels.
Conclusion: Our data suggested agalsidase alfa is effective and the incidence of allergic reactions related to the treatment with agalsidase alfa is low, indicating tolerance to ERT.

P-599

Efficacy of enzyme replacement therapy with agalsidase beta for 17 naïve Fabry disease patients

Tsuboi K[1], Yamamoto H[1], Goto H[1], Ota A[2]

[1]LSD Center, Nagoya Central Hospital, Nagoya, Japan, [2]Genzyme Japan, Tokyo, Japan

Background and objectives: Fabry disease is a lysosomal disease caused by deficiency of alpha-galactosidase activity and the substrate, mainly globotriaosylceramide (Gb3) progressively accumulates in lysosome. In this study, we investigated the efficacy and safety of the enzyme replacement therapy (ERT) in 17 patients (8 males and 9 females) with Fabry disease who were treated with agalsidase beta at our institution.
Material and Methods: We regularly measured plasma Gb3 and lyso-Gb3 at baseline and through ERT, as well as IVSd, LVpwd, LVMi, EF, BNP for cardiac functions and eGFR for renal functions. We also investigated the incidence of adverse events and appearance of infusion reactions related to anti-agalsidase beta IgG antibody.
Results: Parameters related to cardiac function were stabilised in most of the patients. eGFR was stable within the normal range in the patients in G1, G2 and G3a categories according to CKD guideline, but tended to be worse in G4 and G5 categories. Plasma Gb3 and lyso-Gb3 markedly decreased after the initiation of treatment and maintained at low levels.
Conclusion: Our data suggested the earlier initiation of ERT was more effective and ERT with agalsidase beta was well-tolerated.
Conflict of Interest declared.

P-600

Dose- and time-dependent clearance of lysosomal storage in the Mucopolysaccharidosis-IIIB (MPS IIIB, Sanfilippo B) mouse model by intracerebroventricular enzyme replacement therapy with BMN-250, a NAGLU-IGFII fusion protein

Aoyagi-Scharber M[1], Vincelette J[1], Lawrence R[1], Crippen-Harmon D[1], Yip B K[1], Baridon B[1], Prill H[1], Minto W[1], Van Vleet J[1], Vitelli C[1], Adintori E G[1], Strong K M[1], Christianson T[1], Tiger P M N[1], Lo M J[1], Holtzinger J[1], Chen E[1], Fitzpatrick P A[1], LeBowitz J H[1], Bagri A[1], Bullens S[1], Crawford B E[1], Bunting S[1]

[1]BioMarin Pharmaceutical Inc., Novato, United States

Background: Mucopolysaccharidosis-IIIB (MPS-IIIB), caused by heritable deficiency of alpha-N-acetylglucosaminidase (NAGLU) required for lysosomal degradation of heparan sulfate (HS), is a devastating pediatric neurodegenerative disorder with no approved treatment. BMN-250 is an enzyme replacement therapy drug candidate, comprised of NAGLU fused with insulin-like growth factor II for enhanced cellular uptake.
Methods: BMN-250 was administered intracerebroventricularly to MPS-IIIB mice four times over two weeks at 0-100 micrograms/dose. Brain and liver (harvested 1, 7, or 28 days, or monthly for 6 months post-last-infusion) were analyzed for NAGLU, disease-specific HS Non-Reducing Ends (NREs; Sensi-Pro) and other lysosomal markers.
Results: The 100-microgram doses resulted in NAGLU activity above normal levels, clearance of disease-specific HS NREs (98.8±1.9% brain; 100.0±0.0% liver) and reduction of beta-hexosaminidase activity (47.9±5.4% brain; 70.1±6.6% liver), relative to vehicle-treated MPS-IIIB mice. Immunohistochemistry confirmed effective delivery of NAGLU to neurons. At doses above 10 micrograms, reduction of NREs was accompanied by decreases in beta-hexosaminidase activity. Reduction of NREs achievable by the 10-microgram doses

persisted for 2-3 months post-last-infusion before re-accumulation occurred, eventually reaching the vehicle-treated mutant levels; more rapid re-accumulation occurred in liver.
Conclusion: Intracerebroventricular delivery of BMN-250 gives near complete (>98%) clearance of disease-specific HS NREs in brain and liver, warranting clinical evaluation.
Conflict of Interest declared.

P-601

Comparison of endomyocardial biopsy results and cardiac parameters in Taiwanese patients with the Chinese hotspot IVS4+919G>A mutation: data from the Fabry Outcome Survey (FOS)

Niu D M[1], Chang F P[2], Chu T H[1], Yu W C[3], Sung S H[3], Bizjajeva S[4], Hsu T R[1]

[1]Dept of Pediatrics, TVGH, Taipei, Taiwan, [2]Dept of Pathology, TVGH, Taipei, Taiwan, [3]Div Cardiol, Dept of Medicine, TVGH, Taipei, Taiwan, [4]Shire, Zug, Switzerland

Background and objectives: To evaluate cardiac pathology in Taiwanese patients with Fabry disease and the IVS4+919G>A (IVS4) mutation.
Patients and methods: Retrospective analysis of Fabry Outcome Survey data (Shire; extracted January 2015) from patients with IVS4 who underwent endomyocardial biopsy.
Results: Of 24 males and 6 females (median age [Q1; Q3] at biopsy 60.4 [57.4; 64.1] and 61.3 [60.4; 65.1] years, respectively), 23 males (95.8%) and 6 females (100%) received agalsidase alfa enzyme replacement therapy (ERT). Median left ventricular mass index indexed to height (LVMI) within ±6 months of biopsy was 65.3 [52.7; 93.1] and 53.2 [42.0; 55.0] $g/m^{2.7}$ in males and females, respectively. A significant correlation was found between percentage area of globotriaosylceramide (Gb_3) accumulation within cardiomyocytes and LVMI (Spearman's correlation coefficient, 0.45;*P*=0.014), but not between cardiomyocyte diameter and LVMI. A significant negative correlation was found between Gb_3 accumulation and ERT duration (Spearman's correlation coefficient, –0.49, *P*=0.007), and between cardiomyocyte size and ERT duration (Spearman's correlation coefficient, –0.37, *P*=0.048).
Conclusion: Our results provide insight into the pathological characteristics and effects of ERT with agalsidase alfa in Taiwanese patients with cardiac variant IVS4 Fabry disease. Further follow up is recommended to confirm these trends in a larger sample size.
Conflict of Interest declared.

P-602

Pregnancy, enzyme replacement therapy and mucopolysaccharidosis: successful outcome

Guffon N[1], Azzi D[1], Fouilhoux A[1]

[1]Ref Centre of Metab Dis, HFME Hosp, HCL, Lyon, France

Objectives: In our centre, we follow 75 adults MPS with 30 females (18 to 52 years) and 46 males (18 to 71 years). 3 patients live as a couple. 2 patients have had a successful pregnancy (one with MPSVI and one MPSIV) and one MPSII family treated by enzyme replacement therapy (ERT) or by successful engraftment had normal pregnancies
Case Report: In this work, we report 4 patients MPS cases with successful pregnancies (2 females and 2 males). All pregnancies proceeded to normal term with spontaneous vaginal delivery. For our 2 MPS females, the enzyme replacement therapy was not interrupted during pregnancy. For our 2 brothers with MPSII, one receives ERT and did not stop and one has received a bone marrow transplant.
Results: For the two MPS women, the development of the 2 fetuses was normal with fetal data corresponding to gestational age. At birth, the 2 babies had normal physical examination with normal growth parameters. The mothers resumed ERT after no more than 2 infusions missed due to delivery and the immediate post delivery period. For MPSII patients, the development of the fetuses and babies prodeeded without any problem.
Conclusions: These observations show that for patients with MPS, including those on ERT, pregnancy was both possible and safe.

P-603

Changes in plasma biomarkers associated with the inflammatory response in type 1 Gaucher disease patients after one year on therapy with Velaglucerase alfa

Andrade M[1], Gervas J[7], García I[15], Salamero O[2], Martinez-Odriozola P[3], Mendez J A[4], Garcia-Bragado F[5], Calvo C[6], Fernandez-Canal C[8], Sancho-Val I[9], Cano H[10], Perez J[2], Hernandez-Rivas J M[11], Lorenzo D[12], Lopez-Dupla M[13], Callao V[14], Giraldo P[1]

[1]CIBERER. UIT. IISAragon, Zaragoza,, Spain, [2]Vall d'Hebron University Hospital, Barcelona, Spain, [3]Basurto Hospital, Bilbao, Spain, [4]Ourense Hospital, Ourense, Spain, [5]Josep Trueta Hospital, Girona, Spain, [6]San Jorge Hospital, Huesca, Spain, [7]IACS. FEETEG, Zaragoza, Spain, [8]Cabueñes Hospital, Gijon, Spain, [9]Alcañiz Hospital, Alcañiz, Spain,

[10]Arcos del Mar Menor University Hospital, Murcia, Spain, [11]Salamanca University Hospital, Salamanca, Spain, [12]Complejo Hospitalario de Burgos, Burgos, Spain, [13]Joan XXIII Hospital, Tarragona, Spain, [14]Arnau de Vilanova Hospital, Lleida, Spain, [15]Miguel Servet University Hospital, Zaragoza, Spain

Background: We have reported some changes in inflammatory biomarkers related to iron and cytokine profile in Gaucher Disease patients (GD) with severe bone involvement. Now we have explored the changes in the biomarkers of immune response in a cohort of Spanish GD1 after one year on Velaglucerase alfa therapy.
Patients/Methods: 17 naïve or previously treated, symptomatic GD1 patients, > 4 years old, were included in a prospective study. Haematological parameters, liver/spleen size, bone marrow MRI, Chitotriosidase, CCL18/PARC and IL-10, IL-13, IL-4, IL-6, IL-7, Mip1a, Mip1b,TNFα, were evaluated at baseline and after 12 months on velaglucerase alfa (30-60U/kg IV, every two weeks).
Results: M: 9/F:8, mean age: 37.5 years (9-72). 3 splenectomized (17.6%); 3 N370S/N370S, one N370S/L444P and 14 N370S/other. Seven (41.2%) have a previous history of bone disease complications. All patients achieving objective response, no infusion reactions or presence of antibodies were reported, a reduction or stabilization of CT activity and CCL/18 concentration were observed, ferritine concentration reduction, the cytokine profile showed a significant reduction of Mip1α (p=0.027) and TNFα (p=0.023).
Conclusion: Velaglucerase alfa is a well-tolerated therapy in every day clinical practice; in our cohort we found a significant decrease on the inflammatory state reflected through the cytokine reduction.
Conflict of Interest declared.

P-604

Few days earlier enzyme replacement therapy for infantile-onset Pompe disease contribute to better outcomes: 7-year cohort study in Taiwan

Yang C F[1 2], Liao H C[4 5], Huang L Y[7], Chiang C C[4], Ho H C[5], Lai C J[6], Chu T H[1], Yang T F[6], Chuang S Y[6], Hsu T R[1], Niu D M[1 3], Yang C C[2]

[1]Dep Ped, Taipei Veterans General Hosp, Taipei, Taiwan, [2]Envir and Occup Health Sci, Univ Y-M, Taipei, Taiwan, [3]Dep Ped, Sch Med, Univ Y-M, Taipei, Taiwan, [4]Chinese Found health Neona Screen Center, Taipei, Taiwan, [5]Taipei Institute of Pathology, Taipei, Taiwan, [6]Physi Med Rehab, Taipei Veter Gen Hosp, Taipei, Taiwan, [7]Div Nephro, Taipei City Hosp HepingFuyou, Taipei, Taiwan

Background: Pompe disease is one of lysosomal storage disorders characterized by the deficiency of acid α-glucosidase (GAA). Whether outcomes differ between very early (around 10 day-old) and early (around 20-day-old) ERT is undetermined. We analyzed the prognosis of our infantile-onset Pompe disease(IOPD) patients and compared to other IOPD cohort studies.
Methods: In this nationwide program, 729,797 newborns were screened for Pompe disease between Jan. 1, 2008 and Jan. 31, 2015. 16 IOPD were all CRIM-positive, treated and followed in our hospital. We analyzed the outcomes and compared the results with other CRIM-positive IOPD cohort studies.
Results: After 2010, 14 IOPD met this criteria and received their first ERT within 4 hours of admission at a mean age of 10.56 ± 3.4 days. Our patients have better biological, physical and developmental presentations and very lower anti-rhGAA antibodies after 2-year treatment, even comparing to one group which just around 10 days later first-time ERT than us. We also analyzed our 5 IOPD patients with identical mutation and still found that even few days earlier ERT contributes to better outcomes.
Conclusions: Our results indicate that early identification of IOPD patients allows for the very early initiation of ERT, and few days earlier can lead to better patient outcomes.

P-605

Central nervous system manifestations in Fabry disease: comparison of Taiwanese patients with the Chinese hotspot IVS4+919G>A or classical Fabry mutations

Hsu T R[1], Hung S C[4], Lee H J[4], Yu W C[2], Chu T H[1], Yang C F[1], Bizjajeva S[3], Niu D M[1]

[1]Dept of Pediatrics, TVGH, Taipei, Taiwan, [2]Div Cardiol, Dept of Medicine, TVGH, Taipei, Taiwan, [3]Shire, Zug, Switzerland, [4]Dept of Radiology, TVGH, Taipei, Taiwan

Background and objectives: To compare central nervous system (CNS) manifestations in Taiwanese Fabry disease patients with IVS4+919G>A (IVS4) or classical Fabry mutations.
Patients and methods: Retrospective analysis of magnetic resonance imaging (MRI) data from Taiwanese patients in the Fabry Outcome Survey (Shire; extracted January 2015).
Results: Twenty-five patients with IVS4 (19 males) and 12 (4 males) with classical Fabry mutations underwent MRI at median (range) age of 60.7 (45.0-70.4) and 43.0 (18.0-61.4) years, respectively. All patients received agalsidase alfa treatment; 2 classical Fabry patients (16.7%) underwent MRI before treatment start. Median (range) basilar artery (BA) diameter was 2.7 (1.4-4.0) mm in IVS4 and 3.2 (2.3-4.7) mm in

classical Fabry patients. Pulvinar sign occurred in 8 (32%; 7 males) IVS4 and 6 (50%; 3 males) classical Fabry patients. Infarction occurred in 8 (32.0%) IVS4 and 4 (33.3%) classical Fabry patients, and both anterior and posterior circulation stroke in 4 (16%) and 2 (16.7%), respectively. Fazekas scores of 0, 1, 2 and 3 were found for 15 (60.0%), 7 (28.0%), 2 (8.0%), 1 (4.0%) IVS4 patients and 6 (50.0%), 4 (33.3%), 2 (16.7%) and 0 classical Fabry patients.
Conclusion: Similar MRI findings were found in both IVS4 and classical Fabry patients.
Conflict of Interest declared.

P-606

An open-label extension study of velaglucerase alfa enzyme replacement therapy in patients with Gaucher disease in Japan

Ida H[1], Tanaka A[2], Matsubayashi T[3], Murayama K[4], Hongo T[5], Lee H M[6], Mellgard B[7]

[1]The Jikei University School of Medicine, Tokyo, Japan, [2]Osaka City Univ Grad School of Medicine, Osaka, Japan, [3]Hamamatsu University School of Medicine, Hamamatsu, Japan, [4]Chiba Children's Hospital, Chiba, Japan, [5]Iwata City Hospital, Iwata, Japan, [6]Shire, Lexington, United States, [7]Shire, Zug, Switzerland

Background: This phase III extension study assessed the longer-term safety and efficacy of velaglucerase alfa in Japanese Gaucher disease (GD) patients.
Methods:The open-label extension study (ClinicalTrials.gov identifier NCT01842841) enrolled Japanese GD patients who completed the 12-month parent study; patients continued receiving the same dose of every-other-week, intravenous velaglucerase alfa infusions.
Results:Five of six patients from the parent study enrolled: four male patients aged 12 years (GD1), 15 years (GD3), 16 years (GD3), and 27 years (GD1) and one 41-year-old female patient (GD1). Before the parent study, the patients had received 8.9-14.2 years of imiglucerase infusions. All five completed the extension study (63-78 weeks), receiving average doses of 51.5-60.7 U/kg. The most common adverse event was headache (n=3). There were three drug-related non-serious adverse events. Median (range) changes from the baseline of the parent study to 24 months (week 101/week 103) were: haemoglobin -0.10 g/dL (-0.4, 1.6); platelets 5.0×10^9/L (0, 32); liver volume -0.04% body weight (-0.3, 0.3); spleen volume 0.01% body weight (-0.1, 0.2). There were no changes in neurological status. No patient tested positive for anti-velaglucerase alfa antibodies.
Conclusion: There were no safety concerns, and key clinical variables were stable during 2 years of velaglucerase alfa therapy in Japanese GD patients.
Conflict of Interest declared.

P-607

Home infusion of intravenous velaglucerase alfa: experience from velaglucerase alfa clinical trials in patients with Gaucher disease type 1

Elstein D[3], Burrow T A[6], Charrow J[7], Giraldo P[4 5], Mehta A[8], Pastores G M[9], Lee H M[1], Panahloo Z[2], Zimran A[3]

[1]Shire, Lexington, United States, [2]Shire, Zug, Switzerland, [3]Shaare Zedek Med Cent & Hadassah Med Sch, Jerusalem, Israel, [4]CIBER de Enfermedades Raras, Zaragoza, Spain, [5]Miguel Servet University Hospital, Zaragoza, Spain, [6]Cincinnati Children's Hospital, Cincinnati, United States, [7]Ann & Robert H. Lurie Children's Hosp, Chicago, United States, [8]Royal Free Hospital, London, United Kingdom, [9]Mater Misericordiae University Hospital, Dublin, Ireland

Objective: To describe the velaglucerase alfa home infusion experience in clinical trials for Gaucher disease type 1.
Methods: Eligible patients in the trial TKT025EXT and in TKT034 and its extension (HGT-GCB-044) could opt to receive velaglucerase alfa (every-other-week intravenous infusions) at home after receiving initial study infusions in a clinic. Clinic and home infusions were administered by healthcare professionals.
Results: Home therapy was available for 4.57 years in '025EXT' and 5.10 years in '034-044'. 7/8 eligible '025EXT' patients and 28/38 '034-044' patients received ≥1 home infusion. These patients (N=35) received 6%-86% of their scheduled infusions at home (median 68%). The duration of home therapy ranged from 0.11-4.56 years (median 2.73 years), with periodic in-clinic infusions for protocol-mandated evaluations. 1828/2777 infusions (66%) were administered at home. Seven home-therapy patients (20%) experienced 57 infusion-related adverse events (IRAEs) during home infusions, including fatigue, headache, dizziness and hypertension. All IRAEs were mild/moderate and resolved without sequelae. Two patients received premedication to prevent IRAEs. No home-therapy patient reverted to clinic infusions during these trials, and most patients upon transitioning to commercial drug were able to continue home therapy.
Conclusion: Velaglucerase alfa's safety profile permitted initiation of home therapy during clinical trials, giving patients a convenient alternative to clinic infusions.
Conflict of Interest declared.

P-608

Development of anti-drug antibodies in Gaucher disease patients treated in velaglucerase alfa clinical trials

Pastores G M[4], Ben Turkia H[5], Gonzalez D E[6], Ida H[1], Tantawy A A G[7], Lee H M[2], Dinh Q[2], Zimran A[3]

[1]The Jikei University School of Medicine, Tokyo, Japan, [2]Shire, Lexington, United States, [3]Shaare Zedek Med Cent & Hadassah Med Sch, Jerusalem, Israel, [4]Mater Misericordiae University Hospital, Dublin, Ireland, [5]La Rabta Hospital, Tunis, Tunisia, [6]Sanatorio Español, Asunción, Paraguay, [7]Ain Shams University Hospital, Cairo, Egypt

Objective: Describe the development of anti-velaglucerase alfa antibodies (AVA) in the Gaucher disease (GD) enzyme therapy clinical trials.
Methods: Patients on velaglucerase alfa (every-other-week intravenous infusions) were screened for AVA every 6 or 12 weeks.
Results: Across 8 trials, 281 GD1 patients and 4 GD3 patients were assessed for AVA; 4/285 (1.4%) tested positive during trial participation (duration of exposure range 0.1 weeks–7.4 years). One treatment-naïve 27-year-old GD1 male receiving 60U/kg, was transiently positive for neutralizing IgG AVA between weeks 53–89 of treatment. One treatment-naïve 3-year-old GD3 male receiving 60U/kg, tested positive for non-neutralizing antibodies after 13 weeks' treatment. One 'switch' (previously treated with imiglucerase) 13-year-old GD1 female receiving 34U/kg, tested positive for neutralizing IgG AVA after 77 weeks' treatment. One switch 68-year-old GD1 male receiving 30U/kg tested positive for non-neutralizing AVA after 13 weeks' treatment. Both switch patients had high titres of anti-imiglucerase antibodies prior to receiving velaglucerase alfa. No infusion-related or drug-related hypersensitivity reactions were reported for any patient who tested AVA positive; the GD1 patients continued to receive velaglucerase alfa without apparent impact on efficacy.
Conclusion: Velaglucerase alfa had a low seroconversion rate in these trials (< 2%). Development of AVA did not appear to increase occurrence of infusion-related reactions.
Conflict of Interest declared.

P-609

Glucose tetrasaccharide (Glc4) instability in urine: resolved by use of a special collection tube

Prunty H[1], Lock P[1], Yongblah F[1], Cleary M A[1], Harvey K[1], Burke D G[1], Heales S J R[1]

[1]Great Ormond Street Hospital, London, United Kingdom

Urinary Glc4 is a useful biomarker in Pompe disease, being thought to reflect the degree of glycogen storage. However, we have occasionally observed unexpectedly low results. Subsequent studies have indicated that Glc4 degradation occurs at ambient temperature in a small percentage of urines. As transporting samples frozen is not always practical, we investigated whether addition of an ion exchange resin, already used in sample preparation, might also act as a preservative. Pompe urines with suspected Glc4 degradation (n=21) and known infected urines (n=5) were spiked with Glc4, split into both a plain tube and a special resin-containing tube, and left at ambient temperature for 5 days. 15/26 samples showed Glc4 degradation in the plain tubes. No degradation was observed in the resin-containing tubes. Most (but not all) urines with Glc4 degradation showed bacterial growth on culture, but not all infected urines showed Glc4 degradation. Stability therefore appears to be dependent upon the types of organisms present; or may be due to additional, as yet unidentified factors. Regardless of the cause, the resin stabilised Glc4 in all samples studied. This special collection tube therefore represents a practical solution for patients sending samples to the laboratory at ambient temperature via normal post.

P-610

Stability is maintained in adult patients with Gaucher disease type 1 (GD1) switched from velaglucerase to eliglustat: A sub-analysis of the eliglustat, phase 3 ENCORE trial

Giraldo P[1], Pleat R[2], Angell J[2], Rosenbloom B E[3], Ibrahim J[2]

[1]CIBERER, Zaragoza, Spain, [2]Genzyme, a Sanofi company, Cambridge, United States, [3]Cedars-Sinai/Tower Hematol Oncol, Beverly Hills, United States

Introduction: The 12-month ENCORE trial (NCT00943111; Genzyme) found oral eliglustat non-inferior to imiglucerase in maintaining stability in adults with GD1 who had achieved pre-specified therapeutic goals after ≥3 years of enzyme replacement therapy (Cox, *Lancet* 2015:25Mar.pii:S0140-6736(14)61841-9).
Methods: This post-hoc analysis examined safety and efficacy in the 30 ENCORE patients whose treatment at study entry was velaglucerase alfa, 22 randomized to eliglustat and 8 to imiglucerase.
Results: Mean duration of velaglucerase pre-study was 1.34 years; mean dose was 49.9 U/kg/2 weeks. After 12 months of eliglustat, 90% of velaglucerase-transitioned patients

maintained all 4 stability goals; separately, 95% of patients maintained goals for hemoglobin and platelets and 100% of patients for spleen and liver. Mean±SD changes from baseline were: spleen volume (multiples of normal, MN), -1%±8.7; liver volume (MN), 1.6%±7.9; hemoglobin (g/dL), 0.42 ±0.62; platelets, 1%±25.9. Data were comparable in velaglucerase-transitioned patients switched to imiglucerase. Among all velaglucerase-transitioned patients, most adverse events (AEs) were mild/moderate; there were no treatment-related serious AEs and one discontinuation in an eliglustat patient due to an AE (palpitations, moderate, possibly-related). Conclusions: Efficacy and safety results in velaglucerase-transitioned patients were consistent with the full ENCORE trial population. Eliglustat was well-tolerated and maintained clinical stability in patients previously on velaglucerase.
Conflict of Interest declared.

P-611

A desensitization method to maintain ERT in Mucopolysaccharidosis type VI

Kör D[1], Şeker Yılmaz B[1], Bulut D[1], Altıntaş D[1], Onenli Mungan N[1]

[1]Div Metab Dis, Univ Cukurova, Adana, Turkey

Background and objectives: Mucopolysaccharidosis-VI is an autosomal recessive lysosomal storage disorder. The use of ERT, for the treatment of MPS-VI was approved in 2005. A few cases of anaphylactic reactions arising in the context of ERT and desensitization methods have been reported in the literature.
Case report: In the fourth year of ERT in a 9-years old boy with MPS-VI, a generalized rash was seen over the entire body and on the lips following 4-hour enzyme infusion (anaphylactic reaction). We prepared a patient-specific treatment program. As a premedication, 12-hours and 2-hours before the enzyme infusion, infusion of 1 mg/kg IV methylprednisolone and 1 mg/kg IV pheniramine was performed, respectively. Three vials were given consecutively after reconstitution because the duration of enzyme activity in the formulation is 24-hours. The protocol was administered weekly and was well tolerated. In the desensitization protocol, enzyme infusion time was gradually reduced after 6 months without side effects. Our patient has received ERT in 4-hour infusions in the last 6 months. With this protocol, no side effects were seen arising from corticosteroid use.
Discussion/Conclusion: Our desensitization protocol has been beneficial in this case, but more reports of desensitization management are needed to develop a standardized protocol for IARs.

P-612 – Moved to A-100

25. Lysosomal disorders: treatment, enzyme replacement therapy

P-613

First experience with intrathecal administration of baclofen in patient with Hunter syndrome

Bzduch V[2], Horn F[1], Brennerova K[2], Hornova J[2], Petrik M[1], Dubravova D[1], Behulova D[3]

[1]Dept Ped Surg,Comenius Univ Child Hosp, Bratislava, Slovakia, [2]First Dept Ped,Comenius Univ Child Hosp, Bratislava, Slovakia, [3]Dept Lab Med,Comenius Univ Child Hosp, Bratislava, Slovakia

Background**:** The manifestation of Hunter syndrome is multisystemic. Enzyme replacement therapy (ERT) has limited effect on the musculoskeletal system, which primarily affects quality of life. Patients with Hunter syndrome suffer from progressive joint stiffness and contractures with profound loss of joint motion and spasticity with walking on their toes. Over the past two decades remarkable effectiveness of baclofen (selective GABA-B receptor agonist) in the relief of intractable spasticity have been reported.
Method: We used intrathecal baclofen bolus test (BBT) in 10 years old boy with severe Hunter syndrome who had been treated since the age of 3 years with ERT (iduronate-2-sulfatase), but who still suffered from severe musculoskeletal involvement. A bolus dose of 50 μg baclofen was administered by lumbar puncture and further doses were delivered using a surgically implanted catheter and a programmable baclofen pump.
Results: Approximately 4 hours after BBT we observed reduction of spasticity in the upper and lower extremities, improved gait, improvement in activities involving oral motor control and in speech.
Conclusion: We present the first experience with intrathecal administration of baclofen in patient with Hunter syndrome. Beneficial effects of intrathecal baclofen have been observed.

P-614

Effects of oral eliglustat on bone parameters in treatment-naïve patients with Gaucher disease type 1 (GD1): 18-month results from the phase 3, randomized, placebo-controlled ENGAGE trial

Mehta A[2], Mistry P[3], Lukina E[4], Ben Turkia H[5], Baris H[6], Ghosn M[7], Petakov M[8], Danda S[9], Hadjiev E[10], Angell J[1], Peterschmitt M J[1]

[1]Genzyme, a Sanofi company, Cambridge, United States, [2]Royal Free Hospital, London, United Kingdom, [3]Yale University School of Medicine, New Haven, United States, [4]Hematology Research Center, Moscow, Russian Federation, [5]Hôpital La Rabta, Tunis, Tunisia, [6]Rabin Medical Center, Petach Tikvah, Israel, [7]Hôtel-Dieu de France University Hospital, Beirut, Lebanon, [8]Clin Ctr Serbia, Univ Belgrade Sch Med, Belgrade, Serbia, [9]Christian Medical College, Vellore, Tamil Nadu, India, [10]University Hospital Alexandrovska, Sofia, Bulgaria

Background: Safety and efficacy of oral eliglustat, now approved for adults with GD1, were evaluated in the placebo-controlled ENGAGE trial (NCT00891202; sponsored by Genzyme). All primary and secondary endpoints were met in the 9-month primary analysis period (Mistry, *JAMA* 2015;311:695-706), and 39/40 patients entered the open-label extension in which all patients received eliglustat.
Methods: Bone evaluations included changes from baseline in bone marrow infiltration: bone marrow burden (BMB) score and lumbar spine T- and Z-scores.
Results: At baseline, 80% (16/20) of eliglustat patients and 75% (15/20) of placebo patients had marked–severe total BMB score. After 9 months, significant improvements (eliglustat vs. placebo) were observed for spine and femur BMB scores; spine Z-scores also improved. Among patients receiving eliglustat for 18 months (n=18) with bone data available, improvements continued during the extension period (mean changes from baseline: total BMB= 2.15; lumbar spine T-score=+0.19, lumbar spine Z-score=+0.26). For patients switched from placebo to eliglustat (n=20), mean changes from baseline after 9 months of eliglustat were: total BMB=-0.94, lumbar spine T-score=+0.03, and lumbar spine Z-score=+0.03. No new safety concerns were identified.
Conclusions: Patients receiving eliglustat for 18 months continued to show improvement in bone parameters beyond the initial 9-month primary analysis period.
Conflict of Interest declared.

P-615

The predictive value of pharmacogenetics in the identification of Fabry patients eligible for treatment with migalastat

Benjamin E R[1], Della Valle C[1], Wu X[1], Katz E[1], Valenzano K J[1], Bichet D G[2], Germain D P[3], Giugliani R[4], Hughes D A[5], Schiffmann R[6], Wilcox W R[7], Yu J[1], Kirk J[1], Barth J[1], Castelli J[1]

[1]Amicus Therapeutics, Cranbury, United States, [2]Hosp du Sacre-Coeur, Montreal, Canada, [3]Hosp Raymond Poincare, Univ Versailles, Garches, France, [4]Medical Genetics Service, HCPA/UFRGS, Porto Allegre, Brazil, [5]Univ College London, London, United Kingdom, [6]Baylor Research Institute, Dallas, United States, [7]Dept of Human Genetics, Emory Univ, Atlanta, United States

Background: Migalastat is an investigational pharmacological chaperone for Fabry disease (FD). A "GLP-HEK" *in vitro* assay was used to measure increases in FD mutant α-Gal A activity in response to migalastat. An amenable mutant α-Gal A was defined by a relative increase of ≥1.2-fold and absolute increase of ≥3% wild-type in the presence of 10 μM migalastat.
Methods: The predictive value of the assay was assessed based on pharmacodynamic responses to migalastat in Phase 2/3 studies.
Results: A high degree of consistency was observed between the GLP-HEK and white blood cell α-Gal A responses to migalastat from male patients (sensitivity=1.0; specificity=0.875; positive-predictive-value=0.9460; negative-predictive-value=1.0). In Study 011, comparisons of GLP-HEK assay results to clinical responses to migalastat on substrates GL-3 (kidney-globotriaosylceramide) and plasma-lyso-Gb_3 (globotriaosylsphingosine) showed high concordance (sensitivity 0.9286-1.0; specificity 0.6875-1.0; PPV 0.8387-1.0; NPV 0.8462-1.0). In Study 012, after switch from ERT to migalastat, plasma lyso-Gb_3 remained low for ≥18-months in patients with amenable mutations; levels increased in patients with non-amenable mutations. The GLP-HEK assay absolute and relative increases determined from Phase 2/3 amenable mutations (n=51) were not significantly different from all amenable mutations (n=224).
Conclusion: The GLP-HEK assay has high predictive value in identifying patients eligible for treatment with migalastat.
Conflict of Interest declared.

P-616

Outcomes in Fabry disease patients after long-term agalsidase alfa enzyme replacement therapy

Kampmann C[2], Bizjajeva S[1], Beck M[2]

[1]Shire, Zug, Switzerland, [2]Dept of Paed, Univ Med Centre, Mainz, Germany

Background and objectives: To examine effects of long-term agalsidase alfa enzyme replacement therapy (ERT).
Patients and Methods: Retrospective analysis of chart-based data from patients receiving ERT at a single centre in Mainz, Germany.

Results: Mortality was examined in 150 patients who received a median (range) of 5.8 (0.1–12.5) years ERT; Kaplan-Meier estimated median survival was 76.8 years in 83 women and 77.7 years in 67 men. Two women and 9 men died after a median (range) of 6.6 (0.7–11.5) years of ERT; 6 patients had strokes, 4 died with sepsis, and 2 died with ventricular tachycardia. Many had preexisting cardiac, renal, and inflammatory marker abnormalities. In 1 woman and 7 men who died, median (range) estimated glomerular filtration rate values were 50.5 (10.0–96.9) mL/min/1.73m^2) and C-reactive protein values were 9.7 (2.4–466.0) mg/L. Among 16 female and 19 male patients with renal data available and who received a median (range) of 11.1 (9.7–12.6) years of ERT, renal function was generally stable.
Discussion/Conclusion: Estimated median survival in treated patients was 76.8 years in women and 77.7 years in men. Long-term agalsidase alfa ERT appears to have an effect on survival and control progression of Fabry-associated nephropathy.
Conflict of Interest declared.

P-617

Desensitization protocol for galsulfase in two patients after anaphylaxis

Unal O[1], Hafizoglu D[2]

[1]Ankara Hematology Oncology Children's, Ankara, Turkey, [2]Erzurum Training and Research, Erzurum, Turkey

Background: In patients on enzyme replacement treatment (ERT), allergic reactions may occur and usually resolve with different desensitization protocols. The development of antibodies against infused enzyme galsulfase is well described in patients with mucoplysaccharidosis (MPS) type VI patients, and may adversely affect the clinical response to ERT. Here we report our desensitization protocol in two patients who developed allergic reactions, including anaphylactic reaction in one of them, and several protocols had been tried before.
Case Reports: Premedication treatment including steroid was given half an hour before the treatment. Desensitization protocol was prepared according to three bottles, three dilution method. Three intravenous solution was prepared with 250 mL isotonic solution of physiological serum for 15 mg enzyme dose. A solution included 1% of the enzyme dose, B solution incuded 10% of the enzyme dose, and C solution included the remaining dose of the enzyme. Infusion dose was increased in every fifteen minutes for all three solutions. A and B solutions were completed in an hour, and total infusion dose was completed in 6 hours. After first using of protocol, allergic reactions have never developed again in both patients.
Conclusion: Three bottles, three dilution method is found practical and successful desensitization method.

P-618

Pyrimethamine for infantile GM2 gangliosidosis

Salehpour S[1], Tonekaboni S H[1], Pazhouhandeh F[1], Houshmand M[2], Aryani O[2], Yasaei V R[1], Setavand S[1], Rezaei A R[1], Momtazmanesh N[2], Sakhaeian E[1], Sakhaeian E[1]

[1]Genomic Res Cent, Mofid Child Hosp, SBMU, Tehran, Iran, Islamic Republic of, [2]National Inst Genetic Engineer & Biotech, Tehran, Iran, Islamic Republic of

Background and objectives: GM2 gangliosidosis is due to β-hexosaminidase deficiency causing large accumulation of GM2 ganglioside in brain. No enzymatic replacement therapy is currently available. The inhibitors of glycosphingolipid biosynthesis for substrate reduction therapy are associated with serious side effects. Recently the chaperone effect of pyrimethamine on the recovery of β-hexosaminidase activity in cultured fibroblasts of late onset -Sandhoff patients was shown. The aim of this study is to evaluate the effect of oral pyrimethamine one infantile cases of GM2 gangliosidosis.
Methods: Nine children with infantile GM2 gangliosidosis were treated with pyrimethamine, started with 6.25 mg once daily and gradually increased to 25 mg twice daily. Improvement was evaluated by developmental quotient (Bayley's scale- version III), or β-hexosaminidase activity in leukocytes.
Results: Hexosamindase B activity in 5, and hexosamindase A and A-B in 3 patients increased ($p< 0.01$). Six patients showed significant improvement in developmental quotient. Seizure rates increased in 3 patients either for pyrimethamine or the progression of disease itself.
Conclusion: Pyrimethamine may preserve the neurodevelopment of children with infantile GM2 gangliosidosis by increasing the activity of hexosaminidases.

P-619

Survival and neurological outcomes after hematopoietic stem cell transplantation for cerebral X-linked adrenoleukodystrophy, late onset metachromatic leukodystrophy and Hurler syndrome

Saute J A[1], De Souza C F[1], Poswar F[1], Donis C K[1 6], Deyl A V S[3], Burin M G[1], Vargas C R[1 8], Matte U S[5 8], Giugliani R[1 5 6 7 8], Saraiva-Pereira M L[1 4 9], Campos G L[2 7], Vedolin L M[2 7 10], Gregianin L J[3 11], Jardim L B[1 4 6 10]

[1]Med Genet Serv, Hosp Clin Porto Alegre, Porto Alegre, Brazil, [2]Radiology Serv, Hosp Clin Porto Alegre, Porto Alegre, Brazil, [3]Ped Oncol Serv, Hosp Clin Porto Alegre, Porto Alegre, Brazil, [4]Genet Ident Lab - Hosp Clin Porto Alegre, Porto Alegre, Brazil, [5]Gene Ther Lab - Hosp Clin Porto Alegre, Porto Alegre, Brazil, [6]Post Grad Prog Child Adol, UFRGS, Porto Alegre, Brazil, [7]Post Grad Prog Med Sciences, UFRGS, Porto Alegre, Brazil, [8]Gen Mol Biol Department, UFRGS, Porto Alegre, Brazil, [9]Biochemistry Department, UFRGS, Porto Alegre, Brazil, [10]Int Med Department, UFRGS, Porto Alegre, Brazil, [11]Pediatrics Department, UFRGS, Porto Alegre, Brazil

Background and objectives: Hematopoietic stem cell transplantation (HSCT) is the only treatment for late-onset forms of metachromatic leukodystrophy (MLD), mucopolysaccharidosis type I Hurler (MPS-IH), and for cerebral X-linked adrenoleukodystrophy (CALD). We aimed to describe the seven-years´ experience of a university hospital from South Brazil with HSCT for these disorders.
Patients and Methods: We retrospectively reviewed survival and neurological outcomes of 7 CALD, 2 MLD and 2 MPS-IH molecularly confirmed patients, who underwent HSCT between 2007 and 2014. Neurological examinations, magnetic resonance imaging (MRI) and biochemical studies were obtained at baseline and repeated on follow-up visits.
Results: 9/11 transplanted patients were alive after a median of 2.7 years. Two patients (an adult CALD and a MPS-IH) died from procedure-related complications. Clinical picture stabilized in all infantile and a juvenile CALD, in a juvenile MLD, and in a MPS-IH. MRIs of this MPS-IH patient showed improvement of lesions 4 years after HSCT. The exception was the adult MLD patient, who suffered a rapid deterioration 6 months after HSCT.
Conclusion: HSCT outcomes were better when performed early in the diseases course. This was the case of CALD patients; of the pre-symptomatic MLD individual; and of a MPS-IH patient whose MRI lesions were attenuated.
Conflict of Interest declared.

P-620

A higher dose of alglucosidase α in classic infantile Pompe disease positively affects ventilator-free survival and motor outcome: an open-label single-center study

Van Gelder C M[1 2], Poelman E[1 2], Plug I[1 2], Hoogeveen-Westerveld M[1 4], Van der Beek N A M[1 3], Reuser A J J[1 4], Van der Ploeg A T[1 4]

[1]Cent Lys Metab Dis, Erasmus MC, Rotterdam, Netherlands, [2]Dept Ped, Erasmus MC, Rotterdam, Netherlands, [3]Dept Neuro, Erasmus MC, Rotterdam, Netherlands, [4]Dept Clin Genet Erasmus MC, Rotterdam, Netherlands

Background & objectives: Though treatment with alglucosidase a has significantly improved prospects for patients with classic infantile Pompe disease, some 50% of treated infants do not survive ventilator-free beyond the age of 3 years. We investigated whether higher and more frequent dosing improves outcome.
Patients & methods: Eight CRIM positive patients participated. Four received 20 mg/kg every other week (eow) and four received 40 mg/kg/week. Survival, ventilator-free survival, hospital admissions, left-ventricular mass index (LVMI), motor outcome, infusion-associated reactions (IARs) and antibody formation were evaluated.
Results: Currently, all patients are alive (ages 1.7-9.4 years), one became ventilator dependent (20 mg/kg/eow; age 1.7 years). Three patients receiving 20 mg/kg eow learned to walk; two maintained this ability at study end. The loss of motor milestones was preceded by respiratory infections requiring hospital admissions. All patients receiving 40 mg/kg/week acquired and maintained the ability to walk. LVMI normalized in seven patients. In general, a later start of enzyme therapy led to higher peak antibody titers; the number of IARs was not different between groups.
Conclusions: Our data suggests that a dose of 40 mg/kg/week improves ventilator-free survival, decreases respiratory infections, and improves motor outcome over that brought by a dose of 20 mg/kg/eow.
Conflict of Interest declared.

P-621

Nine-year follow-up in a patient receiving migalastat pharmacological chaperone therapy for Fabry disease

Schiffmann R[1], Swift C[1], Barth J[2], Skuban N[2], Castelli J[2]

[1]Baylor Research Institute, Dallas, TX, United States, [2]Amicus Therapeutics, Cranbury, NJ, United States

Objectives: To describe the long-term effects of migalastat in a patient with Fabry disease and an amenable mutation.
Case report: A 37-year-old male patient had an 8-year history of severe acroparesthesia and sensation of stiffness predominantly in the lower extremities that markedly affected his quality of life. He was treated with multiple pain medications including daily oxycodone, tizanidine and baclofen and over-the-counter analgesics. The patient was diagnosed with

Fabry disease following a kidney biopsy showing typical lysosomal inclusions in podocytes. Leukocyte a-Galactosidase A activity (a-gal) was 13.4%-25% of normal resulting from an A143T mutation.
Results: Shortly after diagnosis, the patient began receiving migalastat (various doses) for 4.5 years of treatment, followed by 150 mg every other day for an additional 4.5 years, with no drug-related adverse events observed. a-Gal activity increased 5-fold in leukocytes and 30-fold in skin after 72 weeks of treatment. Cardiac and renal function, as well as urine globotriaosylceramide (GL-3) levels remained normal. Paresthesias and sensation of stiffness progressively improved, and he was able to decrease and then discontinue all his pain medications by the 8th year of therapy.
Conclusions: Long-term migalastat therapy for Fabry disease is safe and may completely reverse symptoms of small-fiber neuropathy.
Conflict of Interest declared.

P-622

Neurological outcome following hematopoietic stem cell transplantation in children with globoid cell leukodystrophy

Ardissone A[1], Moroni I[1], Uziel G[1], Patrini M[1], Farina L[3], Napolitano S[2], Rovelli A[2]

[1]Unit Child Neurology, Besta, Milano, Italy, [2]Pediatric BMT Unit, Dep Ped, Univ Hosp, Monza, Italy, [3]Radiology Unit, Besta, Milano, Italy

Background:Globoid cell leukodystrophy (GLD) is a progressive disease presenting in infancy with different forms. Hematopoietic stem cell transplantation (HSCT) has been demonstrated to correct the metabolic defect and prevent disease progression.
Patients and Methods:Seven children, 6 late onset and 1 early onset, underwent HSCT. Leukocyte enzyme levels, neurological examination, brain MRI, neurophysiological exams were performed before and after HSCT; follow up range: 1 - 13 years.
Results:In four patients HSCT resulted in a sustained normalization of enzyme activity and halted disease progression. One patient developed myoclonus and epileptic seizures without other signs of disease progression. In two patients - one presenting with seizures before HSCT and one with early onset form - clinical and instrumental data worsened. Overall, we observed low transplant related toxicity, stable long-term engrafment and absence of life-threatening complications.
Discussion and conclusion:HSCT in GLD can stop disease progression in late onset phenotype; the early onset or the presence of epilepsy should be considered as a negative prognostic factors. Our report suggests that HSCT represents a possible treatment option: selection criteria must be strict and better defined in order to offer the greatest improvement both in term of survival and of quality of life.

P-623

Intrathecal Cyclodextrine combined with oral Miglustat in early infantile Niemann-Pick type c disease

Tormos M A[1], Felipe A[1], Delgado I[4], Clemente S[5], Carnicer C[2], Dominguez C[3], Perez J[6], Del Toro M[6]

[1]Pediatric Neurol, Hospital Vall d'Hebron, Barcelona, Spain, [2]Metabolic Lab, Hospital Vall d'Hebron, Barcelona, Spain, [3]CIBIM, CIBERER. Hospital Vall d'Hebron, Barcelona, Spain, [4]Radiology Dep, Hospital Vall d'Hebron, Barcelona, Spain, [5]Pharmacy Dep, Hospital Vall d'Hebron, Barcelona, Spain, [6]Hospital Vall d'Hebron, CIBERER, Barcelona, Spain

Background: Niemann–Pick type C (NPC) disease is characterized by abnormal intracellular cholesterol transport, with rapid progressive neurodegeneration and premature death in infantile form. In the last years, 2-hydroxypropyl-β-cyclodextrine has been reported as a possible therapy to reduce neuronal cholesterol accumulation and delay the progression of the disease, especially juvenile forms.
Objectives: To report an early infantile onset patient treated for 14 months with miglustat and intrathecal cyclodextrine.
Case report: A 16 months-old girl born from healthy unrelated parents after an uneventful pregnancy and delivery was referred with progressive neurological regression and splenomegaly. She showed hypotonia and delayed motor development with a NPC disability scale of 11. MRI showed mild leukodystrophy and cerebral atrophy. Metabolic studies confirmed NPC diagnosis with *NPC1* mutation. At the age of 2 she started treatment with oral miglustat and fourth months later with intrathecal cyclodextrin. At that moment disability scale was 14. One year later with both treatments the scale is 15. She has well-controlled epilepsy and shows no signs of dysphagia.
Conclusion: Intrathecal cyclodextrin in combination with oral miglustat has slightly delayed progression of the disease in our patient. Better results are expected in juvenile forms or in early stages of the infantile form.

P-624

Clinical and genetypic characteristics of Gaucher disease and outcomes in Algerian children

Hadji A[1], Sokhal S[1], Boukari R[1]

[1]Pediatric div univ of medicine, Algiers, Algeria

Objectives: To report on the clinical and genotypic characteristics and results of enzyme replacement therapy in Gaucher disease.
Materials and Methods: Retrospective study from 2002 to 2014 concerning 13 children and teens 13 months to 18 years-old-old. The diagnosis is suggested by splenomegaly, bi or pancytopenia, or bone pain and confirmed by enzymatic dosage of ß-glucosidase acid and genetic study. Enzyme replacement therapy (ERT) by Imiglucerase at a dose of 60UI / kg / 15 days was introduced in 2006 in our patients.
Results: 13 children and teens, 9 girls and 4 boys mean age 7 years. The mean age at diagnosis is 3.5 years. Consanguinity is present in 50% of the cases. The diagnosis is confirmed by a very low rate of ß-glucosidase acid and high levels of chitotriosidase. The genetic study confirmed the type of GD: type I (N370S): 4, Type III (L444P): 9. The clinical and biological evolution was favorable.
Conclusion: Gaucher disease is one of the rare treatable metabolic diseases. ERT has transformed the prognosis of severe forms of the disease. The particularity of our series: L444P mutation (type 3) is the most common.

P-625

Long-term safety and efficacy of taliglucerase alfa in pediatric patients with Gaucher disease who were treatment-naïve or previously treated with imiglucerase

Zimran A[1], Gonzalez-Rodriguez D E[2], Abrahamov A[1], Cooper P A[3], Varughese S[3], Giraldo P[4], Petakov M[5], Tan E S[6], Paz A[7], Brill-Almon E[7], Chertkoff R[7]

[1]Gaucher Clin, Shaare Zedek Med Ctr., Jerusalem, Israel, [2]Inst Privado de Hematol e Investig Clin, Asuncion, Paraguay, [3]Univ of Witwatersrand, Johannesburg, South Africa, [4]CIBERER, Hosp Univ Miguel Servet, Zaragoza, Spain, [5]Belgrade Univ Med Sch, Belgrade, Serbia, [6]KK Women's and Children's Hospital, Singapore, Singapore, [7]Protalix BioTherapeutics, Carmiel, Israel

Background/Objectives: Taliglucerase alfa is an enzyme replacement therapy approved for treatment of patients with Type 1 Gaucher disease (GD) and is the first approved plant cell–expressed recombinant therapeutic protein.
Patients/Methods: This extension study of taliglucerase alfa in pediatric patients included those who were either treatment-naïve (n=10) or who were previously switched from imiglucerase (n=5). Patients received taliglucerase alfa 30 U/kg or 60 U/kg (treatment-naive patients) or at the same dose as previously treated with imiglucerase (switch patients).
Results: In treatment-naive patients, taliglucerase alfa 30 and 60 U/kg, respectively, increased mean hemoglobin concentration (+19.7% and +23.3%) and mean platelet count (+23.9% and +156.6%) while also reducing mean spleen volume (-67.8% and -68.9%), liver volume (-37.0% and -34.3%), and chitotriosidase activity (-72.7% and -84.4%) from baseline through 36 total months of treatment. In patients previously treated with imiglucerase, these disease parameters remained stable through 33 total months of treatment with taliglucerase alfa. In both studies, most adverse events were mild/moderate and treatment was well tolerated.
Discussion/Conclusion: These long-term results of taliglucerase alfa in pediatric patients with GD extend the taliglucerase alfa clinical safety and efficacy data set.
The authors wish to acknowledge fellow investigator Dr. Rene Heitner who passed away in January 2012.
Conflict of Interest declared.

P-626

Comparison of taliglucerase alfa 30 U/Kg and 60 U/Kg in treatment-naive pediatric patients with Gaucher disease

Zimran A[1], Gonzalez-Rodriguez D E[2], Abrahamov A[1], Cooper P A[3], Varughese S[3], Paz A[4], Brill-Almon E[4], Lewis D[5], Wajnrajch M[5], Chertkoff R[4]

[1]Gaucher Clin, Shaare Zedek Med Ctr., Jerusalem, Israel, [2]Inst Privado de Hematol e Investig Clin, Asuncion, Paraguay, [3]Univ of Witwatersrand, Johannesburg, South Africa, [4]Protalix BioTherapeutics, Carmiel, Israel, [5]Pfizer, New York, United States

Background/Objectives: Taliglucerase alfa is an enzyme replacement therapy approved for treatment of patients with Type 1 Gaucher disease (GD) and is the first approved plant cell–expressed recombinant therapeutic protein.
Patients/Methods: Pediatric patients were randomized to receive either 30 (n=6) or 60 (n=5) U/kg of taliglucerase alfa every other week. Due to small patient numbers, there were numerical imbalances in disease parameters between the dose groups at baseline but they were clinically comparable with regard to anemia, risk of bleeding, and organ volumes. Mean percentage changes from baseline were used to compare the

response between the dose groups and as a measure of control for numerical imbalances in baseline disease parameters.
Results: Through 12 months, taliglucerase alfa 30 and 60 U/kg, respectively, increased mean hemoglobin concentration (+13.8% and +15.8%) and mean platelet count (+30.9% and + 73.7%), and reduced mean spleen volume (-34.1% and -48.5%), liver volume (-14.5% and -25.0%), chitotriosidase activity (-58.5% and -66.1%), and CCL18 concentration (-50.6% and -52.6%).
Discussion/Conclusion: Although statistical analysis was not possible due to small numbers of patients, both treatment groups demonstrated clinically meaningful improvement from baseline in these disease parameters with numerically greater improvement observed in the 60-U/kg dose group.
The authors wish to acknowledge investigator Dr. Rene Heitner.
Conflict of Interest declared.

P-627

Impact of sebelipase alfa on survival and liver function in infants with rapidly progressive lysosomal acid lipase deficiency

Jones S A[3], Rojas-Caro S[1], Quinn A G[1], Ezgu F[8], Zaki O[9], Gargus J J[6], Hughes J[7], Plantaz D[4], Vara R[5], Eckert S[1], Yang Y[1], Arnoux J B[2], Le Quan Sang K H[2], Brassier A[2], Valayannopoulos V[2]

[1]Synageva BioPharma Corp., Lexington, United States, [2]Univ Hosp Necker Enfants Malades, Paris, France, [3]Univ of Manchester, Manchester, United Kingdom, [4]Hopital Couple Enfant CHU Grenoble, Grenoble, France, [5]Evelina Children's Hospital, London, United Kingdom, [6]Univ of California, Irvine, Irvine, United States, [7]The Children's University Hospital, Dublin, Ireland, [8]Gazi University Faculty of Medicine, Ankara, Turkey, [9]Ain Shams University Pediatrics Hospital, Cairo, Egypt

Background: Lysosomal Acid Lipase (LAL) deficiency in infants is a medical emergency in which infants often present with failure to thrive, massive hepatosplenomegaly, rapidly progressing liver disease and die within the first 6 months of life.
Methods: A phase 2/3 trial assesses the safety and efficacy of sebelipase alfa (SA) in 9 LAL-deficient infants with growth failure. Baseline liver function tests revealed significant underlying liver dysfunction. At baseline, all 9 subjects had diarrhea, vomiting, hepatosplenomegaly, or adrenal calcification.
Results: Six subjects met the primary endpoint of survival at 12 months of age with a mean age of 22 months and 5 continue to receive SA. Deaths (n=4) were unrelated to SA. Three subjects died after receiving ≤4 doses. All subjects demonstrated improved weight gain, improvement of GI symptoms, and reductions in hepatosplenomegaly. One SA-related SAE occurred: an infusion reaction of malaise with tachycardia and fever. The majority of the infusion associated reactions were reported as fever, diarrhea, or vomiting.
Conclusion: Analysis suggests that SA rapidly improves weight gain and many of the disease activity parameters observed in infants with LAL Deficiency. These improvements appear to be accompanied by a substantial survival benefit compared to a matched historical control group.
Conflict of Interest declared.

P-628

A review of the clinical progression in late-onset Pompe disease adult patients following alglucosidase-alfa cessation

Sharma R[1], Silk E J[1], Willis E H[1], Dando S[2], Lachmann R H[2]

[1]MHMU, Salford Royal NHS Foundation Trust, Salford, Manchester, United Kingdom, [2]Charles Dent Metab Unit, UCLH, London, United Kingdom

Background and objectives: Five adult late-onset Pompe patients across two UK Adult Metabolic centres requested to stop enzyme replacement therapy (ERT) with alglucosidase-alfa. These patients were reviewed in clinic on a six monthly basis. The objective of the study was to evaluate the overall clinical response to the cessation of therapy.
Methods: Demographics, reason for stopping and clinic information was collected from this cohort at the time of cessation of treatment and on follow up appointments for muscle strength(dynamometry/MRC), six minute walk test (6MWT), pulmonary functions tests(PFTs), SF36, alglucosidase-alfa and antibodies.
Results: 4M and 1F patients with a mean age 56.3 (46-67) years received ERT for mean 45.5 (33-53) months. PFTs: Two patients showed ≥5% improvement; one patient demonstrated a decline of ≥5%, while others remained stable. SF-36: Four patients completed mental score and showed improvement; physical score was completed by three patients of whom two showed improvement. On 6MWT one declined(≥10%). Overall muscle function/strength reduction was demonstrated in one patient.
Conclusion: All patients who requested to stop ERT still remain off ERT mean 26.3 (12-34) months and all have requested for this to remain the case. Clinical observations remain ongoing in this cohort.

P-629

Different outcome in two sibling with non-classic infantile variant form of Pompe disease

Taurisano R[1], D'Amico A[2], Colafati G S[3], Pichiecchio A[4], De Filippi P[5], Danesino C[5], Catteruccia M[2], Bertini E[2], Dionisi-Vici C[1], Deodato F[1]

[1]Div Metab, Bambino Gesù Child Hosp, Rome, Italy, [2]Un Neuromusc Dis, Bambino Gesù Child Hos, Rome, Italy, [3]Un Neurorad, Bambino Gesù Child Hosp, Rome, Italy, [4]Dep Neurorad, Mondino C.Neur Istitute, Pavia, Italy, [5]Dep Mol Med, Univ Pavia, Pavia, Italy

Background: Many factors are known to influence clinical outcome in Pompe Disease (PD) patients (i.e. age of start of ERT, genotype, CRIM status). Recently Angiotensin converting enzyme (*ACE*) polymorphism has been suggested as a genotype modulator.
Case reports: We describe the clinical outcome of two siblings with non-classic infantile PD (genotype c.572A>G/c.525delT) onERT for 9 years. At diagnosis (4 years and 7 months respectively) both showed hyperCPKemia and mild hypertrophic cardiomyopathy (CMP), but only pt1 have signs of myopathy. They initiated ERT as compassionate therapy at the age of 4.6 years (pt1) and 18 months (pt2). CMP disappeared within 2 years in pt1 and 1year in pt2.Pt1 showed progressive muscular weakness, chronic respiratory insufficiency and at last follow-up she is not able to jump and run; at MRI damage in tongue, gluteus maximus, quadriceps and in the postero-medial compartment of the thigh is evident. Pt2 at the age of 9 has a normal clinical evaluation and muscle MRI. Analysis of ACE polymorphisms revealed DD genotype in pt1 and ID in pt2.
Conclusions: These two cases confirm that early treatment determine a good outcome, but also suggest that ACE polymorphisms could be considered an important modifying factor.
Conflict of Interest declared.

P-630

Miglustat therapy in early infantile Niemann-Pick C patients: a retrospective survival study

Freihuber C[1], Vanier M T[10 11 12], Brassier A[2 12], Broue P[5], Chabrol B[3 12], Eyer D[4 12], Guffon N[6 12], Labarthe F[12 13], Latour P[10 12], Levade T[9 12], Pichard S[7 12], Roche S[5 12], Sevin C[8 12], Valayannopoulos V[2 12], Heron B[1 12]

[1]Dept Neuroped, Trousseau Univ Hosp, APHP, Paris, France, [2]Dept Metab Dis, Necker Univ Hosp, APHP, Paris, France, [3]Dept Neuroped, La Timone Univ Hosp, APHM, Marseille, France, [4]Dept Ped, Univ Hosp, Strasbourg, France, [5]Dept Ped, Child Univ Hosp, Toulouse, France, [6]Dept Metab Dis, HCL Univ Hosp, Lyon-Bron, France, [7]Dept Neuroped, R Debre Univ Hosp, APHP, Paris, France, [8]Dept Neuroped, Bicetre Univ Hosp, APHP, Kremlin Bicetre, France, [9]Lab Metab Bioch, Univ Hosp, Toulouse, France, [10]Lab Gillet-Merieux, CBPE, HCL, Lyon-Bron, France, [11]INSERM U820, Lyon 1 Univ, Lyon, France, [12]CETNL (Comity Eval treat NeuroLipidoses), National, France, [13]Dept Metab Dis, Clocheville Univ Hosp, Tours, France

Background/ objectives: While miglustat can stabilize neurological manifestations in later onset forms of Niemann-Pick disease type C (NP-C), its efficacy in the early infantile neurological form has not been demonstrated. This retrospective study aimed to compare survival between an untreated group and a treated group of early infantile NP-C patients.
Patients/Methods: The study included 27 patients from the French NP-C cohort with a neurological onset < 2 years of age, diagnosed after 1990. Ages are reported as median (range).
Results: The untreated group comprised 16 patients and the treated group 11 patients. Age at diagnosis was 1.73 years (prenatal-4.87) vs 0.34 years (0.04-1.65) and onset of neurological signs occurred at 1.5 years (0.5-1.6) vs 0.75 years (0.4-1.5), respectively. Miglustat was started at 2.01 years of age (0.75-3.6), and median duration of therapy was 2.45 years (0.2-4.7). In this group, 7 patients died at a median age of 4.79 years (2.8-6.8) and all still alive patients with classical early infantile form are less than 6.8 year-old. In the untreated group, age at death was 3.86 years (2.8-6.5).
Conclusion: Miglustat did not allow a significant increase of survival in early infantile neurological form of NP-C.
Conflict of Interest declared.

P-631

Small molecules combination therapy for GM1 gangliosidosis and Morquio B syndrome

Rigat B A J[1], Schulze A[1 2], Mahuran D J[1 2]

[1]GGB Prog, Hosp Sick Child, Toronto, Canada, [2]U of T, Toronto, Canada

Enzyme enhancement therapy (EET) utilizing small molecule(s) offer one of the best hopes of treatment for diseases like LSDs, caused by mutations affecting

protein folding. Our goal is to identify novel EET-agents for GM1-gangliosidosis (GM1) and Morquio-B.
To expand our collection of EET-agents we instigated a high-throughput screening campaign of the Canadian Compounds Collection.
One compound characterized (labeled X, patent pending) produced a strong/specific enhancement of mutant β-galactosidase (β-Gal) in fibroblasts responsive to pharmacological chaperones (PCs). However X did not behave as a classical PC by binding directly to the target β-Gal enzyme. Combination treatment of a classical PC with X, in adult GM1 fibroblast results in up to a 20-fold increase of β-Gal when treated with both drugs, compared with only 8- or 4-fold when treated with each drug separately. Similarly we measured up to a 10-fold increase in fibroblasts derived from a Morquio-B syndrome patient.
We report the identification of a compound that does not bind to β-Gal, but nonetheless specifically enhances mutant β-Gal activity in GM1 cells. Furthermore it act in concert with a classical PC, to increase mutant β-Gal activity in both GM1 and Morquio-B cells.

P-632

Long term immune tolerance experience in a large cohort of CRIM-negative infantile Pompe disease: lessons learned from a global collaboration

Kazi Z B[1], Berrier K[1], DeArmey S[1], Bali D[1], Rosenberg A[2], Kishnani P[1]

[1]Duke University, Durham, United States, [2]US Food and Drug Administration, Silver Springs, United States

Background: Enzyme replacement therapy (ERT) with alglucosidase alfa has improved clinical outcomes in infantile Pompe disease (IPD). Cross-reactive immunological material (CRIM)-negative (CN) patients do poorly on ERT monotherapy due to an immune response. Prophylactic immune tolerance induction (ITI) with rituximab, methotrexate, and IVIG succeeded in achieving immune tolerance; however, longterm outcomes after ITI have not been assessed.
Objectives: This study aimed to evaluate longterm safety and efficacy of ITI in CN IPD.
Methods & Results: Longitudinal ventilator-dependence, cardiac, feeding, and gross motor status was assessed and compared to the ERT monotherapy historical cohort. Of 183 IPD, 17 met inclusion criteria. The median age at ERT + ITI was 3.1 months [0.4 – 11.0]. The median peak and last titers were 200 [0-25,600] at 27 weeks [8-41] and 0 [0-6,400] at 59 weeks [10 – 244] since ERT initiation, respectively. Most patients required only one ITI round. Twelve are currently alive at median age 47.8 months [13.6-103.9] with B-cell recovery. Death was due to disease progression unrelated to ITI. Prophylactic ITI was safely tolerated with longterm immune tolerance and has improved the clinical course of CN IPD.
Conclusions: Our data supports the use of ITI in ERT-naïve setting and may have implications for other therapeutic proteins treated conditions.
Conflict of Interest declared.

26. Glycosylation disorders/CDG, protein modification disorders

P-633

Patient Advocacy Groups as key drivers for research of Congenital Disorders of Glycosylation (CDG) through patient-friendly resources

Ferreira V M R[1], Jaeken J[2], Liddle M[3], Morandat P[4], Vilaseca M A[5]

[1]APCDG-DMR, Lisbon, Portugal, [2]University Hospital Gasthuisberg, Leuven, Belgium, [3]CDG patient advocate, Melbourne, Australia, [4]CDG patient advocate, Paris, France, [5]Guia Metabólica, Barcelona, Spain

There are 7000-8000 rare diseases affecting combined 350 million people worldwide. If we include family members, there is many people directly affected by rare diseases. The majority are children. That is a significant societal health problem. Congenital Disorders of Glycosylation (CDG) are amongst these rare diseases. It is a rapidly growing family of rare genetic diseases with more than 80 members identified since the first description in 1980.
Patients, their families and patient advocacy groups, are of particular relevance for activities in the area of rare diseases. The number of non-profit patient organizations in this area is rapidly growing. Their mission mainly consists of supporting education, research, awareness, and advocacy. The Portuguese Association for CDG and other Rare Metabolic Diseases (APCDG-DMR) is a non-profit organization founded in 2010 by families affected by these diseases.
The APCDG-DMR aims at building a global voice to fight the impact of Rare Metabolic Diseases on affected individuals and their families. The association seeks to make a difference in families' everyday lives, a.o. by developing resources and high standards of care through activities at the national as well as international levels.
Overall, using efficiently limited human and financial resources, the organization has developed several tools that

could be of benefit to other rare diseases as well. This work unravels one key resource implemented to increase knowledge, awareness and dissemination of CDG and foster diagnosis through cooperation among basic scientists, clinicians and family advocacy groups.

P-634

Diagnostic mass spectrometry of intact transferrin and serum N-glycans for identification of CDG-II gene defects

Van Scherpenzeel M[1], AbuBakar A[1], Steenbergen G[1], Zijlstra F[1], Wevers R A[1], Lefeber D J[1]

[1]Radboud UMC, Nijmegen, Netherlands

Subtype identification in the congenital disorders of glycosylation (CDG) type II is a main challenge in CDG diagnostics. The clinical heterogeneity in this group is increasing rapidly with inclusion of skeletal dysplasia syndromes, non-syndromal liver disease, intellectual disability and neurological syndromes. In addition, the underlying biochemical mechanisms in this group are poorly understood, especially defects in Golgi homeostasis.

To subgroup patients with abnormal CDG type II screening result, we applied isofocusing of apoCIII, nanochip-C8-QTOF mass spectrometry of intact serum transferrin and total serum N-glycan profiling using nanochip-PGC-QTOF mass spectrometry. Application in our cohort of patients with an abnormal CDG-II isofocusing profile revealed several subgroups with highly characteristic glycosylation profiles. Some of these unique profiles corresponded with known defects such as MAN1B1-CDG with intellectual disability, obesity and facial dysmorphisms, and SLC35A2-CDG, a neurodegenerative disease. In addition, novel profiles were observed that were different from all known CDG-II gene defects. Subsequently, exome sequencing of such subgroups is being performed, leading to identification of novel CDG-II gene defects.

Our results show the promising addition of QTOF mass spectrometry to the diagnostic repertoire for fast identification of CDG-II subtypes.

P-635

Spectrum of clinical phenotypes in *ALG8* deficiency (congenital disorder of glycosylation CDG-Ih, *ALG8*-CDG)

Karall D[1], Hoeck M[2], Fauth C[3], Lefeber D J[4], Matthjis G[5], Keldermanns L[5], Zschocke J[3], Scholl-Bürgi S[1]

[1]Med Univ of Innsbruck, Pediatr, Met Dis, Innsbruck, Austria, [2]Med Univ of Innsbruck, Pediatr, Neonatol, Innsbruck, Austria, [3]Med Univ of Innsbruck, Div Hum Genetics, Innsbruck, Austria, [4]Radboud Univ Med Center, Neurology, Nijmegen, Netherlands, [5]Univ of Leuven, Center Hum Genetics, Leuven, Belgium

Background: Since 1980, about 80 congenital disorders of glycosylation (CDG) have been reported, ALG8-CDG being less frequently reported, maybe due to its severe multi-organ involvement and often early death.

Case Reports: We report three patients, provide an update on two previously reported, and summarize features of patients reported in literature.

Results: Of 15 ALG8-CDG patients, three were homozygous and 12 compound heterozygous. There were multiple prenatal abnormalities in 6/12 patients. In 13/15, there were symptoms at birth, 9/15 died within 12 months. Birth weight was appropriate in 11/12, only one was small for gestational age. Prematurity was reported in 7/12. Hydrops fetalis was noticed in 3, edemas in 11/13; gastrointestinal symptoms in 9/14; structural brain pathology, psychomental retardation, seizures, ataxia in 12/13, muscle hypotonia in 13/14. Common dysmorphic signs were: low set ears, macroglossia, hypertelorism, pes equinovarus, campto- and brachydactyly (13/15). In 10/11, there was coagulopathy, in 8/11 elevated transaminases; thrombocytopenia was present in 9/9. Eye involvement was reported in 9/14. CDG typical skin involvement was reported in 8/13.

Conclusion: In ALG8-CDG, transferrin isoelectric focusing in serum/plasma shows an abnormal pattern. The diagnosis is confirmed by mutation analysis; all patients reported had mutations/small deletions. Prognosis is generally poor. Diagnosis is important for counselling.

P-636

Congenital Disorders of Glycosylation-Report of further cases from Saudi Arabia

ALTassan R S[1], ALSahlawi Z[1], Imtiaz F[1], Ramzan K[1], Allam R[1], ALSayed M[1]

[1]Med Genetic, King Faisal Specialiest Hosp, Riyadh, Saudi Arabia

Background: Congenital disorders of glycosylation (CDG) are a group of highly heterogeneous mostly autosomal recessive genetic diseases affecting protein and lipid glycosylation. Although Saudi Arabia has a high incidence of autosomal recessive diseases, there are only a few reports of CGD from our country (DPAGT1, ALG2, and ALG6).

Methods: Here we report two new consanguineous families with CGD. The first family has of a 4- year old boy presented with seizure disorder, developmental delay and dysmorphism. His cousin, an 11-month old girl presented with seizure disorder, prolonged fever, chronic diarrhea, and failure to thrive. The second family has a 20-month old girl who presented with hypoglycemia, hepatomegaly and failure to thrive. All three patients had abnormal Carbohydrate-Deficient Transferrin (CDT).
Results: Homozygosity mapping (HZM) in the first family identified a block on chromosome 11q23 where *ALG9* gene is located and ALG9 gene analysis revealed a homozygous missense mutation in exon13 (c.1054C>T, p.E352K). Result of HZM in the second child family identified a block of homozygosity on chromosome Xq23 were ALG13 gene located, gene analysis is pending.
Conclusion: From this report of two different families and high CDT it recommended to screen for CDG in patients with heterogeneous phenotype especially in consanguineous population

P-637

Neurologic manifestations in congenital disorders of glycosylation: a clinical follow-up study of 18 patients

Melo C[1], Bandeira A[2], Garrido C[3], Fortuna A M[4], Gonçalves-Rocha M[5], Figueiroa S[3], Carrilho I[3], Santos M[3], Quelhas D[4], Martins E[2]

[1]Ped Depart, CHMA, Vila Nova de Famalicão, Portugal, [2]Div Metab Dis, CHP, Porto, Portugal, [3]Ped Neurol Depart, CHP, Porto, Portugal, [4]Genetics Depart, CHP, Porto, Portugal, [5]Genetics Unit, Hosp Braga, Braga, Portugal

Background: Heterogeneous neurological manifestations are a frequent finding in patients with congenital disorders of glycosylation (CDG).
Methods: Review of neurological, neuroimaging and genetic features in a cohort of patients with CDG from a large Public Hospital Center.
Results: We followed 18 patients with CDG (median age 15.4 years; 72% males) for a median time of 11.2 years. They were categorized into: *PMM2*-CDG (9), *MAN1B1*-CDG (2), CDG-Ix (4), CDG-IIx (2), *RFT1*-CDG (1).All the patients developed intellectual disability. Movement disorders were present in 8/18 patients: stereotypes (5/18), tics (1/18), dystonia (2/18), tremor (3/18) and choreoatethosis (1/18). All patients with *PMM2*-CDG and *MAN1B1*-CDG showed hypotonia and ataxia. Epilepsy was diagnosed in 3/9 patients with *PMM2*-CDG, 3/4 with CDG-Ix and 1/2 with CDG-IIx. There were no significant progression of neurological symptoms or loss of acquisitions.In the cerebral MRI, the most common abnormality was cerebellar atrophy (9/9 *PMM2*-CDG and 2/3 CDG-Ix). *PMM2*-CDG patients also presented cerebral peduncle atrophy (5/9), cortical atrophy (2/9) and corpus callosum hypoplasia (2/9).
Discussion: Cerebellar clinical and neuroimaging findings were the most common features, mostly in *PMM2*-CDG. However, intellectual disability, epilepsy and movement disorders were also common in all subtypes. Interestingly, stroke-like episodes and progressive polyneuropathy were not found.

P-638

Exome sequencing identifies an atypical case of congenital disorder of glycosylation Iq

Gupta N[1], Kabra M[1], Verma G[1], Bijarnia S[2]

[1]All India Institute of Medical Sciences, New Delhi, India, [2]Sir Ganga Ram Hospital, New Delhi, India

Background: Congenital disorder of glycosylation (CDG) type I is a group of disorders with defect in dolichol linked oligosaccharides leading to multisystem manifestations. We describe a first Indian case of CDG type Iq with pigmentary retinopathyand intellectual disability.
Case report: A young nonconsanguinous couple approached at 6 weeks of gestation for genetic counselling for having a previous child with developmental delay. Their 5 year old male child had severe intellectual disability, seizures, and visual impairment. Examination showed presence of facial coarseness, microcephaly, inverted nipples, central hypotonia, nystagmus and pigmentary retinopathy. His karyotype and chromosomal microarray was normal. Neuroimaging showed mild white matter paucity.
Results: Exome sequencing identified presence of a previously reported W19X mutation in homozygous state in SRD5A3 gene. Serum transferrin isoelectric focusing showed a characteristic type I pattern. Both parents were carriers of the same mutation. Prenatal diagnosis at 16 weeks showed fetus as unaffected.
Discussion and Conclusion: CDG type Iq is a rare disorder characterized by the presence of ocular colobomas, intellectual disability and icthyosis as most striking features. However, the spectrum of clinical manifestations varies from patient to patient even with the similar mutation highlighting the importance of further evaluation of the molecular mechanisms at tissue level.

P-639

MAGT1 deficiency: from magnesium channel defect to a fundamental glycosylation disorder

Rymen D[1 2], Keldermans L[1], Race V[1], Jaeken J[2], Matthijs G[1]

[1]Cent for Hum Gen, Univ Leuven, Leuven, Belgium, [2]Cent Metab Dis, Univ Hosp Gasthuisberg, Leuven, Belgium

Background: Over the years MAGT1 has been swayed back and forward between a role as either a subunit of the oligosaccharyltransferase (OST) complex or as plasma-membrane localized Mg^{2+} transporter. Recent work demonstrated that MAGT1 localizes to the ER, where it aids SST3B in the glycosylation of cysteine-rich proteins. If MAGT1 is indeed an OST subunit, it is obviously also a candidate for unsolved CDG cases.
Methods: A gene panel approach was used to identify the culprit gene in unsolved CDG-I patients. Protein and RNA were extracted from primary patients' fibroblasts. Immunoblotting and qPCR were used to interrogate the steady state and transcript levels of MAGT1 and/or TUSC3.
Results and Discussion: We identified two patients with MAGT1-CDG, both displaying similar clinical features i.e. macrocephaly, intellectual disability and hepatomegaly. This mild phenotype probably results from the observed up-regulation of the MAGT1 homologue TUSC3. Against all odds, sialotransferrin screening demonstrated a type 1 pattern in both patients. Indeed a normal profile was expected, since glycosylation of transferrin is predicted to depend on the catalytic activity of STT3A instead of STT3B. This finding could either point to a flaw in the current model or to the existence of secondary events causing transferrin hypoglycosylation.

P-640

Establishing an international database, evaluating gentype-phenotype correlations and prognosis assessment in PGM1-CDG

Wong S[1], Beamer L[8], Gadomski T[1], Conrad D[9], Honzik T[2], Brocke K[5], Bowling F[6], Cegielska J[4], Kozicz T[7], Lefeber D J[3], Morava E[1]

[1]Tulane University Medical School, New Orleans, United States, [2]Charles University in Prague, Dep Peds, Prague, Czech Republic, [3]Radboudumc, Dep. of Neurology, Nijmegen, Netherlands, [4]The Children's Memorial Health Institute, Warsaw, Poland, [5]Helse Stavanger, Overlege, Norway, [6]Royal Children's Hospital, Brisbane, Australia, [7]Radboudumc Dep. of Anatomy, Nijmegen, Netherlands, [8]University of Missouri, Dep of Chemistry, Columbia, United States, [9]Washington Univ School of Medicine, Gene, Saint Louis, United States

So far no clear genotype-phenotype correlations are found and no explanation is available for the variable clinical features and prognosis in PGM1-CDG. We established a database with 26 patients with PGM1-CDG using a data survey for clinical features, laboratory results, mutation analysis. The patient cohort was divided into "severe, moderate and mild" groups based on the presence of congenital malformation and cardiomyopathy. We validated the assignment of phenotypic groups with a semi-quantitative severity rating-scale, the Tulane PGM1-CDG Rating Scale (TPCRS). Regression-analysis was used to examine the relationships between the TPCRs total score, in vitro enzyme activity, and PGM1 genotype. Associations between clinical features, genotype, and phenotypic group assignment were evaluated by principal component analysis (PCA), to define variables predicting disease severity. Regression-analysis showed that neither PGM1 enzyme activity in fibroblasts (r=-0.265, p=0.233) nor purified enzyme activity assayed in bacteria (r=-0.2664216, p=0.357) has correlation with the total TPCR score. There was positive correlation between the genotype and total TPCR score (r=0.627, p=0.000615). PCA identified variables contributing to 50% variance, and strong predictors of disease severity: genotype, congenital formation, cardiovascular defect and growth-retardation. We pinpointed specific features, which are easily, and early recognizable in the clinical setting and powerful predictors of disease severity.

P-641

Unexpected PMM2-CDG clinical findings in two patients with maternal isodisomy of distal arm of chromosome 16

Medrano C[1], Felipe A[2], Vega A I[1], Del Toro M[2], Desviat L R[1], Girós M[3], Nevado J[4], Ugarte M[1], Macaya A[2], Pérez-Cerdá C[1], Perez B[1]

[1]CEDEM, CBM-SO, UAM-CSIC, CIBERER, IdiPAZ, Madrid, Spain, [2]Hospital Vall d'Hebron, CIBERER, Barcelona, Spain, [3]Instituto de Bioquímica Clínica, CIBERER, Barcelona, Spain, [4]INGEMM, Hosp Univ La Paz, IdiPAZ,CIBERER, Madrid, Spain

Background: The majority of the patients described with congenital disorders of glycosylation (CDG) are due to mutations in *PMM2* gene.
Patients & Results: Out of 40 patients diagnosed of PMM2-CDG in our lab, two were homozygous for c.338C>T (p.Pro113Leu) or c.415G>A (p.Glu139Lys). Mendelian segregation analysis revealed the presence of the mutation only in the maternal allele. SNP array analysis showed a homozygous region spanning from 16p13.3-p12.3 and familial microsatellite marker analysis confirmed a maternal uniparental isodisomy of chromosome 16 (upd(16)mat). Several recessive syndromes have been reported due to upd(16)mat with severe growth

restriction and risk of malformation, while upd(16)pat has been described without additional complications. Both patients presented a very severe phenotype rarely reported for PMM2-CDG. Patient 1 was a six-year old girl born from *in-vitro* fecundation who developed bursitis and cutaneous hyperelasticity besides the PMM2-CDG clinical phenotype. Patient 2 was the first boy from healthy non-consanguineous parents. Cardiopathy was detected at 38 weeks of gestation. He presented neonatally with truncus arterious type II that required surgery at 17 weeks of life and later developed severe PMM2-CDG features.
Conclusion: The clinical findings beyond the PMM2-CDG phenotype suggest that abnormal dosage of the seven imprinted gene products mapping to chromosome 16 could underlie their severe phenotype.

P-642

Secondary disorders of glycosylation in inborn errors of fructose metabolism: Case report

Kasapkara Ç S[1], Kılıç M[1], Keldermanns L[2], Matthijs G[2], Jaeken J[3]

[1]Div Metab Dis, Dr Sami Ulus Child Hosp, Ankara, Turkey, [2]Center for Human Genetics, KU Leuven, Leuven, Belgium, [3]Center for Metabolic Disease, KU Leuven, Leuven, Belgium

Congenital disorders of glycosylation (CDG) is a rapidly expanding group of human multisystemic disorders.The group of congenital disorders of glycosylation (CDG) has expanded tremendously with around 70 distinct disorders described to date.CDG can affect nearly all organs and systems, but there is often an important neurological component.The first-line screening for the *N*-glycosylation diseases is serum transferrin isoelectrofocusing (IEF). It has to be stressed that a normal test result does by no means exclude a CDG. In the case of an abnormal result and as long as the basic defect has not been elucidated, the disease is labeled CDG-x (CDG-Ix when the transferrin IEF shows a type 1pattern, and CDG-IIx when it shows a type 2 pattern).We report here a case of hypotonia, corpus collosum dysgenesis, strabismus, hepatomegaly, abnormal sweating and severe developmental delay since infancy in whom screening for CDG with transferrin isoelectric focussing (TIEF) revealed a type I pattern. Following investigation, the specific defect in glycosylation remains to be identified; hence, a diagnosis of CDG Ix (type unknown) was made.Subsequently a homozygous c.448G>C (p.Ala150Pro) mutation was detected in *ALDOB* gene. In case of an abnormal IEF result, an artifact, a transferrin protein variant or a secondary CDG (galactosemia, fructose intolerance, alcohol abuse, others) should be excluded.

P-643

Clinical exome sequencing in the clinical practice for the genetic diagnosis of congenital disorders of glycosylation

Medrano C[1], Vega A I[1], Ecay M J[1], Ugarte M[1], Pérez-Cerdá C[1], Perez B[1]

[1]CEDEM, CBM-SO, UAM-CSIC, CIBERER, IdiPAZ, Madrid, Spain

Background: Congenital disorders of glycosylation (CDG) are a heterogeneous group of disorders with a wide clinical spectrum caused by genetic defects in the glycosylation pathways.
Methods: In order to identify the gene and disease-causing mutations in a cohort of uncharacterized CDG-patients referred to our lab we performed targeted exome sequencing (TES), whole-exome sequencing (WES) and clinical exome sequencing (CES).
Results: We have been able to characterize 15 out of 39 patients (38%) detecting mutations in 11 different CDG-genes (*RFT1*, *DPAGT1*, *COG7*, *GALT*, *DPM1*, *DOLK*, *PMM2*, *SSR4*, *EXT1*, *SRD5A3* and *ALG1*) and 9 new mutations. We also detected one FOLR1-patient with overlapping clinical features to CDG. The diagnosis rate of TES was lower compared to CES or WES probably due to the limited number of captured gene. Noteworthy, depth of coverage was higher when using panels (TES or CES), making the diagnosis of the patients more accurate. Besides, CES or WES allowed us to identify mutated genes not classified as CDG-genes involved in the pathology of the patient.
Conclusion: In conclusion, CES is a powerful technique for an accurate analysis when dealing heterogeneous disorders comprising a large cohort of genes and with a broad clinical spectrum such as CDGs.

P-644

Individualized drug screening coupled to n-of-1 clinical trial, an innovative approach to drug discovery in rare diseases. Application to creatine transport deficiency

Moreau C[1], Jeoual A[1 3], Briand G[2 3], Dessein A F[2 3], Mention K[2 3], Deprez B[1 4], Dobbelaere D[2 3], Beghyn T[1]

[1]APTEEUS, Lille, France, [2]CRMHM - CHRU, Lille, France, [3]RADEME - Université de Lille, Lille, France, [4]U1177 Inserm - IPL- Univ Lille, Lille, France

Method: We have developed a new technology meant to better meet medical needs in the field of rare and orphan diseases, particularly for inherited metabolic diseases. We bring a highly miniaturized drug discovery technology to the patient's bedside, to test thousands of molecules directly on the patient's primary cells in a couple of days. We screen only marketed drugs, to identify the ones that correct the causative defect of the disease. The best drug candidate is then evaluated in an n-of-1 clinical trial which single-subject design is particularly adapted to rare diseases. Our screening technology uses medically relevant principles that are comparable to those used in diagnostic procedures. It currently addresses creatine deficiency, mitochondrial and peroxisome beta-oxidation disorders.
Results and Discussion: We will disclose our first data on creatine transport deficiency. Among the 1500 tested molecules tested on skin fibroblasts from 4 individual patients, we identified 10 drugs that restore the intracellular concentration of creatine *ex vivo*. One of them, TEE178, is our best candidate for clinical evaluation on the donor patients because of its safety and pharmacokinetics profile.
Conflict of Interest declared.

P-645

Congenital defects of glycosilation (CDG) and Pediatric Intensive Care: 4 cases in 15 years

Serrano-Nieto J[1], Blasco-Alonso J[1], Gil-Gómez R[1], Morales A[1], Yahyaoui R[1], Pérez-Cerdá C[2], Milano G[1], Sierra C[1]

[1]Hospital Regional Universitario, Málaga, Spain, [2]CEDEM, Madrid, Spain

Background and objectives: Congenital disorders of glycosylation (CDGs) may mimic almost any symptom. Its clinical expression varies from extremely severe to very mild (and thus probably underdiagnosed). This case series is devoted mainly to the clinical findings in 4 patients admitted in our pediatric intensive care unit (PICU) for the last 15 years.
Case reports: Three subjects were diagnosed with CDG after admission to PICU (2 status epilepticus and 1 aorctic thrombosis) and the fourth one had been previously diagnosed with CDG and was admitted for status epilepticus management. Cases 1 (6 month-old) and 2 (2 year-old) were diagnosed as CDG-I and are still under massive sequencing in order to find out the genetic mutations. Case 3 (8 days-old) corresponds to a CDG-Ia (PMM2) and case 4 (5 month-old) is a CDG-In (RFT1). Subjects 3 and 4 finally died during PICU admission.
Conclusions: CDG is an emerging pathology and given the clinical heterogeneity should be suspected in any patient with unexplained neurological symptoms, especially if psychomotor retardation, hypotonia, epilepsy and associated coagulation disorders. The affected patients often require control and treatment in the PICU in the debut and/or during evolution.

P-646

ALG1-CDG- Survival into adulthood of a patient with major neurologic presentation

Carvalho J[1], Garcia P[2], Macário M C[1], Robalo C[7], Matthijs G[3], Jaeken J[4], Quelhas D[5], Diogo L[2 6]

[1]2 IPATIMUP and Depart Biology-FCUP, Porto, Portugal, [2]3 S Ped C Hosp Funchal, Funchal, Portugal, [3]4 C for Human Genet, KU Leuven, Leuven, Belgium, [4]5 C Met Dis U H Gasthuisberg, KU Leuven, Leuven, Belgium, [5]1 Unit Bio Genet CGMJM CH Porto, Porto, Portugal, [6]7 S Bioq - Fac Med Univ Coimbra, Coimbra, Portugal, [7]3 Neuroped Unit, Ped Hosp CHUC, Coimbra, Portugal

Background: ALG1-CDG is caused by a defect in the biosynthesis of the N-glycosylation oligosaccharide precursor, and its phenotypic spectrum is expanding, as more patients are reported.
Case report: A 19-years old boy, third child of non-consanguineous parents, presented with seizures at the age of 3 months. Other symptoms included axial hypotonia, peripheral hypertonia, strabismus and psychomotor disability. Recurrent respiratory infections associated with gastroesophageal reflux, *status epilepticus* and coma episodes occurred in the first 3 years of life. Currently, microcephaly, moderate facial dysmorphism, severe intellectual disability (without regression), controlled epilepsy and short stature are the main features. An older sister deceased at the age of three.
Results: First-line etiologic investigation was inconclusive. Brain MRI (13 months) showed generalized cerebral atrophy and myelination delay. Further biochemical investigation revealed a transferrin IEF type 1 pattern. Lipid-linked oligosaccharide analysis showed a normal profile and a very poor incorporation suggesting a defect in early biosynthesis. Recently, two heterozygous mutations have been identified in *ALG1*: p.Ser258Leu (c.773C>T) and p.Arg276Trp (c.826C>T).
Conclusion: Most reported ALG1-CDG patients were severely affected leading frequently to early death. Recent reports reveal a growing number of patients with an apparently static evolution and longer survival such as the present patient.

P-647

SRD5A3-CDG: a novel mutation

Quelhas D[5], Azevedo L[1], Freitas M C[2], Vasconcelos R[2], Sousa D[5], Matthijs G[3], Jaeken J[4], Lacerda L[5]

[1]2 IPATIMUP and Depart Biology-FCUP, Porto, Portugal, [2]3 S Ped C Hosp Funchal, Funchal, Portugal, [3]4 C for Human Genet, KU Leuven, Leuven, Belgium, [4]5 C Met Dis U H Gasthuisberg, KU Leuven, Leuven, Belgium, [5]1 Unit Bio Genet CGMJM CH Porto, Porto, Portugal

Background: SRD5A3-CDG is caused by mutations in the steroid 5α- reductase type 3, a gene involved in dolichol phosphate biosynthesis.
We present two relative patients with a new disease-associated mutation in the *SRD5A3* gene.
Although transferrin isoelectrofocusing (IEF) is not always an effective biomarker for screening SRD5A3-deficient patients, in these here presented it was crucial for the diagnosis.
Case report: A 14 years old male presented severe psychomotor disability, convulsions, hyperreflexia, retinopathy, abnormal eye movements, strabismus, abnormal fat distribution and hirsutism. His 8 years old sister presented a similar but milder clinical phenotype.
Results: Serum transferrin IEF showed a type 1 pattern (increased asialo- and disialo-transferrin isoforms) pointing to a CDG-I defect. Sequencing of the *SRD5A3* gene revealed a homozygous deletion of 1 bp within exon 4 (c.634delT) creating a frameshift at codon Trp212 (p.Trp212GlyfsX46) resulting in a premature stop codon.
Conclusion: With the novel mutation here presented we widen the mutational spectrum of the *SRD5A3* gene and reinforce that serum transferrin IEF remains for a crucial step in the screening of SRD5A3-CDG.

P-648

Severe infantile acute encephalopathy and *COG4* mutation: CDG II

Felipe A[1], Bravo-Garmendia M M B[2], Matalonga L[2], Tort F[2], Giros M[2], Del Toro M[3], Macaya A[3]

[1]Pediatric Neurol, Hospital Vall d'Hebron, Barcelona, Spain, [2]Hospital Clinic, IDIBAPS, CIBERER, Barcelona, Spain, [3]Hospital Vall d'Hebron, CIBERER, Barcelona, Spain

Background : Type II congenital disorders of glycosylation (CDG) are a group of inherited multisystem disorders caused by defects in the processing of the protein-bound glycans in the ER or Golgi compartments. Mutations in members of the Conserved Oligomeric Golgi (COG) complex, cause CDG type II and are being increasingly diagnosed with widespread use of NGS. We report the second patient with *COG4* –associated encephalopathy.
Case report: The son of consanguineous Moroccan parents showed normal development until age 6 months, when he presented with acute encephalopathy during a febrile illness. Evaluation at 3y showed mild dysmorphic features, no visceromegalies, profound retardation, microcephaly and hypotonic tetraparesis. Hyperlactacidemia, hyperammonemia and mild, diffuse white and grey matter loss [r1] on brain MRI were noted. A subsequent dental infection was complicated by obtundation, dehydration, increased serum transaminases, pancytopenia, coagulopathy and hyperammonemia. Study of sialotransferrin isoforms showed a CDG type II profile Whole exome sequencing identified a homozygous c.973T>A variant in *COG4*, leading to a p.F325I substitution. Test of the integrity of Golgi-to-ER trafficking and COG4 expression is underway.
Conclusion: This is the second case linking *COG4* mutations with CDG II, a further metabolic defect presenting as infantile acute neurological deterioration with multiorgan failure and diffuse white and grey matter loss [r1] on brain MRI.

27. Neurotransmitter disorders

P-649

The iNTD registry: A new clinical database of patients with inborn neurotransmitter, pterin and folate disorders

Kuseyri O[1], Garcia-Cazorla Á[2], Kurian M[3], Jeltsch K J[1], Opladen T[1]

[1]Div Child Neur Metab Med,Univ Child Hosp, Heidelberg, Germany, [2]Dep Neur, Hos Sant Joan de Déu, Barcelona, Spain, [3]Neurosc Unit, Univ Coll London, London, United Kingdom

Background:Inherited defects of neurotransmitter (biogenic amines), tetrahydrobiopterin (BH_4) and folate metabolism lead to progressive neurological dysfunction in early infancy. Immediate diagnosis and treatment may result in an improved outcome. Until today there is no standardized systemic evaluation of diagnostic processes, therapeutic approaches and long term outcome of affected patients.
Methods: The new "*International Working Group on Neurotransmitter Related Disorders*" (*iNTD*) provides a platform for the scientific and clinical exchange in the field of neurotransmitter related disorders. It includes 23 metabolic centers from 17 countries worldwide. The newly developed web-based iNTD

patient registry for inherited defects of neurotransmitter, pterin and folate metabolism enables a standardized assessment of the epidemiology, genotype/phenotype correlation and outcome of these diseases, their impact on the quality of life of patients, and current diagnostic and therapeutic strategies. Based on the evaluation of the patient registry recommendations for the clinical and therapeutic management will be developed.
Conclusion: The iNTD network is a growing international initiative to encourage scientific and clinical exchange on neurotransmitter related disorder. Together with the iNTD registry it aims to improve current research, basic knowledge and clinical management strategies considering the rare neurotransmitter related diseases.

P-650

Progressive catecholamine depletion and severely impaired motor control in a hypomorphic tyrosine hydroxylase knock-in (*Th*-ki) mouse

Allegri G[1], Noain D[2], Korner G[1], Ying M[3], Hole M[3], Flydal M I[3], Scherer T[1], Rassi A[4], Fingerhut R[1], Becu-Villalobos D[5], Pillai S[6], Wüest S[8], Konrad D[8], Lauber A[7], Baumann C[2], Bindoff L A[9 10], Martinez A[3], Thöny B[1]

[1]Div Metab, Dept Pediatr, Univ Child Hosp, Zürich, Switzerland, [2]Dept Neurol, Univ Hosp, Zürich, Switzerland, [3]Dept Biomed, Univ Bergen, Bergen, Norway, [4]Div Clin Chem Biochem, Univ Child Hosp, Zürich, Switzerland, [5]Inst Biol Exp Med, Buenos Aires, Argentina, [6]Inst Physiol, Univ Zürich, Zürich, Switzerland, [7]Dept Med, Univ Fribourg, Friburg, Switzerland, [8]Div Endoc, Dept Pediatr, Univ Child Hosp, Zürich, Switzerland, [9]Dept Clin Med, Univ Bergen, Bergen, Norway, [10]Dept Neurol, Haulkeland Univ Hosp Bergen, Bergen, Norway

Background: Tyrosine hydroxylase (TH) is the rate-limiting enzyme in the synthesis of catecholamine neurotransmitter and hormones, and a marker for dopaminergic neurons. Mutations in *TH* gene are associated with the autosomal recessive disorder TH deficiency (THD), which manifests with phenotypes varying from infantile parkinsonism and L-Dopa-responsive dystonia to complex encephalopathy of perinatal onset. The most recurrent mutation in THD patients is *TH*-p.R202H, which is mostly associated to a severe THD phenotype, often concomitant with non-responsiveness to L-Dopa treatment.
Methods & Results: While *Th* knock-out mice are not viable, homozygous *Th*-knock-in mice expressing the *Th*-p.R203H mutation (equivalent to h*TH*1-p.R202H) showed normal survival and food intake but hypotension and growth retardation. Mutant enzyme exhibited impaired inhibitory feedback and stabilizing dopamine binding, which lead to progressive disappearance of TH associated to gradual loss of catecholamines. Unstable mutant TH was expressed in midbrain but specifically absent in the *corpus striatum*. Mutant mice were non-responsive to L-Dopa treatment and showed hypokinesia, reduced motor coordination, altered gait parameters and catalepsy with diurnal fluctuation.
Conclusion: This hypomorphic *Th*-ki mouse model with encephalopathy and impaired motor control thus provides understanding on molecular pathogenic mechanisms for THD, replicates the most severe form of the disease, and provides a platform for the evaluation of novel therapeutics.

P-651

Long-term follow-up of patients with BH4 deficiency in Korea

Lee J[1], Lee D H[1]

[1]Dep Pediatrics, Soonchunhyang University, Seoul, Korea, Republic of

Background: A deficiency of BH4 not only causes the classical phenylketonuric phenotype, but also is the source of neurological symptoms due to impaired syntheses of L-Dopa and serotonin. The treatment of BH4 deficiency usually consists of replacement with BH4 and the neurotransmitters. We performed this study to find out the long-term follow-up clinical symptoms and prognosis of BH4 deficiency.
Methods: Clinical and genetic analysis were done retrospectively from January 1999 to July 2014.
Results: In our study, 207 patients were confirmed to have hyperphenylalaninemia. Among them, 13 patients were BH4 deficiency. 11 patients were PTPS deficiency and one patient was DHPR deficiency. The patients who received delayed treatment, most of our patients suffered from severe psychomotor retardation, hypotonia and seizure. C.259C>T mutation was identified most commonly in *PTPS* gene analysis. A patient with DHPR deficiency had a mental retardation, dystonia, seizure. His seizure semiology was dialeptic feature. His EEG showed generalized spike wave patterns. All treated patients tolerated L-Dopa, BH4 and 5-hydroxytryptophan. Most of the early treated patients had a good tolerance for the drugs.
Conclusion: BH4 deficiency patients who had delayed treatment tend to have severe psychomotor problem and neurologic deficits.

P-652

A patient with 6-pyruvoyl-tetrahydropterin synthase deficiency

Eminoglu F T[1], Kutluk G[2], Tıraş, Teber S[2]

[1]Ankara Univ Hosp, Dept Ped Metab Dis, Ankara, Turkey, [2]Ankara Univ Hosp, Dept Ped Neurology, Ankara, Turkey

6-Pyruvoyl-tetrahydropterin synthase (PTPS) deficiency is the most frequent form of tetrahydrobiopterin (BH4) deficiency related to hyperphenylalaninemia (HPA). PTPS deficiency may not only cause a typical phenylketonuric phenotype, but is also accompanied by various neurological signs and symptoms due to impaired synthesis of catecholamines and serotonin. Our patient was the second child of consanguineous healthy parents. He was born after a 39-week gestation period and an uneventful delivery. A Guthrie test on day 5 revealed HPA (blood Phe 6.5 mg/dl) and on day 10, he was diagnosed as HPA in another hospital. He had been lost in follow-up. At the age of 5 months, he presented with hypotonia. Physical examination revealed blond hair, truncal hypotonia, seizures, abnormal movements, mental and motor retardation. Laboratory findings on admission showed HPA (20.3mg/dl) and 5-HIAA: 50 nmol/L (114-336), HVA: 130 nmol/l (295-932), neopterin: 94 nmol/l (12-30), biopterin: 2 nmol/l (15-40). A BH4 (20 mg/kg) loading test was performed. The plasma PHE fell to normal at the 8 h. The plasma DHPR activity was normal. Genetic analysis revealed homozygous p.A111T mutation. BH4, L-Dopa and 5-hydroxytryptophan were administered. Although this patient in whom replacement therapy had been delayed, has marked improvements in neurological signs and symptoms after initiation of the treatment.

P-653

Modelling dihydrobiopterin reductase (QDPR) deficiency in Zebrafish

Breuer M[1], Opladen T[1], Sauer S W[1]

[1]Inborn Errors of Metab, Univ Hosp, Heidelberg, Germany

Our study focuses on the cofactor tetrahydrobiopterin (BH_4), which is required for the synthesis of neurotransmitters such as dopamine and serotonin. A lack of BH_4 results in severe neurological defects, mental retardation, and hypotonia. Our study aims to unravel the role of BH_4 metabolism in brain development and models the entire pathway in the zebrafish embryo. We use *in situ* hybridization and morpholino knockdowns to analyze the neurological phenotype of the developing embryo. We detected every member of BH_4 metabolism in the developing brain region and specifically to dopaminergic and serotonergic neurons. Especially the recycling factors localize to the high necessity regions in the brain. As example, we target the dihydrobiopterin reductase (QDPR), which recycles dihydrobiopterin (BH_2) back to BH_4 . In contrast to an existing mouse model, down regulation of Qdpr abundance in zebrafish embryos results in defective brain development including microcephaly similar to the phenotype found in affected patients. Our data suggests that QDPR plays a central role in mid- and hindbrain organization. Our study indicates that the zebrafish is a useful model to further investigate defects in BH_4 metabolism. Further, the zebrafish model will be instrumental to develop and test genetic and pharmacological therapeutic strategies.

P-654

Biochemical diagnostic algorithm for aiding in the diagnosis of neurometabolic conditions affecting dopamine metabolism

Aitkenhead H[1], Heales S J R[1]

[1]Chem Path, Gt Ormond St Hosp Children, London, United Kingdom

Background: The diagnosis of neurometabolic conditions affecting dopamine metabolism can be difficult as other more common neurological disorders can present in similar ways. We describe a biochemical diagnostic algorithm that may be helpful to screen patients with neurological disorders, to exclude neurometabolic conditions affecting dopamine metabolism.
Method: Using serum prolactin with paediatric age-related reference intervals and a quantitative method for vanillyllactate (VLA) measurement, it may be possible to select which complex patients should be investigated further by CSF neurotransmitter analysis.
Results: Raised VLA with raised prolactin is suggestive of aromatic amino acid decarboxylase deficiency or a defect in pyridoxal phosphate metabolism. Normal or low VLA with raised prolactin is suggestive of tyrosine hydroxylase deficiency or a defect in biopterin metabolism. Normal or low prolactin with raised VLA is suggestive of liver dysfunction (if 4-hydroxyphenyllactate is also raised) or therapy with L-dopa or dopamine agonists.
Conclusion: Serum prolactin and quantitative urinary VLA results may be useful when used in combination with the clinical picture and CSF neurotransmitter results to help pinpoint the diagnosis in complex cases. This algorithm may also be helpful in reducing the number of monitoring lumbar punctures.

P-655

ERNDIM CSF neurotransmitter pilot scheme: Review of the first year

Pope S A S[1], De Graaf I[2], Weykamp C W[2], Heales S J R[1]

[1]Neurometabolic Unit, Nat Hosp Neurol, London, United Kingdom, [2]MCA Lab, Queen Beat Hosp, Winterswijk, Netherlands

Background/objectives: This was the first year of the ERND IM CSF neurotransmitter pilot scheme. The scheme was set up to monitor the analytical quality and interpretation of the quantitative assay of monoamine metabolites in CSF.
Materials: 4 duplicate lyophilised samples (8 samples in total) were sent to 19 participating laboratories. The samples were made by pooling, spiking and/or diluting human CSF. Laboratories were asked to quantify 4 monoamine metabolites (5-hydroxyindoleacetic acid (5HIAA), homovanillic acid (HVA), 3-methyl dopa (3MD) and 5-hydroxytryptophan (5HTP)) and answer a multiple choice question.
Results: 16/19 laboratories from 15 countries submitted results. 14 laboratories used HPLC with electrochemical detection, while 2 laboratories used LC-MS. The results for HVA and 5HIAA showed good agreement between laboratories and between duplicates (CVs around or less than 10%). However, there was much greater variation for 3MD and 5HTP, with CVs greater than 30%. In terms of interpretation, over 85% of laboratories gave the correct multiple choice answer.
Discussion: HVA and 5HIAA, were accurately and reproducibly reported whereas the minor metabolites, 3MD and 5HTP, showed greater variation. The possible causes of this – less well standardised metabolites, metabolite degradation, concentration dependence – will be investigated during the second year of the pilot scheme.

P-656

Transient hyperphenylalaninemia due to heterozygous mutation in pyruvoyltetrahydropterin synthase (PTS) gene

Nardecchia F[1 2], Artiola C[3], Carducci C[3], Carducci C A[3], Giovanniello T[3], Leuzzi V[1]

[1]Dept of Ped and Child Neurol and Psych, Rome, Italy, [2]Dept of Physiol and Pharmacol, Rome, Italy, [3]Dept of Experim Med, Rome, Italy

Background: Some form of BH4 deficiency can be detected by neonatal screening (NS) programs as they determine hyperphenylalaninemia (HPA). HPAs caused by BH4 deficiency, that account for approximately 2% of individuals with HPA, are inherited in an autosomal recessive manner.
Case report: We report a case of a fullterm newborn diagnosed with HPA by NS. Phenylalanine (Phe) level was 343μM and returned in the normal range without any treatment after 20 days of life. DHPR enzyme activity was normal while pterins in urine and CSF strongly suggested 6-pyruvoyl tetrahydrobiopterin synthase (PTPS) deficiency. CFS neurotransmitters were normal. Molecular analysis of pyruvoyltetrahydropterin synthase (*PTS*) gene detected a new heterozygous mutation (p.Pro46Ala transition) in exon 2. Bioinformatics evaluation of pathogenicity gave controversial results.
Conclusion: To the best of our knowledge this is the first case of HPA due to heterozygous mutation in the *PTS* gene. Follow-up of the patient is needed to establish outcome.

P-657

A rare metabolic disease : succinic semialdehyde dehydrogenase deficiency

Tokatli A[1], Pektas E[1], Yildiz Y[1], Unal O[2], Dursun A[1], Sivri H S[1], Coskun T[1]

[1]Div Metab Dis, Hacettepe Univ Child Hosp, Ankara, Turkey, [2]Div Metab Dis,Hematol Oncol Hosp, Ankara, Turkey

Succinic semialdehyde dehydrogenase deficiency (SSDD) is an autosomal recessive inherited disease known to affect gamma-amino butyric acid (GABA) metabolism. We present a case with increased 4-hydroxybutyrate (gamma hydroxybutyric acid [GHB]) in urine. A 6-year-old boy with consanguineous parents who was seen in our pediatric neurology clinic because of developmental delay, persistant epilepsy and ataxia, was refered to evaluate further for metabolic disease. At first, hypotonia was noticed in the newborn period. On follow up he had poor head control, persistent seizures and ataxia. Seizures frequently occured in the first year of life while the general course of the disease was non-progressive. He was given multiple anticonvulsant drugs. Routine hematologic-biochemical parameters, cerebrospinal fluid (CSF) evaluations, CSF neurotransmitters were within normal limits. Cranial MRI was normal. Urine organic acid analysis, repeated twice, revealed elevated GHB levels. Mutation analysis for *ALDH5A1* gene showed p.Gln43Argfs*50 (c.113_114GCCCG) homozygous mutation. SSDD was thus diagnosed. After the diagnosis was ascertained, anticonvulsant treatment was changed to vigabatrin and the frequency of seizures was reduced dramatically. In conclusion, SSDD should be considered in children with mental retardation, developmental delay, and ataxia. Elevated urine GHB level was the hallmark for SSDD.

P-658

Target prolactin range in treatment of tetrahydrobiopterin deficiency

Porta F[1], Pagliardini V[1], Biamino E[1], Ponzone A[1], Spada M[1]

[1]Dept. Pediatrics, University of Torino, Torino, Italy

Objective: To describe the role of a standardized prolactin (PRL) profile for optimizing treatment in tetrahydrobiopterin (BH4) deficiency.
Methods: 75 PRL profiles in 8 patients with severe BH4 deficiency were related to treatment and clinical outcome. Peripheral PRL was measured in three blood samples collected 1) just before dopaminergic replacement therapy in the morning, 2) 3 hours after the first sample, and 3) 6 hours after the first sample.
Results: Three types of PRL profiles were evident. The understimulation profile, observed in patients on classical L-Dopa therapy (7.5±2.4 mg/kg) and associated with a fluctuant clinical picture (adapted UPDRS 25±12/135±34), was characterized by severe to mild hyperprolactinemia before and after therapy administration. The optimal-stimulation profile (UPDRS 13±6/135±33) was obtained with L-Dopa+ pramipexole therapy (4.5±1.4 mg/kg and 0.014±0.021 mg/kg) and characterized by mild hyperprolactinemia (400-800 mU/l) before treatment administration, followed by steady PRL normalization. The over-stimulation profile (L-Dopa 4.7±1.0 mg/kg, pramipexole 0.031±0.001 mg/kg) showed normal PRL values at all times and was associated to the development of symptoms of dopaminergic overstimulation on the longitudinal follow-up after a period of good clinical compensation (UPDRS 13±6/133±38).
Conclusions: Besides clinical monitoring, a 6-hour PRL profile is useful for optimizing the treatment in BH4 deficiency.

P-659

A *de novo* mutation in *DNM1L* associated with dopaminergic impairment showing infantile parkinsonism and fatal outcome

Diez H[1 2], Girós M[3 4 5], Armstrong J[2 6], Fernández-Marmiesse A[7], Ormazábal A[8], Montoya J[9], Artuch R[2 8], Garcia-Cazorla A[1 2]

[1]Dep Neur Lab Syn, Hosp Sant Joan de Deu, Barcelona, Spain, [2]CIBERER, Madrid, Spain, [3]Sec Err con met, IBC, Barcelona, Spain, [4]Serv Bioq i Gen Mol, Hospital Clínic, Barcelona, Spain, [5]IDIBAPS, Barcelona, Spain, [6]Mol Gen Med Serv, Hosp Sant Joan de Deu, Barcelona, Spain, [7]UDyTEMC, Hosp Clin Univ, Santiago de Compostela, Spain, [8]Neur Clin Bioc De, Hosp Sant Joan de Deu, Barcelona, Spain, [9]Dep Bioc Mol Cel Bio, Uni Zar - IACS, Zaragoza, Spain

Background and objectives: We have previously reported a group of patients with mitochondrial diseases mimicking neurotransmitter defects. Here we report a female patient with a dynamin 1-like gene (*DNM1L*) mutation.
Patient and Methods: The patient showed normal development until 3 months of age, followed by severe rigid-hypokinetic syndrome, brain atrophy and death at18 months of age. CSF neurotransmitters were analyzed by HPLC, brain morphology and metabolites by MRI/MRS, mitochondrial function (enzyme activity; mitDNA) by spectrophotometry, Southern blot, and RT-PCR, and mutations on mitochondrial genes by massive sequencing technology. HeLa human cell line was transduced using lentiviral vectors, protein biochemistry was studied by western-blot and organelle morphology by immunofluorescence.
Results: Reduced levels of CSF dopaminergic metabolites were found, together with brain atrophy, hypomyelination, neurodegeneration markers and increased lactate, suggesting mitochondrial impairment. Muscle biopsy confirmed respiratory chain deficiencies and mitochondrial DNA depletion (60%). Sequencing of 150 mitochondrial genes detected a heterozygous, *de novo* mutation in *DNM1L* gene. Overexpression of this mutation in HeLa cells leads to altered mitochondrial dynamics.
Conclusion: A new mutation in *DNM1L* is associated with severe infantile parkinsonism and signs of mitochondrial encephalopathy

P-660

Model system for fast in vitro analysis of *GABA-T* missense variants

Pop A[1], Struys E A[1], Van Oostendorp J[1], Jansen E E W[1], Roos B[1], Louro P[2], Ramos L[2 3], Mandel H[4], Osaka H[6], Pearl P[5], Gibson K M[7], Salomons G S[1]

[1]Metabolic Unit, VU Univ Med Center, Amsterdam, Netherlands, [2]Med Gen Unit, Ped Hosp, CHUC, Coimbra, Portugal, [3]Fac Health Sciences, Univ Beira Interior, Covilhã, Portugal, [4]Met Unit, Rambam Med Ctr, Tech Fac Med, Haifa, Israel, [5]Neuro, Boston Child Hosp,Harvard Med Sch, Boston, United States, [6]Dept Ped, Jichi Med Univ, Shimotsuke, Japan, [7]Exp Syst Pharm, College of Pharm, WSU, Spokane, Washington, United States

Background: 4-Aminobutyrate aminotransferase [*ABAT*; also GABA-transaminase (*GABAT*)] deficiency is a severe

autosomal recessive disorder of GABA catabolism. The biochemical hallmark is elevated free GABA in brain (detected by GC-MS using cerebrospinal fluid and non-invasive 1H-MRS), impaired *GABA-T* enzyme activity, and *GABA-T* mutations. Recently, five new cases came to our attention: potential pathogenic missense variants in *GABA-T* were mainly detected via exome sequencing. In most cases the proband was deceased prior to molecular analyses, precluding biochemical studies. Since prenatal diagnosis may be requested in the future, rapid diagnostic confirmation of probands is critical.
Methods: We cloned *GABA-T* in pCMV6-AN-GFP. Missense variants were introduced by site directed mutagenesis. We studied *GABA-T* activity using stable isotope labeled substrates and LC-MS/MS in HEK293 transfectants.
Results: The *GABA-T* enzyme activity was impaired for the first two tested alleles. *GABA-T*-GFP fusion protein was detected. These results verified both successful transfection, as well as confirming *GABA-T* deficiency in the proband homozygous for one of these alleles. Our expression system has now been applied to ten novel *GABA-T* missense variants.
Conclusion: Pathogenicity of a novel *GABA-T* missense variant using our *in vitro* model can be confirmed/excluded within one week, facilitating prenatal diagnosis.

P-661

Postmortem diagnosis of GABA transaminase deficiency: a case of a fatal early-onset epileptic encephalopathy

Louro P[1], Garcia P[2], Ramos L[1], Cancelinha C[3], Dinis A[4], Robalo C[5], Veiga R[6], Pina R[7], Rebelo O[8], Diogo L[2]

[1]Med Gen Unit, Ped Hosp, CHUC, Coimbra, Portugal, [2]Metab Dis Unit, Ped Hosp, CHUC, Coimbra, Portugal, [3]Med Ped Unit, Ped Hosp, CHUC, Coimbra, Portugal, [4]Ped Int Care Unit, Ped Hosp, CHUC, Coimbra, Portugal, [5]Neuroped Unit, Ped Hosp, CHUC, Coimbra, Portugal, [6]Neurorad Unit, CHUC, Coimbra, Portugal, [7]Anat Path Unit, CHUC, Coimbra, Portugal, [8]Neuropath Lab, Neur Unit, CHUC, Coimbra, Portugal

Background: GABA transaminase deficiency is a rare autosomal recessive disorder confirmed only in one sibship and one unrelated patient. It is caused by mutations in *ABAT* gene which encodes 4-aminobutyrate transaminase, an enzyme of GABA catabolism.
Case report: We report the case of a boy, deceased at 12 months of age, with early-onset epileptic encephalopathy, severe psychomotor retardation, hypotonia, and central hypoventilation. He was on non-invasive ventilation and tube feeding. There were also signs of premature pubarche, thermal instability, water-electrolyte imbalance, and rapid increase in weight and, to a lesser extent, length and head circumference. Serum total testosterone was elevated (43.3 ng/dL; normal range < 16), as well as serum growth hormone (7.7 ng/mL; normal range < 1). Brain MRI showed decreased myelination and generalized cerebral, cerebellar, and brainstem atrophy, later confirmed by postmortem examination. *ABAT* gene sequencing identified a homozygous variant c.888G>T (p.Gln296His), previously undescribed. MutationTaster, PolyPhen-2 and SIFT predict this variant to be respectively, "disease causing", "probably damaging" and "damaging".
Conclusions: Our patient showed clinical features of GABA transaminase deficiency. *ABAT* gene sequencing identified a homozygous variant, probably pathogenic, allowing a more precise genetic counselling for our patients' family, although further studies will be required to undoubtedly confirm the diagnosis.

28. Disorders of vitamins, cofactors and trace elements

P-662

Pyridoxine-dependent epilepsy due to antiquitin deficiency: clinical, biochemical and outcome in 21 french patients

Gibaud M[1], Nguyen the Tich S[1], Nabbout R[3], Barthez M A[4], Caubel I[5], Chouchane M[6], Doummar D[7], Kossorotoff M[3], Kuster A[8], Lamblin M D[10], Lefranc J[9], Maurey H[11], Roubertie A[12], Schiff M[13], Salomons G[14], Barth M[2]

[1]Dep Ped Neurology, CHU Angers, Angers, France, [2]Genetic Dep, CHU Angers, Angers, France, [3]Dep Ped Neurology Necker-enfants-malades, Paris, France, [4]Dep Ped Neurology Clocheville Hospital, Tours, France, [5]Pediatric Dep CH Lorient, Lorient, France, [6]Pediatric Dep CHU Dijon, Dijon, France, [7]Dep Ped Neurology Hopital Trousseau, Paris, France, [8]Pediatric Dep, CHU Nantes, Nantes, France, [9]Pediatric Dep, CHU Morvan, Brest, France, [10]Dep Clin neurophysiology Roger-Salengro, Lille, France, [11]Dep Ped Neurology Le Kremlin Bicetre, Paris, France, [12]Dep Ped Neurology Hop Gui de Chauliac, Montpellier, France, [13]Pediatric Dep, Robert Debré Hospital, Paris, France, [14]Metabool Lab, VU Med Center, Amsterdam, Netherlands

Background: Pyridoxine-dependent epilepsy due to antiquitin deficiency is a rare form of epilepsy due to an abnormality in the lysine degradation pathway, causing abnormal pyridoxine consumption, responsible of a glutamate/γ-aminobutyric imbalance. In its usual presentation, seizures appear during the neonatal period, are drug-resistant and stop when important doses of pyridoxine are administered, allowing anti-seizure medications to be stopped.

Methods & Results: This study covers 21 multicenter cases of this pathology in France. Collected data were about diagnoses, age at the first seizures and type of seizures, treatment, patients' outcome, EEG, MRI and molecular diagnosis. In most of the cases (12/21), presentation is atypical: seizures start after the neonatal period, or with partial pyridoxine sensitivity, requiring anti-epileptic drugs. Seizures are generally polymorphic. Patients' follow-up shows developmental delay in 15/21 patients. Early pyridoxine administration seems to improve cognitive outcome. Unlike pipecolic acid, α-amino adipic semialdehyde (α-AASA) was elevated in all cases showing excellent sensitivity in this series.
Conclusions: It is generally difficult to diagnose pyridoxine-dependent epilepsy, especially in atypical presentations. We propose to systematically measure α-AASA in patients aged below one year of age, presenting drug-resistant seizures of uncertain or unknown cause, to enable an earlier diagnosis and treatment of this pathology, and improve patients' outcome.

P-663

Clinical and biochemical spectrum of pyridoxine dependent seizures in India

Jalan A B[1], Kudalkar K V[1], Jalan R A[1], Shinde D H[1], Borugale M A[1], Joshi M M[1], Mahakal J M[1], Sonalkar N D[1]

[1]NIRMAN, Div of Biochemical Genetics, Navi Mumbai, India

Background: Pyridoxine Dependent Seizures (PDS) is a treatable yet underdiagnosed entity in India. We hereby report our experience of 12 unrelated patients.
Objective: To analyze the clinical and biochemical spectrum of PDS and develop a strategy to detect PDS at the earliest and treat.
Materials and method: We analyzed 12 cases (6:6 Male: Female). These patients were evaluated clinically and biochemically including Plasma and CSF aminoacids, Carnitine/acyl carnitine profile, GC/MS-SIM of Urine, Pipecolic acid (PIPA) in Plasma, CSF and Urine and AASA in urine in four cases. Molecular studies are under progress.
Result: We found mildly elevated CSF Glycine in 6/8 cases (27.8±16.91 μmol/L), significantly elevated PIPA in 10/11 plasma (14.55±17.34 μmol/L) and 6/8 CSF (8.08±10.27 μmol/L). Urine AASA was elevated in 3 out of 4 cases (34.08±38.93). All the patients were treated with Pyridoxine or Pyridoxine + Folinic acid with good response. One child also received a Lysine restricted Diet.
Conclusion: PDS is a treatable disorder which is not easily detected unless specific tests are performed. We propose screening in Plasma PIPA and if positive, either CSF-PIPA or Urine-AASA. All such suspected patients should receive a trial of Pyridoxine/Folinic acid pending the results. Lysine restricted diet may also be tried.

P-664

Treatment with mefolinate (5-methyltetrahydrofolate), but not folic acid or folinic acid, leads to measurable 5-methyltetrahydrofolate in cerebrospinal fluid in methylenetetrahydrofolate reductase deficiency

Walter J H[1]

[1]Willink Biochemical Genetics Unit, Manchester, United Kingdom

Background: Methylenetetrahydrofolate reductase (MTHFR) is necessary for the remethylation of homocysteine to methionine. The most severe forms of MTHFR deficiency cause a devastating neurological disorder with high mortality in infancy. In addition to hyperhomocystinaemia the disorder is characterised by extremely low levels of CSF 5-methylTHF. In a mouse model treatment with mefolinate (5-methylTHF) but not folic acid, has been shown to decrease mortality (Karp et al, 2008).
Case report: A patient with severe MTHFR deficiency (1% enzyme activity) presented with an encephalopathic illness and respiratory arrest at 24 days of age. Plasma homocysteine was increased at 167 μmol/L and plasma methionine < 2 μmol/L. Treatment with betaine, vitamin B12 and folic acid was started and the patient made a satisfactory recovery. However at 1 month of age CSF 5-methylTHF was unrecordable. Folic acid was changed to folinic acid but there was no increase in CSF 5-methylTHF. Mefolinate 15mg/d was given from 11 months of age and CSF 5-methylTHF increased to 17 nmol/L. Due to a pharmacy error at 18 months of age folinic acid was substituted for mefolinate; at 42 months CSF 5-methylTHF was below detection limits. Reinstitution of mefolinate at 30mg bd increased CSF 5-methylTHF to 26 nmol/L.

P-665

The clinical, biochemical and molecular spectrum observed in 6 Iranian biotinidase deficient patients

Zaman T[1 2], Moarefian S[1], Rahmanifar A[1], Saeed Tehrani F[1], Moradian R[1]

[1]Iranian National Research Unit, Tehran, Iran, Islamic Republic of, [2]IEM department,Tehran Univ, Tehran, Iran, Islamic Republic of

Background; Biotinidase deficiency (BTD) is an autosomal recessive metabolic disorder in which biotin can not be reused or recycled. Complete BTD (activity < 10%) causes seizures, hypotonia, developmental delay or regression, dermatitis, alopecia, hearing loss, ataxia, reduced immune function terminating in coma and death. Partial BTD (activity 10-30%) presents with any of these symptoms but is milder and symptoms are precipitated by stress or infection. Unless compliant with treatment, symptoms may recur.
Patients and methods: A retrospective study identified 5 patients with severe BTD. Three had with convulsion on day three of life with death at day 23, 28 and 7 months. The fourth, diagnosed and treated at 3 months, demonstrated hearing loss, speech retardation and school failure. All patients demonstrated; c.235C>T; Arg79Cys mutation. Case 5 was diagnosed on NBS and treated since 11 days of life. The common P.Cys33 PhefsX36 (c.98_104delinsTCC) mutation Associated with a severe phenotype was identified. The patient is in good condition. Case 6 presented at 2.5 months with refractory convulsions, metabolic acidosis, increased urine 3-OH isovaleric acid. The BT activity was 10-30%. After 6 months of treatment, the seizures ceased and the patient could walk and attended school at 9 years.
Conclusion 3 cases of BTD born before NBS era, died. The one patient identified by newborn screening is normal.

P-666

Multiple congenital malformations in two boys with *HCFC1* mutations mimicking a cobalamin C deficiency

Gerard M[2], Morin G[3], Bourillon A[1], Billette de Villemeur T[4], Ogier de Baulny H[1], Benoist J F[1]

[1]Ref Center Metab Dis, CHU R. Debre, APHP, Paris, France, [2]genetic dept, CHU Caen, Caen, France, [3]Genet Dept, Amiens, Amiens, France, [4]Neuropediatrics, CHU A.Trousseau, APHP, Paris, France

Background: *HCFC1* hemizygous mutations (cblX) explain the majority of clinically and biologically compatible cblC patients without *MMACHC* mutations. Some of the 14 cblX males previously described have a less classic biochemical picture compared to cblC with normal plasma total homocysteine (tHCy). Conversely, severe epilepsy and malformative pattern are largely present (>50 %) in the cblX phenotype.
Case report: We report a family with two maternal half-brothers with a *HCFC1* mutation in the second Kelch domain (c.307T>C). The first boy presented at birth with multiple malformations and rapidly developed untreatable status epilepticus. Metabolic screening revealed methylmalonic aciduria with hyperhomocysteine and low methionine. Despite correction of the biochemical abnormalities under B12 treatment, he died at the age of 4. The second is a male fetus from a medical termination pregnancy because of severe IUGR and malformations. Both presented with dysmorphic features (flat profile, cleft lip for one), increased nuchal translucency, prenatal onset microcephaly and hypospadias.
Discussion: This observation suggests that boys with midline malformations should be investigated for *HCFC1* deficiency which can be easily done by biochemical screening. These observations reinforce the key role of *MMACHC* in the development of the face that has been recently suggested in a zebrafish model.

P-667

Pyridoxal 5-phosphate oxidase deficiency may be associated with a mild epileptic phenotype including later seizure onset and normal developmental milestones

Mastrangelo M[1], Tolve M[2], Blagojevic D[2], Giannini M T[1], Carducci C A[2], Leuzzi V[1]

[1]Pediatr. Neurol.- Univ.-Sapienza, Rome, Italy, [2]Dep. Exp. Med.- Univ. Sapienza, Rome, Italy

Background. Reported here is the case of a 28 month old male with a novel mild epileptic phenotype of PNPO deficiency.
Patient history. This patient presented at the age of 20 months with a clonic status epilepticus with right deviation of gaze which was interrupted with intravenous phenobarbital. Intercritical sleep electroencephalogram revealed mild bilateral spikes, sharp waves and slow waves in the fronto-temporal regions. Brain MRI and metabolic investigations were normal. He was subsequently dosed on phenobarbital and pyridoxine. In the following months brief seizures of staring were noted but increased pyridoxine stopped them. At the age of 24 months phenobarbital was gradually suspended without problems.
Methods and Results. Direct *PNPO* gene sequencing revealed the homozygous mutation c.347G>A (p.Arg116Gln). The pathogenic role of this mutation, that had been previously considered as a polymorphism, was confirmed by Ensembl (http://www.ensembl.org/index.html) Mupro (http://www.ics.uci.edu/~baldig/mutation.html), SIFT (http://blocks.fhcrc.org/sift/SIFT.html), ConSEQ v1.1 (http://conseq.bioinfo.tau.ac.il/), Mutation Tasting (http://www.mutationtaster.org/), Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), Meta SNP (http://snps.biofold.org/meta-snp/) and ESE finder (http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi).

Conclusions. The present case expands the epileptic phenotype of PNPO deficiency to milder manifestations (including a later seizure onset, a responsiveness to pyridoxine and no abnormalities in developmental milestones) and supports the pathogenic role of the mutation c.347G>A (p.Arg116Gln).

P-668

Cobalamin C deficiency leading to pulmonary hypertension and renal thrombotic microangiopathy in a young adult

Grangé S[2], Tebani A[1], Rives A[6], Sauvètre G[5], François A[3], Benoist J F[6], Vianey-Saban C[7], Guerrot D[4], Tamion F[2], Bekri S[1]

[1]Metab Biochem Rouen University Hospital, Rouen, France, [2]Intensive Care Rouen University Hospital, Rouen, France, [3]Pathology Rouen University Hospital, Rouen, France, [4]Nephrology Rouen University Hospital, Rouen, France, [5]Internal Medic Rouen University Hospital, Rouen, France, [6]Biochemistry Robert Debré-APHP, Paris, France, [7]Metab Biochem Lyon University Hospital, Lyon, France

Background: Cobalamin C (cblC) deficiency is a defect in vitamin B12 metabolism caused by mutations in the *MMACHC* gene leading to methylmalonic aciduria with homocystinuria. The clinical phenotype is heterogeneous ranging from severe neonatal presentation to adult onset form. Systemic symptoms were reported including neurological, thromboembolic, ocular, renal, and pulmonary events. A few cblC patients have been reported to have renal thrombotic microangiopathy (rTMA) or pulmonary hypertension (PAH). The combined occurrence of rTMA and PAH has been linked to cblC deficiency only in children.
Case report: An 18-year old patient was admitted to intensive care with rTMA, PAH and acute renal failure. His 17 year old brother died a few years ago with similar systemic disorders, without diagnosis.
Results: Renal biopsy showed rTMA with ischemic glomerular collapse, foot process effacement, and tubulointerstitial fibrosis. Total homocysteine, methylmalonic acid and methylmalonylcarnitine levels were elevated in plasma, suggesting cbl C deficiency. This diagnosis was confirmed by molecular study of the *MMACHC* gene. Hydroxocobalamin treatment resulted in a rapid clinical improvement.
Discussion: The combination of these two rare clinical conditions should suggest a cblC deficiency and implies a prompt initiation of hydroxycobalamin treatment in young adult patients. Thus, the guidelines for rTMA management of adult patients could be accordingly adjusted.

P-669

Cobalamin C disease with hypopigmentated cutaneous findings: A unique case

Tumer L[1], Arhan E[2], Okur I[1], Aydın K[2], Hirfanoglu T[2], Karaoglu A[1], Ozturk Z[2]

[1]Div Pediatr Metabolism, Gazi Univ Hosp, Ankara, Turkey, [2]Div Pediatr Neurology, Gazi Univ Hosp, Ankara, Turkey

Background: Disorders of intracellular cobalamin metabolism have a variable phenotype and CblC disease is likely the most common. The infantile presentation is the most frequently recognized form. Infants may present with failure to thrive, poor feeding, and hypotonia or with an acute metabolic derangement. Untreated infants may have multi-organ involvement, neurologic deterioration, seizures and encephalopathy.
Case report: Here we reported a 4 months old female infant presented with infantile spasms and hypopigmented macules. She also had failure to thrive, poor feeding, and hypotonia. Initial metabolic tests revealed combined methylmalonic acidemia and hyperhomocystinemia with normal vitamin B_{12} and folate levels. MRI of the brain showed cerebral atrophy and delay of myelinization and EEG showed multifocal epileptic discharges. Genetic testing revealed a previously identified pathogenic mutation, c.394C>T in the *MMACHC* gene and confirmed the diagnosis of CblC defect.
Discussion/Conclusion: Although some reports have shown vitiligo and vitamin B_{12} deficiency association, to our knowledge this is the first report of CblC defect with hypopigmented skin lesions. This case demonstrates that defects or deficiencies of cobalamin should be remembered in the differential diagnosis of hypopigmentated skin lesions, and the role of CblC defect should be identified in pathophysiology of skin lesions.

P-670

Partial biotinidase deficiency with late-onset severe cutaneous manifestations

Sivri S[1], Yildiz Y[1], Pektas E[1], Ciki K[3], Alehan D[2], Dursun A[1], Tokatli A[1], Coskun T[1]

[1]Div Metab Dis, Hacettepe Univ Child Hosp, Ankara, Turkey, [2]Div Cardiology,Hacettepe Univ Child Hosp, Ankara, Turkey, [3]Dept Pedtr, Hacettepe Univ Child Hosp, Ankara, Turkey

Partial biotinidase deficiency (PBD) has a wide spectrum of presentations from asymptomatic individuals to involvement of multiple organs. Here, we present a case with late-onset

severe cutaneous symptoms which was diagnosed with PBD and successfully treated with biotin.
14-year-old female patient presented to the pediatric clinic with a 6-month history of perioral cheliosis and xerosis cutis, which had rapidly progressed to generalized and severe erythematous squamous lesions causing pain, pruritus and cutaneous bleeding. Numerous cutaneous ointments, systemic steroids and supplementation of B-complex vitamins and zinc had provided only limited benefit. Infectious and rheumatologic screening tests were normal. Skin punch biopsy showed non-specific icthyosiform changes. Metabolic investigations revealed normal urinary porphyrins, normal serum and urinary amino acid levels and slightly increased urinary excretion of pyruvic acid, lactic acid and 3-hydroxy isovaleric acid. Biotinidase activity was found to be 1.3 U/L, consistent with PBD. *BTD* gene sequencing showed compound heterozygous c.511G>A and c.1330G>C mutations, the co-occurrence of which has not been reported previously. She displayed a dramatic reponse to 5mg/day biotin therapy.
Biotinidase deficiency should be excluded in patients with chronic dermatoses, especially if newborn screening had not been performed; since easy, cheap and effective therapy is readily available.

P-671

Mutations causing biotinidase deficiency in children detected by newborn screening in south eastern Turkey

Seker Yilmaz B[1], Kor D[1], Ceylaner S[2], Oktem M[3], Ceylaner G[2], Bulut D[1], Altinsu T[4], Onenli Mungan N[1]

[1]Div Metab Dis, Univ Cukurova, Adana, Turkey, [2]Intergen Genetic Lab, Ankara, Turkey, [3]Duzen Lab, Ankara, Turkey, [4]Governmental Public Health Office, Adana, Turkey

Background and Objectives: Biotinidase deficiency (BD) is an autosomal recessive inborn error of metabolism characterized by neurologic and cutaneous symptoms and can be detected by newborn screening (NS). NS for BD was implemented in Turkey in 2008.
Materials and Methods: 101 patients, identified among the infants screened by the NS were later confirmed through measurement of serum biotinidase activity. We also performed *BTD* mutation analysis to characterize the genetic profile.
Results: Twenty-six mutations were identified. The most commonly found variants were c.470G>A (p.R157H), c.1595C>T (p.T532M) and c.1330G > C (p.D444H) with allele frequencies of 24.1%, 20.6%, and 19.2% respectively. Four novel pathogenic variants were identified; c.956C>T (p.S319F), c.592_594delGTC (p.198delV), c.192_193insCATC (p.L69Hfs*24) and c.419G>A (p.W140*). Although all of the patients were asymptomatic on treatment with biotin, just one of them, who has the novel c.419G>A homozygous mutation, became symptomatic during an episode of acute gastroenteritis with a presentation of ketosis and metabolic acidosis. Among the screened patients, 65 have partial and 45 have profound BD.
Conclusion: We determined the mutation spectra of BD from the southeastern part of Turkey. The results of this study add four more novel mutations to the total number of mutations described as causing BD.

P-672

Molybdenum cofactor deficiency (*MOCOD*); expanding the phenotypic spectrum to the prenatal period

Hofstede F C[1], Van der Crabben S N[2], Pistorius L[3], Wamelink M M C[4], De Sain-van der Velden M G M[2], De Vries L S[5], Van Hasselt P M[1]

[1]Dep Metab Dis, Univ Med Center, Utrecht, Netherlands, [2]Dep Med Gen, Univ Med Center, Utrecht, Netherlands, [3]Dep Obst Gyn, Univ Med Center, Utrecht, Netherlands, [4]Dep Clin Chem, Met Unit, VU Med Center, Amsterdam, Netherlands, [5]Dep Neon, Univ Med Center, Utrecht, Netherlands

Introduction: Molybdenum cofactor deficiency is a rare inborn error of metabolism, characterized by neonatal onset of seizures and encephalopathy.
Case report: We describe a case in which cerebral cysts were observed prenatally. Diagnosis was suspected when both ultrasound and prenatal MRI revealed characteristic bilateral cerebral cysts, ventriculomegaly and cerebellar atrophy as observed in MOCOD, albeit postnatally. In addition, a sibling had died of 'perinatal asphyxia'. Amniotic fluid analysis showed elevated levels of S-sulfocysteine and taurine, while cysteine was decreased. Diagnosis of MOCOD was confirmed by urine analysis - revealing elevated levels of xanthine – and genetic analysis - homozygous mutations (c.418+1 G>A) in *MOCS1*.
Review: We performed a structured review of cases with MOCOD, aiming to delineate the timing of cystic lesions. Sufficiently detailed description of clinical features and neuroimaging were available in 53 cases, including our own. In three cases, antenatal onset of cystic formation was documented by imaging. In an additional 4 cases cysts or a "Dandy Walker variant" were documented on day 1, strongly indicating antenatal onset.

Conclusion: Antenatal onset of cerebral damage in MOCOD is present at a higher frequency than currently appreciated.
Conflict of Interest declared.

P-673

Metabolic consequences of vitamin B6 deprivation

Ramos R[1], Gerrits J[1], Van der Ham M[1], Pras-Raves M L[1 2], Fung Y[1], Albersen M[1], Bosma M[1], Prinsen H C M[1], Jans J J[1], Verhoeven-Duif N M[1]

[1]Dept Med Gen, Univ Med Center (UMC), Utrecht, Netherlands, [2]Acad Med Center, Amsterdam, Netherlands

Background & Objectives: Pyridoxal-5'-phosphate (PLP), the metabolically active form of vitamin B6, is an essential cofactor for more than 140 metabolic reactions. The metabolic consequences of nutritional inadequacies and genetic defects leading to vitamin B6 deficiency are difficult to predict. Because the classical picture of vitamin B6 deficiency is characterized by severe neonatal seizures, we aim to explore the metabolic consequences of altered vitamin B6 availability in neuronal cells.
Materials & Methods: Mouse neuroblastoma (Neuro2A) cells were cultured in the absence and in the presence of pyridoxal (100 and 1000 nmol/L). At different time points, the intracellular metabolic consequences of altered vitamin B6 availability, using a targeted (UPLC-MS/MS) and a new untargeted (Direct Infusion High Resolution Mass Spectrometry-DIMS) methodology, were investigated.
Results: The combined methodologies showed altered intracellular amino acid, organic acid (TCA cycle intermediates) and neurotransmitter concentrations upon altered vitamin B6 availability. In addition, new metabolites were identified as being vitamin B6 dependent.
Discussion & Conclusion: With the combination of targeted and the new untargeted approach, we shed light on the complex metabolic network that depends on vitamin B6 availability.

P-674

Nitrous oxide laughing gas: not so funny!

Desprairies C[1], Schiff M[1], Benkerrou M[1], Imbard A[1], Pichard S[1], Lorrot M[1], Gaumetou E[1], Mleki I[1], Sauvé Martin H[2], Holvoet L[1], Ithier G[1], Missud F[1], Benoist J F[1]

[1]Robert Debré Univ. hosp., Paris, France, [2]Margency hosp., Margency, France

Background: Nitrous oxide (N_2O) laughing gas, is a commonly used anaesthetic drug. It was shown that N_2O inhibits intracellular vitamin B12 action by oxidizing its cobalt ion metabolism.
Methods: We present a ten-year old girl with sickle cell disease who exhibited signs of intracellular vitamin B12 metabolism dysfunction due to massive and long-term N_2O exposure. This patient had a multifocal and extremely painful osteomyelitis due to poor sickle cell control. She therefore was treated with N_2O at least 2 hours a day during seven months. At that time, due to the potential toxic effect of long-term N_2O on B12 metabolism and because the pain could have been exacerbated by N_2O-induced vitamin B12 dysfunction, a metabolic workup was performed. Total homocysteine plasma concentration was 155 μM (N < 15 μM), with low methionine and high urinary methylmalonate excretion contrasting with normal vitamin B12 level. Such biochemical findings confirmed inhibition of intracellular vitamin B12 metabolism. After withdrawal of N_2O and oral vitamin B12, metabolic abnormalities disappeared.
Conclusion: Caution is in order with long-term N_2O exposure due to the risk of secondary vitamin B12 dysfunction. This is particularly true in sickle cell disease where homocysteine accumulation is an additional thromboembolic risk factor.

P-675

Riboflavin responsive multiple acyl-CoA dehydrogenation deficiency associated with flavin-sensitive variant FAD synthase proteins

Mosegaard S[1], Giancaspero T A[2], Galluccio M[3], Leone P[2], Boneh A[4], Tyni T[5], Gregersen N[1], Olsen R[1], Barile M[2]

[1]Res Unit Mol Med, Aarhus Univ Hosp, Aarhus, Denmark, [2]Dept of Bioscience, Biotech and Biopharm, Bari, Italy, [3]Dept of Biology, Ecology and Earth Scien, Arcavacata di Rende, Italy, [4]Murdoch Children's Research Institute, Melbourne, Australia, [5]Dept of Pediatric Neurology, Helsinki, Finland

The human FAD synthase (FADS), the product of the *FLAD1* gene, is an important part of the riboflavin metabolic pathway. FADS converts FMN into FAD. FMN and FAD are essential cofactors of numerous flavoprotein dehydrogenases involved in energy metabolism and in other functions crucial for regulation of cell life. Multiple acyl-CoA dehydrogenation deficiency (MADD) is an autosomal inherited disease characterized by dysfunction of multiple mitochondrial flavoprotein dehydrogenases, which use the electron transfer flavoprotein (ETF) or its dehydrogenase (ETFDH) for delivery of electrons to the respiratory chain. Riboflavin responsive forms of

MADD (RR:MADD) have been explained by flavin-sensitive ETF/ETFDH variant proteins, or by variant riboflavin transporters, which cause impaired cellular uptake of riboflavin. In this study we investigated the disease-causing nature of two *FLAD1* gene variations identified in two RR:MADD patients. Protein stability studies of recombinant p.Ser495del and p.Arg530Cys FADS proteins showed that the variant FADS proteins had decreased resistance to proteolytic degradation and decreased enzyme activity. Addition of flavin prevented proteolytic degradation, even if it did not restore the enzyme activity. Our results provide a molecular explanation for the response to riboflavin observed in the patients.

P-676

Inhibition of pyridoxal kinase reduces GABA concentrations and results in seizures in zebrafish larvae

Albersen M[2], Bosma M[2], Mensink S[2], Ramos R[2], Prinsen H C M[2], Jans J J M[2], Tessadori F[1 2], Bakkers J[1], Van Haaften G[2], Verhoeven-Duif N M[2]

[1]Cardiac Dev Genet, Hubrecht Instit-KNAW, Utrecht, Netherlands, [2]Dept Med Genet, Univ Med Center, Utrecht, Netherlands

Background & Objective: We studied the phenotypic and metabolic consequences of inhibition of pyridoxal kinase (PDXK) in zebrafish larvae, used here as an *in vivo* model for vitamin B6 deficiency.
Materials & Methods: Zebrafish larvae at 3 days post fertilization were treated with ginkgotoxin, a compound which structurally resembles vitamin B6 and interferes with B6 metabolism through competitive inhibition of PDXK. Locomotion of zebrafish larvae was monitored and relevant metabolites were quantified by mass spectrometry. The effects of supplementation with pyridoxal phosphate (PLP) in the medium were subsequently studied.
Results: Treatment of zebrafish larvae with ginkgotoxin resulted in a seizure-like swimming pattern, characterized by hyperactivity and asymmetrical movement of the fins. Concentrations of PLP in larval homogenates decreased whereas concentrations of pyridoxal increased. In addition, GABA concentrations decreased rapidly. Supplementation with PLP resulted in immediate normalization of locomotion and PLP concentrations, but not of GABA.
Conclusion: Zebrafish larvae are a suitable *in vivo* model to study disorders affecting vitamin B6 metabolism on phenotype and metabolite level. Inhibition of PDXK by ginkgotoxin causes seizure-like behavior in zebrafish larvae, which can be reversed by PLP.

P-677

Strongly increased PMP/PLP ratios in *in vitro* and *in vivo* models of PNPO deficiency: potential implications for treatment

Albersen M[2], Chi W[3], Bosma M[1], Jans J J M[1], Zhuang X[3], Verhoeven-Duif N M[1]

[1]Dept Med Genet, Univ Med Center, Utrecht, Netherlands, [2]Dept Med Genet, Univ Med Center, Utrecht, Netherlands, [3]Dept Neurobiol, Univ Chicago, Chicago, United States

Background & Objective: The systemic pyridoxal phosphate (PLP) deficiency in patients with pyridox(am)ine phosphate oxidase (PNPO) deficiency results in a range of organ involvement. Despite its capability to reverse the epilepsy, current treatment with vitamin B6 fails to prevent intellectual disability. To optimize treatment, we studied the consequences of PNPO deficiency and treatment with vitamin B6 *in vitro* and *in vivo*.
Materials & Methods: In mouse neuroblastoma (Neuro2A) cells, *PNPO* was silenced by RNA interference. The *in vitro* effects of treatment with different B6 vitamers were compared to those in fruit fly with a homozygous c.95C>A (p.Ala32Asp) missense *PNPO* mutation.
Results: Besides decreased concentrations of pyridoxal and PLP, Neuro2A cells showed increased pyridoxamine phosphate (PMP) concentrations and thus a strongly increased PMP/PLP ratio after silencing of *PNPO* and treatment with B6. In mutant fruit fly, similar effects were observed.
Conclusion: PNPO deficiency does not only result in decreased concentrations of PLP, but also in strongly increased PMP/PLP ratios. The phenotypical consequences of this observation are unknown, but the PMP/PLP ratio is likely important for transamination reactions. Since PM(P) is highly present in our nutrition, this might have implications for the dietary advice given to PNPO deficient patients.

P-678

Structural characterisation of human methionine synthase, a cytosolic client enzyme for cobalamin cofactor

Kopec J[1], Kiyani W[1], Vollmar M[1], Froese D S[1], Yue W W[1]

[1]Structural Genomics Consortium, Oxford, United Kingdom

Background: Methionine synthase (MS; EC 2.1.1.13), encoded by the *MTR* gene, converts homocysteine (Hcy) to methionine as part of S-adenosyl-methionine (AdoMet) biosynthesis. MS is one of the two human enzymes that require

cobalamin as an essential cofactor, with inherited *MTR* mutations causing the rare cobalamin disorder *cblG* type. The MS catalysed reaction involves cobalamin in the methylated form, generated by a second cofactor 5–methyl-tetrahydrofolate (MTHF). MS also necessitates an AdoMet-dependent reactivation step to regenerate Cbl during catalysis. Such highly complex chemistry is mediated by the multi-domain architecture of the MS protein, comprising four individual binding domains for Hcy, MTHF, Cbl and AdoMet.
Methods & Results: To facilitate structural studies, we purified full-length and truncated human MS by *E. coli* and insect cell expression. This allows the crystal structure determination of the N-terminal half of MS (aa 16-657), revealing the binding modes for Hcy and MTHF. We further demonstrate, using limited proteolysis, solution scattering, and thermostability methods, that full-length MS exists predominantly in two conformations, dictated by different ligand-bound states, that differ in the relative orientations of the four binding domains.
Conclusion: Our data support a model whereby MS accommodates multiple enzyme reactions and substrates within a single polypeptide by means of its conformational plasticity.

P-679

Increased PLP and GABA concentrations may explain behavioral changes in pyridoxal phosphatase deficiency

Albersen M[2], Jeanclos E[3 4], Bosma M[2], Raab A[3], Ramos R[2], Jans J J M[2], Gohla A[3 4], Verhoeven-Duif N M[1]

[1]Dept Med Genet, Univ Med Center, Utrecht, Netherlands, [2]Dept Med Genet, Univ Med Center, Utrecht, Netherlands, [3]Dept Pharmacol, Univ Würzburg, Würzburg, Germany, [4]Rudolf Virchow Center Exp Biomed, UW, Würzburg, Germany

Background and Objective: The first step in vitamin B6 degradation, the hydrolysis of pyridoxal phosphate (PLP) into pyridoxal, is dependent on pyridoxal phosphatase (PDXP). We studied the consequences of a deficiency of PDXP *in vitro* and *in vivo*.
Materials and Methods: In mouse neuroblastoma (Neuro2A) cells, *PDXP* was silenced by RNA interference and the effects of treatment with different B6 vitamers were studied. In mice, a whole body *PDXP* knock-out was created by EIIa-Cre deletion and the consequences for B6 vitamers, neurotransmitters and behavior were studied.
Results: Neuro2A cells showed increased concentrations of PLP after silencing of *PDXP* and treatment with pyridoxamine, pyridoxine, pyridoxal or even when no B6 vitamer was given. In homogenates of different *PDXP* knock-out mouse brain regions, concentrations of PLP were threefold higher and GABA increased with 20%. *PDXP* knock-out mice were viable, but showed signs of anxiety and depression.
Conclusion: A deficiency of PDXP results in strongly increased PLP concentrations *in vitro*, regardless of treatment. In PDXP-deficient mice, the increased PLP and GABA concentrations may explain the observed behavioral changes.

P-680

Antiquitin expression in cultured fibroblasts following treatment with folinic acid and ascorbic acid

Crowther L[1], Meili D[1], Simmons T L[1], Plecko B[1]

[1]Div Neuro, Univ Child Hosp, Zurich, Switzerland

Background and objectives: To investigate a possible explanation for folinic acid responsiveness in antiquitin deficiency, we aimed to determine whether folinic acid treatment affects antiquitin expression in cultured cells.
Materials and Methods: We examined the expression of antiquitin in commercially available untreated cultured fibroblasts and under treatment with folinic acid and another vitamin, ascorbic acid. Change in expression was analysed following treatment with folinic acid (1 – 100 μM) (Western blot), or ascorbic acid (50-100 μM) (Western blot and qPCR).
Results: Expression of antiquitin did not change after treatment with folinic acid. Ascorbic acid treatment led to an increase in antiquitin expression by Western blot, and a 2.4 fold increase by qPCR.
Discussion/Conclusion: Surprisingly, we found that ascorbic acid, but not folinic acid treatment, increased antiquitin expression in cultured fibroblasts. To test these results in a more relevant model, we are currently developing a cell culture model of antiquitin deficiency in astrocytes with the E427Q mutation (earlier nomenclature E399Q) reported in approximately 30% of published alleles. We will examine whether ascorbic acid increases antiquitin expression in this cell model, and whether this translates into increased antiquitin activity in astrocytes with the E427Q mutation.

P-681

The human journey of vitamin B_{12}: a structural perspective

Kopec J[1], Froese D S[2], Fitzpatrick F[1], Forny P[2], Baumgartner M R[2], Yue W W[1]

[1]SGC, University of Oxford, Oxford, United Kingdom, [2]Div Metabolism, Univ Child Hosp, Zurich, Switzerland

While Vitamin B_{12} serves as the cofactor for only two human enzymes (methylmalonyl-CoA mutase and methionine synthase), an intracellular pathway of seven known proteins has evolved for the uptake, processing and delivery of the appropriate cofactor form to the two enzymes. Inherited defects are reported in all seven proteins, giving rise to methylmalonic aciduria and homocystinuria. Nevertheless, the biochemical function of the seven proteins and molecular basis of their genetic defects remain poorly understood. Since 2010, the Structural Genomics Consortium has provided a structure coverage of five B_{12} pathway proteins (PDB codes: 3BIC, 2WWW, 3RMU, 3SOM, 4CCZ), resulting in new lessons for the B_{12} pathway, including:
adaptation of an ancient nitroreductase fold to endow a protein with B_{12}-binding and protein-interacting functionalities;
accommodation of multiple enzyme reactions and substrates within a single polypeptide, via long-range conformational dynamics;
involvement of protein-protein interactions in B_{12} trafficking, which are modulated by ligand (substrate/cofactor) binding and disrupted by disease mutations;
occurrence of destabilising missense mutations that lead to reduced functional protein, and a proof of concept for small molecule rescue.
The above illustrate the utility of protein-based structural and biochemical characterisation towards a mechanistic understanding and therapeutic advancement of metabolic disorders.

P-682

The diagnostic value of the vitamin B6 profile in epileptic encephalopathies

Mathis D[1], Abela L[2], Albersen M[3], Bürer C[4], Hartmann H[5], Hersberger M[1], Verhoeven-Duif N M[3], Plecko B[2]

[1]Div Clin Chem Biochem, Univ Child Hosp, Zurich, Switzerland, [2]Div Child Neurology, Univ Child Hosp, Zurich, Switzerland, [3]Dep Med Genetics, Univ Med Center (UMC), Utrecht, Netherlands, [4]Div Metabolism, Univ Child Hosp, Zurich, Switzerland, [5]Dep Pediatrics, Hannover Med School, Hannover, Germany

Goal: We wanted to further investigate if inborn errors of metabolism that can lead to vitamin B6 dependent epilepsies (Antiquitin, PNPO and TNSALP deficiency) have distinct plasma vitamin B6 profiles.
Methods: The B6 vitamers, pyridoxal-5'-phosphate (PLP), pyridoxal (PL), pyridoxamine (PM), pyridoxine (PN) and pyridoxic acid (PA) were quantified by LC-MS/MS in plasma of children with Antiqutin (n=18), PNPO (n=5) and TNSALP (n=2) deficiency. Reference values were obtained from 150 plasma-samples of children (0-18 years) without any neurologic abnormalities.
Results: The vitamin B6 profiles of patients with Antiquitin deficiency on pyridoxine showed an unspecific increase of PLP, PL, PA and partly PN. However patients with PNPO deficiency on pyridoxine or PLP had clearly elevated PM and PM/PA ratio compared to controls and to patients with other inborn errors of vitamin B6 metabolism. Plasma of patients with congenital hypophosphatasia (TNSALP deficiency) had a clear elevation of PLP that was higher than in any other patient on pyridoxine.
Discussion: These results suggest a diagnostic value of plasma vitamin B6 profiles for patients with suspected PNPO deficiency and congenital hypophosphatasia, but not with Antiquitin deficiency. This method could be helpful in the differential diagnosis of patients with vitamin B6 dependant disorders.

P-683

Wilson Disease. Further evidence of phenotypic heterogeneity in two sisters

Fiumara A[1], SAPUPPO A[1], Brafa Musicoro V[1], Lepori M B[2], Loudianos G[2], Raudino F[1], Bertino G[3]

[1]Reg Ref Centre Metab Dis, Univ Catania, Catania, Italy, [2]Mol Clin Med, Univ Cagliari, Cagliari, Italy, [3]Hepat Unit, Univ Catania, Catania, Italy

Wilson disease (WD), is an autosomal recessive disorder caused by mutations in the *ATP7B* gene on 13q14.3. Defective protein leads to low ceruloplasmin blood levels and copper accumulation in liver, basal ganglia and cornea with clinical evidence of liver disease, tremors, dysarthria, dystonia and psychiatric signs. Haemolytic anemia, hypertransaminasemia, renal tubular acidosis are other common findings. We report on a series of 21 patients, aged 8 to 52 years with a long follow up under treatment with penicillamine, zinc or both. A striking phenotypic variability was observed in two sisters carrying the same genotype (c.3207C->A(p.H1069Q) / c.3904-2A->G). Although both started to present with signs at age 10 years, onset was characterized by neurological signs in the first (tremors, motor incoordination, language and cognitive impairment) whilst liver involvement is the only sign in the other. After a 20 year follow-up the former is severely affected (MRI evidence of basal ganglia copper deposits and hyperchogenic liver, piastrinopenia), while the latter still has moderate liver enlargement.This phenotypic variability could be explained by the intervention of modifier genes (*CTR1*, *ATOX1*, *MURR1*) regulating copper metabolism in the presence of defective ATP7B protein function. Further

investigations on their role might have a profound impact also on therapy.

P-684

Identification of new protein members of the methionine synthase interactome

Bassila C[1], Ghemrawi R[1], Flayac J[2], Guéant J L[2], Coelho D[2]

[1]Inserm U954, Vandoeuvre-lès-Nancy, France, [2]Nancy Univ Hosp and INSERM U954, Vandoeuvre-lès-Nancy, France

Background: The recent identification of several genes involved in vitamin B12 or cobalamin (Cbl) processing suggests that new protein-protein interactions could take part in the regulatory mechanisms of Cbl metabolism. Our previous results described a novel interaction between methionine synthase (MS) and MMACHC and the regulation of MMACHC activity by MS isoforms.
Objective: Our goal is to further characterize the MS interactome.
Methods: Transcription, splicing and protein expression of the already known or putative protein partners of MTR were evaluated by RT-PCR, western blot, immunohistochemistry and confocal microscopy with HepG2 cells and with fibroblasts from patients with cblC and cblG inherited defects of Cbl metabolism.
Results: Our data confirm that MS interacts with methionine synthase reductase (MSR) and with MMACHC, which also interacts with MMADHC. Novel interactions include MMAD HC with MS, MMADHC with MSR and MSR with MMAC HC.
Conclusions: Our data support the hypothesis of a large multiprotein complex composed of at least MS, MSR, MMACHC and MMADHC suggesting that mutations affecting one of the corresponding genes could disturb the interactions involving the other members of the MS interactome.
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P-685

Cobalamin X (*HCFC1* deficiency) mimicking nonketotic hyperglycinemia with increased CSF glycine and methylmalonic acid-a case report

Scalais E[1], Weitzel C[6], De Meirleir L[2], Maertens G[7], Shaikh T[3], Coughlin C[3], Swanson M[3], Friederich M[3], Gunter S[4], Helbling D[4], Wendt-Andrea J[4], Acquaviva-Bourdain C[5], Van Hove J[3]

[1]Ped Neuro, Centre Hosp Luxembourg, Luxembourg, Luxembourg, [2]Ped Neuro, UZ Brussels VUB, Brussels, Belgium, [3]Depart Ped, Univ Colorado, Denver, United States, [4]Depart Ped, Med Coll Wisconsin, Milwaukee, United States, [5]Maladies Héréd Métab, CHU, Lyon, France, [6]Dep Ped, Centre Hop Luxembourg, Luxembourg, Luxembourg, [7]Lab, UZ Brussels, VUB, Brussels, Belgium

The proband presented in the neonatal period with hypotonia with preserved reflexes, tonic, clonic and myoclonic seizures and irritability. He developed refractory epilepsy and severe neurocognitive impairment without microcephaly. Early DWI showed hyperintensity of the internal capsule and corona radiate, and later T2-weighted MRI showed hyperintensity in the internal capsule, periventricular and cerebellar white matter. Metabolic screening showed elevated CSF glycine with elevated CSF/plasma glycine ratio (0.24, normal < 0.05), indicative of nonketotic hyperglycinemia and not of ketotic hyperglycinemia. The glycine cleavage enzyme activity in liver biopsy was severely reduced. Sequencing did not identify a mutation in *AMT*, *GLDC* or *GCSH*, and fibroblasts showed normal lipoylation. Subsequent metabolite analysis identified persistently elevated CSF and urinary methylmalonic acid and plasma homocysteine, which on hydroxocobalamin treatment improved biochemically but not clinically. Exome sequencing identified a known pathogenic sequence variant in *HCFC1*, c.344C>T p.Ala115Val. A review of another patient with cobalamin X with a similar mutation identified mildly elevated CSF glycine. Biochemical CSF abnormalities are compatible with a primary neurological disorder. Putative binding regions for *HCFC1* were identified near several genes of the glycine cleavage enzyme, providing a potential mechanistic link between *HCFC1* mutations and elevated glycine.
Conflict of Interest declared.

P-686

6 novel mutations in Menkes disease in russian patients

Kamenets E A[1], Demina N A[1], Milovanova N V[1], Zakharova E Y[1]

[1]FSBI «Res Cent Med Gen», Moscow, Russian Federation

Background: Menkes disease (MNK) is a rare X-linked recessive disorder characterized by generalized copper deficiency and caused by mutations in gene *ATP7A* that encodes one subunit of transmembrane copper-transporting ATPase.
Objective: To define the *ATP7A* mutation spectrum in Russian patients.

Materials and methods: We examined 8 male patients (1 m – 3 y) with early retardation in growth and severe neurologic impairment. DNA samples were extracted from whole blood. Coding sequences of *ATP7A* were analyzed by direct sequencing.
Results: In all patients hemizygous defects of *ATP7A* were found. Five of them are single base substitutions included 3 unknown mutations: *c.3288C>A*, *c.4052G>C*, *c.4112C>G*. Three of them are undescribed small deletions: *c.1744_1745del2*, *c.3751_3753del3*, *c.4148_4152del5*.
Conclusion: Most of the detected mutations are unique for each family. 5 of the 8 alterations (62,5%) occurred in exons 19 – 22 of the gene (1220 – 1409 amino-acid residues of the protein including the metal binding domain) so we recommend study of this region as a first step of *ATP7A* genetic analysis.

P-687

S-Sulfocysteine excitotoxicity provides the molecular basis of neurodegeneration in molybdenum cofactor deficiency

Schwarz G[1], Kumar A[1], Dejanovic B[1], Semtner M[3], Fusca D[2], Arjune S[1], Kloppenburg P[2], Meier J C[3], Abdel A A[1]

[1]Inst. Biochem. Univ. Cologne, Cologne, Germany, [2]Inst. Zoology Univ. Cologne, Cologne, Germany, [3]MDC Molecular Medicine, Berlin, Germany

Background: Molybdenum cofactor deficiency (MoCD) is an autosomal recessive inborn error of metabolism characterized by neurodegeneration and mostly death in infancy. MoCD patients present with intractable neonatal seizures, progressive encephalopathy, facial dysmorphisms, feeding difficulties and other symptoms. Biochemically, MoCD results in the loss of sulfite oxidase (SO) activity, which leads to the accumulation of sulfite, S-sulfocysteine (SSC), thiosulfate, taurine and depletion of cystine.
Methods & Results: Here, we show that SSC, is a major cause of neurodegeneration in MoCD. Similarly to glutamate, SSC causes a significant increase in postsynaptic excitatory currents, followed by cellular signalling events, which we have studied in cultured hippocampal and cortical neurons. Pharmacological intervention of excitatory signalling pathways, resulted in suppression of SSC-mediated cytotoxicity. In a tungsten-induced MoCD mouse model, SSC accumulated in urine and brain, which was accompanied by weight loss, weakened motoric movements and neuronal cell death. Additionally, SSC treatment impaired the inhibitory synaptic transmission via the major postsynaptic scaffolding protein gephyrin, which further exaggerated the imbalance between excitation and inhibition in neuronal circuits.
Conclusions: Our results provide a plausible molecular mechanism for the rapidly progressing and intractable neurodegeneration in MoCD and suggest alternative anticonvulsive treatments to prevent acute neuronal cell death in MoCD.

P-688

MEDNIK syndrome: clinical and biochemical delineation of the response to long-term zinc therapy - A case report

Martinelli D[1 2], Dionisi-Vici C[1]

[1]Div Metab Dis, Bambino Gesu' Child Hosp, Rome, Italy, [2]Sect Translational Neuroscience, NIH, Bethesda, United States

Background: MEDNIK syndrome – acronym for mental retardation, enteropathy, deafness, neuropathy, ichthyosis, keratodermia - is caused by mutations in *AP1S1* gene, encoding the sigma 1A subunit of adaptor protein complex-1 (AP-1). AP-1 directs the intracellular trafficking of several transmembrane proteins, including the copper-transporting ATPases, ATP7A and ATP7B. We recently identified disordered copper metabolism in MEDNIK syndrome, a picture combining abnormalities of both Menkes and Wilson diseases, partially ameliorated by zinc acetate therapy.
Methods: To confirm our previous observation, we performed serial clinical and biochemical evaluations in one Italian MEDNIK syndrome patient under zinc therapy. Length of treatment was 4 years.
Results: Our patient presented at diagnosis with hepatopathy, hypocupremia, hypoceruloplasminemia, increased urinary copper excretion, liver copper overload, intrahepatic cholestasis. At the end of the follow-up she showed striking improvement of hepatopathy and enteropathy, with complete normalization of serum transaminases and liver copper content. However, skin abnormalities worsened during the follow-up, and she showed stagnation of cognitive development.
Conclusions: The above findings pose new questions about the clinical and biochemical effects of AP-1 dysfunction and the possible therapeutic options. Chronic zinc acetate may protect affected patients from hepatic copper overload, but some copper may be required for normal brain growth and maturation.

P-689

New insights into the frequency of molybdenum cofactor deficiency from the Exome Aggegation Consortium

Schwarz G[1], Lal D[3], Arjune S[1], Mayr S[1], Havarushka N[1], Nuernberg P[2]

[1]Inst. Biochem. Univ. Cologne, Cologne, Germany, [2]Center for Genomics Univ. Koeln, Cologne, Germany, [3]Harvard Med School, Broad Inst., Boston, United States

Background: Molybdenum cofactor deficiency (MoCD) is an autosomal recessive inborn error of metabolism characterized by neurodegeneration and mostly death in infancy. MoCD was discovered in 1978 (Duran et al. JIMD) and since than more than 150 cases have been reported in the literature. A recent study by Mechler et al. (Genetics in Medicine, 2015) highlighted a great discrepancy between the age of first onset of symptoms of MoCD and the mean time of diagnosis, showing a gap of more than two months. Given the poor survival of patients, the severe neonatal presentation, and the general lack of awareness, it is likely that the number of affected patients is underestimated.
Methods & Results: Given the recent success in treating MoCD type A patients with cyclic pyranopterin monophosphate, we investigated the frequency of pathogenic mutations in the *MOCS1* gene. We screened exomes of approximately 60,000 unrelated individuals for severe *MOCS1* mutations as well as previously reported pathogenic mutations, of which we found more than 50% in the ExAC database. Following a bioinformatics approach, we developed a prediction score for unknown *MOCS1* mutations, 50 of which were functionally investigated.
Conclusion: The results provide strong evidence for a disease incidence of MoCD higher than previously anticipated.
Conflict of Interest declared.

P-690

Extensive characterization of 14 CblC (Cobalamin C)-defective patients: clinical signs and neurocognitive outcome, biological, molecular and ophthalmologic findings

Paviolo M[2 3], Garcia N[3 4], Imbard A[2 3], Delouvrier E[1], Rigaudière F[2 5], Rigal O[2 3], Ogier de Baulny H[2 3], Benoist J F[2 3], Schiff M[2 3]

[1]Ophthalmology Unit, Hosp R Debré, Paris, France, [2]University Paris-Diderot, Sorbonne, Paris, France, [3]Ref Cent for Metab Dis, R Debré Hosp, Paris, France, [4]Center for Molec Diseases, DMCP, CHUV, Lausanne, Switzerland, [5]Optht Unit, Lariboisière Univ Hosp, Paris, France

Objectives: We retrospectively report clinical, biological, ophthalmological and outcome findings in 14 CblC patients (5 females, 9 males) with *MMACHC* mutations.
Patients & Results: The age of presentation varied from 7 days to 20 years. Time between first symptoms and diagnosis ranged from 2 days to 1 year. Six patients had neonatal onset, 6 between age 1 month and 1 year and 2 at early adult age. Two patients died before 4 months of age. Age at final evaluation ranged from 1 year to 32 years. Hypotonia, feeding difficulties, failure to thrive, nystagmus and bicytopenia with macrocytic anemia were the most frequent symptoms at initial presentation in neonatal and early onset forms. Other less common symptoms (< 50%) were HUS, hypertension, liver disease, cardiomyopathy, interstitial pneumopathy and hypothermia. All patients exhibited hallmarks of defective remethylation at diagnosis. All neonatal and early-onset patients presented abnormal ophthalmological findings. Characteristic maculopathy was present in 43% of patients and diffuse retinopathy in 50%. Neurocognitive outcome was poor with intellectual disability ranking between mild to severe for 85% of patients.
Conclusion: While marked hematological and metabolic response was observed with treatment including parenteral hydroxocobalamin, the neurocognitive and ophthalmological outcome worsened independent of therapy.

29. Miscellaneous

P-002

Bone mineral density in children with inborn errors of metabolism on protein or galactose restricted diet compared to children on non-restricted diets

Baluyot M[1], Alcausin M M L[1 2], Chiong M A[1 2], Estrada S C[1 2]

[1]Inst Hum Gen Nat Inst Health Univ Phil, Manila, Philippines, [2]Dept Ped Phil Gen Hosp Univ Phil, Manila, Philippines

Background: Decreased bone mineral density (BMD) has been reported among patients with inborn errors of metabolism but has not yet been evaluated amongst our patients in the Philippines. This study aimed to determine if there is a difference in the BMD of children on protein and/or galactose-restricted diets (cases) and the BMD of children with non-restricted diets (controls). The study also aimed to determine whether a correlation of BMD and dietary intake exists.
Methods: Cases aged 5–18 years and age- and sex-matched controls were recruited from a tertiary hospital and from private clinics of metabolic specialists. Demographic data, biochemical parameters, dietary recall and BMD were performed on both groups.
Results: Sixteen patients (2 PKU, 5 galactosemia, 9 MSUD) and matched-controls participated. Paired t test and multiple linear regressions have shown that BMD measures of cases were lower. Higher total caloric and calcium intake were associated with higher BMD measures. On the other hand,

protein intake was shown to be negatively associated with BMD measures.
Conclusion: This study has shown that BMD is lower among patients with galactosemia, PKU and MSUD. The results in our study seem to support the theory that poor dietary compliance is related to worse bone health.

P-005

Telemedicine – is this a safe, cost effective solution?

Green D L[1], Bleakly C[1], Hendriksz C[1], Thompson L[1], Kempshall S[1], Anand I[1]

[1]Mark Holland Metab Un, Salford Royal Hos, Salford, United Kingdom

Introduction: One of the challenges facing our service is finite budget, increasing population with rare conditions and the geographic spread of our patient cohort. We believe that one of the options to support the changing needs of our patients within financial restraints will be the improved use and adoption of healthcare technologies such as telemedicine. Telemedicine allows healthcare professionals to provide patient care from a remote location allowing patients to receive care closer to home. Aiming to provide better patient experience and prevent unnecessary costs.
Method: To maintain and establish professional relationships, a metabolic dietitian and specialist nurse attends the clinic, taking the secure video conferencing equipment which allows a video link into the specialist centre. Patient's experience and DNA rates of the video clinic have been used to assess the success.
Results: The results of the patient satisfaction are encouraging with 91% patients able to speak easily and openly and about sensitive issues they had. 73% of patients would rather have a video consultation with their consultant than travel into the specialist centre, due to the distance and financial cost as they feel they can get the support from the specialist team they require closer to home.

P-691

Optimized gene therapy for mice with Canavan disease using Kozak consensus sequence

Matalon R[1], Gessler D[3], Li J[2], Su Q[2], Gao G[2]

[1]University of Texas Medical Branch, Galveston, United States, [2]University of Massachusetts Medical Scho, Worcester, United States, [3]University of Heidelberg Medical School, Heidelberg, Germany

Background: Canavan Disease (CD) is a severe leukodystrophy caused by mutations of the aspartoacylase (*ASPA*) gene, leading to N-acetylaspartate (NAA) accumulation. We previously reported that intravenous recombinant adeno-associated virus (rAAV) successfully restored *AspA* activity in the KO mice (i.e. CD mice). The trial led to partial restoration of motor function and increased life span.
Methods & Results: In the present study, we optimized gene therapy with Kozak sequence with *hAspA*. cDNA with enhanced therapeutic expression produced a more potent CD gene therapy and a better tool to study the physiologic role of AspA.We have created expression cassettes with *hAspA* together with different Kozak sequences (i.e. HKz and FKz). *In vitro* and *in vivo* data show increased hAspA activity and decreased NAA. Mice treated with rAAVFKz*hAspA* perform equally to WT mice on inverted screen and balance beam and significantly (>50% in average) better than WT at p28, p90 and p180 on rotarod. Data indicates that highly expressed *hAspA* has beneficial effect on the CD treated mice. Another Canavan strain Nur7 treated similarly resulted in the same level of improvement.
Conclusions: The cause of improvement may be caused by improved utilization of NAA products beyond myelin formation.Supported by: NIH 5R01NS07699103

P-692

Energy expenditure in chilean children with maple syrup urine disease (MSUD)

Campo, Pérez K[1], Cornejo, Espinoza V[1], Castro, Chaves M G[1], Hamilton, Viollier V[1], Cabello, Andrade J F[1], Arias, Pefaur C[1]

[1]INTA, U. de Chile, Santiago, Chile

Background: Maple syrup urine disease (MSUD) is caused by a blockage of the catabolic pathway of branched chain amino acids leading to neurological damage-induced by leucine and metabolites accumulation. Expenditure and energy requirements information is limited.
Objective: To determine if basal/total energy expenditure (BEE/TEE) agrees with recommendations of energy in MSUD children, and whether it´s related to nutritional status.
Methods: case-control study between MSUD (n=16) and healthy children (n=11) aged 6-18 years. Current nutritional status, daily energy intake, physical activity and BEE by indirect calorimetry (BEEr) and predictive equations were assessed; STATA 2012 ($p< 0,05$).
Results: When comparing the energy expenditure variables there was no significant difference between groups (*P=0,5212*; *0,5052*; *0,4897*). Moreover, compared to BEEr,

equations underestimate: in the study group 9,5% vs 10,4 % and in control group 7,5% vs 9,4% according to BEE WHO and Schofield, respectively (*P=0,0008*; *0,0208*). WHO equation has lower average calorie difference, higher concordance coefficient and association than Schofield equation for each group, compared to indirect calorimetry; being the best predictor of the BEE for MSUD group.
Conclusion: MSUD children energy recommendations are according to energy expenditure so the use of FAO/WHU/UNU 2001 equation is a clinically and statistically feasible tool for its determination.

P-693

Application of whole exome sequencing to a rare inherited metabolic disease with neurological and gastrointestinal manifestations: a congenital disorder of glycosylation mimicking glycogen storage disease

Park H D[1 2], Choi R[1 2], Woo H I[2 3], Choe B H[4], Park S[5], Yoon Y[5], Ki C S[1 2], Lee S Y[1 2], Song J[6], Kim J W[1 2]

[1]Dept Lab Med, Samsung Medical Center, Seoul, Korea, Republic of, [2]Dept Lab Med, SKKU School Med, Seoul, Korea, Republic of, [3]Dept Lab Med, Samsung Changwon Hosp, Changwon, Korea, Republic of, [4]Dept Pediatr, KNU School Med, Daegu, Korea, Republic of, [5]Dept Lab Med, Konkuk Univ Coll Med, Seoul, Korea, Republic of, [6]Dept Lab Med, SNU Coll Med, SNUBH, Seongnam, Korea, Republic of

Background: Rare inherited metabolic diseases with neurological and gastrointestinal manifestations can be misdiagnosed as other diseases. This study aims to provide evidence to recommend the utility of whole exome sequencing (WES) in diagnosis of rare inherited metabolic diseases.
Methods: A 4-month-old female baby presented with poor weight gain, repeated seizure-like episodes, developmental delay, and hepatomegaly with abnormal liver function test results. Liver biopsy revealed moderate fibrosis with a suggested diagnosis of glycogen storage disease (GSD). WES was performed in the patient. Carbohydrate-deficient transferrin assay was done by capillary electrophoresis.
Results: No mutations were identified by next generation sequencing panels for GSD (including 21 genes). WES revealed compound heterozygous mutations of *PMM2*: c.580C>T (p.Arg194*) and c.713G>C (p.Arg238Pro) which mutations were associated with congenital disorder of glycosylation Ia (CDG-Ia: PMM2-CDG). These were confirmed by Sanger sequencing. Parents of the patient were carriers of CDG-Ia. Patient showed an increase in asialotransferrin and disialotransferrin (30.8%, reference range: < 1.3%).
Conclusions: We successfully applied exome sequencing to diagnose the first reported Korean patient with CDG-Ia, which was misdiagnosed as GSD. WES may prove to be the preferred strategy for analysis of clinical features that do not readily suggest a specific diagnosis.

P-694

Autism and inherited metabolic disease

Ersoy M[1]

[1]Child Metab Dis, Sadi Konuk Res&Tra Hosp, Istanbul, Turkey

Background: Autism spectrum disorders (ASD) are neurodevelopmental disabilities characterized by pervasive impairment in reciprocal socialization, qualitative impairment in communication, restricted interests and repetitive behaviours. 10% of the cases have a known genetic or inherited metabolic disorder (IMD), whereas majority of cases have primary autism with unknown etiology.
Objectives: To evaluate the incidence and the importance of IMD in ASD.
Methods and patients: 46 pediatric patients diagnosed with ASD were evaluated for IMD. We prospectively analyzed the results of a metabolic workup (plasma uric acid, creatinine, homocysteine, amino acids, carnitine/acylcarnitines together with urinary mucopolysaccharides and organic acids) and brain MRI routinely.
Results: Median age of the patients was 42 (25-87) months; 31 of them (67%) were male. Three of them (6%) had 3-methylglutaconic aciduria; two of them (4%) had Krebs cycle metabolites in urine. One (2%) had low plasma uric acid and one (2%) had high plasma proline levels. Cerebellar hypoplasia were found in 3 patients (6%). One had typical signs of Rett syndrome.
Conclusion: Since they have relatively high incidence and the chance to be able to be treated, we suggest to evaluate the metabolic workup of each patient diagnosed with ASD.

P-695

Qualitative urinary organic acid analysis: 10 years of quality control

Peters V[1], Bonham J[2], Hoffmann G F[1], Scott C[2], Langhans C D[1]

[1]Univ Child Hosp, Heidelberg, Germany, [2]Dep Clin Chem, Sheffield, United Kingdom

Background: Over the last 10 years, 90 urine samples from patients and controls were circulated to different laboratories worldwide. The participants were asked to analyze the samples and to direct the report to a non-specialist pediatrician.
Results: The performance for the detection of fumarase deficiency, glutaric aciduria type I, isovaleric aciduria, methylmalonic aciduria, mevalonic aciduria, phenylketonuria and propionic aciduria was excellent (98-100%). Detection improved for tyrosinaemia type I (39% in 2008; >80% in 2011/2014), maple syrup urine disease (85% in 2005; 98% in 2012), hawkinsinuria (62% in 2010; 88% in 2014) or aminoacylase I deficiency (43% in 2009; 73% in 2012). When the findings are unambiguous, the reports were mostly clear. However, when they were less clear, the content and quality of the reports varied greatly. It was notable that very few participants suggested subsequent referral to a specialist center and evidence suggests that this may result in poorer outcomes in patients with inherited metabolic disorders.
Conclusion: The technical reliability of qualitative organic acid analysis and interpretation has improved. However, the content of reports shows considerable diversity of practice and deserves careful scrutiny. EQA providers such as ERNDIM may be well placed to facilitate this discussion.

P-696

Is long-term ketogenic diet treatment hepatotoxic for children with intractable epilepsy?

Arslan N[1], Guzel O[2], Yılmaz U[2], Calik T[2], Akisin Z[2]

[1]Div Metab Dis, Dokuz Eylul Univ, Izmir, Turkey, [2]Div Pediatr Neurol, Behcet Uz Child Hosp, Izmir, Turkey

Aim: The aim of this study was to evaluate the hepatic side effects of KD.
Methods: 121 patients (mean age: 7.45±4.21 years), receiving KD for at least one year for intractable epilepsy due to different diagnosis (congenital brain defects, GLUT-1 deficiency, West syndrome, aminoacidopathies, tuberosclerosis, hypoxic brain injury, etc) were included in the study. Serum biochemistry and abdominal ultrasonography were performed before and every three months during diet.
Results: The mean duration of KD was 15.4±4.1 months. Body mass index, aminotransferase, bilirubin and albumin levels of all these patients were within normal ranges. Cholelithiasis was detected in one patient at 12 months of treatment. This seven year-old-girl was treated for West syndrome. Hepatosteatosis was detected in three patients at sixth months of treatment. Two of these patients were treated with KD for the primary diagnosis of tuberosclerosis and one for Landau Kleffner syndrome. Although, hypercholesterolemia appeared at third months of therapy in three of these patients (total cholesterol levels were 256, 270 and 272 mg/dL), mean cholesterol levels were not significantly different from other patients without liver side effects.
Conclusion: Long-term KD may stimulate hepatosteatosis and gallstone formation. This is the first study in the literature documents hepatic side effects of KD treatment in epileptic patients.

P-697

Genotype and phenotype analysis in Taiwanese patients with osteogenesis imperfecta

Lin H Y[1 2 3 4 7], Lin S P[1 2 3 4], Chuang C K[2], Su Y N[5], Chen M R[1 3 4], Chiu H C[1], Niu D M[6]

[1]Dep Pediatrics, Mackay Memorial Hospital, Taipei, Taiwan, [2]Dep Med Res, Mackay Memorial Hospital, Taipei, Taiwan, [3]Dep Medicine, Mackay Medical College, New Taipei City, Taiwan, [4]Mackay Jun Col of Med, Nurs Management, Taipei, Taiwan, [5]Dep Obs Gyn, Taipei Medical University, Taipei, Taiwan, [6]Dep Ped, Taipei Veterans General Hosp, Taipei, Taiwan, [7]Inst Clin Med, National Yang-Ming Univ, Taipei, Taiwan

Background: Osteogenesis imperfecta (OI) is a congenital disorder characterized by increased bone fragility and low bone mass.
Methods: Seventy-two patients with OI types I, III, or IV (27 males and 45 females; age range at last follow-up, 0.2-62 years) were checked for the presence of *COL1A1* or *COL1A2* mutations using polymerase chain reaction/denaturing high performance liquid chromatography (PCR/DHPLC).
Results: Thirty-seven *COL1A1* and *COL1A2* mutations were identified in these 72 patients, including 28 *COL1A1* mutations and 9 *COL1A2* mutations. Fifteen of them were novel mutations. Medical records revealed that 72 OI patients could be classified into types I (n = 42), III (n = 5), and IV (n = 25). Thirty patients had helical mutations (caused by the substitution of a glycine within the Gly-X-Y triplet domain of the triple helix), and 42 had haploinsufficiency mutations (caused by frameshift, nonsense, and splice-site mutations). Compared with haploinsufficiency, patients with helical mutations had more severely damaged skeletal phenotypes, including lower height and bone mineral density, poorer walking ability, more frequent manifestations of dentinogenesis imperfecta and scoliosis ($p < 0.05$).
Conclusion: The database of genotype and phenotype is expected to promote better genetic counseling and medical care of Taiwanese OI patients.

P-698

Effect of purified α1-antitrypsin (AAT) on expression of AAT in normal (PiMM) and AAT deficient (PiZZ) primary human hepatocytes

Karadagi A[1], Johansson H[1], Zemack H[1], Jorns C[1], Gramignoli R[3], Stokkeland K[2], Strom S[3], Ericzon B G[1], Nowak G[1], Ellis E[1]

[1]Div Transplant Surg, Karolinska Inst, Stockholm, Sweden, [2]Dep Med, Huddinge, Karolinska Inst, Stockholm, Sweden, [3]Dep Lab Med, Div Pat, Karolinska Inst, Stockholm, Sweden

Background: Increasing circulating α1-antitrypsin (AAT) is the main goal of augmentation therapy for treatment of severe AAT deficiency (AATD). We have used primary human hepatocytes to evaluate the effect of purified AAT on mRNA expression of Serpina1 gene (AAT) in primary human hepatocytes.
Methods: Primary human hepatocytes were isolated from liver tissue collected from liver resection and from AATD patients undergoing liver transplantation, PiMM (n=12) and PiZZ (n=2). Hepatocytes were cultured on EHS-matrigel and subsequently treated with purified AAT and/or Oncostatin M (OSM) days 1-5. Expression of Serpina1 mRNA was analyzed by real-time qPCR.
Results: OSM increased Serpina1 expression and increased AAT levels in culture media. Addition of purified AAT down regulated Serpina1 expression dose dependently in both PiMM and PiZZ primary human hepatocytes.
Conclusions: We show that OSM up regulate mRNA expression of Serpina1 and purified AAT down regulate Serpina1 in primary human hepatocytes, both PiMM and PiZZ. This suggests that augmentation therapy, besides targeting elastase activity in lungs, also down regulates Serpina1 expression and production of PiZZ in hepatocytes. By negative feedback regulation, augmentation therapy may also display protective effects on liver in patients with severe AATD and suggests that AATD patients may benefit from hepatocyte transplantation.

P-699

Dihydrolipoamide dehydrogenase deficiency diagnosed by using new generation sequencing technology

Inci A[1], Tumer L[1], Okur I[1], Olgac A[1], Sarı S[2], Ciftci B[1], Topcu A B[1], Ezgu F S[1]

[1]Div Pediatr Metab Dis, Gazi Univ Hosp, Ankara, Turkey, [2]Div Pediatr Gastro Dis, Gazi Univ Hosp, Ankara, Turkey

Background: Dihydrolipoamide dehydrogenase (DLD) deficiency is a rare autosomal recessive disorder The clinical presentations are varied and may include Reye-like syndrome, hepatic failure, encephalopathy and myopathy.
Methods: 6.5 month old male boy presented with recurrent episodes of hepatic failure associated with elevated serum ALT and AST (ALT 1105 U/L, AST 1224 U/L), markedly elevated prothrombin time (PT), activated partial thromboplastin time (APTT) and INR (PT/PTT/INR 25.3sn/35.2sn/ 2.15), hypoglycemia (serum glucose 25 mg/dl) with metabolic acidosis and hyperammonemia (300 mg/dL.) He had moderate hepatomegaly with 5 cm palpable. Tandem mass spectrometry showed decreased serum glycine levels and plasma amino acid chromatography showed increased valine, leucine and isoleucine levels. All laboratory tests were normal between episodes. Screening tests for metabolic disorders causing hepatic failure were within normal limits. To diagnose our patient we used next generation sequence technology (NGST) and found DLD deficiency.
Results: Here in we describe a DLD deficiency with recurrent episodes of liver failure, hypoglycemia and increased levels of branched-chain aminoacids.
Conclusion: He would be the first case of DLD deficiency with early presentation of hepatic form and diagnosed by NGST. This method has provided the investigation of 500 inborn errors of metabolism in a one chip.

P-700

A metabolic disease as an underlying cause of a child psychiatric disorder: literature review

Eyskens F[1], Simons A[2]

[1]Div Metab Dis, Univ Hosp Antwerp, Antwerp, Belgium, [2]UKJA, Antwerp, Belgium

Background: Many patients who visit a centre for hereditary metabolic diseases remarkably also suffer from a child psychiatric disorder. Those child psychiatric disorders may be the first sign or manifestation of an underlying metabolic disease. Lack of knowledge of metabolic disorders in child psychiatry may lead to diagnoses being missed. Patients therefore are also at risk for not accessing efficacious treatment and proper counselling.
Aims: To review the literature for the combination of child psychiatric disorders such as attention deficit hyperactivity disorder (ADHD), autism, psychosis and learning disorder and metabolic diseases.
Method: A systematic review of the literature was conducted by performing a broad search on PubMed, using the terms “ADHD and metabolic disorders“, "autism and metabolic

disorders", "psychosis and metabolic disorders", "learning disorders and metabolic disorders", and "eating disorders and metabolic disorders". Based on inclusion criteria (concerning a clear psychiatric disorder and concerning a metabolic disorder) 4441 titles and 248 abstracts were screened and resulted in 74 relevant articles.
Results: This systematic review provides child and adolescent psychiatrists with an overview of metabolic disorders associated with child psychiatric symptoms, their main characteristics and the recommended diagnostic work-up.

P-701

Juvenile hemochromatosis type 2b: a case report

Aydemir Y[1], Yuce A[1], Talim B[2], Pietrangelo A[4], Hizal G[1], Berberoglu-Ates B[1], Demir H[1], Saltik-Temizel I N[1], Bayhan T[3], Ozen H[1]

[1]Hacettepe Univ,Div Pediatr Gastroenterol, Ankara, Turkey, [2]Hacettepe Univ, Div Pediatr Pathol, Ankara, Turkey, [3]Hacettepe Univ, Div Pediatr Hematol, Ankara, Turkey, [4]Univ Hosp Modena,Div Int Med and Met Dis, Modena, Italy

Background: Juvenile Hemochromatosis (JH) type 2b is a rare, autosomal recessive disorder characterized by severe iron overload in first three decades.
Case report: 12-year-old boy presented with hypertransaminasemia and hyperechogenic solid lesion in the liver for 2 months. He had 2 cm hepatomegaly. Ultrasonography showed hepatomegaly with grade 1 steatohepatitis and hyperechogenic lesion consisted with hemangioma. Laboratory examination showed increased levels of alanine aminotransferase: 257 IU/L, aspartate aminotransferase: 91 U/L, ferritin: 1029ng/ml, transferin saturation: %99.6. Only mild hepatic iron overload was detected with magnetic resonance imaging. Liver biopsy showed hemosiderin pigment deposit particularly in periportal hepatocyte. HFE (hereditary hemochromatosis) mutations were negative. HAMP (hepcidin antimicrobial peptide) sequence analysis revealed a homozygous missense mutation in exon 3 (c.208T>C (p.C70R)). The patient was diagnosed with juvenile hemachromatosis type 2b and treated with phlebotomy weekly until ferritin level was lower than 100ng/ml. His ferritin level and liver transaminases were normal at the second and fifth months of treatment respectively.
Conclusion: JH must be considered in patients with hepatic, cardiac and endocrine manifestations especially in the presence of high ferritin and transferin saturation levels. If HFE mutations are negative, non-HFE mutations must be screened to diagnose JH particularly in patients under 30 years old.

P-702

Wasted time devasted children: Effect of war on inborn errors of metabolism

Aktuglu Zeybek A C[1], Kiykim E[1], Zubarioglu T[1], Cansever M S[2], Cam H[1], Aydin A[1]

[1]Div Nutr Metab Dis, Ist Univ Cer Med Fac, Istanbul, Turkey, [2]Cent Lab, Ist Univ Cer Med Fac, Istanbul, Turkey

Background: War, not only by trauma but also by prevention of getting appropriate health care, forcing to migration, increases the mortality and morbidity rates. Inborn errors of metabolism (IEM) consists a disease group that is usually ignored during the chaotic time.
Patients and results: Four patients from Syria and Iraq were admitted to Pediatric Metabolic Diseases unit upon suspicion of IEM. First patient was a $5^{9/12}$ years-old female diagnosed as argininemia at 2.5 years of age at Syria but insufficient medical and dietary treatment resulted in severe neurodevelopmental delay and spastic paraplegia upon admission. Second patient was diagnosed as PTPS deficiency at 1 months of age during Turkish nationwide screening programme. Lack of social security led to delay in medical treatment with the consequence of neurodevelopmental delay. Third patient was a 6 months-old female with hepatomegaly and hypoglycemia, referred from Syria and was diagnosed as GSD type 1. Fourth patient was from Iraq referred because of hepatomegaly and was diagnosed as Fanconi-Bickel syndrome at 1 year of age.
Conclusion: All patients were coming from countries suffering from war and were both late diagnosed and treated because of insufficient health care. The effect of war is definitely devasting on IEMs.

P-703

Molecular diagnosis of lactic acidosis by using a clinical exome-sequencing panel

Navarrete R[1], Oyarzabal A[1], Vega A[1], Bravo-Alonso I[1], Merinero B[1], Pérez-Cerdá C[1], Ugarte M[1], Perez B[1], Rodríguez-Pombo P[1]

[1]CEDEM, CBM-SO, UAM-CSIC, CIBERER, IDIPAZ, Madrid, Spain

Background: Lactic acidosis (LA) results from the accumulation of lactate and protons in the body fluids and is often associated with poor clinical outcome. Conditions such as heart failure, severe trauma or sepsis account for the majority

of cases of hyperlactacidemia, but this condition also includes a plethora of congenital metabolic disorders that disrupt normal pyruvate metabolism and/or mitochondrial function, making the diagnosis very laborious.
Methods: Massive exome sequencing allows a cost-effective analysis of a large number of genes. In this study, we present data relative to the massive-parallel sequencing of a cohort of 27 patients with a hallmark of LA, with or without Leigh syndrome, using the Illumina® clinical-exome TruSight™ One sequencing panel. Sanger sequencing confirmed clinically significant deemed variants in patients. Parental samples, when available, were also analyzed by means of Sanger.
Results: In eight out of the 27 patients we yield the molecular diagnosis, with mutations in *PDHA1* (2), *PDHX* (1), *ACAD9* (1), *NDFUS4* (1), *TK2* (1), *SLC19A3* (1), and *ATPAF2* (1) genes. The overall rate of a positive molecular diagnosis has been 29%.
Conclusion: Thus, we conclude that, for an accurate molecular diagnosis of LA, extensive clinical and biochemical diagnostic workups based in the biochemical profiles or functionality tests are needed.

P-704

Molecular genetic study of congenital adrenal hyperplasia in Serbia: novel p.Leu129Pro and p.Ser165Pro *CYP21A2* gene mutations

Stojiljkovic M[1], Milacic I[1], Barac M[2], Milenkovic T[3], Ugrin M[1], Klaassen K[1], Skakic A[1], Jesic M[4], Joksic I[5], Mitrovic K[3], Todorovic S[3], Vujovic S[2], Pavlovic S[1]

[1]IMGGE, University of Belgrade, Belgrade, Serbia, [2]Clinical Center of Serbia, Belgrade, Serbia, [3]Mother and Child Health Care Institute, Belgrade, Serbia, [4]University Children's Hospital, Belgrade, Serbia, [5]University Clinic for Gynecology, Belgrade, Serbia

Congenital adrenal hyperplasia (CAH) is an autosomal recessive disease characterized by impaired adrenal steroidogenesis and most often caused by *CYP21A2* gene mutations. Here, we reported complete spectrum and frequency of *CYP21A2* gene mutations in 61 unrelated patients with classical and nonclassical CAH from Serbia. Combining direct sequencing of whole *CYP21A2* gene and PCR-SSP for detection of *CYP21A1P*/*CYP21A2* chimeras, we identified 18 different pathogenic alleles - two of them novel. Mutation detection rate was highest in patients with SW-CAH (94.7%). The most prevalent mutation was intron 2 splice-site mutation, c.290-13A/C>G (18.5%). Other mutation frequencies were: *CYP21A1P*/*CYP21A2* chimeras (13%), p.P30L (13%), p.R356W (11.1%), p.G110fs (7.4%), p.Q318X (4.6%), p.V281L (4.6%), p.I172N (2.8%), p.L307fs (2.8%), p.P453S (1.9%), etc. Mainly, frequencies were similar to those in Slavic populations and bordering countries. However, we found 6.5% of different alleles with multiple mutations, frequently including p.P453S. Effects of novel mutations, c.386T>C (p.Leu129Pro) and c.493T>C (p.Ser165Pro), were characterized *in silico* as deleterious. The effect of well-known mutations on Serbian patients' phenotype was as expected.
Conclusion: The first comprehensive molecular-genetic study of CAH patients from Serbia revealed two novel *CYP21A2* mutations. This study is valuable for strategy delineation for pre-, peri- and postnatal diagnosis of CAH in our population.

P-705

Clinical utility of a new metabolic NGS panel in diagnostic service

Heptinstall L[1], Ghosh A[1], Schlecht H[1], Bhaskar S[1], Urquhart J[1], Broomfield A[1], Morris A[1], Jameson E[1], Schwahn B[1], Walter J[1], Ramsden S[1], Jones S[1], Banka S[1 2]

[1]Centre Genomic Med, St Mary's Hosp, Manchester, United Kingdom, [2]Inst of Hum Dev, Univ of Manchester, Manchester, United Kingdom

Methods: Using next generation sequencing (NGS) we have developed a 226 gene diagnostic panel for inborn errors of metabolism (IEMs) to complement traditional metabolic screening investigations. The genes selected prioritise disorders with predominant neurodevelopmental phenotypes that are either genetically heterogeneous or where traditional investigations are invasive, expensive or have poor availability. Using biochemical and clinical phenotype we have created overlapping 'sub-panels' to facilitate variant analysis and reporting. These subpanels include amino acid and neurotransmitter defects; organic acidaemias and vitamin-related disorders; disorders of fatty acid oxidation or ketone metabolism or with hyperammonaemia; carbohydrate metabolism defects; lysosomal disorders and neuronal ceroid lipofuscinoses; and peroxisomal disorders.
Results: Successful implementation of a stringent laboratory validation process has ensured that that this targeted NGS assay is robust, sensitive and specific. Additionally, the design includes all known intronic mutations in the genes on the panel. 79 patients have been analysed over the last 6 months with 43 reports issued, and we identified definite causative mutations in 55%.
Conclusion: These cases demonstrate that this approach helps to achieve a high rate of accurate diagnoses for IEMs, it can be cost-effective and facilitates a better understanding of molecular basis of rare IEMs.

P-706

Importance of early diagnosis and start of treatment in Mevalonate Kinase Deficiency (MKD) patients

Burnyte B[1 2], Peciuliene S[2], Gudaitiene R[2], Rusoniene S[3], Songailiene J[1], Liubsys A[2], Cimbalistiene L[1], Drazdiene N[2]

[1]Dep Hum Med Genet, Vilnius Univ, Vilnius, Lithuania, [2]Neonatology Centre, Vilnius Univ, Vilnius, Lithuania, [3]Pediatrics Centre, Vilnius Univ, Vilnius, Lithuania

Background: Mevalonate kinase deficiency (MKD) is an autosomal recessive autoinflammatory disorder caused by a mutation in the mevalonate kinase (MVK) gene. Two phenotypes of MKD are delineated according to the level of enzymatic deficiency; however, a wide spectrum of intermediate phenotypes has been reported.
Case report: The patient was born at 33 weeks by C-section due to hydrops fetalis with a weight of 2676 g. Apgar score 8/8. Immediately after birth hypotonia, hepatosplenomegaly, metabolic acidosis, severe anemia and intense acute phase reaction was observed. From the 2nd week of life failure to thrive, lethargy and short recurrent febrile episodes with bloody diarrhoea started. Metabolic screening revealed an increased urinary excretion of mevalonic acid lactone, supporting the MKD diagnosis. Genetic testing showed a homozygous nonsense mutation within the *MVK* gene. Treatment with the anti-IL-1 agent anakinra was started at 2 months of age continuously (1,5 mg/kg/day). After 3 days systemic inflammatory reaction markers returned to normal. After 1 month of treatment cessation of febrile episodes, weight gain and improvement of psychomotor development was noticed.
Conclusions: Early diagnosis of MKD prevents incorrect therapeutic procedures, reduces the psychological stress and is important to the quality of life.

P-707

Two years experience of selective screening for organic acidurias (OA) and amino acidopathies (AA) in Pakistani pediatric population

Sherazi N[1], Khan A H[1], Jamil A[1], Mehar R[1], Jehan F[1], Yousufzai N[1], Jafri L[1], Siddiqui I[1], Ghani F[1], Afroze B[2]

[1]Bio Genetic Lab, Aga Khan Univ Hosp, Karachi, Pakistan, [2]Dept Peds&child hlth, Aga Khan Univ Hosp, Karachi, Pakistan

Background: Diagnosis of IEM in Pakistan is challenging due to resource constraints and limited technical expertise.
Aim: To determine frequency of OA and AA in high-risk patients.
Methods: Urine, plasma and CSF for OA and AA analyzed at first ever diagnostic biochemical genetic laboratory in Pakistan. Two years (2013-14) data was reviewed by pathologist and metabolic physician.
Results: Eighty-eight cases (4.7%) were diagnosed including OA (n=49), AA (n= 29) and others (n=10) from 1866 specimens analyzed. Among OA, cobalamin defect and methylmalonyl-CoA mutase deficiency were diagnosed in 9 and 8 cases respectively and MMA was suspected in 3 cases. Five cases of MHBD, 4 each of PPA and HMG-CoA lyase deficiency, 3 cases each of IVA, biotinidase / holocarboxylase deficiency, fructose-1, 6-biphosphatase deficiency, fumarase deficiency and 2 cases each of, EMA and GA I were reported. AA included; MSUD (n=9), CBS (n=6), UCDs (n=6), hyperphenylalaninemia (n=5), hyperprolinemia (n=3). Others reported included 2 cases each of alkaptonuria, Canavan's disease, SUCL deficiency and one case each of DPD deficiency, GA II, NKH, AADC.
Conclusions: Study shows frequency of OA and AA in the high-risk Pakistani pediatric population and can be utilized for planning and providing services for patients with IEM, including newborn screening.

P-708

Exome sequencing results in unknown genetic, metabolic/neurometabolic disorders

Kilic M[1], Ozgul R K[3], Kilic E[2], Yilmaz D Y[3], Kavak P[4], Yuceturk B[4], Demirci H[4], Sagiroglu M[4]

[1]Div Metab Dis, Dr Sami Ulus Child Hosp, Ankara, Turkey, [2]Div Genet Dis, Hemato-Onco Child Hosp, Ankara, Turkey, [3]Div Metab Dis, Hacettepe Univ Child Hosp, Ankara, Turkey, [4]BİLGEM, TUBİTAK, Kocaeli, Turkey

Objective: Our aim was to identify diseases and related genes with exome sequencing in unknown patients.
Patients and Methods: A total of five families with two affected individuals, in each individual patient with similar clinical findings were taken to study.
Results: The first family of patients had heterezygous p.Asp975Asn;c.2923G>A mutation in *CACNA1H* gene and 'altered channel function, epilepsy' diagnosis was made as a autosomal dominant model. The second family of patients had homozygous p.Gly69Argfsx10 mutation in the *C19orf12* gene and 'Mitochondrial-membran protein associated neurodegeneration' was diagnosed. The third family of patient's had duplication on chromosome 14 nucleotide positions 20182017-20404268 were found and 14q11-q22 deletion syndrome was

diagnosed. The fourth family patients were found 17p13.1 deletion syndrome (CHR17: 9943008-10416015 duplication). The fifth family patients had mutations in the *MRPS24* (mitochondrial ribosomal protein) gene. This gene has not previously identified and further studies are needed to prove the disease.
Conclusion: New generation DNA sequencing methods are very important diagnostic tools in unknown patients.

P-709

Therapeutic management in pediatric intensive care unit of inherited metabolic diseases of intoxication by proteins: retrospective study of 53 cases

Abily-Donval L[1 2], Joffre C[3], Lesage F[3], Oualha M[3], De Saint Blanquat L[3], Renolleau S[3], Valayannopoulos V[2], De Lonlay P[2], Dupic L[3]

[1]Dpt of Neonatal Med, Rouen Univ Hosp, Rouen, France, [2]Div Metab Dis, Necker Univ Hosp, Paris, France, [3]Ped Intensiv Care Unit, Necker Univ Hosp, Paris, France

Background: Inherited metabolic diseases of intoxication by proteins-IMDP- (aminoacidopathies, organic acidurias, urea cycle defects) induce the accumulation of neurotoxic compounds. Acute phase management requires two complementary ways:
1- to block endogenous catabolism and proteolysis, and promote anabolism.
2- to remove toxic metabolites using metabolite scravengers (MS) or continuous venovenous extra-renal dialysis (CVVH).
Material & Methods: We studied a cohort of 53 newborns affected by IMDP and we compared the outcome of the patients treated with MS or with MS associated with CVVH.
Results: The average age and weight at admission were respectively 12.8 days and 3076g. The PRISM severity score was comparable in both groups. Among hyperammonemic patients, 41% were treated with MS only (97% of them received sodium benzoate, which was given before the transfer to intensive care in one third of them). For patients with maple syrup urine disease no MS is available, and 93% had CVVH.
Conclusion: The CVVH is an effective technique for the management of IMDP at diagnosis. MS are also effective but underused treatments. The early use of MS before transfer to a specialized unit may prevent the use of CVVH. The impact of dialysis on neurological prognosis should be evaluated.

P-710

Prenatal diagnosis on 143 cases with organic and fatty acid diseases by biochemical and gene analyses with amniocentesis

Yamaguchi S[1], Hasegawa Y[1], Bo R[1], Yamada K[1], Kobayashi H[1], Taketani T[1], Fukuda S[1]

[1]Dept of Ped, Shimane Univ School of Med, Izumo, Shimane, Japan

Background: Expanded newborn screening (NBS) using MS/MS is becoming widespread. Organic acidaemias (OAs), fatty acid oxidation disorders (FAOD) and amino acidemias are screened in the NBS. However, it is known that a small part of cases are fatal in early infancy despite any intensive treatments. For such cases, only prenatal diagnosis may be an optional measure. We report an experience of prenatal diagnosis for 143 cases with the severest type of OAs or FAODs.
Methods: Amniotic fluid collected at around 16 weeks of gestation was centrifuged. The supernatant was used for acylcarnitine (AC) and organic acid analyses using MS/MS and GC/MS, respectively. The cell pellet was subjected to gene analysis. Microsatellite marker was analyzed in the "affected cases" to exclude the contamination of mother derived DNA.
Results and Discussion: MS/MS and GC/MS identified 43 subjects affected with OAs among the 112 cases screened. Two cases among the 10 subjects were affected with FAOD by gene analysis. Some OAs or urea cycle disorders were identified in the 5 subjects out of 21 cases whose probands died of unknown reasons. C16-OH, C18-OH and C18:1-OH were significantly increased in amniotic fluid from an affected case of TFP deficiency.

P-711

***RARS*: a novel disease-causing gene for Pelizaeus–Merzbacher disease**

Nafisinia M[1 2], Sobreira N[3], Gold W A[2 7], Riley L G[2 7], Ouvrier R[4 5], Boehm C[3], Uhlenberg B[6], Christodoulou J[1 2 7]

[1]Genetic Metabolic Disorders Research Un, Sydney, Australia, [2]Discipline of Paediatrics & Child Healt, sydney, Australia, [3]Institute of Genetic Medicine, Johns Hop, Baltimore, United States, [4]TY Nelson Dept of Neurology and Neurosu, sydney, Australia, [5]Discipline of Paediatrics and Child Heal, Sydney, Australia, [6]Berliner Centrum für Seltene Erkrankung, Berlin, Germany, [7]Discipline of Genetic Medicine, Sydney, Sydney, Australia

Background: Pelizaeus-Merzbacher disease (PMD) is a rare Mendelian disorder characterized by central nervous system hypomyelination, caused by mutations in several genes.
Case Reports: We report two siblings with a clinical diagnosis of PMD but negative for mutations in*PLP* and*GJC2* genes.

The affected male had motor and expressive delay, bilateral horizontal nystagmus, an action tremor, head titubation, an ataxic gait and moderate cognitive impairment. His brain MRI scan was consistent with a dys- or demyelinating process in the central and non-myelination in the peripheral white matter. His sister was less severely affected.
Methods & Results: Whole exome sequencing uncovered a homozygous missense mutation in the arginyl-tRNA synthetase (*RARS*) gene(c.5A>G, p.Asp2Gly) in both affected siblings. RARS attaches arginine to its cognate tRNA and therefore plays an integral part in protein synthesis. RARS protein levels were reduced by 80% in patient fibroblasts compared to controls. We then tested whether they could function without arginine. Patient cells cultured in limited arginine at 30°C, showed a significant reduction ($p< 0.001$) in viability compared to control cells indicating their inefficiency to synthesise protein
Conclusion: A larger cohort screening program has led to the identification of a novel compound heterozygous mutation (c.1367C>T, p.Ser456Leu and c.1846-1847delTA) in the *RARS* gene. Our findings provide further evidence suggesting that *RARS* is a novel PMD causing gene.

P-712

Paeonol can stimulate SIRT1 via activation of the NAD+ salvage pathway

Potthast A B[1], Das A M[1]

[1]Ped Metab Dis, HMS, Hannover, Germany

Background: Paeonol is a phenolic compound from peonys, which can activate the NAD^+-dependent deacetylase SIRT1 in vivo and in vitro. Activation of SIRT1 can be beneficial in different disorders. The NAD^+ salvage pathway is localized in the nucleus involving the enzymes Nampt and Nmnat to regenerate NAD^+ from NAM. In own experiments, we measured higher NAD^+ levels after treatment with paeonol. Considering the co-localisation of SIRT1 and Nampt as well as the increased NAD^+ levels, we examined the effect of paeonol on the NAD^+ salvage pathway.
Methods: Fibroblasts from healthy donors were incubated for 7 days with paeonol and analysed at Nampt transcript-, protein-, and enzyme-levels, intracellular NAD+ was measured spectrophotometrically. Afterwards, the same parameters were measured after siRNA induced knock-down of Nampt.
Results: Paeonol treatment leads to an activation of Nampt on all measured levels. Transcript levels were increased up to 140%, protein levels up to 115% and Nampt activity was increased by 75%.
Conclusion: The activation of SIRT1 by paeonol is at least partly ascribed to the activation of Nampt respectively the NAD^+ salvage pathway. Further studies should investigate an influence of paeonol on the other nuclear sirtuins (SIRT6, SIRT7).

P-713

Neurometabolic disorders associated with early childhood epilepsy: A single center experience in Saudi Arabia

Mohamed S[1 2], El Melegy E[2], Talaat I[2], Hosny H[2], Abu-Amero K K[3]

[1]King Saud University Medical City, Riyadh, Saudi Arabia, [2]Saad Specialist Hospital, Alkhobar, Saudi Arabia, [3]Opth Lab, King Saud University, Riyadh, Saudi Arabia

Background: We aimed to identify the metabolic causes of epilepsy presenting in the first 2 years of life and to describe their characteristics.
Methods: This retrospective study was conducted at Saad Specialist Hospital, Alkhobar, Saudi Arabia. All patients aged under 2 years at onset of epilepsy caused by metabolic disorders were reviewed. The International League against Epilepsy (ILAE) definition was used, and febrile convulsion was excluded.
Results: Out of 221 children diagnosed with epilepsy in the first 2 years of life at our hospital, 24 had a metabolic disease. The characteristics of these 24 children included the following: consanguinity in 18 patients (75%), developmental delay in 13 (54%), generalized tonic-clonic seizures in 10 (42%), infantile spasms in 4 (17%), myoclonic seizyres in 7 (29%), and focal seizures in 3. The main diagnoses included the following: peroxisomal disorders (3), nonketotic hyperglycinemia (3), Menkes disease (2), neuronal ceroid lipofuscinosis (2), biotinidase deficiency (2), and mitochondrial disorder (2). The remaining patients had lysosomal storage disease, aminoacidopathy, fatty acid oxidation defects, and organic aciduria. Seizure freedom was achieved in one-third of patients in this cohort.
Conclusion: A wide range of metabolic disorders caused different types of epilepsy. Myoclonic seizures and infantile spasms were especially identified in this cohort.

P-714

Do the depression and anxiety levels of parents differ in different Inherited Metabolic Disorders (IMD)?

Bülbül S[1], Arslan,Nur N A[2], Gündüz,Mehmet M G[3], Ünal, Özlem Ö Ü[3]

[1]Kirikkale Univ, Div Metab Dis,, Kırıkkale, Turkey, [2]Dokuz Eylül Univ, Div Metab Dis, İzmir, Turkey, [3]Ankara Child Ed & Re.Hosp, Div Metab Dis, Ankara, Turkey

Objectives: The chronic pediatric illness constitutes a substantial risk factor for psychological disorders of the parents. The objective of this study was to investigate the level of parental depression and anxiety.
Methods: Demographic and disease variability in depression, anxiety and risk perceptions in 129 parents of children with phenylketonuria (37%), amino acid disorders (18,8%), carbohydrate metabolism disorders (12,7%), lysosomal storage diseases (9,7%) and 36 healthy controls were determined. Beck Depression Inventory (BDI) and State-Trait Anxiety Inventory I-II (STAI- I and II) were used to assess the depression/anxiety levels.
Results: No significant difference was found between inherited metabolic disease groups regarding parental depression, anxiety and risk perception scores. The differences were significant between the patient and the control groups ($p< 0.01$). Existence of depression and anxiety did not vary with the childs' and parents age. Significant correlations were found between the BDI and STAI- I and II levels of parents ($r=0.572$ and $r=0.674$, $p>0.001$). In addition, risk perception and BDI/STAI-I and II levels were significantly correlated ($r=0.259$ $p=0.005$, $r=0.289$ $p=0.001$, $r=0.253$ $p= 0.006$).
Conclusion: Determination of depression and anxiety in parents caring for children with IMD may help health professionals to identify those who need special attention and family support.

P-715

Liver transplantation in paediatric patients with metabolic disease: 20 years' experience in Melbourne, Australia

Yaplito-Lee J[1], Jones R[3], Peters H[1], Herd L[2], Boneh A[1], Hardikar W[2]

[1]Depart of Metab Med, Royal Children Hosp, Melbourne, VIC 3052, Australia, [2]Gastroenterology, Royal Children Hosp, Melbourne, VIC 3052, Australia, [3]VIC Liver Transplant Unit, Austin Hosp, Heidelberg, VIC 3084, Australia

Background: Liver transplantation is a robust treatment option for several metabolic disorders. We report our experience in paediatric metabolic patients.
Methods: All medical records of patients managed by the metabolic department who underwent liver transplantation (1995–2015), were reviewed. Clinical and transplant data were collected.
Results: Twelve patients were transplanted: UCD (5), familial hypercholesterolaemia (4), GSD IB (1), MSUD (1) and tyrosinaemia I (1), constituting 22% of UCD and 80% of hypercholesterolaemia patients transplanted in Australia and New Zealand (Transplant Registry 2013). The age at transplant was 3 years to 14.5 years. The main indication was failure of medical treatment. The median period between active listing and transplantation was 211 days. Split liver transplantation was performed in 7/12 patients. Cold ischaemic time was 5.3 hours to 11.4 hours. Median hospital and intensive care stay was 19.5 and 5 days respectively. Follow-up period was 2 months to 13.4 years. Complications included: hepatic thrombosis (4), CMV infections (2), biliary leak (2), rejections (2), acute renal failure, ischaemic liver resection, intra-abdominal bleed and passenger lymphocyte syndrome (1 each). No patient required a second transplantation. All patients are on a normal diet; growth and development are satisfactory.
Conclusion: Liver transplantation was performed successfully in our patients and should be considered early when maximal medical management fails.

P-716

Parent coping and the behavioural outcomes of children diagnosed with inherited metabolic disorders

Brown A[1], Crowe L[1 2], Anderson V[1 2], Boneh A[3]

[1]Child Neuropsy, Murd Child Res Inst, Melbourne, Australia, [2]Dep of Psych, Roy Child Hosp, Melbourne, Australia, [3]Metab Res, Murd Child Res Inst, Melbourne, Australia

Background and objectives: Inherited metabolic disorders (IMD) differ in their pathogenesis and clinical presentation, yet families often share similar stressful experiences concerning diagnosis, ongoing treatment and outcome. Children with IMD are susceptible to poor neurodevelopment and may be more vulnerable to their environment, and family life may therefore impact their behavioural outcomes. In this study we aimed to explore the relationship between the level of parental coping and management, and their children's behavioural functioning.
Methods: Parents of children with confirmed IMD ($n = 22$) completed the Kessler 10 Psychological Distress Scale, the Family Management Measure and the Parent Experience of Childhood Illness questionnaires. Parents rated their child's behavioural and emotional functioning on the Strengths and Difficulties questionnaire. Scores were compared with normative data.
Results: 16/22 parents were coping well; 2/22 and 4/22 reported moderate or high levels of psychological distress, respectively. Exploratory analysis found that parent coping variables

were related to children's internalising (emotional) symptoms, whereas family management was related to children's externalising behaviours.
Conclusions: Parental coping impacts on the child's internalising symptoms whereas family management may impact on their externalising behaviours. Early identification of issues in these domains may enhance referral for therapeutic interventions and family support programs.

P-717

A longitudinal, prospective, long-term registry of patients with hypophosphatasia

Kishnani P[1], Langman C[2], Linglart A[3], Mornet E[4], Ozono K[5], Rockman-Greenberg C[6], Seefried L[7], Bedrosian C[8], Fujita K P[8], Cole A[8], Högler W[9]

[1]Duke University Medical Center, Durham, NC, United States, [2]Northwestern Univ and Lurie Child Hosp, Chicago, IL, United States, [3]Paris-Sud Univ, APHP and INSERM U1169, Paris, France, [4]Centre Hospitalier de Versailles, Le Chesnay, France, [5]Osaka Univ, Graduate School of Medicine, Osaka, Japan, [6]University of Manitoba, Winnipeg, MB, Canada, [7]University of Würzburg, Würzburg, Germany, [8]Alexion Pharmaceuticals, Cheshire, CT, United States, [9]Birmingham Children's Hospital, Birmingham, United Kingdom

Background & Objectives: Hypophosphatasia (HPP) is a rare, inherited metabolic disease characterized by bone mineralization defects and systemic manifestations including seizures, respiratory insufficiency, muscle weakness, nephrocalcinosis, and pain. The biochemical hallmark of HPP is low serum alkaline phosphatase activity, resulting from loss-of-function mutations in the gene encoding tissue non-specific alkaline phosphatase. The rarity of HPP, combined with its variable expressivity, presents considerable challenges for diagnosis and understanding of the disease. Here, we describe the design of a Registry that will enable better understanding of the epidemiology and clinical course of HPP.
Patients & Methods: This multinational, observational, prospective, long-term registry will enroll ≥ 500 patients (excluding participants in Alexion-sponsored clinical trials). Sites will conduct the study in accordance with local regulations. Available patient data will be collected retrospectively via chart review at baseline and thereafter at intervals ≤6 months in the course of routine clinical care. Data collected will include patient demographics; method of diagnosis; HPP disease history; family history; clinical manifestations; HPP-specific medical and laboratory assessments; and genotype. Standardized questionnaires will be used to quantify patient-reported burden of disease, functional status/disability, and quality of life.
Conclusion: This HPP registry will provide a detailed, longitudinal, multidimensional profile of patients with HPP.
Conflict of Interest declared.

P-718

Does Aicardi-Goutière syndrome present with high neopterin and biopterin?

Shinde D H[1], Jalan A B[1], Joshi M M[1], Borugale M A[1], Mahakal J M[1], Sonalkar N D[1], Jalan R A[1], Kudalkar K V[1], Damle S V[2], Merchant R H[3]

[1]NIRMAN, Div of Biochemical Genetics, Navi Mumbai, India, [2]Dept of Life Sci, K C College, Mumbai, India, [3]Dept of Paediatrics, Nanavati Hospital, Mumbai, India

Background: Aicardi-Goutiére syndrome (AGS) is a heterogeneous, autosomal recessive, progressive encephalopathy, presenting in early infancy. Recent studies showed increased production of Neopterin and Biopterin in CSF of AGS patients and its variants as a response to inflammation.
Objective: To study Pterins – Neopterin, Biopterin and Folate levels in CSF of AGS patients diagnosed clinically and radiologically.
Materials and method: We studied 6 patients, all males. All of them had microcephaly, seizures and calcifications of basal ganglia. Diagnosis was supported by elevated IFN-α in CSF. CSF IFN- α was more than that of serum. CSF samples of these patients were also analysed for Pterins and 5-MTHF using UHPLC (Agilent) with florescent detection.
Result: All patients had elevated Neopterin levels, Mean 5002 [130.82 – 17,723.63 (NR9-20)] and normal Biopterin levels Mean 11.87 [0.36-29.63 (NR 10-30)]. 4/6 had low folates [50.62±22.39 (NR= 63-129nmol/l)].
Conclusion: CSF neopterin and biopterin have been reported to be elevated in AGS due to various reasons, but in our cohort of 6 patients, Neopterin was found high in all the patients and normal Biopterin. Also 4/6 patients had low folate levels. A possibility of Folinic acid substitution exists in AGS patients with low Folates.

P-719

Dunnigan syndrome - an unusual cause of severe and premature cardiovascular events

Sequeira S[1], Moldovan O[2], Alves A C[3], Medeiros A M[3], Ferreira A C[1], Bourbon M[3]

[1]Metab Dis Unit, D. Estefânia Hosp, CHLC, Lisbon, Portugal, [2]Genet Unit, Sta Maria Hosp, CHLN, Lisbon, Portugal, [3]I&D Unit, Dr. Ricardo Jorge INS, Lisbon, Portugal

Introduction: Dunnigan syndrome is the most prevalent form of familial partial lipodystrophy (FPLD2) with an estimated prevalence of 1:15.000. The children affected with FPLD2 may present hyperlipidemia and selective loss of fat in the lower limbs and trunk and accumulation of fat in the face, neck and supraclavicular fossa. The affected adults may have metabolic syndrome and therefore an increased risk of cardiovascular events.
Case Report: We report a family in which death of a 52 year-old male due to an episode of myocardial infarction led to the investigation of the extended family. The lipid levels and abnormal phenotype suggested the diagnosis of Dunningan syndrome. The sequencing of lamin A/C (LMNA) gene was performed by PCR and direct sequencing of all exons. The index patient revealed a heterozygous missense mutation, p.R482W. All family members were subsequently screened for this mutation, confirming the heterozygous status in four additional relatives, including two children. One of these children, a 17-year- -old girl, despite having normal glucose and lipid levels already presents signs of a partial LD.
Comments: The high prevalence of premature and severe cardiovascular complications make the early diagnosis of this condition essential for the treatment strategy and anticipation of disease progression.

P-720

Next generation sequencing role in diagnosis of mitochondrial disease in Serbian patient

Djordjevic M[1], Stojiljkovic M[2], Sarajlija A[1], Kecman B[1], Skakic A[2], Pavlovic S[2]

[1]Mother and Child Health Institute, Belgrade, Serbia, [2]IMGGE, Univ Belgrade, Belgrade, Serbia

Introduction: Clinical manifestations of oxidative phosphorylation disorders are heterogeneous and complex. It is difficult to suggest a type of mitochondrial disease according solely to clinical and biochemical findings.
Case report: A female child was admitted at the age of 11 months due to prolonged fever of unknown origin. Since two months of age, hypoactivity and hypotonia were present. At admission, we observed frontal bossing and hypertelorism and lactic acidosis with increased lactate/pyruvate ratio. Brain MRI showed cytotoxic edema pronounced in basal ganglia, with lactate peak in spectroscopy. Echocardiography revealed mild hypertrophic cardiomyopathy. Later on, acidosis and neurological deterioration progressed. Despite mechanical ventilation and supportive care, the child died at 12 month of age. Next generation sequencing (NGS) panel with 4813 genes was used postmortem to detect mutations. Two disease-causing mutations were found in *SCO2* gene.
Conclusion: Due to heterogeneity of mitochondrial diseases NGS could play a key role for the diagnosis of patients with this type of inborn errors of metabolism. This is the first patient that has been genetically diagnosed with mitochondrial disease by NGS in Serbia. Considering the high mortality of these diseases, NGS gives promises of providing exact genetic cause necessary for future prenatal diagnosis.

P-721

Inborn errors of metabolism in the molecular era: will biochemical analyses become a second-tier test?

Piazzon F B[1 2], Monteiro F P[1], Costa L S A[1 2], Bueno C[2], Kitajima J P[1], Borges R L[1], Dellamano M[1], Freitas E L[1], Santos B A C[1], Corrêa I[1], Kok F[1 2]

[1]Mendelics Genomic Analysis, Sao Paulo, Brazil, [2]Neurometabolic Clinic, FMUSP, Sao Paulo, Brazil

Background: Molecular analysis using next-generation sequencing (NGS) technology has already had a huge impact on clinical practice and is a fast and reliable way to provide diagnosis for rare, atypical or genetically heterogeneous conditions.
Methods and Results: In a series, we performed 698 clinical whole exome sequencing (WES) and identified 61 different IEM in 73 individuals, 31 of them without a previous suspicion of IEM. Of the 42 patients in which an IEM was suspected, only 7 had a confirmed specific clinical suspicion. Diagnosed conditions belonged to the following group of diseases: LSD (10), peroxisomal (1), mitochondrial (12), amino acids (6), CDG (5), GSD (2), neurotransmitter (2), nucleotides (3), metal (4), organic & FAO (4), and other (13). Among the IEM diagnosed, many had only a couple of reports, as the X-linked SSR4-CDG, the purine synthesis ATIC deficiency, the inositol glycan anchor synthesis defects *PGAP1*, *PGAP2*, and *PIGO*, the ceramide synthesis deficiency *CERS1* and the isoleucine metabolism *ECHS1* deficiency. A biochemical confirmatory test was performed when appropriate.
Conclusion: In countries with limited access to biochemical tests, WES might overcome most of diagnostic hurdles related to turnaround time, quality, cost, availability and dependence on overseas service provider. With a single test, we may answer several different questions.
Conflict of Interest declared.

P-722

Protective role of *CNDP1* genotype on renal survival in children with glomerulopathies

Peters V[1], Kebbewar M[2], Janssen B[2], Hoffmann G F[1], Möller K[3], Wygoda S[4], Charbit M[5], Fernandes-Teixeira A[6], Urasinski T[7], Jeck N[8], Zschocke J[9], Schmitt C P[1], Schaefer F[1], Wühl E[1]

[1]Univ Child Hosp, Heidelberg, Germany, [2]Univ Hosp, Heidelberg, Germany, [3]Univ Children´s Hosp, Hamburg, Germany, [4]Urban Hosp, Leipzig, Germany, [5]Hospital Necker, Paris, France, [6]Hospital S Joao, Porto, Portugal, [7]Pomeranian Acad Med, Szczecin, Poland, [8]Dep Ped, Univ Hosp, Marburg, Germany, [9]Div Human Gen, Med Univ, Innsbruck, Austria

Background: The *CNDP1* Mannheim allele (homozygosity for the five-leucine allele) reduces the susceptibility for diabetic nephropathy in diabetic patients. To evaluate the impact of the Carnosinase (*CNDP1*) polymorphism on the course of pediatric kidney disorders, we prospectively followed the long-term clinical outcome of 272 children with non-diabetic glomerulopathies, renal hypodysplasia or tubulointerstitial disorders.
Results: Among children with glomerulopathies patients homozygous for the five-leucine allele were less susceptible for end-stage renal disease (p=0.05). Within 5 years 39% of the glomerulopathy patients carrying the *CNDP1* Mannheim allele lost 50% glomerular filtration rate or progressed to end-stage renal disease, as compared to 77% of the patients with other *CNDP1* genotypes (p=0.06). In contrast, renal failure progression was independent of *CNDP1* genotypes in children with renal hypo/dysplasia or tubulointerstitial kidney disease.
Conclusion: The *CNDP1* Mannheim allele may have a nephroprotective effect in children with glomerular disorders but not in other pediatric nephropathies. The protective role of the *CNDP1* Mannheim allele appears not to be restricted to patients with diabetic nephropathy.

P-723

Can the same platform of TMS for newborn screening of amino acid and acylcarnitine be used for investigation of symptomatic patients? A 4-year-experience

Piazzon F B[2], Garcia L R G[2], Arita D Y[3], Iskandar M A M[2], Martins H H[2], Silva E N[2], Bueno C[1], Kok F[1], Hadachi S M[2]

[1]Neurometabolic Clinic, FMUSP, Sao Paulo, Brazil, [2]Newborn screening Lab APAE de SAO PAULO, Sao Paulo, Brazil, [3]Department of Medicine, UNIFESP, Sao Paulo, Brazil

Background: Amino acid (AA) and acylcarnitine (AC) profile by tandem mass spectrometry (TMS) is a well-established technology for newborn screening (NBS), but its usefulness in diagnosing symptomatic patients beyond neonatal period is not well determined.
Methods: APAE-SP, a non-profit institution pioneer in NBS in Brazil, offers AA and AC profile by TMS for patients with suspicion of IEM around the country.
Results: In the last 4 years, we have screened 2,178 symptomatic individuals and we were able to diagnose 18 cases of IEM (0.8%), further confirmed by other methods, including molecular analysis. Confirmed IEM is at least 16 times more frequent in this population than in NBS. AA disorders were detected in 7 patients (PKU in 1, UCD in 4, MSUD in 1 and NKH in 1), organic acidemias in 7 (MMA in 3, GA1 in 3, and HMG-CoA lyase deficiency in 1) and fatty acid oxidation disorders in 4 individuals (CUD in 2 and MADD in 2).
Conclusions: Even knowing that AA and AC profile by TMS using paper filter is not ideal for investigation of IEM in symptomatic patients, in countries like Brazil, with severe access constrains to more specialized biochemical tests, it can be a fast and reliable alternative.
Conflict of Interest declared.

P-724

A case of metaphyseal chondromatosis with high levels of D-2-hydroxyglutaric acid

O'Byrne J J[2], Fitzsimons P E[1], Unger S[6], Croft J M[5], Mayne P D[1], Moylett E[3], Mc Donnell C[4], Lynch S A[2]

[1]Paed. Lab. Med. Temple St Child Uni Hosp, Dublin, Ireland, [2]Dept. of Genetics OLCH, Crumlin, Dublin, Ireland, [3]Dept. of Paed. University College Hosp, Galway, Ireland, [4]Dept. of Paed. Temple St Child Uni Hosp, Dublin, Ireland, [5]Dept. Clin. Chem. Sheffield Child Hosp, Sheffield, United Kingdom, [6]Dept. Med. Genet. Centre Hosp Uni, Vaudois, Lausanne, Switzerland

Case Report: A developmentally appropriate thirteen month old girl, born to non-consanguineous Irish parents, presented with an asymmetrical crease in the midshaft of her right arm and relative short stature.
Radiology of both arms followed by a skeletal survey revealed bilateral symmetrical irregularities of the metaphyses of the humeri (proximal), femora (proximal and distal), and tibiae (proximal and distal) with less marked changes in the fibulae,

feet and phalanges. Irregular chondral dysplastic changes in the left iliac blade were also noted. The features fitted within the Ollier's spectrum. The epiphyses were spared; the skull, vertebrae, ribs and clavicles were unremarkable and bone age was normal. Metaphyseal chondromatosis (MC) was clinically suspected.
Urinary organic acids demonstrated a marked increase in excretion of
2-Hydroxy glutarate (2-HGA) confirmed as D-enantiomer with atypical slight increases in α-ketoglutarate and fumarate. Overall pattern supported the diagnosis of MC-D-2-HGA. The prognosis is variable and not possible to predict due to the somatic mosaic nature of the disorder.
Conclusion: This case highlights the unusual combination of characteristic skeletal and metabolic abnormalities and the importance of performing urine organic acid analysis in patients presenting with generalized enchondromatosis.

P-725

Low plasma aromatic L-amino acid decarboxylase (AADC) activity in children and adolescents with high functioning autism

Footitt E J[1 2], Mills P B[1], Skuse D[1], Hargreaves I P[3], Allen G[3], Heales S J R[1 2 3], Clayton P T C[1 2 3]

[1]UCL Institute of Child Health, London, United Kingdom, [2]Great Ormond Street Hospital, London, United Kingdom, [3]National Hospital Neurology & Neurosurg, London, United Kingdom

Background: This study investigated whether hyperserotonaemia is a feature of children and adolescents with high-functioning autism as has previously been demonstrated in studies of children with more severe forms of autism.
Patients & Methods: In 22 individuals with high-functioning autism and 22 age-matched controls we determined whole blood serotonin and other variables which might determine serotonin synthesis from tryptophan: plasma amino acids, B6 vitamers and plasma activity of aromatic L-amino acid decarboxylase (AADC) measured using L-dopa as substrate (DOPA decarboxylase [DDC] activity).
Results: The most significant finding was that the individuals with high-functioning autism had lower levels of plasma DDC activity than the controls (p=0.003). Sequencing of the *AADC* gene revealed no mutations in 5 patients studied. Recent work has identified L-type calcium channel subunits as a locus for psychiatric disease including autism. Results were considered in light of the observation that AADC in body fluids may be present in exosomes; microvesicles released from cells by a calcium dependent mechanism.
Conclusion: We hypothesise that various apparently unrelated observations in autism including hyperserotonaemia, its association with integrin-β3 variants and with calcium channels can be unified in a scheme whereby peripheral findings in autism and abnormal central synaptic plasticity may be understood by impaired calcium-induced secretion of exosomes.

P-726

Designing tools for multiorgan-directed neonatal gene therapy in neuro-hepatotropic inherited metabolic diseases: interest of an ubiquitous promoter "elongation factor 1α"

Baruteau J[1 2], Hanley J[3 4], Buckley S M[1], Gissen P[3 4 5], Howe S J[4], Karda R[1], Mills P B[5], Ng J[1], Perocheau D[1], Rahim A A[6], Suff N[1], Waddington S N[1]

[1]Gene Transfer Technology Group, UCL, London, United Kingdom, [2]Metab Med Dep, Great Ormond Street Hosp, London, United Kingdom, [3]MRC Lab for Mol Cell Biol, UCL, London, United Kingdom, [4]Mol Immun Unit, Inst Child Health, UCL, London, United Kingdom, [5]Clin Mol Genet, Inst Child Health, UCL, London, United Kingdom, [6]School Pharmacy, UCL, London, United Kingdom

Background: Expanding progress in viral vectors is achieving to make gene therapy (GT) a realm in monogenic diseases. Animal studies and trials have demonstrated efficacy of adeno-associated-virus (AAV) in correcting diseases affected by a single impaired organ (eye, muscle, liver). Transduction of the correct cell type relies on the AAV serotype and promoter used. In early-onset debilitating inherited metabolic diseases (IMD), neonatal systemic GT prevents early-onset decompensation, long-term sequellae, immune sensitisation, and targets the brain crossing the blood-brain barrier. We aimed to target two metabolic key-organs simultaneously, the brain and the liver, using a safe promoter suitable for human translation.
Methods: We assessed Green-Fluorescent-Protein (GFP) expression with the Elongation-Factor-1α promoter (EF1α). An AAV2/8-EF1α -GFP-WPRE construct was injected intravenously in P0 CD1 mice, and compared with AAV2/9-CMV-GFP-WPRE. GFP expression was assessed by fluorescence microscopy, GFP ELISA, immunohistochemistry, qPCR.
Results: At 5 weeks, EF1 α promoter resulted in persistent hepatic expression with a sinusoidal distribution and an exclusive diffuse neuronal expression. Additionally, muscle, heart and skin were transduced.
Conclusion: When injected systematically in the perinatal period, an AAV2/8-EF1α construct is suitable for targeting

neurons and hepatocytes. This finding is of interest for various IMD including lysosomal disorders and mitochondrial diseases with nuclear genetic defects.

P-727

Validation of an NGS-panel for routine diagnosis of lysosomal and peroxisomal disorders

Blomqvist M[1], Lindgren J[1], Olsson L[2], Månsson J E[1], Asin Cayuela J[1]

[1]Dept Clin Chem, Sahlgrenska Univ Hosp, Gothenburg, Sweden, [2]Bioinf Core Fac, Sahlgrenska Academy, Gothenburg, Sweden

Background: Today´s routine diagnosis of lysosomal and persoxisomal disorders is highly demanding regarding resources, leading to long response times. Using a next-generation sequencing (NGS) panel as a screening tool would improve time- and cost efficiency of the diagnostic process.
Methods: 20 patients with lysosomal or peroxisomal disorders and one commercial reference control were included in the validation. The custom made panel targets exons and flanking regions of 90 genes. Library construction was done using SureSelect^{QXT} (Agilent). Prepared libraries were sequenced on the MiSeq instrument (Illumina®). Secondary analysis was performed on-instrument with MiSeq Reporter and variants were filtered with Illumina Variant Studio.
Results: High quality data was obtained with excellent coverage for all targeted regions (20x target coverage of 100% and an average coverage of ca 500x). Expected pathogenic mutations (SNVs and small insertions/deletions) were found in 20 of 20 patients. In the commercial reference containing 281 variants, the panel showed a sensitivity of 100% and a specificity of > 97.5%.
Conclusion: Our NGS-panel will be a useful tool for the detection of pathogenic mutations in lysosomal and peroxisomal disorders, thereby by-passing the diagnostic odysseys that these patients often go through before they get their diagnosis.

P-728

Awareness amongst medical doctors of inherited metabolic disorders

Bülbül S[1], Arslan,Nur N A[2], Dursun, Osman O D[1], Gündüz, Mehmet M G[3], Dursun, Esra E D[1]

[1]Kirikkale Univ, Div Metab Dis,, Kırıkkale, Turkey, [2]Dokuz Eylül Univ, Div Metab Dis, İzmir, Turkey, [3]Ankara Child Ed & Re.Hosp, Div Metab Dis, Ankara, Turkey

Objectives: Considering the fact that inherited metabolic disorders (IMDs) are not easy to diagnose, this study was planned to assess physicians' knowledge of IMDs.
Methods: A questionnaire including 23 questions probing general knowledge on IMDs and a case of phenylketonuria (PKU) was prepared and was sent via e-mail to the physicians other than specialists and assistants in pediatrics. Among 190 physicians, 126 (66%) [(34%) male,(66%) female] answered the given questions.
Results: 17 of the participants were academics, 24 were specialists in other areas of medicine, 50 were assistants and 35 were family physicians. 113 (89%) participants stated that their knowledge was good. However approximately 20% and 35% claimed that birth weight (low/high) and cigarette/alcohol consuming could cause IMDs. When they were asked to list at least three IMDs, 82 (72%) could list three but 7 (6%) couldn't list even one. In addition, 10% could not list which disorders were screened in Turkey. The case of PKU was correctly diagnosed by only 77% of the participants, although it is screened in Turkey since 1983.
Conclusion: Awareness of IMDs is low and it is obvious that physicians should get more familiar with the clinical aspects of IMDs to decrease late diagnosis.

P-729

Vacuolar storage material in a family with juvenile parkinsonism and mutations in *FBXO7*

Serdaroglu E[2], Ozgul R K[1], Yalnizoglu D[2], Madeo M[3], Malandrini A[4], Klee E[5], Li Y[5], Jepperson T N[3], Oguz K K[6], Yucel-Yilmaz D[1], Patterson M[7], Kruer M C[3], Dursun A[1]

[1]Div Metab Dis, Hacettepe Univ Child Hosp, Ankara, Turkey, [2]Div Pediatric Neurol, Hacettepe Univ, Ankara, Turkey, [3]Children's Health Research Center, Sioux Falls, United States, [4]Dep Med, Surg and Neuro, Univ of Siena, Siena, Italy, [5]Medical Informatics, Mayo Clinic,, Rochester, United States, [6]Dep of Radiology, Hacettepe Univ Hosp, Ankara, Turkey, [7]Mayo Clinic, Rochester, United States

Background and objectives: Mutations in several genes are known to cause autosomal recessive early-onset forms of parkinsonism. We report a consanguineous Turkish family in whom multiple members presented with dementia, psychosis, and parkinsonism.
Case report: Four siblings were analysed. Patient 1 had a decline in expressive speech after 14 years. He had hypomimia, supranuclear gaze palsy, tremor, bradykinesia, and cogwheel rigidity. He developed parasomnias, sialorrhea, dysphagia, impaired volitional head control, and spasticity. Patient 2 had been mildly delayed and showed intellectual decline after the 3rd

grade. He exhibited downgaze palsy and mild rigidity. He developed gait impairment, dysphagia, bradykinesia, hypomimia, and cogwheel rigidity. Patient 3 had moderate intellectual disability and normal neurological examination. Patient 4 was unavailable for examination.
Methods and Results: Analysis of patient fibroblasts revealed autofluorescence and accumulation of PAS-positive material. Electron microscopy revealed numerous vacuoles with either granular or lamellar accumulations. Whole exome sequencing identified a pathogenic homozygous p.R498X mutation in the Skip-Cullin-Fbox E3 ubiquitin ligase FBXO7.
Discussion/Conclusion: Abnormalities of autophagy-lysosomal function have been implicated in lysosomal storage diseases and neurodegenerative diseases. Our findings indicate additional overlap between autosomal recessive forms of parkinsonism and disorders of lysosomal metabolism.
This study was partially supported by TÜBİTAK (111S217).

P-730

Value of Whole Exome Sequencing in the diagnosis of neurometabolic disorders with unusual clinical presentation

Selim L A[1], Mahmoud I G[1], Gleeson J[2], Rolfs A[3]

[1]Cairo University Children Hospital, Cairo, Egypt, [2]Rockefeller University, New York, United States, [3]Rostock University, Rostock, Germany

Background: Whole Exome sequencing(WES) is a recent technique for sequencing all the protein–coding genes in a genome. It consists in selecting the subset of DNA (exons) that encodes proteins, and sequencing that DNA using any high throughput DNA sequencing technology.
Subjects and Methods : 5 patients were included in the study, their ages ranged from 3 to 15 years. Main clinical presentation included epilepsia partialis continua, epileptic encephalopathy, neurodegenerative disease course, persistent hyperlactic acidemia and severe neurodevelopmental delay. Brain MRI, metabolic screening, urine organic acid profile, and specific enzyme assay or mutational analysis for certain mitochondrial syndromes (MELAS, MERRF) were performed as dictated by the clinical picture of the patients. Finally WES was performed for identification of the specific mutation.
Results : Whole exome sequencing identified homozygous mutation for Krabbe disease in the patient presenting with epilepsia partialis continua, Gaucher disease in the patient with epileptic encephalopathy, Nieman Pick type C in 2 patients and mitochondrial depletion syndrome-13 in the last patient.
Conclusion : Whole exome sequencing technique should be widely use for the diagnosis of difficult neurometabolic cases with unusual presentation as this help to initiate a properly timed therapeutic intervention.

P-731

Elevated pipecolic acid in cerebro-facial-ocular skeletal syndrome

Trujillano D[1], Abou Jamra R[1], Brandau O[1], Berger J[2], Wiesinger C[2], Lampe C[3], Rolfs A[4], Scarpa M[3]

[1]Centogene AG, Rostock, Germany, [2]Center for Brain Research, Viena, Austria, [3]Horst Schmidt Kliniken, Wiesbaden, Germany, [4]Albrecht-Kossel-Institute, Rostock, Germany

We performed whole exome sequencing in a female pre-term patient with polyhydramnion, multiple arthrogryposis, brain atrophy, atrium septum defect, arterial hypertonia, severe hypotonia, and tubulopathy, and increased plasma pipecolic acid. We identified a homozygous variant in *ERCC2* (c.1279A>G p.Thr427Ala) not previously described in the public variant databases, which affects a strongly conserved position. Mutations in *ERCC2* cause the autosomal recessive cerebro-facial-ocular skeletal syndrome (COFS) that is characterized by severe IUGR, microcephaly, facial dysmorphisms, joint contractures, and profound developmental delay. Because of the genetic results and the clinical overlapping, we considered that our patient has COFS. However, in the approximately 20 cases that were published so far, no elevation of pipecolic acid has been reported. Extensive re-evaluation of the whole exome sequencing data did not reveal any relevant findings in genes of peroxisomal or mitochondrial diseases. This, and unchanged levels of very-long chain fatty acids (VLCFA), phytanic acid, plasmalogens, peroxisomal ß-oxidation, and unremarkable peroxisomal staining of fibroblast cultures suggest that elevated plasma pipecolic acid levels might represent a further symptom of COFS.
Conflict of Interest declared.

P-732

Rett-like clinical picture as an entry to detect inborn errors of neurotransmission

Armstrong J[1 2], Vidal S[3], Brandi N[1], Gerotina E[3], O'Callaghan M[2 4], Perez-Poyato M S[5]

[1]Mol.& Genet Med Serv. H.Univ.St Joan Déu, Esplugues de Llobregat, Barcelona, Spain, [2]CIBER de Enfermedades Raras (CIBERER), Valencia, Spain, [3]FundaciónSant Joan de Déu, Esplugues de Llobregat, Barcelona, Spain, [4]NeurologyUnit,

Hospital Sant Joan de Déu, Esplugues de Llobregat, Barcelona, Spain, [5]Neuroped.Unit, H.Univ.Marqués Valdecilla, Santander, Spain

Background: Rett syndrome (RTT) is a developmental disorder of early onset that affects almost exclusively girls with a prevalence of 1/15000. *MECP2* is the most common responsible gene (X-linked dominant inheritance). Other genes such as *CDKL5* and *FOXG1* have been also reported. However, in 30% of patients, the etiology remains unknown.
Methods: A cohort of patients fulfilling criteria of Rett/Rett-like clinical picture without genetic diagnosis was studied by next-generation sequencing platforms (NGS): a gene panel including 17 genes related to Rett-syndrome and GABA/glutamatergic transmission (HaloPlexTarget technology, *Enrichment System*, *for Illumina Sequencing*), and WES. All NGS results have been verified by Sanger sequencing and studied in the parents.
Results: We found four patients affected with 4 mutations in genes that encode GABAergic and glutamatergic pathways: 1) *GRIN2B*: the subunit 2 of the N-methyl-D-aspartate receptor; 2) *GABBR2*: a subunit of the brain-expressed G protein coupled $GABA_B$receptor; 3) *SLC6A1* or GAT-1: voltage-dependent GABA transporter; 4) *ALDH5A1*: encoding for succinic semialdehyde dehydrogenase.
Conclusion: Clinical presentation of RTT patient can be due to mutations in neurotransmitter genes. Both glutamatergic and GABAergic pathways have been involved in the pathophysiology of RTT.

P-733

Neuroimaging findings in patients with GLUT1 deficiency syndrome

Larshina E A[1], Mikhailova S V[2], Andreeva L P[3], Zakharova E Y[1]

[1]Research Centre for Medical Genetics, Moscow, Russian Federation, [2]Russian Pediatric Regional Hospital, Moscow, Russian Federation, [3]Saratov Regional Children's Hospital, Saratov, Russian Federation

Background: Glucose transporter type 1 deficiency syndrome (GLUT1-DS) is the result of impaired glucose transport into the brain. Most GLUT1-DS patients present with seizures resistant to antiepileptic treatment and/or with developmental delay, ataxia and dystonia. According to the literature, brain MRI usually does not show any abnormalities in GLUT1-DS patients.
Case reports: Here we report the MRI findings in 3 patients with GLUT1-DS. In all patients, diagnosis was confirmed by direct sequencing of *SLC2A1* gene. Three different new *de novo* mutations were detected: c.679+2T>C, c.115-2T>G, p.Glu247Ter in heterozygous state in our series of 3 patients.
Results: All patients have diffuse white matter changes described as "leukoencephalopathy". Two patients had abnormalities affecting the subcortical U-fibers, similar to L-2-hydroxyglutaric aciduria.
Conclusion: The finding of leukoencephalopathy, especially with subcortical white matter changes, in a child with neurological involvement should prompt a thorough evaluation to exclude GLUT1-DS.

P-734

A completely new approach to the diagnosis of inborn errors: development of a 450-gene (all metabolic disorders) next-generation sequencing panel

Ezgu F[1], Ciftci B[1], Topcu B[1], Okur I[1], Inci A[1], Olgac A[1], Karaoglu A[1], Biberoglu G[1], Tumer L[1], Hasanoglu A[1]

[1]Div Metab Dis, Gazi Univ Fac Med, Ankara, Turkey

Background: Next-generation DNA sequencing provides cheaper and higher-throughput alternative compared to traditional Sanger sequencing, enabling sequencing hundreds and thousands of genes, even the whole genome in parallel. For this study, nearly all the genes (450 genes) listed in SSIEM 2012 classification were included in a custom next-generation sequencing panel to investigate its efficacy and reliability for the diagnosis.
Patients and Methods: Three patients with clinical and laboratory findings highly suggestive of an inborn error but who did not have a definite diagnosis even after a significant number of metabolic tests, individual enzyme or genetic assays were investigated with this panel. Semiconductor sequencing technology was used for the analysis.
Results: Two patients with hepatopathy were diagnosed with mtDNA depletion syndrome (homozygous p.S52C mutation in *DGUOK* gene) and dihydrolipoamide dehydrogenase deficiency (p.T183M and p.G229C mutations in *DLD* gene) and one patient with developmental delay and hearing loss with 3-methylglutaconic aciduria (homozygous p.G642fs mutation in *SERAC1* gene). All the patients were tested simultaneously. The analysis took two days and the interpretation of the results took one day.
Conclusion: The 450-gene metabolic disease panel is a promising diagnostic tool and could be considered before exome sequencing in the diagnostic algorithm for inborn errors.

P-735

***DNM1* mutations: from exercise-induced collapse syndrome to a metabolic cause of severe epileptic encephalopathy**

Lourenco C[1], Leprevost C[1], Almeida M[1], Nobre L[1], Marques Jr W[1]

[1]Univ of Sao Paulo, Ribeirao Preto, Brazil

Background: Dynamins are large GTPases that belong to a protein superfamily that includes classical dynamins, dynamin-like proteins, mitofusins and guanylate-binding proteins/atlastins. They are involved in many processes including budding of transport vesicles, division of organelles, cytokinesis and pathogen resistance
Objective: To report two Brazilian patients with *DNM1* mutations as cause of their severe epileptic encephalopathy.
Methodology: After excluding the main metabolic causes of epileptic encephalopathy, whole exome sequencing (WES) was performed
Results: De novo mutations in *DNM1* gene were identified in two non-related patients with intractable seizures. Patient showed a complex movement disorder with choreoatethotic movements and severe language delay.
Conclusions: *DNM1* was first identified as a cause of exercise induced collapse in dogs, but it is also implicated in human disease a rare cause epileptic encephalopathy, showing the important role of dynamins in the neurotransmission process and synaptic vesicle endocytosis in brain developmental.

01. Inborn errors of metabolism in adult

A-001 - Withdrawn

02. Novel diagnostic/laboratory methods

A-002

Comparison of non-derivatization and derivatization tandem mass spectrometry research methods for analysis of amino acids, acylcarnitines, and succinylacetone in dried blood spots

Thibert V[1], Xie X[2], Kozak M[2]

[1]Thermo Fisher Scientific France, Courtaboeuf, France, [2]Thermo Fisher Scientific, US, San Jose, United States

Background: Flow injection tandem mass spectrometry (FIA-MS/MS) has been frequently used to analyze amino acids (AA), acylcarnitines (AC), and succinylacetone (SUAC) in dried blood spots (DBS) for inborn errors of metabolism research. We conducted a comprehensive study to evaluate and compare nonderivatization and derivatization tandem MS methods on a triple quadrupole mass spectrometer.
Methods: AAs, ACs and SUAC were extracted in a single extraction resulting in significant reduction in labor and time. The extracts were analyzed by Thermo Scientific™ TSQ Endura™ triple quadrupole mass spectrometer. Quantification of 12 AAs, 18 ACs, and SUAC was achieved using a meta-calculation software, iRC PRO™. The off-line automated tool streamlined data processing of peak area, concentration and user-defined formulas.
Results & conclusion: For each method, we evaluated within-run precision by means of ten successive, independent measurement of DBS samples (n=10), which was inferior to 10% for both methods, and run-to-run precision by means of ten independent measurement in seven different test series (n=70), which was inferior to 16.5% for both methods. We also compared quantitative results of multianalytes from nonderivatization and derivatization methods. Quantitative differences were lower than 5% for 12 AAs and SUAC, and were inferior to 15% for 18 ACs. Therefore the two methods were highly correlated.

A-003

Direct and fully automated extraction/analysis of Dried Blood Spots (DBS)

Gaugler S[1]

[1]CAMAG, Muttenz, Switzerland

Background: The full automation of dried blood spot (DBS) sample preparation and online LCMS analysis has been shown previously [Loppacher et al.]. In this study, further improvement in terms of flexibility of the analysis method has been performed. The fully automated DBS-MS 500 was coupled to an auto collector to enable biological assays or derivatization and dilution steps prior the analysis.
Methods: The DBS-MS 500 from CAMAG was coupled to a robotic auto-collection system and carry over was determined according to ICH standards.
Results: Experiments of DBS-LC-MS/MS methods have shown that the carry-over from spiked samples at the upper limit of quantification (ULOQ) to blank samples was below one ppm. Also the carry-over from DBS-Auto collect methods could be minimized to below one ppm from spiked samples to blanks.

03. Newborn screening

A-004 moved to P-736

A-005

The first index case with coinsidence of phenylketonuria, polysplenia syndrome and methylene tetrahydrofolate reductase mutation from newborn screening

Ozer I[1], Çam S[2], Durakbaşa Ç[3], Kıral A[4]

[1]Ped Metab Dis, Goztepe Edu Res Hosp, Istanbul, Turkey, [2]Ped Gastroent Dis, Goztepe Edu Res Hosp, Istanbul, Turkey, [3]Ped Surg, Goztepe Edu Res Hosp, Istanbul, Turkey, [4]General Ped, Goztepe Edu Res Hosp, Istanbul, Turkey

Background and objectives: Newborns are lead to the metabolism centers for a specific illness with newborn screening. This application can provide early diagnoses for some rare comorbidities.
Patients and Methods: She born as mature baby. Jaundice started at the second day. She is guided to child metabolism center at the 8th day with 3.9 mg/dl phenylalanine (phe). She is second child of first cousin marriage's parents. Family history is unremarkable. Physical examination revealed that jaundice, organomegaly in the left, systolodiastolic murmur at the right and deep sacral dimple.
Results: Her control phe level was found 34 mg/dl. She hospitalized for tetrahydrobiopterin (BH4) challenge test and nutrition therapy. BH4 challenge test was found 64% responsive. During this hospitalization, whose acholic feces has noticed, and diagnosed extrahepatic biliary tract atresia, gallbladder agenesis, situs inversus totalise, Patent Ductus Arteriosus, Methylene Tetrahydrofolate Reductase C677T heterozygote mutation by the laboratory evaluation. We started supplementation of fat soluble vitamins, ursodeoxicholic acid, folic acid. She was operated for extrahepatic biliary tract atresia. Multiple accessory spleens was seen in peritoneal cavity during the operation.
Conclusion: The comorbidity is first time defined and offered for the purpose of the early diagnoses contribution in rare situations of newborn screening.

A-006

Current state of newborn screening in Slovenia

Smon A[1], Groselj U[1], Zerjav Tansek M[1], Bicek A[2], Oblak A[2], Zupancic M[1], Krzisnik C[3], Repic Lampret B[1], Murko S[1], Hojker S[2], Battelino T[1 3]

[1]UMC Ljubljana, Univ Child Hosp Ljubljana, Ljubljana, Slovenia, [2]UMC Ljubljana, Dep Nuclear Med, Ljubljana, Slovenia, [3]Univ Ljubljana, Faculty of Medicine, Ljubljana, Slovenia

Background: Newborn screening (NBS) for metabolic diseases is an important public health programme, because an early identification of affected children can help to prevent disabilities. Newborn screening in Slovenia started in 1979 with screening for phenylketonuria (PKU), congenital hypothyroidism (CH) started 2 years later in 1981.
Results: From 1993 to 2012 57 cases were diagnosed positive for PKU, which is up to 6 positive cases of PKU per year. Between 1991 and 2012 there were from 3 to 16 positive cases of CH annually, amounting to a total of 184 patients with CH. Incidences of PKU and CH in Slovenia are 1:6769 and 1:2323, respectively.
Conclusion: NBS programme in Slovenia has been successfully implemented in the public health programme and has been beneficial for a significant number of affected newborns. As tandem mass spectrometry has not yet been implemented into NBS programmes in southeastern Europe, a pilot study for expanding NBS is currently running in Slovenia. It will make an important contribution in designing the optimal strategy for screening Slovenian newborns for inborn errors of metabolism and also significantly contribute to the evaluation of the incidences of each inborn error of metabolism in our population.

A-007

Newborn screening using the DBS-MS 500 from CAMAG

Gaugler S[1]

[1]CAMAG, Muttenz, Switzerland

Background: A routine neonatal screening procedure requires that a health professional takes a few drops of blood from the baby's heel, applies them onto a filter paper and sends such prepared samples to a laboratory for a number of analytical tests. The sample preparation taking place before analysis may be labor intensive, time-consuming and not very precise due to carry-over when processed with traditional "punch-and-elute" methodology. Application of the CAMAG DBS-MS 500 direct sample elution system offers fast, efficient and highly reliable sample handling. In addition, this fully automated system eliminates the need for any manual intervention between samples. Recent applications of the fully automated DBS analysis with the DBS MS 500 were developed for phenylketonuria (PKU), medium chain acyl-CoA dehydrogenase deficiency (MCAD), propionic acidemia (PA) and maple syrup urine disease (MSUD).

Methods: The fully automated DBS-MS 500 system from CAMAG was online coupled to a Waters MS system for the detection of PKU, MCAD, PA and MSUD. The analysis was performed at the Children hospital in Zürich, Switzerland.
Results: A reference study showed similar results as obtained by the reference kit. All four genetic disorders could be detected from DBS cards and outliners or spiked samples were correctly recognized.

04. Dietetics and nutrition

A-008

Intravenous and enteral ketogenic diet in a patient with cow milk allergy

Desloovere A[1], Verloo P[1]

[1]Div Metab Dis, Univ Child Hosp, Ghent, Belgium

Case: An 8 month old boy presented to our metabolic unit with difficult-to-control epilepsy. He was referred to us for initiating a ketogenic diet. Two major problems interfered with establishing a normal ketogenic diet: he had a severe cow milk allergy and was hospitalised on the intensive care for status epilepticus without the ability to use the gastrointestinal tract completely.
We calculated a MCT ketogenic diet with total parenteral nutrition (TPN) and a small amount of tube feeding consisting of a semi-elemental feed. A classic ketogenic diet was impossible because commercially available products contain cow milk protein. We successfully established ketosis with the combination of parenteral nutrition and a small amount of enteral feeding, which was increased over time.
Conclusion: Here we present an adapted to protocol to bring a kid into ketosis with a combination of parenteral en enteral feeding. An adapted MCT ketogenic diet with a semi-elemental feed was later the introduced when enteral feeding was well tolerated.

06. Phenylketonuria: general

A-009

Nutritional evaluation and metabolic profile in children affected by phenylketonuria (PKU): case-control study

Moretti F[1], Verduci E[1], Paci S[1], Salvatici E[1], Zuvadelli J[1], Bellini S[1], Riva E[1]

[1]Cl Dept of Ped, San Paolo Univ Hosp, Milan, Italy

Introduction: vegetables, fruits, low-protein-foods and phenylalanine-free aminoacidic mixtures mostly make up PKU patients' nutrition.
Objectives: to evaluate PKU children's nutritional supply issues and metabolic profile compared to a control group.
Methods: 20 PKU and 20 healthy subjects (age and sex matched) were recruited. Caloric and macronutrient intakes, glycemic index (IG) and load (CG) and daily energy density (DE) breaking down 3 days food diaries were determined; low-protein foods' IG, plasma lipid profile, glucose and insulin levels evaluated. HOMA and QUICKI were studied for sensitivity and insulin resistance.
Results: PKU patients, compared to healthy comparisons, show lower caloric (p=0,048) and protein intakes (p< 0,001) with higher carbohydrate consumption (p=0,004). Mean CG seems higher at lunch (53,58 vs. 40,24; p=0,011) and dinner (53,67 vs. 45,98; p=0,047). Mean daily IG and CG didn't differ significantly. Mean solid foods DE was lower (p=0,032) as insulin levels (4,61 vs. 6,04 mU/L, p=0,05) and HOMA indices (0,87 vs 1,18; p=0,050), while QUICKI (0,40 vs. 0,37; p=0,050) were higher.
Conclusions: PKU children's supply seems better aligned with healthy population reference values if compared to controls; that could result in better glucidic and insulinemic pattern. It's still however necessary to focuse on daily glucidic, mainly fiber, supply.

A-010

Vitamin D levels in an adult PKU population

Green D L[1], McLoughlin M[1], McDarby S[1], Ripley S[1]

[1]Mark Holland Metab Un, Salford Royal Hos, Salford, United Kingdom

Vitamin D impairs the absorption of dietary calcium and phosphorus, which in adults can give rise to bone pain and tenderness as a result of osteomalacia, osteoporosis or increased risk of fractures. In addition observational studies have suggested that low 25-hydroxyvitamin D values are associated with an increased risk of cancer, infections, autoimmune diseases and CVD.
Hypponen (2007) looked at Vitamin D levels in British adults at age 45 years. Their average Vitamin D levels were 41.1nmol/L in winter. National Surveys suggest approximately one fifth of the adult population in the UK may have low vitamin D status. Of our adult PKU population (175), we audited the notes of 122 patients reviewed in clinic from Oct 2014 to March 2015. 85.3% had Vitamin D levels measured, 16 patients (15.4%) had a serum 25OHD < 30nmol/L and 21 patients (20.2%) had serum 25OHD of 30–50nmol/L.

The Vitamin D levels between patients on and off diet were not statistically significantly, nor for patients on PKU protein substitutes was there any statistical significance between the vitamin D levels and the product used within our patient cohort. However 35.6% of our patient cohort could be considered as either Vitamin D deficient or depleted.

A-011

Marmara metabolism group consensus report for large neutral amino acids

Ozer I[1], Zübarioğlu T[2], Önal H[3], Gökçay G[4], Sağlam H[5], Cesur Y[6], Taş I[6], Aydın A[2], Balcı M C[4], Kıykım E[2], Aktuğlu Zeybek Ç[2], Demirkol M[4]

[1]Ped Metab Dis, Goztepe Edu Res Hosp, Istanbul, Turkey, [2]Ped Metab Dis, Ist Uni Cerrahpaşa Med F, Istanbul, Turkey, [3]Ped Metab Dis, Kan Sult Süleyman Hosp, Istanbul, Turkey, [4]Ped Metab Dis, Ist Uni Istanbul Med Fac, Istanbul, Turkey, [5]Ped Metab Dis, Uludağ Uni Medical Fac, Bursa, Turkey, [6]Ped Metab Dis, Bezmialem Vakif Uni, Istanbul, Turkey

Background: Phenylketonuria (PKU) treatment with large neutral amino acids (LNAAs) is not accepted yet. We discussed and tried to create an action plan about LNAAs using practice in the 6 child metabolism centres in the Marmara region from Turkey.
Materials and Methods: The reasons for choosing or refusing to use LNAA in metabolism centres in our region are determined with the usage experiences in clinical and laboratory data.
Results: LNAA is used only for 0.3% PKU patients at two of the six centres. LNAA is used for patients during their diet treatment and it is observed that the phenylalanine (phe) has decreased and the tyrosine has increased and the other amino acids have no change, and the weight and appetite control is affected positively. The second centre has used free nutrition and LNAA, but they haven't observed any positive finding except decrease of phe.
Discussion: Although a promising treatment, there is no clinical data for the long-term use.There is not yet enough information regarding the use for pregnant women, lactating mothers or newborns.
Conclusion: We plan long-term neuro-psychiatric and laboratory investigations, to be able to provide satisfactory replies to the existing questions about LNAAs.

A-012

First day phenylalanine levels in the offspring may reflect maternal phenylketonuria

Unal O[1], Celen G[2]

[1]Ankara Hematology Oncology Children's, Ankara, Turkey, [2]Erzurum Training and Research, Erzurum, Turkey

Background: Elevated maternal phenylalanine (Phe) concentrations during pregnancy are teratogenic, may result in microcephaly, developmental delay, and birth defects.
Case Report: A two week old boy was referred to us with 10 mg/dL Phe level from the neonatal screening program. He was term, and the second offspring of the family. It was learned that the blood sample had been taken on the first day. On physical examination, microcephaly was noticed. Mother also seemed to have borderline intellectual disability. The first baby of the family had also been evaluated for hyperphenylalaninemia, his phe level had been found normal, but he had developed intellectual disability and microcephaly. Family history of the mother revealed that, she had a sister and a brother with severe mental retardation and phenylketonuria but because she appeared normal, her phenylalanine level had not been checked. Blood Phe level of the mother was found to be 30 mg/dL, the baby's was normal.
Conclusion: Our case indicates two important points. First, there may be phenotypic variability in the same family, blood phe level should be checked of women born before screening programs at childbearing age, if they have a family history. Second, first day high blood phe levels may indicate maternal PKU.

A-013 – moved to P-739 Phenylketonuria: general

A-014

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

Boury A[1]

[1]Dep Ped Neur, Univ Hosp Ghent, Ghent, Belgium

There are several problems associated to the management of patient with phenylketonuria (PKU). Correct information could be one of the affecting factors on social integration of these patients.
There is an important need for clear and correct information about PKU for pediatric patients and their network.
Based on interviews 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 (a boy aged 10 years), so the reader is able to witness

which events, feelings and thoughts a child with PKU can experience through daily life.
This recognition can be very meaningful in accepting their diagnosis and feeling more confident. By adding essential medical and diet related information, this book aims to be helpful in explaining this quite unknown and rare disease to the environment of the patient. Earlier research assessed the importance of psycho-education to enhance therapy compliance.
Developing an educational book for children and adolescents with PKU and their environment could not only stimulate their common knowledge, it could also have a positive effect on the patient's therapy compliance, self-esteem and social integration.

A-015

Metabolic group study for consensus development on the management of phenylketonuria

Demirkol M[1], Balcı M C[1], Ozer I[2], Aktuglu Zeybek A C[3], Saglam H[4], Yasar C[5], Onal H[6], Kıykım E[3], Erdol S[4], Yesil A[1], Cansever M S[3], Zubarioglu T[3], Türkoğlu Ü[1], Gokcay G[1]

[1]Ped Nutr Metab, Ist Med Faculty Ist Univ, Istanbul, Turkey, [2]Ped Metab Dis, Goztepe Edu Res Hosp, Istanbul, Turkey, [3]Div Nutr Metab Dis, Ist Univ Cer Med Fac, Istanbul, Turkey, [4]Div Ped Metab, Uludag Univ, Bursa, Turkey, [5]Bezmialem Vakif Univ Div Ped Nutr Metab, Istanbul, Turkey, [6]Ped Metab Dis, Kan Sult Süleyman Hosp,, Istanbul, Turkey

Background: Evidence-based guidelines for the management and of phenylketonuria (PKU;OMIM261600) are lacking in Turkey. Different PKU management strategies of six metabolic centres caring for a population of 20 million residents in the Marmara region cause confusion.
Objectives: During 2014-March 2015 Marmara Metabolic Group organized eight meetings aiming to develop a regional consensus on PKU management.
Methods: Blood phenylalanine (phe) level for treatment initiation and follow-up, laboratory methods for phe determination, frequency of monitoring, clinical visits, nutritional treatment, pharmacological interventions and management across lifespan of PKU patients (n:1734) were evaluated and compared among centres. Relevant literature was reviewed.
Results: Recommendation for treatment was 600μmol/L in five and 480μmol/L in one centre. Target blood phe level was < 360μmol/L during first 10 years of life in five and < 240μmol/L in one centre. Phe monitoring frequency was once a week in the first three years of life in three centres and at longer intervals in others. BH4 loading test in the newborn period, monitoring of phe level from dried-blood spot and nutritional and/or pharmacological treatments were performed in all centres.
Conclusion: We observed great disunity regarding management of PKU among centres. Regional studies to develop common practice may be a step toward nation-wide concensus development.

07. Phenylketonuria: treatment, BH4

A-016

Prevalence, frequency and phenotype of BH4 deficiencies identified in a Neonatal Screening Program for hyperphenylalaninemia

Souza C A A[1 2], Soares R D L[1 2], Alves M R A[1], Kanufre V C[1 2], Norton R C[1 2], Starling A L P[1 2], Aguiar M J B[1 2]

[1]Nupad, Fed Univ Min Ger, Belo Horizonte, Brazil, [2]HC, Fed Univ Min Ger, Belo Horizonte, Brazil

Introduction: Tetrahydrobiopterin (BH_4) deficiencies are caused by defects in enzymes of synthesis and regeneration of the cofactor BH_4. Deficiencies of the enzymes: GTPCH I, PTPS, DHPR and PCD, present with hyperphenylalaninemia, identified by newborn screening for phenylketonuria.
Objectives: To present the prevalence and clinical characteristics of the BH_4 deficiencies with hyperphenylalaninemia.
Methods: Descriptive study based on the records of patients accompanied by the Newborn Screening Program in Minas Gerais.
Results: The prevalence was 2.1 to 1 million newborns (10 patients) and the frequency was 1.71% among hyperphenylalaninemias: PTPS deficiency (4 patients) 40%, DHPR (3 patients) GTPCH I (3 patients) 30% each one, PCD (no patient). Six patients were diagnosed by clinical history and four by systematic research. First symptoms occurred between two and four months of age. After starting treatment, patients coming from the neonatal screening, had rapid improvement (1 month) and better psychomotor development compared with patients diagnosed by clinical history (3 months).
Conclusions: The prevalence of BH_4 deficiency in Minas Gerais was higher than in the literature, but the frequency, among hyperphenylalaninemias, was similar. If not treated, they lead to developmental delay, movement disorders, seizures and premature death. The research by newborn screening for phenylketonuria is justified.

08. Sulphur amino acid disorders

A-017

Trimethylaminuria (Fish odor syndrome) in a adolescent boy

Kilic M[1], Celik-Babalioglu P[2], Tokgoz-Cuni B[2]

[1]Div Metab Dis, Dr Sami Ulus Child Hosp, Ankara, Turkey, [2]Dr. Sami Ulus Child Hosp, Ankara, Turkey

Background and objectives: Trimethylaminuria is a rare inherited disorder due to decreased metabolism of dietary-derived trimethylamine by flavin-containing monooxygenase 3.
Case report: A twelve-year-old adolescent male patient was admitted to hospital for the malodor of urine and sweat. The complaint of the patient increased of eating meat. There was no discoloration of the urine. Parents had a distant kinship. There was a history of levothyroxine treatment for hypothyroidism and varicocele operation. Physical examination findings were normal. Laboratory findings were unremarkable except for the mild TSH elevation and low vitamin B12 levels. In the *FMO3* gene the c.1160G>A (p.R387H) homozygous mutation was detected and a diagnosis of 'Trimethylaminuria' (Fish odor syndrome) was made. A diet restricted in foods that contain choline, use of acidic soap, taking frequent showers and 3x250 mg oral metronidasole treatment for two weeks in case of heavy smell conditions were advised.
Conclusion: Direct molecular genetic analysis should be considered in patients with suspicious Trimethylaminuria.

A-018 - Withdrawn

09. Other amino acid disorders

A-019

Hereditary tyrosinemia type 1, effectiveness of early treatment

Sadaoui B[1], Arrada Z[1]

[1]Consult. Metab. Dis., Dep. of Ped. H.Dey, Algiers, Algeria

We only have recent experiences with metabolic diseases in our country, and our challenge is to establish the screening and develop the research.We wish to present 4 cases of HT1 with different clinical presentation, where the early treatment given from suspicion of HT.

Case study 1: 2 years old boy, with liver failure and nephrocalcinosis at the age of 3 months, without liver nodules at MRI, currently has a good evolution under diet and medication.
Case study 2: 1 year old girl, sister of the 1st case, without clinical expression the diagnosis was made at birth by targeted screening, but she has a liver nodule at segment 6, without signs of hepatocellular carcinoma, currently controlled by treatment.
Case study 3: 8 months old girl, presented early at 3 months with severe liver failure and strong suspicion of a hepatocellular carcinoma at MRI. She died recently after waiting for a liver transplant.
Case study 4: 8 months old girl, brother died in the same clinical table, she presented with severe liver failure and severe rickets, it was difficult to control these problems by symptomatic treatment, but she well improved after specific treatment for HT1, unfortunately she has multiple hepatic nodules at liver ultrasonography and CMV acute infection.

A-020

Drug development for paediatric patients with inborn errors of metabolism (IEM) – A development program for an oral suspension of nitisinone for hereditary tyrosinemia type 1 (HT-1)

Rudebeck M[1], Svensson L[1], Sahlberg M[1], Olsson B[1]

[1]Swedish Orphan Biovitrum (Sobi), Stockholm, Sweden

Background: Development of new medicines is associated with high costs and lengthy timelines which may limit new development of paediatric formulations. However, access to and administration of suitable dosage forms for children with IEMs are crucial, especially in diseases diagnosed early in life. We present an example of recent development of a new formulation of a drug with well-established efficacy/safety profile; nitisinone, currently available as capsules for treatment of HT-1.
Methods: Nitisinone is not stable in solution and has a bitter taste. The formulation work aimed at an easy to administer, stable oral suspension with a sweet fruity taste.
Two clinical studies were performed:
· A bioequivalence study vs. the capsule in healthy volunteers.
· An acceptability study investigating the taste/palatability of the suspension in paediatric HT-1 patients (see P-010).
Results: The suspension was demonstrated to be bioequivalent to the capsule form, and well accepted in paediatric HT-1 patients.
Conclusion: For a new dosage form of an established drug, the pharmaceutical development can be challenging. However, small-scale and efficient clinical development programs can provide the required scientific and regulatory evidence to

support approval. Careful consideration to the development of paediatric formulations is important to meet the needs of paediatric patients with IEMs, including HT-1.
Conflict of Interest declared.

A-021

Clinical case report: maple syrup urine disease treatment

Kanufre V C[1], Cruz A F[1], Lopes J C C[1], Alves M C[1], Valadares E R[1]

[1]HC, Fed Univ Min Ger, Belo Horizonte, Brazil

Objective: To highlight the importance of specific biochemical tests and the relevance of specialized staff in the patient's lifelong monitoring.
Case report: The patient was admitted to the emergency room of a public hospital, at 9 days old, with a suspicion of maple syrup urine disease (MSUD) due to the fact that the child has a sister of five years old with confirmed diagnosis of MSUD. She presented with muscle hypertonia, dystonia, spontaneous pseudo-babinski, dehydration, depressed fontanelle, impaired breastfeeding and sweet odor in urine. The diagnosis was confirmed by biochemical tests. The treatment was started with diet and isoleucine and valine supplementation. Throughout the treatment, skin lesions typical of isoleucine deficiency were observed. Due to the unavailability to perform testing, isoleucine and valine supplementation were increased, resulting in worsening of the symptoms. Biochemical analyses then revealed leucine deficiency and the diet was corrected. The child evolved with improvement of his clinical status and levels of branched chain amino acids.
Discussion: The difficulty in accomplishing specific biochemical tests requires decisions based on the child's clinical condition. This situation reinforces the need for efficient laboratory control for proper treatment of patients with MSUD, in addition to dietary and clinical monitoring, ensuring the success of long-term treatment.

A-022

MSUD presenting as an episodic ataxia

Verloo P[1], Verhelst H[2]

[1]Div Metab Dis, Univ Child Hosp, Ghent, Belgium, [2]Div Ped Neur, Univ Child Hosp, Ghent, Belgium

Case: A three-year-old child presented to the pediatric neurology department because of an episodic ataxia. The symptoms started age of 18 months, and noticeably appeared during catabolic episodes and could be stopped with feeding. Work-up in the referring hospital showed a MRI with marked symmetrical T2 hyperintensities in the capsula externa, globus pallidus and cerebellar white matter. However these abnormalities were not recognised as being suggestive for MSUD. Upon referral, plasma leucine was 1381 μmol/L, isoleucine 601 μmol/L and valine 1179 μmol/L. A trial therapy with thiamin didn't reduce plasma leucine. When a low-protein diet was introduced, symptoms quickly got better, plasma leucine normalised and MRI abnormalities disappeared.
Conclusion: MSUD should be included in the differential diagnosis of episodic ataxia, especially when the symptoms appear during moments of catabolism.

10. Urea cycle disorders

A-023

Continuous renal replacement therapy for nenatal hyperammonemia due to ornithine transcarbamylase deficiency

Han J Y[1]

[1]Div Ped Neu, Univ St.Mary's Hop, Daejeon, Korea, Republic of

Background: In patients with severe hyperammonemia, measures that reduce blood ammonia should be employed to minimize permanent brain injury. We performed continuous renal replacement therapy (CRRT) for rapidly clear ammonia in ornithine transcarbamylase deficiency patient.
Case: A 4 days old male (3.25kg) developed poor feeding, lethargy and vomiting. He was transferred to our hospital, He had apnea and cyanosis and decreased in level of consciousness. He was intubated and admitted to natal intensive care unit. Initial laboratory studies revealed ammonia 1099 μmol/L, bicarbonate of 12 mEq/L, creatinine 0.8 mg/dL, arterial pH 7.27, pCO2 50.7 mmHg, lactate 97.3 mg/dL, and ionized calcium 1.67 mEq/L. Medical treatment with sodium benzoate, soduim phenylacetate, arginine 10% dextrose and protein restriction was started. Surgery placed internal jugular dialysis catheter and CRRT was initiated. Ammonia level was decreased rapidly after CRRT (ammonia 2 μmol). The patient regained alert consciousness and normal respiration 2 days later. CRRT was discontinued after 29 hours and ammonia level rebounded peak to 120 μmol/L.
Conclusion: When ammonia level is significantly elevated, CRRT is requisite to obtain rapid clearance in combination with medical treatment. We propose that CRRT is preferred method in treatment of hyperammonemic encephalopathy.
Conflict of Interest declared.

A-024

Clinical and biochemical features, molecular diagnosis and management of a Moroccan female with *OTC* gene mutation

Dahri S[2], Talbaoui H[2], Kriouile Y[3], Acquaviva C[1], Vianey-Saban C[1], Chabraoui L[2]

[1]Centre de Biologie et de Pathologie Est, Lyon, France, [2]Biochem Univ Hosp Lab of Rabat, Rabat, Morocco, [3]Neuropediatr Univ Hosp of Rabat, Rabat, Morocco

Ornithine trancarbamylase deficiency (OTCD) is the most frequent urea cycle error. For this X-linked disease, the clinical features in females are highly variable even within a family, depending on the X-inactivation pattern in liver. We present a 26 months old girl who presented with a 10-day history of finger tremors followed by vomiting and behavioral troubles. Investigations revealed hyperammonemia (350 μmol/l, high plasma glutamine (2200 μmol/l) with normal citrulline and arginine levels and an enhanced orotic aciduria (42 μmol/mmol creatinine), being suggestive of OTCD. DNA analysis revealed a heterozygous mutation in the exon 3 (c.275G>A, p.Arg92Gln, R92Q). The administration of a low protein diet (1g/kg/day), sodium benzoate, sodium phenylbutyrate and L-arginine led to improvement of biological and clinical states. But liver cytolysis (?necrosis/persistent dysfunction) has been noted. The mutation (c.275G>A) was explored, by sequencing of the exon 3 of OTC gene, in the parents, brother and 2 sisters. No carrier was identified suggesting that the detected mutation could be a de novo mutation.

A-025

Recurrent vomiting and somnolence in a 18-month old girl: ornithine transcarbamylase deficiency due to de novo heterozygous mutation

Grkovic S[1], Djordjevic M[1], Sarajlija A[1], Kecman B[1]

[1]Mother and Child Health Care Inst, Belgrade, Serbia

Background: Ornithine transcarbamylase (OTC) deficiency is the most common urea cycle defect.
Case report: A female child was evaluated at the age of 18 months in our institution because of recurrent vomiting and somnolence. A comprehensive clinical and laboratory evaluation revealed a respiratory alkalosis, mild lactic acidemia, normoglycaemia, elevated transaminases and hyperammonemia 191 μmol/L (reference range 11-51 μmol/L). Metabolic evaluation found increased urinary excretion of orotic acid 1600 mmol/mol creatinine. In the majority of female patients the mutation appears de novo and the mothers are usually not a disease carrier, as was the case in our patient.
Conclusion: This case underlines that the diagnosis of a OTC deficiency in females should be considered in the differential diagnosis of recurrent idiopathic vomiting in combination with unexplained neurological symptoms.

11. Organic acidurias: branched-chain

A-026

Replacement of ethyl acetate with acetonitrile greatly improves extractability of derived urinary organic acids before GC/MS

Habbal M Z[1]

[1]Ameican University of Beirut, Beirut, Lebanon

Background: Keto-acids play an important role in the metabolism of branched-chain amino acids. Since the keto group is difficult to extract, it has been derived by several reagents to increase volatility. One such derivative is O-(2,3,4,5,6)-pentafluorobenzylhydroxylamine.
Method: following the preparation of O-PFBO derivatives of keto- acids, they were extracted along with non-keto acids with acetonitrile once. Solid NaCl was added to achieve separation of the phases. The upper phase was evaporated, silylated and injected into GC/MS.
Results: 27 organic acids, mostly observed in IEM were added to a urine sample at a final concentration of 10.0 mm/mole creatinine. The samle was halved and extracted with ethyl acetate or acetonitrile.The following extractabilities were obtained assuming that of ethyl acetate 100%: glyoxylic (180), α-ketoisovaleate-A form (425), α-ketoisocaproate-A (220), succinylacetone (145), pyroglutamic (150), citric (350). The S-form of keto-acids, as well as other acids, were similarly extracted. Orotic acid was not extracted with ethyl acetate. The QC of the derivatives ranged from 91-99 %.
Conclusion: Acetonitrile extraction of derived urinary acids offers much better extraction than ethyl acetate which is used twice. This specifically applies to PFBO derivatives of keto-acids (A-form). Citric acid represents a special case also. Orotic acid, most important in diagnosis of OTC deficiency, was also very clear by our modification.

A-027

Patient with MSUD presenting with diabetic ketoacidosis

Olgac A[2], Okur I[2], Akbas E D[1], Doger E[1], Ezgu F S[2], Bideci A[1], Tumer L[2]

[1]Div Ped Endocrinology, Gazi University, Ankara, Turkey, [2]Div Ped Metabolism, Gazi University, Ankara, Turkey

Introduction: MSUD is an inborn error of metabolism caused by branched-chain α-ketoacid dehydrogenase deficiency resulting in the accumulation of the branched chain amino acids (BCAA). Patients may present with acute ketoacidosis, lethargy and coma. Other features include developmental delay, hematological abnormalities and pancreatitis. We report a patient with MSUD who presented with acute metabolic decompensation and hyperglycemia who was further diagnosed as Diabetes Mellitus type 1 (DM 1).
Case report: 2.5 year old girl, followed-up for MSUD in our clinic presented for routine follow up. Compliance to low leucine diet was good. Her only complaint was short periods of staring, which were present in the last few days. On laboratory analyses, high amounts of blood glucose (487 mg/dl) and ketoacidosis were detected. Plasma amino acid analyses showed very high amounts of BCAA's. Acute metabolic decompensation was managed by high amounts of energy supply, insulin and formulas free of BCAA's. Glutamic acid decarboxylase and insulin antibodies were found positive and the child was diagnosed as DM1.
Conclusion: In some organic acidurias, presence of ketoacidosis in combination with hyperglycemia mimicking diabetic ketoacidotic coma may be confusing. Also, the presence of an organic aciduria may cause diabetic conditions to be overlooked in such patients.

A-028

Propionic acidemia and humoral immune deficiency

Souza C F M[1], Poswar F O[1], Refosco L F[1], Sitta A[1], Wajner M[1 4], Jobim M S L[3], Taniguchi A N R[2], Vairo F P[1], Schwartz I V D[1 4]

[1]Med Genet Serv, Hosp Clin Porto Alegre, Porto Alegre, Brazil, [2]Clin Hemat Serv, Hosp Clin Porto Alegre, Porto Alegre, Brazil, [3]Immun Serv, Hosp Clin Porto Alegre, Porto Alegre, Brazil, [4]Univ Fed Rio Grande do Sul, Porto Alegre, Brazil

Background and objectives: Propionic acidemia (PA) is an organic acidemia caused by deficiency of propionyl-CoA carboxylase. Immune deficiency is an unusual presentation of PA. We report a case of a patient with PA associated with humoral immune deficiency.
Case report: A 6-month-old male was referred for investigation of immune deficiency. He had a history of three hospitalizations due to pneumonia, sepsis, seizures and pancytopenia. In the last admission, he presented low levels of CD19 positive cells (152/mcL) and immunoglobulins (IgA: 15 mg/dL; IgG: 363.3 mg/dL; IgM: 61 mg/dL; IgE: 4 mg/dL). He received intravenous immunoglobulin (IVIG) and was transferred to our center. It was noticed microcephaly, low weight and length, axial hypotonia and ankle clonus. Acylcarnitines profile and organic acids were compatible with PA diagnosis and specific treatment was started, including IVIG infusions. Bone marrow biopsy showed only reactive changes. After 28 days of treatment, the CD19 positive cells increased to normal levels (1019/mcL).
Discussion/Conclusion: The association of PA and humoral immune deficiency is rare but there is little experience in the use of IVIG in PA. The present case reinforces the need of considering the diagnosis organic acidurias in patients with immune deficiency, pancytopenia and neurological findings.

12. Organic acidurias: others

A-029

3-methylglutaconic aciduria and cerebellar vermis hypoplasia in a non-syndromic autistic boy: A new entity?

Ersoy M[1], Yılmaz S[2]

[1]Child Metab Dis,Sadi Konuk Res&Tra Hosp, Istanbul, Turkey, [2]Dep Child Psych, Sadi Konuk Res&Tra Hosp, Istanbul, Turkey

Background and objectives: Autism spectrum disorders (ASDs) are neurodevelopmental disabilities characterized by pervasive impairment in reciprocal socialization, impairment in communication, restricted interests and repetitive behaviours. Inherited metabolic diseases (IMDs) have been evaluated with a growing interest as the underlying cause of autism. 3-metylglutaconic aciduria (3-MGA) is the biochemical marker of a heterogeneous group of IMDs, mostly associated with mitochondrial dysfunction. Cerebellar hypoplasia/atrophy is one of the important finding in autism as seen in IMDs.
Case report: A four-year-old boy was referred with autistic signs and progressive ataxia. Physical examination was normal except ataxic gait. Brain MRI revealed cerebellar vermis hypolasia and atrophy. Urinary organic acids analysis showed persistent elevation of 3-MGA, methylglutaric, glutaric and 2-oxoglutaric acids. The patient was started on antioxidant therapy. He was evaluated using the Childhood Autism Rating Scale (CARS) before and after three and sixth months of treatment. Although he showed improvement on autistic symptoms, his ataxic gait didn't showed any improvement or deterioration.

Discussion: Autism associated with cerebellar hypoplasia/atrophy and 3-MGA is yet an undefined entity to our knowledge. Whole-exome sequencing analysis is expected to clarify the underlying genetic defect.

A-030

A novel mutation for L-2 hydroxyglutaric aciduria in a 7 year old patient

Olgac A[1], Tumer L[1], Ezgu F[1], Biberoglu G[1], Hasanoglu A[1]

[1]Div Met Dis, Gazi University, Ankara, Turkey

Background and objectives: L-2-hydroxyglutaric aciduria (L2HGA) is an autosomal recessively transmitted inborn error of metabolism characterised by neurological manifestations, including mild to moderate psychomotor retardation, cerebellar ataxia, macrocephaly, and epilepsy. The disease causing gene is *L2HGDH*. To date, nearly 50 different mutations have been defined.
Case Report: 7 year old boy was referred to our department due to dystonic movements and walking disability. He was the first child of consanguineous parents. Physical examination revealed macrocephaly and ataxia. Psychometric tests revealed mild retardation in motor functions and social behaviour. Complete blood count and biochemical parameters were within normal limits. Urinary organic acid examination showed high excretion of L-2-hyroxyglutaric acid. Cranial MRI showed high signal intensity in cerebral hemispheres, subcortical white matter, caudate and lentiform nuclei. A novel c.368A>G homozygous mutation was detected in *L2HGDH* gene analysis. Riboflavin treatment was initiated.
Conclusion: L-2 hydroxyglutaric aciduria must be considered in the differential diagnosis in dystonic patients especially in countries with high rates of consanguineous marriages. A novel mutation for *L2HGDH* is also reported in this case report.

A-031

Evaluating laboratory performance in external quality assessment schemes for urine organic acids by GCMS

Jafri L[1], Shirazi N[1], Jamil A[1], Jehan F[1], Mehar R[1], Yousufzai N[1], Afroze B[1], Khan A H[1]

[1]Aga Khan University Hospital, Karachi, Pakistan

Background: Biochemical genetics laboratory (BGL) differs from clinical chemistry laboratory in interpretation that is necessary to make its results meaningful.

Objective: To evaluate the performance of BGL for analysis/interpretation of urinary organic acid (UOA) through external proficiency testing.
Materials and Methods: BGL started participation in ERND IM since 2013 (18 samples from 3 surveys) and College of American Pathologist (CAP) (4 samples from 2 surveys) in 2014. Scoring/sample in ERNDIM is on analytical and interpretative performance and recommendations for further testing (maximum score 4).
Results: In ERNDIM, satisfactory diagnosis (score 4) was provided for 3-methyl crotonyl CoA-carboxylase enzyme deficiency, IVA, MMA, malonicacidurias, GAII, 3-MGA, L-Dopa agonist treatment, hyperphenylalaninemia, GAI and II, PPA and PKU. In a sample scored 3, majority participants were unable to associate a metabolite with diagnosis but weren't penalized indicating educational role of ERNDIM. Two samples misdiagnosed the disease. In one diagnosis of MSUD was missed due to mild excretion of metabolites and in another orotic acid peak wasn't determined by GCMS. Both the samples were challenging and posed difficulties for laboratories. In CAP, GA I, neuroblastoma, PPA and PKU were correctly identified.
Conclusion: Evaluation by CAP and ENDRIM has allowed BGL to monitor its performance and intensify their insight.

A-032

Ethylmalonic encephalopathy with a homozygous mutation in C.554T>G in the exon 5 of *ETHE1* gene

Lougani S[1], Moussa L[1], Neggazi K[1], SAADI A[1], Chaoch M[1], Tiranti V[2], Ameur Elkhedoud W[1], Bendib M[1]

[1]Neurology, Benaknoun 16000, algiers, Algeria, [2]Div Mol Neurogenetics,Carlo Besta 20126, Milano, Italy

Introduction: Ethylmalonic encephalopathy (EE) is an autosomal recessive severe metabolic disorder of infancy affecting the brain, the gastrointestinal tract and peripheral vessels, and it is caused by a defect in the *ETHE 1* gene (chromosome 19 q13.32).
Case report: We report a detailed clinical and genetic study of a **4**years old Algerian patient with ethylmalonic encephalopathy who presented with development delay, feeding difficulties, failure to thrive, microcephaly, intermittent episode of diarrhea, recurrent petechiae and ecchimoses, orthostatic acrocyanosis, tetraparesis and epilepsia. Brain MRI showed increased signal on T2 – weighted images in basal ganglia. Organic acid analysis in urine revealed increased excretion of ethylmalonic and methylsuccinic acids. The genetic sequencing of the *ETHE1* gene found a missense homozygous

mutation in c.554T>G resposnsible for the aminoacid change p.Leu185Arg in the protein.
Discussion: No more than 30 cases of EE had been described worldwide (from Mediterranean basin and the Arabic peninsula). Our patient had classical phenotype of the disease and the mutation found has already been described but in a compound heterozygote whereas in our patient this mutation is in a compound homozygote which has not been reported before.
Conclusion: Ethylmalonic encephalopathy is a rare and severe metabolic disorder; we report the first Algerian case.

A-033

The acute management in intercurrent illness of isovaleric acidemia associated with typhoid fever

Widjaja N A[1], Puspitaningtyas E[1], Irawan R[1]

[1]Ped Dept, Airlangga Univ/Soetomo Hosp, Surabaya, Indonesia

Background: Isovaleric acidemia (IVA) is characterized as abnormal metabolism resulting from a deficiency in isovaleryl-CoA dehydrogenase. In the chronic form, affected patient are at risk of episodes of acute metabolic crisis usually due to intercurrent illnesses during periods of stress catabolic in infections. Typhoid fever is an endemic disease in tropical country caused by salmonella typhii infection. During acute metabolic crisis, adequate caloric intake with glucose infusion and protein restriction are required.
Methods: Reported survival IVA case in acute episodes metabolic illness associated with Typhoid fever
Results/Case Report: A 7-year-old girl with IVA recurrent presented prolonged fever, vomiting, diarrhea and somnolens. Metabolic acidosis, increased anion gap, hyperammonemia, elevated serum lactate and elevated Typhidot-M were noted. Isovalerylcarnitine levels, and markedly increased urine isovalerylglycine concentration were noted from Shimane laboratory Japan. During acute metabolic illness the patient received high caloric glucose infusion therapy and protein restriction. Third generation cephalosporins antibiotic was administered. The patient has remained in a stable condition on dietary treatment with low leucine diet and high dose carnitine treatment.
Conclusion: Recurrent acute metabolic illness is usually present in IVA survivors. It can be caused by infections such as typhoid fever. Adequate caloric infusion therapy and protein restriction must be administered during acute metabolic crisis.

A-034

Associated Glutaric aciduria type 1 and celiac disease

Kuyum P[1], Cakar S[1], Ozturk Y[2]

[1]Dept Pediatrics, Dokuz Eylul Univ S Med, Izmir, Turkey, [2]Div Ped Metab Dis,Dokuz Eylul Univ S Med, Izmir, Turkey

Case Report: Glutaric aciduria type 1 (GA I) is a congenital metabolic disease that develops from glutaryl-coenzyme A dehydrogenase deficiency and show symptoms such as acute impaired consciousness and acquired motor skills mostly as a result of interrupting stress; spastic type cerebral palsy, choreoathetosis, dystonia or mental retardation in previously healthy child and sometimes slowly progressing motor function disorder or isolated macrocephaly.Celiac disease (CD), which is triggered by gluten exposure, is a commonly seen autoimmune enteropathy that causes diarrhea, failure to thrive. In this paper, an association of GA I and celiac disease in 4-year-old girl was reported.
Conclusion: GA I may result in severe neurological sequel after neurological attack. Neurologically affected children may have feeding problems effecting growth potentials and neurological problems and difficulties in special dietary management of GA I caused growth retardation as well as celiac disease. Association of Celiac disease and Glutaric aciduria type-1 should be kept in mind.

13. Carbohydrate disorders

A-035

Clinical, laboratory data, molecular features, and follow up of 3 Iranian GSD type IIIa patients

Zaman T[1 2], Moarefian S[1], Saeed Tehrani F[1], Moradian R[1]

[1]Iranian National Research Unit, Tehran, Iran, Islamic Republic of, [2]IEM department,Tehran Univ, Tehran, Iran, Islamic Republic of

Background: Glycogen storage disease (GSD) type III (Cori-Forbes Disease), which accounts for 24% of GSD cases, is an autosomal recessive inherited disorder characterized by fasting hypoglycemia, growth retardation, hepatomegaly, progressive myopathy and cardiomyopathy, caused by deficiency in glycogen debranching enzyme encoded by *AGL* gene, located on chromosome 1p21. There are two major subtypes: IIIa affecting both liver and muscle (80-85%) and IIIb only liver (15%). Currently, other than symptomatic management of hypoglycemia and diet intervention, there is no definitive therapy.

Methods and Patients: This is a retrospective study on the GSD III patients (2006-2014).
Results: Male/female: 2/1, consanguinity: 2/3, age at diagnosis: 16±7.2 months, first presentations: 1/3 abdominal distension, 2/3 motor delay, 3/3 hepatomegaly, 2/3 seizure, 3/3 fasting hypoglycemia:52.3±11.5mg/dL with no response to glucagon, ABG and fasting lactate: normal/ after glucose challenge: 23.6±1.5 mg/dL, high total Chol:252±41 ,CPK:881 ±808 U/L, SGOT:357±230 U/L, SGPT: 328±97 U/L and low alanine:129±10 μmol/L. abdominal sonography: 3/3, increase in liver size, normal spleen and kidneys. After 5.8±3.4 years hypoglycemia and hepatic involvement improved but mild or asymptomatic cardiomyopathy (1/3 mild HCM, 2/3 LVH) and myopathy (CPK; 1548± 1462) developed gradually. One patient was homozygous for c.1468 A>C (p.Ser490Arg) and one for c.1592C>G (p.Thr531Arg) mutation (debranching enzyme activity: 3.4 nmol/24hr/mgHb (8-34) in the last case).
Conclusion: These patients' treatment, while controlling hypoglycaemia, has had a modest effect on liver function and no effect onmyopathy and cardiomyopathy.

A-036

Galactosemia case with a novel mutation

Kilic M[1], Zenciroglu A[3], Goksun E[2], Yilmaz D Y[2], Ozgul R K[2]

[1]Div Metab Dis, Dr Sami Ulus Child Hosp, Ankara, Turkey, [2]Div Metab Dis, Hacettepe Univ Child Hosp, Ankara, Turkey, [3]Newborn Unit, Dr. Sami Ulus Child Hosp, Ankara, Turkey

Background and objectives: Classical galactosemia is an autosomal recessive disorder of carbohydrate metabolism, due to a severe deficiency of the enzyme, galactose-1-phosphate uridyltransferase (GALT).
Case report: Fourteen days old male patient was referred to our hospital with a diagnosis of cholestasis, E. Coli sepsis and suspicion of metabolic disease. At the end of first day, poor sucking and respiratory difficulty were noticed. On the second day jaundice was seen and phototheraphy was started. On the fifth day laboratory examination showed elevated levels of transaminases, total and direct bilirubin and thrombocytopenia. E. Coli was present seen in blood culture. Eye examination was normal. GALT activity was slightly reduced. Molecular analyses showed homozygous c.200delG; p.Arg67ProfsX19 mutation in *GALT* gene. Galactosemia diagnosis was made. He was well after the galactose-restricted diet.
Conclusion: This case reports a new mutation for galactosemia.

A-037

Genotype-phenotype characteristics of Turkish patients with glycogen storage disease type I at a single centre

Kilic M[1], Ozbudak P[3], Demir E[3], Kasapkara Ç S[1], Tanyildiz H G[4], Aytekin C[5], Özbay-Hosnut F[2], Sahin G[2], Karaer K[6], Ceylaner S[6]

[1]Div Metab Dis, Dr Sami Ulus Child Hosp, Ankara, Turkey, [2]Ped Gastro Dis, Dr Sami Ulus Child Hosp, Ankara, Turkey, [3]Dr. Sami Ulus Child Hosp, Ankara, Turkey, [4]Ped Hem Dis, Dr. Sami Ulus Child Hosp, Ankara, Turkey, [5]Ped Immun Dis, Dr. Sami Ulus Child Hosp, Ankara, Turkey, [6]Intergen, Ankara, Turkey

Background and objectives: Glycogen storage disease type I (GSD-I) consists of two subtypes: GSD-Ia and GSD-Ib. In this study, we aimed to show genotype-phenotype characteristics of our GSD-I patients.
Patients and Methods: Six patients with a diagnosis of GSD-I were enrolled in the study.
Results: The patients were between 5.5-30 months old. Half of the patients (3/6) were Ia. 2 were male. Consanguinity was present in 5/6. Five presented with hypoglycaemia in the neonatal period without an established diagnosis. Their second admission to the hospital with hypoglycemia was seen at average age of three-months old. The main clinical and laboratory findings were hypoglycemia, transaminitis, metabolic acidosis, hyperuricemia, hypertriglyceridemia, elevated levels of lactate, pyruvate, hepatomegaly, doll like face, neutropenia and recurrent infections. One patient had bilateral cataracts removed. All GSD-Ia patients had the same c.247C>T homozygous mutation. In GSD-Ib, two of patients had homozygous c.1042_1043 delCT, while the other had homozygous c1211_1212delCT mutation. Microcephaly and developmental delay were seen as an important chronic sequelae in patients with poor metabolic control.
Conclusion: GSD-I should be considered in the differential diagnosis of hypoglycemia patients even in the newborn period.

A-038

Hepatic glycogen storage diseases in Macedonian patients: A single centre experience

Kostovski A[1], Zdraveska N[1]

[1]Univ Child Hosp, Medical faculty, Skopje, The F.Y.R of Macedonia

Background: The aim of the study was to investigate the characteristics of hepatic glycogen storage diseases (GSD) diagnosed and followed at a single center. There are no previous reports of GSD in Macedonia.
Methods: We retrospectively reviewed the charts of patients diagnosed with GSD at Department for Gastroenterohepatology,University Children Hospital Skopje during a 5 year period (2010-2015). Clinical manifestations, laboratory results, treatment, and prognosis were analyzed.
Results: Five patients were included. The diagnosis and GSD subtypes were confirmed by enzyme activity tests and/or gene analysis. GSD I was diagnosed in 1 patient, IIIa in 1 patient, VI in 2 patients, and IXb in 1 patient. Types other than GSD I constituted 80% of the patients. The median age at presentation was 28±5.5 months. Hepatomegaly was the main clinical manifestation. Elevated transaminases were observed in all patients, and hyperlactacidemia in 80% of the patients. The duration for follow-up was 27±19.1 months. No mortality was observed and liver transplantation was performed in 20%. Molecular analysis revealed novel mutations in 3 patients.
Conclusion: Hepatic GSDs comprise significant etiology of hepatomegaly. Types other than GSD I were present in most of our patients.Clinical suspicion is initial thorough evaluation for prompt diagnosis of hepatic GSDs.

A-039

Successful dietary management of severe hyperlipidemia in patients with glycogen storage disease type 1

Kecman B[1], Djordjevic M[1], Skakic A[2], Stojiljkovic M[2], Sarajlija A[1], Grkovic S[1]

[1]Mother and Child Health Institute, Belgrade, Serbia, [2]IMGGE, Univ Belgrade, Belgrade, Serbia

Background: Key metabolic features of glycogen storage disease type I (GSD I) are recurrent hypoglycemia and lactic acidosis. However, accompanying hyperlipidemia can cause serious complications.
Case reports: Patient 1 is the boy who manifested with symptomatic hypoglycemia (1 mmol/L) at 5 months of age. Clinical and metabolic work-up revealed typical findings for GSD I. We registered extremely high serum concentrations of total cholesterol (21 mmol/L) and triglycerides (87 mmol/L). Genetic analysis in this patient revealed homozygousity for 1042_1043delCT in SLC37A4 gene so diagnosis of GSD type Ib was established. Patient 2 was admitted for hepatomegaly and skin xanthomas at 7 months of age. The girl had serum cholesterol of 40.3 mmol/L and triglyceridemia of 35 mmol/L. Homozygousity for R83C in G6PC gene confirmed the diagnosis of GSD type Ia. Administration of diet according to current guidelines for GSD I showed success in significant reduction of hyperlipidemia in both patients. Xanthomas in patient 2 have subsided over the course of several weeks.
Conclusion: Hyperlipidemia may pose substantial risk for complications in GSD I. Proper dietary treatment is essential for good metabolic control in this disease.

14. Disorders of fatty acid oxidation and ketone body metabolism

A-040

Primary systemic carnitine deficiency: two turkish cases with two novel *SLC22A5* mutations

Seker Yilmaz B[1], Kor D[1], Kucukosmanoglu O[4], Bulut D[1], Ceylaner G[2], Oktem M[3], Onenli Mungan N[1]

[1]Div Metab Dis, Univ Cukurova, Adana, Turkey, [2]Intergen Genetic Lab, Ankara, Turkey, [3]Duzen Lab, Ankara, Turkey, [4]Div Ped Cardiol, Univ Cukurova, Adana, Turkey

Background and Objectives: Systemic primary carnitine deficiency (CDSP, OMIM #212140) is an autosomal-recessive disorder of fatty acid oxidation caused by mutations in the *SLC22A5* gene, encodes the high-affinity carnitine transporter, OCTN2 expressed in muscle, heart, kidney and fibroblasts.
Case Report: Here we report two Turkish boys presented with cardiac insufficiency after an acute infection. Echocardiogram showed dilated left ventricle with reduced systolic function with myocardial thickening in both of the patients. Neurological examination including the muscle strength and reflexes were totally normal. Plasma free-total carnitine levels were extremely low. Genetic analysis for *SLC22A5* gene were performed and p.F200Lfs*4(c.597_597delG) homozygous and p.G168D(c.503G>A)/ p.F200Lfs*4(c.597_597delG) compound heterozygous mutations were detected, respectively. After confirmation of the diagnosis, they were both placed on treatment with L-carnitine (100 mg/kg/day, p.o.) plus digoxin, diuretics, and vasodilators. The treatment resulted significant improvement in the cardiac functions. Even though we could not detect the urinary carnitine levels, mild increase in the serum carnitine levels were observed with carnitine supplementation.
Conclusion: CDSP is a treatable disorder of fatty acid oxidation. In case of early diagnosis and early initiation of the treatment, favorable outcome could be provided. p.F200Lfs*4(c.597_597delG) mutation should be considered in Turkish patients with pure cardiac phenotype.

A-041

Screening for inherited metabolic disorders in patients with familial Mediterranean fever

Kiykim E[1], Aktuglu Zeybek A C[1], Barut K[2], Cansever M S[3], Zubarioglu T[1], Kasapcopur O[2], Aydin A[1]

[1]Div Nutr Metab Dis, Ist Univ Cer Med Fac, Istanbul, Turkey, [2]Div Rheum, Ist Univ Cer Med Fac, Istanbul, Turkey, [3]Cent Lab, Ist Univ Cer Med Fac, Istanbul, Turkey

Background: Familial Mediterranean fever (FMF) is an autosomal recessive auto-inflammatory disease, presenting with recurrent episodes of fever and polyserositis. Diagnosis of FMF is may be challenging especially in pediatric population. Mitochondrial fatty acid oxidation disorders and porphyrias can present with periodic abdominal and muscle pain. The aim of the present study is determine the inherited metabolic disorders in differential diagnosis of Turkish pediatric FMF patients.
Methods: 174 patients who were diagnosed as FMF enrolled the study. In all patients, a fasting dry spot blood sample was taken for acyl-carnitine analyses by tandem mass spectrometry. Fresh, light-protected spot urine test was performed for porphobilinogen screening. Second-tier test with urine organic acid analysis and urine porphyrin metabolites were performed if pathologic findings were detected in acyl-carnitine profile or in porphobilinogen screening, for confirmation.
Results: None of our patients was diagnosed with porphyria; two patients with fatty acid oxidation defect, one with multiple acyl-CoA dehydrogenase deficiency and one with possible medium-chain acyl-CoA dehydrogenase deficiency were detected during the study.
Conclusion: Our data revealed that screening for porphobilinogen for pediatric FMF patients is unnecessary, but an investigation of acyl-carnitine analyses can be helpful for the differential or additional diagnosis of FMF in developing countries.

A-042

Is there any effect of acylcarnitines on proinflammatory process in obese children

Biberoglu G[1], Derin Genc B[1], Inci A[1], Doger E[2], Okur I[1], Ezgu F S[1], Tumer L[1]

[1]Div Ped Metabolism,Gazi Univ Medical Fac, Ankara, Turkey, [2]Div Ped Endocrin,Gazi Univ Medical Fac, Ankara, Turkey

Background: Incomplete beta-oxidation process of fatty acids in mitochondria is a feature of insuline resistance. It is proposed that medium chain acylcarnitines are effective on potential activation of proinflammatory pathways. The aim of the study was to evaluate the medium chain acylcarnitine levels of obese children and to investigate whether if these acylcarnitines are biomarkers for insuline resistance in obese children or not.
Methods: This study includes 43 obese children and 40 healthy control children. Plasma acylcarnitine levels were measured by tandem mass spectrometry. Results: C10, C12, C14 acylcarnitine levels were found higher in obese children than control (p=0.009, p=0,010, p=0,016 respectively).
Conclusion: It is predicted that C10, C12, C14 acylcarnitine levels are increased in obese children.This may be due to incomplete beta-oxidation process. And these acylcarnitines may be responsible from proinflammatory process.

A-043

Screening of free carnitine and acylcarnitine status in patients with familial Mediterranean fever

Kiykim E[1], Aktuglu Zeybek A C[1], Barut K[2], Zubarioglu T[1], Cansever M S[3], Alsancak S[4], Kasapcopur O[2], Aydin A[1]

[1]Div Nutr Metab Dis, Ist Univ Cer Med Fac, Istanbul, Turkey, [2]Div Rheum, Ist Univ Cer Med Fac, Istanbul, Turkey, [3]Cent Lab, Ist Univ Cer Med Fac, Istanbul, Turkey, [4]Dep Bio, Duzen Lab Group, ıstanbul, Turkey

Background: Familial Mediterranean fever (FMF) is an autosomal recessive disease characterized by recurring self-limited serositis. Carnitine is an important molecule in cellular energy metabolism. Secondary carnitine deficiency can be detected in chronic diseases by either renal loss or increased needs. We hypothesized that FMF patients could have lower free carnitine levels than their controls due to increased need of carnitine because of recurrent auto-inflammation.
Methods: 205 patients with Familial Mediterranean fever and 50 healthy subjects were enrolled to the present study. A fasting dried blood sample was taken for performing free carnitine and acyl-carnitine ester levels with tandem mass spectrometry from children in both groups.
Results: Screening of acyl-carnitine profile revealed increased free carnitine ($p< 0,001$), C16-OH ($p< 0,05$) and C18:2-OH ($p< 0,05$) carnitine levels, while decreased C2 ($p< 0,001$), C3 ($p< 0,001$), C4 ($p< 0,001$), C5:1 ($p< 0,05$), C6 ($p< 0,001$), C8 ($p< 0,05$), C10:1 ($p< 0,001$), C10:2 ($p< 0,05$), C3DC ($p< 0,05$), C4DC ($p< 0,001$), C5DC ($p< 0,001$), C16:1 ($p< 0,05$), C4-OH ($p< 0,001$), C18:1-OH ($p< 0,01$) carnitine levels in FMF patients in comparison to control group.
Conclusion: In this study; some of acyl-carnitine profile variations were detected in FMF patients, however we were not able to define secondary carnitine deficiency, therefore usage of carnitine in all patients with FMF is not recommended.

A-044

Determination of free carnitine and acyl-carnitine status in patients with juvenile idiopathic arthritis

Kiykim E[1], Aktuglu Zeybek A C[1], Barut K[2], Zubarioglu T[1], Cansever M S[3], Alsancak S[4], Kasapcopur O[2], Aydin A[1]

[1]Div Nutr Metab Dis, Ist Univ Cer Med Fac, Istanbul, Turkey, [2]Div Rheum, Ist Univ Cer Med Fac, Istanbul, Turkey, [3]Cent Lab, Ist Univ Cer Med Fac, Istanbul, Turkey, [4]Dep Bio, Duzen Lab Group, ıstanbul, Turkey

Background: Juvenile idiopathic arthritis is the most common rheumatic disease in childhood and mainly present with auto-inflammation in joints and other tissue. Carnitine is an important molecule in cellular energy metabolism. Secondary carnitine deficiency can be detected in chronic diseases by either renal loss or increased demand. In the present study we evaluated free carnitine and acyl-carnitine status of patients with juvenile idiopathic arthritis.
Methods: 114 patients with a diagnosis of JIA and a healthy 50 individuals served as control group, were included to the study. A fasting blood sample was taken for free carnitine and acyl-carnitine esters with tandem mass spectrometry from children in both groups.
Results: Screening of acyl-carnitine profile revealed free carnitine, C14, C14:2, C16, C16-OH, and C18 carnitine levels were higher, while C2, C3, C4, C6, C8, C10, C10:1, C10:2, C3DC, C4DC, C5DC, C4-OH and C18:1-OH carnitine levels were lower in JIA patients in comparison to the control group. Total acyl-carnitine levels ($p< 0,001$) and acyl-carnitine to free carnitine ratio ($p< 0,001$) were also lower in JIA patients.
Conclusion: In the present study we were not able to define secondary carnitine deficiency in JIA patients, therefore routine carnitine supplementation is not recommended in all patients with JIA.

A-045

Challenge to detect the suspected of carnitine palmitoyl transferase-1 (CPT-1) deficiency with limited resources

Gultom L C[1], Sjarif D R[1]

[1]Div. Nutr & Metab Dis, Univ of Indonesia, Jakarta, Indonesia

Background: CPT-1 deficiency is one of fatty acid oxidation defects (FAOD) which is difficult to diagnose in limited resources.
Case: A 17-year-old boy was transferred to our division because of unknown etiology of sepsis after difficulty case discussion by Pediatric Teams. He suffered prolonged fever, recurrent abdominal pain and vomiting for a month. He also lost 3 kg in two weeks before hospitalization. These symptoms occurred 1-2 times/year, induced by fever, illness or fatigue, usually happening in the fasting month (Ramadhan), and disappeared with/without treatments. Physical examination revealed severe malnourished without hepatomegaly. There were leukocytosis, leucocyturia, recurrent metabolic acidosis with elevated anion gap, and decreased glomerular filtration rate caused by increased level of ureum and creatinine. The liver function test, procalcitonin, ammonium, and lactate levels were elevated. Creatine kinase was highly elevated but blood ketone and β-hydroksibutirate were low. Hypoglycemia was not found probably due to the used of parenteral nutrition. We suspected FAOD and Tandem MS report was strongly suspected of CPT-1 deficiency.
Conclusion: CPT-1 deficiency is established based on history of disease, clinical and laboratory findings in limited resources. In this case, the tandem MS report confirmed the diagnosis.

A-046

Two novel mutations in clinically detected MCADD

Smon A[1], Groselj U[1], Repic Lampret B[1], Zerjav Tansek M[1], Battelino T[1 2]

[1]UMC Ljubljana, Univ Child Hosp Ljubljana, Ljubljana, Slovenia, [2]Univ Ljubljana, Faculty of Medicine, Ljubljana, Slovenia

Background: First two cases of medium chain acyl-CoA dehydrogenase deficiency (MCADD) in Slovenia were recently identified.
Patients: Patients were a 15 months old girl and 20 months old boy. Based on clinical features and primary biochemical results a metabolic disorder was suspected.
Results: Analysis of organic acids in urine showed elevated metabolites typical for fatty acid oxidation defects. Hexanoylglycine was not elevated. The results of acylcarnitine analysis in dried blood spots (DBS) showed elevations of C6, C8, C10 and C10:1 in both patients, characteristic for MCADD. For confirmation, Sanger sequencing of *ACADM* was performed. Reference sequence was NM_001127328.1. The girl was a compound heterozygote with a known mutation in exon 4 (c.256dupT, p.Trp86Leufs*23) and a previously unreported very likely disease-causing variant in intron 9 of *ACADM* (c.861+2T>C). The boy was a compound heterozygote with a known mutation in exon 11 (c.997A>G, p.Lys333Glu) and a previously unreported very likely disease-causing variant in exon 7 (c.527_533del, p.Ile176Thrfs*19).

Discussion: We also measured acylcarnitines in DBS, taken from both patients at birth. The quantified analytes were compared to the 99th percentile of 4000 newborns analysed in a pilot screening programme. Patients' acylcarnitines were characteristically elevated and would be detected with expanded newborn screening.

A-047

Medium/short chain 3-hydroxyacyl-coA dehydrogenase deficiency *M/SCHAD*: A case report

Kendirci M[1], Kardas F[1], Ustkoyuncu P[1], Gokay S[1], Akyildiz B N[2], Tekerek N[2]

[1]Div Metab Dis, Erciyes Univ, Medical Fac, Kayseri, Turkey, [2]Div Intensive Care, Erciyes Univ, Med F, Kayseri, Turkey

Background: Medium/short chain3-hydroxy acyl-CoAdehydrogenase deficiency is a worldwide rare autosomal recessive disorder of beta-oxidation. The patients have hypoglycemia due to hyperinsulinemia. Encephalopathy, myoglobinuria, cardiomyopathy, hypotonia, sudden infant death, increased creatine kinase activity and dicarboxylic aciduria are common clinical findings. M/SCHAD deficiency is caused mutations in the gene encoding M/SCHAD (HADH on 4q25).
Case report: Sixteen-month-old female was admitted to our clinic with complaints of confusion, vomiting, fever and tachypnea following vaccination. She was a child of Turkish descent distant relative parents. On physical examination, mild dehydration, pallor, tachypneic respiratuar pattern and confusion were observed. Laboratory investigations revealed hypoglycemia, ketosis, increased liver enzymes and creatin kinase activity. Metabolic screening by gas chromatography mass spectrometry (GC-MS) showed significant lactic aciduria and ketosis. Acylcarnitine analysis by liquid chromatography- mass spectrometry (LC-MS/MS) demonstrated significant elevation of C4-OH acyl-carnitine to 1,62mmol/L (N: 0-0,7). The case was diagnosed as M/SCHAD deficiency. Carnitine, Coenzyme Q and riboflavin therapies were initiated and her diet was arranged. Metabolic and physical examinations were normal on follow up.
Conclusion: Finding related to SCHAD deficiency was onset at the early period of life and irreversibl problems were developed due to hypoglycemia. Early diagnosis and appropriate therapy will save the life.

A-048

Systemic primary carnitine deficiency - early treatment with a good outcome in first case from India!

Bijarnia-Mahay S[1], Deswal S[4], Hara K[3], Shigematsu Y[2], Verma I C[1]

[1]Center Med Genet, Sir Ganga Ram Hosp, New Delhi, India, [2]Dept Health Science & Pediatr,Univ Fukui, Fukui, Japan, [3]Dept of Ped, NHO Kure Med Centre,, Aoyama-Cho, Kure City, Hiroshima, Japan, [4]Dept of Ped, PGIMER & Dr RML Hospital, New Delhi, India

Background: Carnitine uptake defect across plasma membrane due to *SLC22A5* gene mutations causes a deficiency of intra-cellular free carnitine, preventing entry of long chain fatty acids into mitochondria disrupting energy production through fatty acid oxidation (FAO). Carnitine supplementation reverses harmful effects on organs like heart, muscle and liver.
Case report: We report a 9-month-old boy who presented with jaundice, failure to thrive for two months, mild motor developmental delay and frequent breathing problems since 3 months of age. The family was non-consanguineous. Previous sibling died at 2 years because of breathing problem and cardiomegaly. Proband had cardiomegaly with biventricular hypertrophy with LVEF 55%, liver dysfunction and raised triglycerides. Free carnitine level on MS/MS analysis was low (1.98 nmol/ml) with normal acylcarnitine levels. Secondary carnitine deficiency was ruled out.
Result: Genetic analysis revealed compound heterozygous mutations in SLC22A5 gene, p.Glu452Lys (previously reported), and c.231delC (novel frame-shift) confirming systemic primary carnitine deficiency (SPCD). Child responded to oral carnitine supplementation with improved LVEF to 75% and normalization of liver function and triglycerides on follow up after 3 months.
Conclusion: Accurate diagnosis and treatment improved outcome in the child. To our knowledge, this is the first report of SPCD with mutations in SLC22A5 gene from India.

A-049

A case of severe rhabdomyolysis in early childhood: *LPIN1* gene mutation

Zubarioglu T[2], Aktuglu Zeybek A C[2], Kiykim E[2], Cansever M S[3], Demirkol D[4], Aydin A[1]

[1]Div Nutr Metab, Ist Univ, Cerr, Istanbul, Turkey, [2]Div Nutr Metab Dis, Ist Univ Cer Med Fac, Istanbul, Turkey, [3]Cent Lab, Ist Univ Cer Med Fac, Istanbul, Turkey, [4]Div PICU, Koc Univ, Istanbul, Turkey

Background: Recurrent episodes of life-threatening rhabdomyolysis attacks in childhood can be caused by inborn errors

of glycogenolysis, mitochondrial fatty acid beta-oxidation and oxidative phosphorylation. However, in approximately half of the patients, any defect of these pathways cannot be detected despite detailed metabolic investigations. Autosomal recessive *LPIN1* gene mutations have recently been described as a novel cause of rhabdomyolysis. Rhabdomyolysis shows often a recurrent course of disease and a high mortality especially in early childhood period. Episodes were triggered predominantly by febrile illnesses, fastening or intense exercise.
Case report: In this report, a 4 years old female patient who was presented with severe rhabdomyolysis and followed up in a pediatric intensive care unit (PICU) was reported. Diagnostic investigations including complete blood count, liver and kidney function tests, blood lactate and ammonia levels, acylcarnitine profile by tandem mass spectrometry, blood quantitative amino acid chromatography, urine organic acid analysis were normal. Molecular analysis of *LPIN1* gene was performed in terms of the early onset of rhabdomyolysis and requirement of hospitalization in intensive care unit and a homozygote mutation was detected.
Conclusion: In conclusion, *LPIN1* mutations should be considered in any child presenting with severe rhabdomyolysis. Each episode should be aggressively treated at a center with PICU.

16. Mitochondrial disorders: nuclear encoded

A-050

PolG deficiency revealed by valproic acid in a 3-year-old child

Torre S[5], Cassinari K[1], Abily-Donval L[5], Tebani A[1], Guerrot A M[4], Gaignard P[2], Marret S[5], Slama A[2], Allouche S[3], Bekri S[1]

[1]Metab Biochem Rouen University Hospital, Rouen, France, [2]Bicêtre Hospital, AP-HP, Kremlin Bicêtre, France, [3]Caen University Hospital, Caen, France, [4]Genetics Rouen University Hospital, Rouen, France, [5]Neonatal Medecine Rouen University Hospi, Rouen, France

Background: Alpers' syndrome is a rare mitochondrial disease linked to *POLG* gene mutation. This disease affects children and is characterized by a clinical triad: psychomotor decline, drug-resistant epilepsy and hepatic failure. Patients are typically asymptomatic at birth and may have a normal development for a variable period.
Case report: A male patient born preterm to non-consanguineous parents following a pregnancy conceived after IVF presented with a malformative uropathy and a transient renal insufficiency. His height-weight and psychomotor developments were normal in the first three years of life, then an atypical absence epilepsy appeared and was treated using valproic acid (VPA). Initially, this treatment succeeded to control the seizures. But 7 months later, the patient presented with an acute neurometabolic disorder and a hepatic failure that led to death.
Results: Metabolic investigations and molecular studies concluded to POLG deficiency.
Discussion: In this patient, Alpers' syndrome was precipitated by the fulminant liver toxicity of VPA and its deleterious actions at the mitochondrial level. This fatal consequence may be due to the impairment of VPA-induced liver regeneration in POLG deficient cells. This condition should prompt us to consider the generalization of *POLG* testing prior to VPA treatment in order to identify at-risk patients.

A-051

Rare cause of congenital lactic acidosis: A case of TMEM70 - ATP synthase deficiency

Çoker M[1], Canda E[1], Güneş S[2], Altun Köroglu Ö[4], Tanyalçın T[3], Kültürsay N[4]

[1]Div Metab Dis, Ege Univ Med Faculty, İzmir, Turkey, [2]Div Neonatol,İzmir Univ MedikalPark Hosp, İzmir, Turkey, [3]Tanyalçın Laboratory, İzmir, Turkey, [4]Div Neonatology, Ege Univ Med Faculty, İzmir, Turkey

Nuclear encoded mutations in the Transmembrane Protein 70 (TMEM70) gene are a most common cause of ATP-synthase deficiency. Common symptoms include congenital lactic acidosis, hypertrophic cardiomyopathy, hypotonia, hyperammonemia, and facial dysmorphism. We presented a case with TMEM70, ATP-synthase deficiency.
A 1800 g female infant was born at 32 week of gestation to first cousin parents. The patient developed a sudden worsening of the general condition, encephalopathy and respiratory depression during her stay in intensive care unit. Lactic acidosis (blood gas LA: 11.6 mmol/L) and hyperammonemia (172 mg/dl) were found. Dysmorphic facial features (long philtrum, thin lips, and arched brows) were observed in physical examination. Echocardiography releaved hypertrophic cardiomyopathy on the seventh day of life. Urine organic acid analysis revealed 3-methylglutaconic acid, 3-OH isovalerate (mild), 2-OH isovalerate and lactic aciduria. Heterozygous c272delG(p.Asp91metfs*4) mutations of the TMEM70 gene were found in the parents. Genetic analysis of the patient is still in progress. Lactic acidosis and hyperammonemia attacks were observed in follow-up.
TMEM70 deficiency and ATP-synthase deficiency must be considered in the presence of congenital lactic acidosis attacks,

hyperammonemia, hypertrophic cardiomyopathy, dysmorphic facial features and 3-methylglutaconic aciduria. Effective treatment of acute attacks affects the prognosis.

A-052

Mitochondrial DNA depletion syndrome: A case with DGUOK mutation

Kilic Yildirim G[1], Durmus Aydogdu S[1], Tekin N[2]

[1]Osmangazi Uni, Med Fac Dept of Ped Metab, Eskişehir, Turkey, [2]Osmangazi Uni, Med Fac Dept of Ped Neona, Eskişehir, Turkey

Introduction: Mitochondrial DNA depletion syndromes are characterized with the reduction in mtDNA content and impaired energy production. Mutations in the DGUOK gene lead to hepatocerebral form with severe feeding difficulties, progressive cholestatic liver disease, hypoglycemia, lactic acidemia, and neurological abnormalities usually in the early life. We present the clinical, laboratory and genetic characteristics in a Turkish newborn with the DGUOK mutation.
Case report: A 4 day old boy patient was referred with the suspicion of metabolic disease due to poor sucking, respiratory difficulty, hypoglycemia, metabolic acidosis and hyperammonemia after circumcision operation. He was feeding with protein free diet. He had normal serum ammonia, lactic acid and mildly eleveted liver function tests on admission. He was fed with gradually increasing natural protein. Tandem MS and urinary organic acid analysis were normal. In the follow-up he developed progressive hepatopathy, coagulopathy, lactic acidosis and eleveted serum α-fetoprotein and tyrosine levels. We couldn't determine succinylacetone in the repeted urine organic acid analysis. The rotary nystagmus was developed at the age of 30 days. We determined p.E44K (c.130G>A) homozygous mutation in the DGUOK gene.
Conclusion: DGUOK gene mutations should be considered in the differential diagnosis of the patients with liver dysfunction, especially in the presence of rotatory nystagmus.

A-053

Deoxyguanosine kinase deficiency in a Turkish infant with a novel mutation

Gunduz M[1], Ozaydin E[1], Bastemur M[1], Kirsaclioglu C T[1], Unal O[1]

[1]Ankara Hematology Oncology Children's, Ankara, Turkey

Background: Deoxyguanosine kinase (DGUOK) deficiency is a frequent cause of mitochondrial DNA depletion syndrome associated with a hepatocerebral phenotype. We report a 5-month old baby diagnosed with DGUOK deficiency with a novel mutation.
Case Report: Five month-old baby was referred to us with growth retardation and cholestasis. He was the third child of a three-degree consangious marriage. The patient presented with liver dysfunction, hypotonia and nystagmus. Laboratory tests showed abnormal coagulation tests, international normalized ratio:3.2, prothrombin time:31 second, activated partial thromboplastin time: 59 second, alanine aminotransferase (ALT):127 U/L, aspartate aminotransferase (AST):531 U/L, γ-glutamyltransferase (GGT):130 U/L. Tandem-mass spectrometry revealed elevations of alanine and tyrosine. Urine organic acid analysis showed elevated lactate level. Blood lactate levels were 38-50 mg/dl (N: 4.5-19.8). Transferrin isoelectric focusing was normal. Urinary succinylacetone level was negative. Serum α-fetoprotein level was >5400 ng/ml.
Conclusion: This novel mutation in the DGUOK gene was related with poor prognosis. MRI findings and nystagmus in an infant with liver failure should lead the clinicians to the diagnosis of DGUOK deficiency.

17. Mitochondrial disorders: mtDNA

A-054

A case with Kearns Sayre Syndrome and definition of an atypical mutation

Köse M[1], Canda E[1], Kagnıcı M[1], Bozkurt Gökçe S[2], Erturk L[1], Kalkan Ucar S[1], Kalkan Ucar S[1], Ceylaner S[1], Ozyurek R[3], Çoker M[1]

[1]Div Metab Dis, Ege Univ Med Faculty, İzmir, Turkey, [2]Div Pediatrics Dis, Ege Univ Med Fac, İzmir, Turkey, [3]Div Cardiol Dis, Ege Univ Fac, İzmir, Turkey

Kearns-Sayre Syndrome (KSS) is a mitochondrial disorder characterized before the age of 20 years by the emergence of progressive external ophthalmoplegia, pigmentary retinopathy, with other heterogeneous clinical manifestations. A 7-year-old male presented to our emergency department with syncope. Electrocardiogram revealed complete AV Block. Further physical examination revealed ataxic gait and dropping eyes with diplopia. Ophtalmological studies showed retinitis pigmentosa. Suspecting KSS, we performed mitochondrial DNA molecular genetic analysis. A deletion of 1.3 kb in RNR1-RNR2 was detected in leucocytes which was shorter than expected. It is planned to show mitochondrial heteroplasmy level especially in muscle tissue however due to patient's pace-maker and high risks of anesthesia it could not

be performed. Generally, mitochondrial DNA deletions were associated with KSS but the size and position of these deletions differ among patients. Illustrating this, we described a patient who has typical features of KSS but carrying an atypical short deletion of mitochondrial DNA.

A-055

Mitochondrial respiratory chain disorders in the Old Order Amish population

Vockley J[1], Ghaloul Gonzalez L[1], Goldstein A[1], Dobrowolski S[1], Walsh Vockley C[1], Irani A[1], Wayburn B[1], Morton H[2]

[1]University of Pittsburgh, Pittsburgh, United States, [2]Clinic for special children, Strasburg, United States

Background: Mitochondrial DNA mutations have not previously been reported in an Old Order Amish community. We describe an Amish family with the *MTTL1* mitochondrial gene mutation m.3243A>G that causes MELAS syndrome.
Case Report: The first patient from the Mercer County Amish community presented at age 15 years with 74% heteroplasmy in saliva. Several members of the maternal pedigree exhibited variable clinical problems consistent with m.3243A>G, but had not been genetically evaluated.
Methods: Targeted assessment was performed for m.3243A>G using high resolution melt profiling on urine and blood from 15 family members.
Results: The mutation was found in 13 individuals. Heteroplasmy levels were higher in urine than in blood, but similar in saliva and blood. Heteroplasmy in a 2 year old was similar in blood and urine supporting a decline in mutation levels in blood with time. The degree of heteroplasmy in urine correlated more closely with clinical severity, concurring with recommendation of urine as the best non-invasive sample to measure m.3243A>G heteroplasmy.
Discussion: Our findings represent the first report of mitochondrial respiratory chain disorders in the Amish community, and suggest that it may be under diagnosed. Testing for these disorders is warranted in Amish individuals with suggestive symptoms.

A-056

Clinical variability of mitochondrial encephalopathy, lactic acidosis, and stroke-like (MELAS) syndrome

Lee S[1], Yu J[1]

[1]Dep Pediatr, Dankook Univ Hosp, Cheonan, Korea, Republic of

Background: Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome is a subgroup of mitochondrial encephalomyopathy. The early clinical manifestations of MELAS is variable, therefore it is important to suspect the disease in a patient with multiple organ dysfunction. Here are two cases presenting MELAS syndrome, but the early symptoms and outcomes are different.
Case reports: Case 1 was brought to the hospital with the respiratory arrest and asystole. After resuscitation, vital sign were recovered, but his cognition and motor function were severely damaged. Brain MRI and muscle biopsy confirmed MELAS syndrome, although extensive genetic tests did not reveal any mitochondrial mutations. Past medical history showed that he had a failure-to-thrive, deafness, and optic atrophy.
Case 2 was a male patient with epilepsy. Two years after the diagnosis, severe headache and blurred vision occurred. On examinations, there was a left homonymous hemianopsia with consistent MRI abnormality and mildly elevated lactate. Genetic test confirmed the MELAS syndrome caused by the mutation m.3243 A>G. After the diagnosis he suffered several stroke-like episodes, but his general condition is good with normal daily activity.
Conclusion: The early diagnosis and proper education with supportive care may be very important for the better outcome of patients with MELAS syndrome.

A-057

Mitochondrial hepatopathy as an unusual presentation of mtDNA mutation

Kathom H[1], Avdjieva-Tzavella D[1], Mihailova S[2], Tincheva R[1]

[1]Clin Gen Dep, Univ Hosp of Paed, Sofia, Bulgaria,
[2]Dept Clin Immun, Sofia, Bulgaria

Background: Mitochondria are cellular organelles responsible for energy production. Since they are under dual control of nuclear DNA and mitochondrial mtDNA, mutations in genes of both genomes are associated with inherited mitochondrial disorders. In general, clinical manifestations include multisystem involvement such as brain, muscle, heart, or kidney, with acute or chronic liver dysfunction, sometimes in the presence of lactic acidosis.
Methods: Case study of a 3y 5mo old female with initial presentation of lactic acidosis and disproportionate central nervous system (CNS) involvement. In 18-months period of clinical observation, the child presented many times only with lactic acidosis. In the last 8 months a mild chronic liver failure

was present in times, and between acute episodes. Direct sequencing of mtDNA and NGS analysis of 139 mitochondrial nuclear genes was performed.
Results: The patient was found to be carrier of a heteroplasmic mtDNA alteration at 5277T>C, in a gene that encodes ND2 subunit of NADH dehydrogenase. NGS analysis found heterozygous SCO1:c.590C>T variant and heterozygous deletion of 3 nucleotides C8ORF38:c.557_559delTTT.
Conclusion: Mitochondrial hepatopathies are usually the result of mutations in nuclear mitochondrial genes, while in our case we have a late presentation of chronic liver disease as a result of mtDNA mutation.

A-058

Patient with *DGUOK* gene mutation diagnosed by using new generation sequence technology scanning 500 metabolic diseases

Okur I[1], Inci A[1], Tumer L[1], Olgac A[1], Sari S[2], Ciftci B[1], Topcu A B[1], Turkyilmaz C[3], Ezgu F S[1]

[1]Dept Ped Metab Nutr, Gazi Univ Hosp, Ankara, Turkey, [2]Dept Ped Gastroenter, Gazi Univ Hosp, Ankara, Turkey, [3]Dept Neonatology, Gazi Univ Hosp, Ankara, Turkey

Background: Deoxyguanosine kinase (DGUOK) deficiency is a frequent cause of mitochondrial DNA depletion syndromes and characterized by progressive liver failure, hypotonia, nystagmus and neurologic impairment.
Case report: A male child was born 2240 g and evaluated in our neonatal intensive care unit as intense family history of suspected metabolic diseases. Physical examination was unremarkable. Tandem MS and plasma amino acid chromatography showed increased methionine, phenylalanine, tyrosine, alanine levels. The enzyme analysis and genetic tests for galactosemia and tyrosinemia were all normal. Ferritin was 543 mg/dl, respectively. Ophtalomological examination revealed bilateral cataract. Cholestasis and progressive liver failure developed. Liver and pancreas magnetic resonance imaging revealed no iron overload accumulation and liver biopsy showed microvesicular steatosis. Lactate was elevated to 14.9 mM (n≤ 2 mM). Since our patient was not diagnosed by a thorough biochemical and molecular analysis, we used next generation sequencing technology (NGST) to investigate 500 inborn diseases of metabolism in a chip and found DGUOK mutations.
Discussion: He would be the first case of DGUOK deficiency diagnosed by NGST. This method has provided the investigation of 500 inborn errors of metabolism in a one chip.

18. Other disorders of energy metabolism, creatine disorders

A-059

Mitochondrial deficiency in two siblings with Cockayne syndrome

Mexitalia M[1], Susanto J C[1], Utari A[1], Purwanti A[1]

[1]Pediatrics Dep, UNDIP / Dr. Kariadi Hosp, Semarang, Indonesia

Background: Cockayne syndrome (CS) is a rare autosomal recessive disorder characterized by growth failure, impaired development of the nervous system, photosensitivity, and premature aging. Recent study has implicated mitochondrial dysfunction in the pathogenesis of this disease.
Methods: To describe two siblings with CS type I and some characteristics of mitochondrial disfunction.
Results / Case Report : The characteristics of the children were dwarfism with microcepahly and progeria-like appearance of face. The first child (A) was boy 9.5 year old and his brother (B) was boy 4.5 year. Both of them had normal fetal growth until 2 years old, then the growth were failure and abnormality were revealed. A's weight was 8.2 kg, height 93.5 cm, meanwhile B's weight was 8.4 kg and height 85 cm. They had severe mental abnormality, xeroderma pigmentosum in skin with photosensitivity, pigmented retinopathy, and deafness. Laboratory findings revealed metabolic acidosis with increased of anion gap and lactic acidosis. Their MRI revealed calcification of bangsal galia. They were given nutritional therapy with isocalorie milk, bicarbonate, CoQ10, and L-carnitine.
Conclusion: The prognosis of Cockayne syndrome was poor due to the progressively degenerate of central nervous systems until death in the first or second decade of life.

20. Lipid and lipoprotein disorders, porphyrias

A-060

Abetalipoproteinemia: A case report

Kilic Yildirim G[1], Durmus Aydogdu S[1], Eren M[2], Tekin T[3]

[1]Osmangazi Uni, Med Fac Dept of Ped Metab, Eskişehir, Turkey, [2]Osmangazi Uni, Med Fac Dept of Ped Gastr, Eskişehir, Turkey, [3]Osmangazi Uni, Med Fac Dept of Ped, Eskişehir, Turkey

Introduction: Abetalipoproteinemia is a rare autosomal recessive inherited disorder of lipoprotein metabolism with

multisystem involvement. The mutations in the microsomal triglyceride transfer protein (*MTP*) gene led to severe deficiency of apoB-containing lipoproteins. The disease is characterized by failure to thrive, fat malabsorption, acanthocytosis, reduced plasma levels of TC, LDL-C and apoB in infancy. We presented a patient who was diagnosed abetalipoproteinemia with the symptoms of growth retardation and chronic diarrhea.
Case report: Twenty-two months old female patient was presented symptoms with inadequate weight gain and chronic diarrhea. Her weight was < 5.p, she had normal ophthalmological and neuromuscular findings. Hemoglobin was 10.8 g/dl, peripheral blood picture revealed acanthocytes. Serum transaminases were mildly elevated. TC 68 mg/dl, HDL-C 62 mg/dl, LDL-C 1 mg/dl, triglyceride 7 mg/dl and apoB 2 mg/dl. There was no fat in the stool. She had low level of serum vitamin A, normal vitamin E and D. Lipid profiles of parents were normal. Genetic analysis showed c.309_309delA/p.T618Nfs*7 and c.1852-1853insA compound heterozygous mutations in the *MTP* gene. Medium chain fat rich diet and fat-soluble vitamins treatment was started.
Conclusion: Because abetalipoproteinemia is preventable disease, it should be considered in the etiology of the patients with growth retardation and chronic diarrhea.

21. Peroxisomal, sterol and bile acid disorders

A-061

X-linked dominant condrodysplasia punctata: clinical phenotype in an affected female

Plaiasu V[1], Ochiana D[1], Motei G[1], Coltoiu A[2]

[1]Genetics Department-IOMC Alfred Rusescu, Bucharest, Romania, [2]Dermatology Div-IOMC Alfred Rusescu, Bucharest, Romania

Background: Conradi-Hünermann-Happle syndrome, or X-linked dominant chondrodysplasia punctata, is a rare genetic disorder characterized by skeletal dysplasia, stippled epiphyses, cataracts, transient ichthyosis and follicular atrophoderma in a mosaic pattern. Mutations in the gene encoding the emopamil-binding protein (*EBP*) have been identified as an underlying cause. An abnormal sterol profile with increased levels of sterol precursors 8(9)-cholestenol and 8-dehydrocholesterol is associated with this disorder.
Case report: Our patient was referred for examination to the Division of Genetics at age of 4 years 9 mo. The girl is the third child of a nonconsanguineous family, with facial dysmorphism, dental dystrophy, prenatal and postnatal growth failure, alopecia, abnormal skin pigmentation on the trunk and legs, with ichthyosis, without body asymmetry. Investigations revealed normal peripheral blood karyotype, normal ophthalmologic exam, radiographs were found to reveal stippled epiphyses and abnormal vertebral bodies. Sterol analysis and molecular investigation were not performed.The clinical and radiographic findings of this case followed a course consistent with chondrodysplasia punctata.
Conclusions: This case presentation highlights the evolution of clinical findings over time in this X-linked dominant form of chondrodysplasia punctata.*Acknowledgments*: *This work was supported by Objective 3.3 of Romanian Ministry of Health Program VI and by PN-II-PT-PCCA-2013-4-133 grant*

22. Lysosomal disorders: mucopolysaccharidoses, oligosaccharidoses

A-062

p.258delY: A novel mutation of *IDUA* gene with atypical cardiac involvement in an infant with Mucopolysaccaridosis-I

Ersoy M[1], Akyol M B[2], Yaroglu Kazancı S[3]

[1]Child Metab Dis,Sadi Konuk Res&Tra Hosp, Istanbul, Turkey, [2]Div Pedi Cardio, Sadi Konuk Res&Tra Hosp, Istanbul, Turkey, [3]Depart Pedia. Sadi Konuk Res&Tra Hosp, Istanbul, Turkey

Background and objectives: Mucopolysaccharidosis type I (MPS-I) is an autosomal recessive disorder caused by deficiency of α-l-iduronidase which leads to a wide spectrum of clinical severity due to to accumulation of the heparan / dermatan-sulphate. Cardiovascular involvement which includes hypertrophic cardiomyopaty, thickened valves, coronary artery disease is the major cause of death.
Case report: A 3-month-old boy was referred to hospital to identify the etiology of cyanosis which was developed during feeding. She had coarse face, her liver was enlarged. Cardiac auscultation revealed a grade II /VI pansystolic murmur . The blood pressure upper extremities were 110/79 mm/Hg right and 101/43 mm /Hg left, while the lower extremities were 99/88 mm/Hg right; 98/44 mm/Hg left, respectively. ECHO showed coarctation of arcus and decenden aorta in addition to thickening of the mitral valve with left ventricular hypertrophy. Cardiac angiography showed left ventricular trabeculation without coronory anomaly. A diagnosis of Hurler syndrome was established on the basis of the deficient leukocyte α-L-iduronidase enzyme activity, and clinical features. The molecular analysis

revealed a novel homozygous deletion in exon 6 of *IDUA* gene (p.Y258del) c[772_774delTAC];[772_774delTAC].
Conclusion: We demonstrate the first case of MPS-I with non-compaction cardiomyopathy together with aorta coarctation due to a novel mutation.

A-063

A carrier detection of Hunter syndrome (MPSII) in Mongolia

Doljoo Z[1], Tumurkhuu M[1], Jav S[1], Ichinkhorloo P[1], Puntsag M[2], Tsedenbal N[1], Bazarragchaa M[1]

[1]Dep Cellular Biology,Biochemistry, MNUMS, Ulaanbaatar, Mongolia, [2]Dep Molecular Biology, Genetic, IVM, Ulaanbaatar, Mongolia

Background**:** Mucopolysaccharidosis type II is a X chromosomal recessive disorder caused by deficiency of iduronate-2-sulfatase. *IDS* gene is located in Xq28 consisting of 9 exons, and small deletions and point mutations account for 50% of all cases. A determination of carriers is very essential in genetic counseling.
Method**:** Iduronate-2 sulfatase enzyme activity was checked by tandem mass spectrometry. *IDS* gene mutation was analyzed in Hunter syndrome patients by direct sequencing. We used MLPA assay to detect X chromosome deletion in a patient for whom we could not amplify *IDS* gene.
Results**:** Iduronate-2 sulfatase deficiency was detected in three patients. p.R468W mutation leading to a replacement of arginine to tryptophan was detected in one patient. His mother carries the mutant allele, as well as his three sisters. MLPA assay could not detect large deletions in one patient, suggesting small deletion in the patient.
Conclusions**:** This is a first genetic analysis of MPS cases in Mongolia. We detected p.R468W in the patient with severe phenotype of Hunter syndrome. We also confirmed four female cases of this patient's family as carriers.

A-064

Long-term clinical course of a patient with mucopolysaccharidosis type IIIB

Lee J H[2], Kim J H[1]

[1]Div Ped, Hanyang Univ Guri Hosp, Guri, Korea, Republic of, [2]Div Ped, Samsung Changwon Hosp, Changwon, Korea, Republic of

Case report: Mucopolysaccharidosis type III (MPS III) is a rare genetic disorder caused by lysosomal storage of heparan sulfate. MPS IIIB results from a deficiency of the enzyme alpha-N-acetyl-D-glucosaminidase (NAGLU). Affected patients show behavioral changes, progressive profound mental retardation, and severe disability since 2 to 6 years. We reported a case of a patient with MPS IIIB with a long-term follow-up duration. He showed normal development until 3 years, subsequently he presented behavioral change, sleep disturbance, and progressive motor dysfunction. He has been hospitalized due to recurrent pneumonia and epilepsy with severe cognitive dysfunction. The patient has compound heterozygous c.1444C>T (p.R482W) and c.1675G>T (p.D559Y) mutation. Since individuals with MPS IIIB have less prominent facial features and skeletal changes, long-term clinical course for diagnosis is important. Although there are no effective therapies yet in MPS IIIB, early and accurate diagnosis can offer important information for family planning in at-risk families.

A-065 – moved to P-737

A-066 – moved to P-738

A-067

Clinical and radiologic findings of Morquio A disease and spondylo-epi-metaphyseal dysplasia of two prepubertal girls

Mexitalia M[1], Wahyuni F M[2], Susanto J C[1], Purwanti A[1]

[1]Pediatrics Dep, UNDIP / Dr. Kariadi Hosp, Semarang, Indonesia, [2]Radiology Dep, UNDIP / Dr. Kariadi Hosp, Semarang, Indonesia

Background: Spondylo-epimetaphyseal dysplasia (SEMD) are heterogeneous group of disorders characterized by the abnormality of vertebral, ephyseal and metaphyseal. One of the differential diagnosis of SEMD is Morquio syndrome.
Method: To differentiate clinical and radiological findings between SEMD and Morquio A in 2 prepubertal girls.
Results / Case Report: Case 1 was 11 years girl with mildy coarse facies, progressive musculoskeletal abnormality including kyphoscoliosis and pectus carinatum, without mental retarded. Radiological studies revealed oar shape ribs, flared iliac wing with inferior tapering and steep acetabular roof, proximal pointed of metacarpals and platyspondyly. Biochemical testing revealed large glycosaminoglycans and low galactose-6-sulphate sulphatase activity, confirming diagnosis of Morquio A. Enzyme replacement therapy (ERT) could not be given due to the cost. Case 2 was 13 years girl with mental

retarded and deafness, juvenille cataract, myopia and retinal detachment, waddling gait, short trunk, genu valgum, kyphoscoliosis, and spared hand-feet. Radiological showed prominent metaphyseal flaring with epiphyseal dysplastic of the long bones and sclerotic, platyspondyly in cervical and thoracal region, and hypoplasia of iliac wing. Biochemical testing showed mild elevated glycosaminoglycans. The diagnosed was suspected Strudwick type SEMD.
Conclusion: Both diseases were very rare. Prompt investigation to establish the diagnosis should be made, and start ERT if possible.

A-068

A novel aspartylglucosaminuria mutation in a patient with co-existence of Gaucher disease

Kiykim E[1], Aktuglu Zeybek A C[1], Gorukmez O[3], Zubarioglu T[1], Gunes S[4], Cansever M S[2], Aydin A[1]

[1]Div Nutr Metab Dis, Ist Univ Cer Med Fac, Istanbul, Turkey, [2]Cent Lab, Ist Univ Cer Med Fac, Istanbul, Turkey, [3]Div Med Gen, Sev Yil Tra Res Hosp, Bursa, Turkey, [4]Dep Ped, Ist Univ Cer Med Fac, Istanbul, Turkey

Background: Lysosomal storage disorders (LSDs) are a group of rare inherited metabolic disorders. The clinical phenotypic spectrum encompasses overlapping features of variable severity with diagnostic challenges especially if two diseases appear in the same patient.
Case report: A $3^{8/12}$ years old boy was the second born child of consanguineous Turkish parents. He had recurrent respiratory infections, gingivitis, diarrhea, hepatosplenomegaly, hypertelorism, broad short nose, micrognathia and mild mental retardation, initially diagnosed as Gaucher disease (GD). *GBA* gene analysis was performed by using direct sequencing and a homozygote missense mutation c.1223C>T/p.Thr408Met was defined. The patients' complaints, physical examination and laboratory findings that could not be explained solely by GD led other lysosomal storage disorders to be considered as differential diagnosis. Aspartylglucosaminuria was considered as an additional diagnosis and deficient enzyme activity was detected (aspartylglucosaminidase: 0 umol/l.h; Normal range: 10-60 umol/l.h). Direct sequencing revealed a novel homozygous frameshift mutation c.1017_1018delTG in the *AGA* gene.
Conclusion: Two rare inherited metabolic disorders can be found in a single patient especially in populations with high consanguinity rates. Additional diseases should also be considered in patients with indistinguishable clinical findings that could not be explained by a single disease especially in case of parental consanguinity.

A-069

Mucopolysaccharidosis type VI Maroteaux-Lamy Syndrome: A rare case report

Juliaty A[1 2]

[1]Pediatrics Department Hasanudin Universi, Makassar, Indonesia, [2]Pediatrics Department Airlangga Universi, Surabaya, Indonesia

Background: Mucopolysaccharidosis type VI (Maroteaux-Lamy syndrome) is a rare autosomal recessive disorder, a lysosomal storage disease that is characterized by changing of facial feature, systemic clinical manifestations and significant functional impairment. Diagnosis in mucopolysaccharidosis type VI is often delayed.
Methods: To describe a rare case of mucopolysaccharidosis type VI (Maroteaux-Lamy Syndrome), affecting a 12-year-old Indonesian child.
Results/Case Report: A boy, twelve years old, with a dysmorphic face, facial coarseness and growth retardation that was noted from the age two years old. Opacification of the bilateral cornea, high arch palate, enlarged protruded tongue, abnormal dentition, contracture claw hands, hepatomegaly and hernia scrotalis permagna was observed. A diastolic heart murmur, with cardiomegaly and cardiac valves disorder was revealed from cardiac examination. Laboratory results from the Biochemical genetic laboratory at National Taiwan University revealed large glycosaminoglycans excretion in urine, enzyme assay in leukocytes revealed a specific deficiency in arylsulfatase B activity. Thus the diagnosis of mucopolysaccharidosis type 6 (Maroteaux-Lamy Syndrome) was confirmed.
Conclusion: Early detection of the disease and appropriate management through a multidisciplinary approach is recommended to improve the quality of life.

A-070

A case of α-mannosidosis

Canda E[1], Köse M[1], Kağnıcı M[1], Kalkan Ucar S[1], Çoker M[1]

[1]Div Metab Dis, Ege Univ Med Faculty, Izmir, Turkey

Background: α-mannosidosis is a rare autosomal recessive lysosomal storage disorder. Defective α-mannosidase activity causes a lysosomal accumulation of mannose-rich oligosaccharides. Main clinical features include severe mental retardation, deafness, cataract, dysostosis multiplex and hepatosplenomegaly.

Case report: A 3-year old female patient born to first cousin parents presented with neuromotor developmental delay. In physical examination, her weight was in 3-10 percentile, height was in 3-10 percentile, head circumference was in 75-90 percentile, and coarse facial features, moderate hepatomegaly and severe neuromotor developmental delay. The examination of the skeletal system revealed findings compatible with dysostosis multiplex. Bilateral hearing loss was found. Brain MRI showed hyperintense areas in bilateral deep parieto-occipital white matter. α-mannosidase enzyme activity was found low (2.6 μmol/g/h; N: 100-800). A homozygous c.283G>C p.(Ala95Pro) mutation was found in the *MAN2B1* gene.
Conclusion: Although α-mannosidosis is a rare disorder, it must be considered for patients with dysmorphic findings, developmental delay, skeletal deformities and organomegaly. The successful outcomes of enzyme replacement therapy raise hopes in the treatment of patients.

A-071

Growth curves in mexican children with mucopolysaccharidosis I receiving enzyme replacement therapy

Ruiz-Cruz E D[1], Márquez-Gutierrez M A[1], Vega-Ramírez M E[2], Franco-Ornelas S J[2]

[1]Div of Med Genet, CMN La Raza, IMSS, Mexico, Mexico, [2]Div Intern Med Pediatr, CMN la Raza IMSS, Mexico, Mexico

Background and Objectives: Mucopolysaccharidosis I (MPS I) is caused by a genetic deficiency of lysosomal α-iduronidase. The disease occurs in severe infantile type (Hurler) and attenuated types (Hurler/Scheie and Scheie). Short stature is commonly observed, this may be attributed to a combination of structural, metabolic and endocrine abnormalities. We present growth curves of 5 children treated with enzyme replacement therapy (ERT).
Patients and Methods: Four patients had a classical Hurler form and one had an attenuated Hurler/Scheie phenotype. ERT was initiated at age from 2 to 7 years. The curves were generated for: weight for age, height for age; weight for height; BMI for age; head circumference for age; height velocity (cm/year/age)
Results: Only one patient maintained a height and weight within standard growth percentiles, while other patients had severe stunting.
Conclusions: According to the literature patients with Hurler who received ERT grow below average, whereas those with type I are below expectations for their age.

A-072

Mucopolysaccharidosis Type VII at an Early Age: A good candidate for investigational enzyme replacement therapy

Karaoglu A[1], Ezgu F S[2], Biberoglu G[2], Olgac A[1], Inci A[1], Okur I[1], Tumer L[1]

[1]Gazi University Hospital, Ankara, Turkey, [2]Div Pediatr Met Dis, Gazi University, Ankara, Turkey

Introduction: Mucopolysaccharidosis type VII (MPS VII, Sly syndrome), is a rare autosomal recessive lysosomal storage disease. The deficiency of lysosomal β-glucuronidase enzyme activity leads to the accumulation of glycosaminoglycans (GAGs). Two month old girl presented with hyperpigmentation on hands, edema of feet and diagnosed as MPS VII. The early age of this patient was considered as a good candidate for investigational enzyme replacement therapy (ERT).
Case report: Two month old infant presented with complaints of hyperpigmentation of hands, edema of feet. Her mother had three miscarriages and parents were non- consanguineous. Her physical exam revealed caput quadratum, rhizomelic shortening of limbs, hyperpigmentation on hands, and edema of feet. Her enzyme activity for lysosomal β-glucuronidase showed 0.69 (129.9 ± 45.8) nmol/saat/mg protein which was very low. And MPS 7 was diagnosed.
Discussion: ERT is available for MPS I, II, IVA and VI. Fow et al. reported a 12 year old boy with MPS VII who was treated with investigational ERT. We assume that our patient will benefit from this ERT because of her early age. As there is no effective treatment for MPS VII, this investigational ERT will provide us to effectively screen the patient for outcome and adverse reactions of the treatment.

A-073

Bilateral tarsal tunnel syndrome in mucopolysaccharidosis type VI

Barth A L[1], Silva D S[1], Esposito A C[1], Bellas A R[1], Salomão J F M[1], Horovitz D D G[1], Llerena J C[1]

[1]Instituto Fernandes Figueira/Fiocruz, Rio de Janeiro, Brazil

Background: Tarsal Tunnel Syndrome (TTS) is a compressive peripheral neuropathy of the tibial nerve or one of its branches. Patients report numbness, burning or tingling in the toes, sole of the foot or heel. This may be accompanied with nocturnal awakening, worsening of symptoms as day goes on or after prolonged standing or walking. Diagnosis is based on history

and clinical examination; imaging exams suggest and electrophysiologic studies confirm the diagnosis.
Patient: Our mucopolysaccharidosis-VI (MPS-VI) patient was diagnosed at 2yrs8mo and started enzyme replacement therapy (ERT) 3 months later. She presented cervical compressive myelopathy at 5yrs6mo, and carpal tunnel syndrome at 7yrs. At 8yrs, complaints about tingling in the soles and pain on the heels after short distances began. Physical examination showed positive Tinel's sign and worsening of symptoms after feet dorsiflexion. Electrophysiologic study revealed focal neuropathy of posterior tibial nerves on ankle region confirming bilateral TTS. Patient underwent surgical intervention for nerve entrapment release. This exemplifies another peripheral compressive neuropathy, rather than the well known carpal tunnel syndrome, in a MPS-VI patient.
Conclusion: Patients under ERT are living longer and with better quality of life, giving time for glycosaminoglycans to deposit in other structures not commonly reported, inducing new symptoms in MPS patients.

A-099

Biodistribution of idursulfase in cynomolgus monkeys after intrathecal-lumbar administration

Chung J K[1], Mascelli M A[1], Brown E[1], Crooker R[1], Palmieri K J[1], McCauley T G[1]

[1]Shire, Lexington, MA, United States

Background: Enzyme replacement therapy with intravenous idursulfase (recombinant iduronate-2-sulfatase [I2S]) is approved for the treatment of Hunter syndrome. Intravenous administration does not, however, treat the neurological manifestations, due to its low CNS bioavailability. Intrathecal-lumbar (IT-L) administration of I2S delivers it directly to the CNS. This study investigates the CNS biodistribution of IT-L administered I2S in cynomolgus monkeys.
Methods: Twelve monkeys were administered I2S in one 30 mg IT-L injection. Brain, spinal cord, liver and kidneys were collected for I2S concentration and enzyme activity measurement at 1,2,5,12,24 and 48 hours following administration.
Results: Tissue ELISA confirmed I2S uptake to the brain, spinal cord, kidneys, and liver in a time-dependent manner. In spinal cord and brain, I2S appeared as early as 1 hour following administration, and peak concentrations were observed at ~2 and ~5 hours. I2S appeared in liver and kidneys 1 hour post IT-L dose with peak concentrations between 5 and 24 hours. Liver I2S concentration was ~10-fold higher than kidney. The enzyme assay confirmed intracellular I2S bioactivity.
Conclusions: The I2S localization and enzyme activity in the CNS, following intrathecal administration, demonstrates that IT-L treatment with I2S may be considered for further investigation as a treatment for Hunter syndrome patients with neurocognitive impairment.
Conflict of Interest declared.

23. Lysosomal disorders: sphingolipidoses

A-074

A novel mutation in a patient with early infantile type GM1 gangliosidosis

Erdöl S[1], Dorum S[1], Saglam H[1]

[1]Uludag Univ, Div Metab Dis, Bursa, Turkey

Background: GM1 gangliosidosis is a sphingolipid metabolism disorder due to lysosomal acid β-galactosidase deficiency. Specific oligosaccharide pattern in urine and decreased β-galactosidase enzyme activity in white blood cells (WBCs) are important for diagnosis. There is no currently available effective therapy.
Case Report: Hypotonia, failure to thrive, a mild hepatosplenomegaly, cherry red spot and a mild dysostosis multiplex were detected in a girl with mild hyperphenylalaninemia at the age of 3 months. Urinary oligosaccharides pattern were consistent with GM1 gangliosidosis and beta-galactosidase activity was extremely low in WBCs [3.6 mmol/gram/h (N=100–400)]. A GLB1 gene analysis showed a p.M480V (c.1438A>G) (homozygous) mutation, which was previously unidentified. This mutation was considered as the cause of disease according to the Polyphen-2, SIFT, and mutation Taster data.
Conclusion: Mutation analysis is an important tool in confirming the diagnosis of metabolic diseases and in genetic counseling. We reported this novel mutation in this case with GM-1 gangliosidosis as a contribution to the literature.

24. Lysosomal disorders: others

A-075

Two cases of Pompe's disease

Kilic M[1], Tokgoz-Cuni B[2], Polat E[2], Ertugrul I[3], Akcaboy M[2], Zorlu P[2]

[1]Div Metab Dis, Dr Sami Ulus Child Hosp, Ankara, Turkey,
[2]Dr. Sami Ulus Child Hosp, Ankara, Turkey,
[3]Ped Card Dis, Dr. Sami Ulus Child Hosp, Ankara, Turkey

Background and objectives: Pompe disease is a lysosomal storage disorder in which acid alpha-glucosidase is deficient or absent.
Case report:
Case 1: Six months old male patient was referred to hospital with respiratory difficulty. He was observed and treated in the intensive care unit. On physical examination, hypotonia, hepatomegaly, respiratory failure were seen. Laboratory examination showed elevated levels of ALT, AST, CK. Cardiomegaly was seen on telecardiography. Echocardiography showed left ventricular hypertrophy and hypertrophic cardiomyopathy. α-glucosidase level was 0.1 μmol/l/h (>3.3). A homozygous mutation (c.2662G>T; p.E888*) was found on the *GAA* gene. He died in spite of all supportive treatment before starting ERT.
Case 2: Four months old male patient was referred to hospital with respiratory difficulty. He was in the intensive care unit. On physical examination, hypotonia, hepatomegaly, respiratory failure were seen. Laboratory examination showed elevated levels of ALT, AST, CK. Cardiomegaly was seen on telecardiography. Echocardiography showed left ventricular hypertrophy and hypertrophic cardiomyopathy. α-glucosidase level was 0 μmol/l/h (>3.3). The mutations c.1064T>C (p.Leu353Pro) and c.2262G>T (p.Glu888Term) were found on the *GAA* gene. Enzyme replacement therapy was started with supportive therapy.
Conclusion: Pompe disease should be considered in the differential diagnosis of infant patients with respiratory failure and hypotonia

A-076

Cases of neuronal ceroid lipofuscinosis

Çoker M[1], Canda E[1], Kağnıcı M[1], Köse M[1], Tosun A[2], Polat M[3], Kalkan Ucar S[1]

[1]Div Metab Dis, Ege Univ Med Faculty, İzmir, Turkey, [2]Div Ped Neurol, Adnan Menderes Univ, Aydın, Turkey, [3]Div Ped Neurol, Celal Bayar Univ, Manisa, Turkey

Neuronal ceroid lipofuscinosis (NCL) is an autosomal recessively inherited lysosomal storage disorder characterized by severe neurodegeneration. Four cases of different NCL including clinical and laboratory findings are presented.
Case 1: An eight year old male patient presented with resistant seizures (onset at 3 years) and acquired disability. Cranial MRI revealed a progressive cerebral and cerebellar atrophy. Tripeptidyl-peptidase enzyme activity was found at 0 nmol/spot*45h (normal: 0.1 - 1.2).
Case 2: A seven year old female patient presented with ataxia and seizures. Cranial MRI revealed diffuse cerebral and cerebellar atrophy. MFDS8 gene analysis was made for CLN7 and c.1234_1235dupTT was found.
Case 3: 10 year old female patient presented with progressive vision loss. The gene analysis for CLN3 revealed a homozygous IVS7+5G>T mutation.
Case 4: A four year nine month old male patient presented with seizures and loss skills. Cranial MRI revealed cerebral and cerebellar atrophy. Tripeptidyl-peptidase enzyme activity was found at 0.01 nmol/spot*45h (normal: 0.1 - 1.2).
NCL must be considered for patients presenting with neurodegenerative findings. Early identification of patients is of importance for new treatment options for late-onset infantile NCL.

A-077

Novel small deletion of *GALC* gene in a young Russian boy with Krabbe disease

Jourkova N V[1], Pushkov A A[1], Savostyanov K V[1], Nechaeva N L[1], Kuzenkova L M[1]

[1]Scientific Centre of Children Health, Moscow, Russian Federation

Background and objectives: Krabbe Disease is an autosomal recessive disorder resulting from a deficiency in an enzyme known as β-galactocerebrosidase. We present the clinical case of a young Russian boy with a novel small deletion in the *GALC* gene.
Case report: Patient birth : 2013. Apgar score: 9. At the age of 4 months, after vaccination, he began to loose his skills. At age of 6 months, after six days of hight temperature, he was taken to hospital. The first MRI of the brain revealed periventricular white matter T2-hyperintense areas, hyperintensity subcortical white matter. The boy had developmental regression, mental degeneration, progressive spasticity. An MRI of the brain show symmetric lesions of brain supra- and subtemporal localization. Ophthalmologic examination show optic atrophy. EEG pattern was abnormal. The results of β-galactocerebrosidase activity measured in two different samples of DBS revealed 0.20 and 0.18 mkmol/l/hr, respectively (in control persons: 1.0-4.0 mkmol/l/hr). The bidirect sequencing of all coding exons of *GALC* gene revealed a small deletion: *c.2037_2040del* (*p.Phe679Leufs*9*).
Conclusion: Finally, we would like to emphasize that cooperation and exchange of information between parents and specialists is a key issue in the diagnosis of rare and difficult neurological diseases, particularly if the clinical picture is inconclusive.

A-078

Clinical spectrum of Fabry disease In Egyptian children : report of 6 cases

Al Menebawy N M[1], Girgis M Y[1], Selim L A[1], Mahmoud I G[1], El Badawy A I[1], Abdelmoneim M[2]

[1]Div metabolic dis, Cairo univ Child hosp, cairo, Egypt, [2]clinical and Chem path dep, Cairo Univ, cairo, Egypt

Background: Fabry disease, a rare X-linked lysosomal storage disorder, is caused by deficiency of the enzyme α-galactosidase A.The symptoms, including neurological, gastrointestinal, renal, ophthalmological and dermatologic manifestations, start in childhood and adolescence, cause life-threatening complications with end-stage renal disease, cardiomyopathy and high incidence of stroke.
Subjects and methods: 6 male patients with confirmed Fabry disease with age ranging from 7 to 21 years were included in the study. All the patients were subjected to thorough general and neurologic examination, basic laboratory investigation, including urine microalbuminuria, echocardiography, dermatologic examination for the presence of angiokeratomas. Brain MRI and MRA were performed for patients presenting with stroke as well as vasculitis profile. Enzyme assay for enzyme alpha galactosidase A performed on leucocytes confirmed the diagnosis.
Results: Main clinical signs were angiokeratoma (4 cases), severe acroparesthesia and chronic neuropathic pain (1 case), microalbuminuria (1 case), recurrent strokes with alternating hemiplegia, intermittent claudication (1 case). Neither hypertrophic cardiomyopathy, nor ocular manifestations were detected in our patients.
Conclusion: Although the signs and symptoms of Fabry disease generally appear during childhood, the diagnosis is often missed. The earliest symptoms of Fabry disease in children are usually pain and angiokeratomas. Recurrent stroke should alert pediatrician to perform enzyme assay for Fabry disease.

25. Lysosomal disorders: treatment, enzyme replacement therapy

A-079

Histologic examination of the effect of a proprietary recombinant human acid α-glucosidase co-administered with a pharmacological chaperone on glycogen reduction in disease-relevant muscles of Pompe mice

Lun Y[1], Xu S[1], Gotschall R[1], Schilling A[1], Soska R[1], Frascella M[1], Garcia A[1], Feng J[1], Chang K[1], Do H[1], Valenzano K J[1], Khanna R[1]

[1]Amicus Therapeutics, Cranbury, United States

Background: Pompe disease is an inherited lysosomal storage disorder that results from a deficiency in acid a-glucosidase (GAA) activity, and is characterized by progressive accumulation of lysosomal glycogen in cardiac and skeletal muscles. Enzyme replacement therapy (ERT) using alglucosidase alfa is the only approved treatment available for Pompe disease. While providing some clinical benefits, the infused enzyme shows insufficient uptake into key disease-relevant muscles.
Methods & Results: Here we compared the effects of a proprietary recombinant human GAA (designated ATB200) to that of alglucosidase alfa (herein designated 'rhGAA') *in vivo* by histologic means. We show that ATB200 is more effective than rhGAA at reducing glycogen in key muscle tissues of male *Gaa* knock-out (KO) mice following repeat bi-weekly bolus intravenous (IV) injections. Importantly, co-administration of ATB200 with a pharmacological chaperone (PC) resulted in even greater glycogen reduction compared to ATB200 alone. Likewise, LAMP1 levels in these tissues also decreased, suggesting a reversal of lysosomal proliferation.
Results: Taken together, these data demonstrate that ATB200 leads to better tissue targeting and substrate reduction in *Gaa* KO mice, which can be further improved by combination with a PC, thus warranting further investigation of this next-generation treatment for Pompe disease.
Conflict of Interest declared.

A-080

Hematological manifestations and enzyme replacement therapy outcomes of Gaucher disease: experience of eighteen years

Zubarioglu T[1], Kiykim E[1], Cansever M S[2], Aktuglu Zeybek A C[1], Aydin A[1]

[1]Div Nutr Metab Dis, Ist Univ Cer Med Fac, Istanbul, Turkey, [2]Cent Lab, Ist Univ Cer Med Fac, Istanbul, Turkey

Background: Gaucher disease (GD) is caused by deficiency of lysosomal enzyme acid β-glucosidase which results in the accumulation of glucosylceramide in cells. In this study, hematological findings and enzyme replacement therapy outcomes of GD patients were reported.
Methods: Thirty-six patients who were diagnosed as GD at Division of Metabolism of Cerrahpasa Medical Faculty between 1996 and 2014 were conducted to study.
Results: Hematological findings are the presentation symptoms in 36 % of 36 patients (n=13). Among these patients, 3 patients had pancytopenia, 8 had bicytopenia and 2 had isolated thrombocytopenia. Enzyme replacement therapy (ERT) with

imiglucerase was started at the dose of 30 IU/kg/2 weeks. Six of these 13 patients could not be followed up and did not receive enzyme replacement therapy regularly. In these patients only a partial response was noted. However of 7 patients who were followed-up and received ERT regularly, complete recovery of the clinical findings were achieved.
Discussion: Regular ERT had resulted in full recovery in hematological findings in the long term follow up of all patients.Although all clinical findings in GD do not respond to ERT, hematological findings are among those that can respond with full recovery, resulting in increase in quality of life.

A-100

Comorbidities and use of concomitant medications in adults with Gaucher disease (GD): a MarketScan™ US claims database analysis

Pleat R[1], Underhill L[1], Ross L[1], Nalysnyk L[1], Ibrahim J[1], Tcherny-Lessenot S[1], Wang L[1]

[1]Genzyme, a Sanofi company, Cambridge, United States

Introduction: Safe and effective use of eliglustat, a new oral treatment for adults with GD type 1, necessitates monitoring the use of concomitant medications metabolized through CYP2D6/3A pathways.
Methods: Using a US claims database (MarketScan™), comorbidities and prescriptions of strong and moderate CYP2D6/3A inhibitors and strong CYP3A inducers were analyzed retrospectively in 168 adults being treated with enzyme replacement therapy and/or miglustat for GD. Comorbidities were analyzed ≤6 months before the first GD prescription, concomitant medication usage during the 6 months after.
Results: Twelve (7.1%) and 6 (3.6%) patients were prescribed medications for comorbid respiratory infections and depression/anxiety disorders, respectively. Cardiac arrhythmias were rare; no cardiac disease was reported. Moderate CYP3A inhibitors were prescribed in 12% of patients, strong CYP2D6 inhibitors in 5.4%, and strong CYP3A and moderate CYP2D6 inhibitors each in 2.4%. Antibiotics/antifungal agents for acute use (< 15 days) were the most-commonly prescribed moderate CYP3A inhibitors; antidepressants used chronically (≥15 days) the most-commonly prescribed strong CYP2D6 inhibitors. Two (1.2%) patients reported simultaneous chronic use of CYP2D6/3A inhibitors; there were no reports of simultaneous acute use.
Conclusions: Use of CYP2D6 and CYP3A inhibitors in patients treated for GD was limited. Moderate CYP3A inhibitors were prescribed most frequently for acute use.
Conflict of Interest declared.

26. Glycosylation disorders/CDG, protein modification disorders

A-081

Our patients with congenital disorders of glycosylation

Köse M[1], Canda E[1], Kagnici M[1], Kalkan Ucar S[1], Çoker M[1]

[1]Div Metab Dis, Ege Univ Med Faculty, İzmir, Turkey

Congenital disorders of glycosylation (CDG) are being recognized as a rapidly growing and complex group of disorders.
Case 1 is a 11 month of age boy who was first referred with cardiac tamponade. Further analyses showed hypetrophic cardiomyopathy and resistant lactic acidosis. Mitochondrial investigations did not detect any finding pointing mitochondrial diseases. Type I abnormality was detected in IEF. Lipid linked oligosaccharid (LLO) analyse showed Mannose peak.
Case 2 is a 12 month of age boy with neuromotor retardation, hypertrophic cardiomyopathy and inverted nipples Cerebellar hypoplasia was detected in MRI. Homozygous c.691G>A mutation in PMM2 gene was identified in molecular analyse.
Case 3 is an 8 month of age girl with liver failure.
Case 4 is an 2 month of age girl with congenital cataract, dysmorphic features and liver dysfunction. IEF Analyse showed Type I abnormality in both of patients. Molecular genetic analyse of cases except case 2 did not resulted yet.We described the clinical cases of four infants which three of them is CDG Ix and one of them is CDG 1a

A-082

CDG incidence in unknown genetic-metabolic/ neurometabolic disorders

Kilic M[1], Kasapkara Ç S[1], Kilic E[2], Jaeken J[3]

[1]Div Metab Dis, Dr Sami Ulus Child Hosp, Ankara, Turkey, [2]Div Genet Dis, Hemato-Onco Child Hosp, Ankara, Turkey, [3]Cent Met Dis, Univ. Hosp. Gasthuisberg, Leuven, Belgium

Background and objectives: Congenital disorders of glycosylation (CDG) are genetic diseases due to defects in the synthesis or the attachment of the glycan moiety of glycoproteins and glycolipids. In this study, we aimed to find out CDG incidence in patients with unknown diagnosis in spite of multidisciplinary genetic, neurology, metabolism approach and was seen also in other clinics according to the required symptomatology.
Patients and Methods: 100 patients with unknown disease were taken to study. Initial metabolic and genetic work-up

were normal. Serum transferrin isoelectric focusing (TIEF) test was run (Dr. Jaekeen-Belgium).
Results: Two cases result were appropriate for CDG type I pattern. However, in the first patient homozygous *ALDOLASE B* gene mutation was determined and Hereditary Fructose Intolerance (HFI) was diagnosed and it was found secondary glycosylation defect. This patient had mental motor retardation and dysmorphic facial features with due to concomitant disease which continues to investigate. The second case had microcephaly, autism and mental retardation. Molecular analyses of this patient going on.
Conclusion: Although CDG is a very rare disease, the incidence was found 1% in unknown genetic-metabolic/neurometabolic disorders. CDG should be looked for in any unexplained genetic-metabolic/neurometabolic disorders.

28. Disorders of vitamins, cofactors and trace elements

A-083

Evaluation of vitamin B12, methylmalonic acid and homocysteine levels in obese adolescents

Turan R[2], Akis M[3], Kuyum P[1], Cakar S[1], Kant M[3], Arslan N[1], Islekel H[2 3]

[1]Div Metab Dis, Dokuz Eylul Univ, Izmir, Turkey, [2]Depart Mol Med, Dokuz Eylul Univ, Izmir, Turkey, [3]Depart Med Biochem, Dokuz Eylul Univ, Izmir, Turkey

Aim: The aim of this study was to evaluate vitamin B12, methylmalonic acid (MMA) and homocysteine levels in obese adolescents and to compare them with healthy peers.
Patients and methods: 56 obese adolescents (mean age 14.0 ±1.7 years) and 41 healthy controls (14.8±1.8 years, p=0.061) were enrolled in the study. Obesity was defined as a body mass index exceeding 95th percentile. Vitamin B12, homocysteine and MMA levels were measured.
Results: The mean vitamin B12 level was not different between obese (199.4±84.8 pmol/L) and healthy (226.9±102.4 pmol/L) adolescents (p=0.152). MMA and homocysteine levels were higher in obese adolescents (MMA: 0.41±0.43 and 0.29±0.14 μmol/L respectively, p=0.050, homocysteine: 12.3±8.3 and 9.0 ±2.7 μmol/L respectively, p=0.008). Blood B12 level was negatively correlated with MMA (r=-0.329, p=0.001) and homocysteine (r=-0.428, p=0.000) levels. There was no significant correlation between alanine aminotransferase and HOMA-IR levels with vitamin B12, MMA and homocysteine levels.
Conclusion: Subclinical B12 deficiency is higher in obese adolescents than healthy controls. MMA and homocysteine are better indicators of in vivo vitamin B12 status than serum B12 measurement in obese adolescents.

A-084

5,10-Methylenetetrahydrofolate reductase deficiency with different pictures

Çoker M[1], Köse M[1], Canda E[1], Kagnici M[1], Smith D E C[2], Mendes M I[2 3], Kalkan Ucar S[1], Salomons G S[2]

[1]Div Metab Dis, Ege Univ Med Faculty, İzmir, Turkey, [2]Dept Clin Chemist,Metab Unit, VU Univ, Amsterdam, Netherlands, [3]Metab and Genet Group, Univ Lisbon, Lisbon, Portugal

5,10-Methylenetetrahydrofolate reductase (MTHFR) deficiency, the most prevalent inborn error of folate metabolism, has variable clinical manifestations from asymptomatic to severe psychomotor retardation, microcephalus and seizure. Four patients having non-classical homocystinuria were evaluated with respect to their symptoms for MTHFR deficiency. The diagnosis was confirmed by impaired or very much reduced MTHFR enzyme activity and the presentence of pathogenic mutations in the *MTHFR* gene. All patients presented with severe CNS disease symptoms. Betaine and folinic acid therapy has been started immediately after diagnosis. One patient had ataxia, myoclonus, oculomotor appraxia and peripheral nerve involvement for 11 years; he has been diagnosed at 16 years old. Mean age at diagnosis of the other three severe MTHFR deficiency cases is 3.13±1.34 months (1-7 months). Two of them have died at 7 and 10 months of age. The last patient; our longest follow-up patient; with an early diagnosis and a severe course of the disease (encephalopathy on newborn period) is currently improving under treatment. He is now 8.5 years old. We presented 4 cases of MTHFR deficiency. Our results illustrate that inborn errors of folate metabolism such as MTHFR deficiency should be include in the differential diagnosis of infants and children with unexplained neurological findings

A-085

Reappearance of neurological symptoms in a patient with a remethylation defect due to a cerebral folate deficiency

Eyskens F[1], Verloo P[2]

[1]Div Metab Dis, Univ Hosp Antwerp, Antwerp, Belgium, [2]Div Metabol Dis, Univ Hosp Gent, Gent, Belgium

Introduction: Formation of methylcobalamin is disturbed in the cblE and cblG disorders. The most common clinical findings are megaloblastic anaemia and neurological disease, including poor feeding, failure to thrive, developmental delay, nystagmus, hypotonia, ataxia, seizures and blindness.

Cerebral folate deficiency can be defined as any neurological syndrome associated with low CSF 5-methyltetrahydrofolate (5MTHF) in the presence of normal folate metabolism outside the nervous system.
Clinical case: we report a boy, first child of non-consanguineous parents, who presented with an encephalitis associated with pancytopenia at the the age of 1 year. Hyperhomocysteinemia (150 μmol/L) in the absence of methylmalonic aciduria, led to the diagnosis of cbl G deficiency. Under treatment with hydroxycobalamin, folic acid and betaine he completely recovered. At the age of 6 years he developed behavior abnormalities and episodes of visual impairment. Epilepsy and cardiac disease was excluded. Biochemical parameters in plasma and urine were and remained normal. Examination of the CSF revealed a low 5MTHF consistent with the diagnosis of cerebral folate deficiency. Under folinic acid supplementation his clinical condition improved, especially the irritability and the behavior problems.
Conclusion: we report a patient with a hyperhomocysteinemia due to a remethylation defect complicated by a (most probably) acquired cerebral folate deficiency.

A-086 Abstract withdrawn by the author

A-087

Cobalamin (Cbl) D deficiency: A case report

Kendirci M[1], Kardas F[1], Ustkoyuncu P[1], Gokay S[1], Per H[2], Kacar Bayram A[2]

[1]Div Metab Dis, Erciyes Univ, Medical Fac, Kayseri, Turkey, [2]Div Child Neuro, Erciyes Univ, Medical F, Kayseri, Turkey

Background: Cobalamin is a cofactor in several metabolic pathways and also essential for the development of human beings. It is required for methylmalonic acid and homocysteine metabolism. Cobalamin (Cbl) D deficiency is a rare, autosomal recessive inherited disease which may present with different clinical features. It may cause isolated methylmalonic aciduria or homocystinuria but also combined. The responsible gene is MMADHC located on choromosome 2q23.2.
Case: An eleven year old boy was admitted to the clinic with complaints of convulsions, and mental-motor retardation by his parents. He was not able to walk and the seizures were not under control by drug treatment. Plasma homocysteine levels and methylmalonic acid in urine organic acid analysis were elevated. Vitamin B12 levels were in the normal range. Molecular genetic analysis showed a C.748C>T homozygote mutation in the MMADHC gene. The diagnosis of CblD deficiency was made. Betaine (3g/day), carnitine (100mg/kg/day), folic acid (5mg/day), hydroxycobalamin (1mg/week, I.M.) were started. The results of the tretment were successful. Now, he can walk with the help of his mother, and the seizures are under control.
Conclusion: We have to consider cobalamin defects in patients with seizures and mental-motor retardation. Plasma homocysteine and urine methylmalonic acid levels with genetic analysis will help to confirm the diagnosis.

A-088

The clinical predictive power in terms of the clinical results and family history of the MTHFR thermolabile variants

Ozer I[1], Ozen F[2]

[1]Ped Metab Dis, Goztepe Edu Res Hosp, Istanbul, Turkey, [2]Div Gen Dis, Goztepe Edu Res Hosp, Istanbul, Turkey

Background and objectives: : Methylenetetrahydrofolate reductase (MTHFR) catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a cosubstrate for homocysteine remethylation to methionine. Although the type of the thermolabile variant (OMIM236250) has a higher enzyme activity, it has a loss of functionality with high temperature and it may cause stroke, myocardial infarction, abortion and convulsions.
Materials and Methods: MTHFR mutations were determined and the predictive value of genotype definition was investigated with the possible clinical symptoms and family history in terms of MTHFR in accordance with the results of the patients homocysteine and methionine levels.
Results: We evaluated 1700 patients with MTHFR C677T and A1298C mutations. It was found to be highly associated with the sacral dimple, marfanoid features of the patients and recurrent abortion, and early cerebral and cardiac infarctions in the patient family history.
Discussion: Even before concluding further laboratory evaluation by the clinical results, it was diagnosed right nearly almost of the patients. Even though by basic clinical evaluation, MTHFR can be recognized easily, most of the patients are diagnosed late or wrongly.
Conclusion: MTHFR is a common inborn error of folate metabolism. It must be learned and managed by not only metabolism specialist but also family physician.

29. Miscellaneous

A-089

The inborn errors of metabolism emergency folder: making life easier for health professionals and families

Robinson J[1]

[1]Dept Med Gen, Syd Child Hosp, Sydney, Australia

Inborn errors of metabolism (IEM) are rare conditions that can present severe risks of metabolic decompensation for affected children. These risks can be exacerbated by intercurrent illnesses which may cause the parents to present the child to an emergency department (ED) whose staff have no familiarity with the condition. Providing that information promptly is critically important to medical outcomes. The treating physician requires clear, simple instructions specific to the individual IEM. Calculations of dose and rate of medication delivery are particularly problematic since they must be carried out both quickly and accurately. Based on my experience working in the Sydney Children's Hospital (SCH) ED, management of complex medical conditions was made substantially easier when parents provided plans listing the name of the condition, the name of the health professional involved, and his or her recommended management strategy. From this, the idea of systematically providing an Inborn Errors of Metabolism Emergency Folder (IEMEF) to parents was born. Staff and parents have reacted enthusiastically to the folder system. The success of the method depends on the parents presenting with the folder, thus education is essential. Every new metabolic patient at SCH receives both a customised folder and education about using it.

A-090

Application of sigma metrics for the assessment of quality assurance for plasma amino acid analysis in biochemical genetics laboratory in Pakistan

Jafri L[1], Yousufzai N[1], Sherazi N[1], Jamil A[1], Jehan F[1], Mehar R[1], Afroze B[1], Khan A H[1]

[1]Aga Khan University Hospital, Karachi, Pakistan

Background: Quality is assessed on sigma scale with 3 sigma as the minimum allowable sigma and 6 being world-class quality goal. We aim to present sigma-metrics of plasma amino acids (AA) observed in our Biochemical Genetics Laboratory (BGL).

Methods: Internal quality control (IQC) data of AA run in BGL was analyzed from 2013 -2014. Two years mean from IQC and proficiency testing (PT) results were utilized to establish coefficient of variation (CV) and bias respectively. Bias (%): (mean of all laboratories using same instrument-BGL AKU mean)/(mean of all laboratories using same instrument)*100. Sigma metrics were then calculated: $\sum(\sigma)$= (total allowable error – bias%)/CV.

Results: Overall CV of individual AA ranged from 3.46-11.2%. The laboratory mean and PT target mean showed < 1% variation for all amino acids. Sigma values elicited for alanine, cystine, leucine, methionine, proline, ornithine, taurine, threonine and valine were 6. Plasma asparginine, histidine, glutamine and aspartate elicited < 3 on sigma-scale. We achieved sigma metrics of the range 3.1–5.9 for remaining amino acids. Of all the amino acids evaluated the average sigma level was 4.8.

Conclusion: Satisfactory sigma metrics were achieved for all AA. The AA below3 sigma must be evaluated with discretion and strict quality control checks.

A-091

Netherton syndrome: *SPINK5* gene mutation found through whole exome sequencing

Seker Yilmaz B[1], Kor D[1], Ceylaner S[2], Bulut D[1], Yilmaz M[3], Onenli Mungan N[1]

[1]Div Metab Dis, Univ Cukurova, Adana, Turkey, [2]Intergen Genetic Lab, Ankara, Turkey, [3]Div Ped Allergy, Cukurova Univ, Adana, Turkey

Background and Objectives: Netherton Syndrome (NS) is a rare hereditary autosomal recessive disorder which presents with ichthyosiform erythroderma, bamboo hair and atopic features. It is caused by mutations in *SPINK5* gene, which encodes the serine protease inhibitor LEKTI.

Case Report: A 6-months-old male child, born of second-degree consanguineous marriage, was referred to our clinic for evaluation of diffuse scaling erythroderma. The prenatal and perinatal history was unremarkable. The child was normal at birth, but developed widespread erythema and desquamation at the first week of life, which gradually progressed all over the body in a span of 6-months. The child had recurrent respiratory infections. He had delayed developmental milestones with growth retardation. Scalp hair, eyebrows and eyelashes were sparse. Hepatosplenomegaly was detected as an

unexpected finding. Whole exome sequencing was performed for the differential diagnosis and the patient's genomic DNA disclosed a IVS2+5 G>T (c.81+5G>T) homozygous mutation in the *SPINK5* gene. Parents were heterozygous for the same mutation. During the follow up, laboratory evaluation revealed elevated Ig E levels and multiple food allergies.
Conclusion: We would like to highlight the importance of NS in the differential diagnosis of colloidan baby. Hepatosplenomegaly could be the part of the atypical clinical course.

A-092

Coexistence of pseudohypoaldosteronism and cholelithiasis, presenting with metabolic acidosis, dystrophy and vomiting in a neonate

Karavanaki K[1], Sdogou T[1], Kakleas K[1], Soldatou A[1], Skouma A[3], Papathanasiou A[2]

[1]2nd Dep of Ped, Univ Child Hosp'P&A Kyr', Athens, Greece, [2]Dep of Ped End, Univ Child Hosp'P&A Kyr', Athens, Greece, [3]1st Dep of Ped, Univ Child Hosp 'Ag Sof', Athens, Greece

Context: Cholelithiasis in childhood and adolescence is uncommon and is usually attributed to congenital haemolytic disorders or obesity. Gallstone formation in infancy is very rare and is mainly associated with total parenteral nutrition, congenital biliary diseases or administration of specific drugs. An extremely rare form of cholelithiasis occurs with pseudohypoaldosteronism (PHA). In these patients gallstone formation has been attributed to dehydration and salt-wasting, starting from fetal life.
Case report: A neonate with PHA presented with dystrophy, vomiting, hyponatraemia, hyperkalaemia, metabolic acidosis and gallstone formation. Plasma renin activity and aldosterone concentrations were elevated and urinary Na excretion was increased. An abdominal ultrasound scan revealed a normal pylorus, but multiple gallbladder stones. Electrolyte abnormalities were corrected with the addition of sodium chloride 15%, administered initially intravenously and subsequently orally at each meal. Gallstones automatically subsided at the age of six months. The infant continued with oral sodium chloride administration for the following months, until the second year when it was discontinued. His growth and development were absolutely normal.
Conclusions: Infants with PHA, even without signs of salt wasting, should be investigated for cholelithiasis. Inversely, in infants with pertinent electrolyte abnormalities and cholelithiasis, PHA should be considered among the possible diagnoses.

A-093

Pelizaeus-Merzbacher Disease (PMD) and Mitochondrial Dysfunction

Fitzsimons P E[1], Mayne P D[1], Shadani K[2], Robinson I[3], Lynch B[2]

[1]Paed. Lab. Med. Temple St Child Uni Hosp, Dublin, Ireland, [2]Dept. of Neurol.Temple St Child Uni Hosp, Dublin, Ireland, [3]Dept of Radiol. Temple St Child Uni Hosp, Dublin, Ireland

Background: Pelizaeus-Merzbacher disease (PMD), a rare X-linked neurodegenerative leukodystrophy is caused by mutations in the *PLP1* gene, encoding the main component of myelin, proteolipid protein 1. PMD typically manifests in infancy with nystagmus, hypotonia, cognitive impairment; progressing to severe spasticity and ataxia. Reduced white matter is observed as thinness of the corpus callosum or general decrease in myelination. Pelizaeus-Merzbacher like disease (PMLD), an autosomal recessive disorder is caused by mutations in *GJA12* encoding connexin47 and *HSPD1* gene encoding mitochondrial chaperonin Hsp60.
Case Report: A 9 month old boy born to non-consanguineous Irish parents presented from birth with head bobbing, head lag, hypotonia and nystagmus. Clinical and radiological findings were suggestive of PMD. Molecular genetics confirmed duplication of the *PLP1* gene. However, urine organic acid analysis revealed a persistently raised ethylmalonic acid (EMA) of 36 and 57 μmol/mmol cr (ref < 20). Although the link between mitochondrial derangements and neurodegenerative diseases is known, increased EMA excretion has only been documented in some patients with PMLD caused by mutations in *HSPD1* gene, not in PMD.
Conclusion: This case highlights both PMD and PMLD should be considered in the differential diagnosis of elevated EMA excretion, particularly in infants presenting with nystagmus, head lag and hypotonia.

A-094

Clinical experience in an Inherited Metabolic Diseases Unit of a high complexity hospital in Medellin, Colombia, between 2010 to 2014

Ortiz B L A[1], Garcia C L A[2]

[1]child neurol,, San Vicente Fund Hosp, medellin, Colombia, [2]Metabolica Nutrit Program, medellin, Colombia

Objective: Inherited metabolic diseases are hereditary disorders with a low occurrence but bad outcome if

untreated. Organic acidurias OA and aminoacidopathies AP are due to abnormal byproduct that causes cell dysfunction. The main objective of this study was to show the clinical data of patients followed in the Inherited Metabolic Disease Unit of the Child Hospital's San Vicente Fundacion, Medellin-Colombia, between 2010 to 2014.
Methods: Retrospective study of patients assessed by pediatrics, nutritionist, child neurologist and genetics. Feeding history, anthropometry, social issues, biochemics, prescriptions and comorbidities were analyzed.
Results: Propionic-isovaleric-glutaric type 1-methylmalonic OA, homocistinuria and MSUD were diagnosed. 10 patients had a complete medical record. Age of patients was 2 months to 11 years old, 60% were female, 40 % had normal nutritional status, 20% had chronic malnutrition and 10% had obesity. 10% have died because of the primary disease.
Conclusion: OA and AP are diseases treated mainly by food exclusion and specialized nutritional formula prescription. Patients have their clinical debut in the first ten years but a timely diagnosis and treatment may prevent irreparable recurrence. In this study, most of the patients had some grade of malnutrition but they could reach adolescence and prevent metabolic crisis.
Conflict of Interest declared.

A-095

Clinical and psychophysiological aspects of l-dopa treatment in ataxia-telangectasia patients: preliminary observations

Daniela, d'agnano D D[1], Daniela, Mannarelli D M[2], Vincenzo, Leuzzi V L[1], Caterina, Caputi C C[1], Francesco, Fattapposta F F[2]

[1]dep Ped Child Neur Psich, Sapienza univ, Roma, Italy, [2]dep Neurol, Psych, Sapienza Univ, Roma, Italy

Background: Ataxia-teleangiectasia (AT) is a rare pleiotropic disorder presenting with early onset ataxia and a striking combination of cerebellar and extrapyramidal symptoms. Despite of the well known cerebellar neurodegeneration, a dysregulation of nigrostriatal pathway has not been clearly demonstrated. Present paper report on the clinical and psychophysiological evaluation results of L-DOPA treatment in A-T patients.
Methods: 4 subjects were treated for 8 weeks with the dopaminergic drug (melevodopa/carbidopa); dosage was tried up to 8 mg/kg. Ataxia was assessed by the International Cooperative Ataxia Scale, parkinsonism by the Unified Parkinson Disease Rating Scale. Variations occurring in dopaminergic pathway were monitored by the contingent negative variation, a psychophysiological phenomenon influenced by dopaminergic activity. Assessment of adaptive behavior and laboratory tests were performed.
Results: 3 patients completed the trial and experienced a progressive improvement in UPDRS score. No significance variations were detected in ICARS score and VABS. CNV evaluation showed a progressive restoration of the wave that initially appeared disrupted. Treatment was well-tolerated, no side effects occurred.
Conclusions: These preliminary results call for a wide-scale controlled study on efficacy of L-DOPA treatment on movement disorders in A-T patients and provide new data for a better understanding of pathophysiology of nigrostriatal involment in A-T.

A-096

The experience of erythrocyte-delivered dexamethasone in ataxia-teleangiectasia

D'Agnano D[1], Micheli R[2], Alessandro P[5], Luciana C[3], Mauro M[4], Vincenzo L[1]

[1]dep Ped Child Neur Psich, Sapienza univ, Roma, Italy, [2]Unit child neur, psich, Spedali Civili, Brescia, Italy, [3]dep clinic molecular med, Sapienza univ, Roma, Italy, [4]dep biomol sci, Carlo Bo univ, Urbino, Italy, [5]dep clin exp sci, ped spedali civili, Brescia, Italy

Background: Ataxia-telangiectasia (AT) is a rare neurodegenerative disease presenting with early onset ataxia, oculocutaneous telangiectasia, immunodeficiency and radiosensitivity. Nowadays no therapies are available. In a phase II study, 6 monthly infusions of autologous erythrocytes loaded with dexamethasone were effective in improving the neurological impairment in AT patients. We report on the result of an additional 24 month-period treatment.
Methods: After the end of first trial, 4 subjects continued monthly dexamethasone infusions for further 24 months; their clinical outcome was compared with that of 7 age-matched subjects who had stopped the treatment. Serial assessment of ataxia (by International Cooperative Ataxia Rating Scale) adaptive behaviour (by Vineland Adaptive Behaviour Scales), clinical and laboratory tests were performed, safety profile was evaluated.
Results: Patients in the extended study experienced a continuous neurological improvement while controls showed a progressive neurological deterioration (according to the natural history of AT). The delivery system proved to be safe and well-tolerated, none of the side effects associated with the chronic administration of corticosteroids was observed.
Conclusions: These promising results call for a wide-scale controlled study on protracted treatment of AT patients with dexamethasone loaded erythrocytes; the erythrocyte-delivered seem to be a safe and effective system to administer corticosteroids.

A-097

Whole exome sequencing in clinical context identifies treatable intellectual disability

Brandau O[1], Trujillano D[1], Rolfs A[1], Abou Jamra R[1]

[1]Centogene, Rostock, Germany

Background: Whole exome sequencing (WES) in the context of clinical and genetic work-out is becoming an essential diagnostic.
Methods: We have performed WES in 400+ families with different disorders, mostly including intellectual disability.
Results: One impressive lesson out of this large dataset is that at least in 8 families we were able to identify variants in genes that probably lead to a treatable form of intellectual disability (based on the well-curated website http://www.treatable-id.org/). That means that in these cases appropriated therapy may lead to fully normal development if timely applied, or at least to relief of or abated symptoms. These mutations were in the genes *SLC19A3* (in two families; p.Trp59Arg as well as p.Thr422Ala, lethal early-infantile encephalopathy), *SLC6A8* (p.Asn549Thr, cerebral creatine deficiency), *IDS* (p.Ser149Ala, mucopolysaccharidosis II), *NPC2* (p.Cys93Phe, Niemann-Pick disease type C2), *PDHA1* (p.Trp421Serfs*6, pyruvate dehydrogenase E1-alpha deficiency), *PCCB* (p.Arg292Gln, propionic acidemia), and *ETHE1* (p.His198Profs*23, ethylmalonic encephalopathy).
Conclusion: These results clearly demonstrate that such metabolic disorders may be overseen although programs of newborn screening are applied, and may only be detected via pan-analyses such as WES. These results also demonstrate the importance of clarifying the genetic causes of intellectual disability rapidly and as early as possible since therapy options are available for a notworthy part.
Conflict of Interest declared.

A-098

Atypical presentation of Lowe syndrome

Brennerova K[1], Šalingova A[2], Škodova J[2], Gerinec A[3], Bzduch V[1]

[1]I.st Paed Dep, Univ Child Hosp, Bratislava, Slovakia, [2]Dep Lab Med,Univ Child Hosp, Bratislava, Slovakia, [3]Paed Ophtal Dep, Univ Child Hosp, Bratislava, Slovakia

Objective: Lowe syndrome is a very rare, X-linked disorder, caused by mutations in the OCRL gene. It involves multiple anatomic systems, particularly the eyes (congenital cataract), central nervous system (mental retardation, seizures), and kidneys (proximal tubulopathy). The first symptoms of tubulopathy generally develop during the first months of live and lead to failure to thrive.
Case report: We report a case of 5 year old boy who was admitted to the hospital at the age of 3 months due to congenital cataract surgery. For the shortened upper limbs and hypotonic syndrome in the differential diagnosis considered a rhizomelic disease. Mental and motoric development of the boy was retarded. Up to 1.5 years of life a mild metabolic acidosis with aminoaciduria and decreased concentration of L-carnitine in serum appeared. Brain MRI showed periventricular cysts. Despite the negative ocular findings in his mother, molecular - genetic test for Lowe syndrome was done; confirmed mutation c.2083C>T (Arg695Ter)
Conclusion: Congenital cataract is part of several inherited metabolic disorders. The slow proximal tubulopathy development of our patient and absence of lens opacities of his mother led to later confirmation of correct diagnosis.