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25. Miscellaneous

O-001

DISCOVERY OF A HUMAN 3-HYDROXYPROLINE DEHYDRATASE, THE FIRST OF A NOVEL SUBFAMILY OF PROLINE RACEMASE-LIKE PROTEINS

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A family of eukaryotic proline racemase-like genes has recently been identified. Many members of this family, including a human protein called C14orf149 lack a cysteine residue at the 300 position which is criticial for the racemase activity. The function of these enzymes has remained unresolved until now. We demonstrate that human C14orf149 catalyzes the degradation of trans-3-hydroxy-L-proline to delta-1-pyrroline-2-carboxylate (P2C). This is the first enzyme of this subclass of proline racemase-like genes for which the enzymatic activity has been resolved. It is also the first human enzyme that acts on 3-hydroxyproline. A mutant enzyme in which the threonine-300 in the active site is mutated back into cysteine, regains 3-hydroxyproline epimerase activity. This result and the evolutionary relationship between enzymes of the proline racemase-like family, suggests that a single mutation gave rise to this subclass of enzymes. Presumably, human C14orf149 serves to degrade 3-hydroxyproline that is released by the degradation of proteins that contain this amino acid such as collagen IV, an important structural component of basement membrane. Interestingly, the 14q23 locus containing the C14orf149 gene has been linked to familial aneurisms.

O-002

WERNICKE ENCEPHALOPATHY IN CLASSIC MAPLE SYRUP URINE DISEASE (MSUD) DECOMPENSATION

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A classic-MSUD patient was admitted at the age of 10 yrs because of severe neurological deterioration (ataxia, hallucinations, drowsiness) during severe decompensation due to gastroenteritis. Despite previous decompensation episodes, his neurological status was unremarkable. On admission, plasma leucine level was 1400 μmol/l, and BCAA-free aminoacid mixture (2 g/kg/day) was immediately started. After 12 hours a brain MRI was performed, showing a picture resembling Wernicke encephalopathy (WE), characterized by FLAIR and DWI symmetric hyperintensity at the level of midbrain, hypothalamus, mamillary bodies and thalami. As WE is attributed to thiamine deficiency, thiamine supplementation (1 g/day i.v.) was started, with complete clinical and neuroradiologic recovery. A second classic-MSUD patient previously admitted with the same clinical picture and treated by analogous dietary adjustments, was retrospectively recognized with WE-like MRI lesions. The patient recovered completely even without thiamine supplementation.
As WE pathophysiology seems to be mainly due to α-ketoglutarate dehydrogenase (α-KGDH) inhibition, we hypothesized a possible common pathogenesis of neuronal damage between the two disorders. Interestingly, α-ketoisocaproic acid was shown to selectively inhibit α-KGDH in brain mitochondrial preparations (Amaral AU, 2010). These findings might explain the neuroradiological picture of WE in MSUD and its reversibility with decompensation treatment, furthermore MSUD should be included as a cause of WE.

O-003

COGNITIVE FUNCTION IN TYROSINAEMIA TYPE III: IS DIETARY INTERVENTION IMPORTANT?

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Background: Tyrosinaemia type III (4-hydroxyphenylpyruvate dioxygenase deficiency) is extremely rare and the cognitive outcome is not well defined.
Objectives: (i) to document cognitive profiles in tyrosinaemia III; (ii) to explore the relationship between dietary treatment and cognitive outcome.
Methods: Cross-sectional study of 11 patients with tyrosinaemia III (4 males, 7 females), age range 2–17 years (median 8) using age-appropriate cognitive tests. A questionnaire was completed by school relating to behaviour and special educational needs. Age at which dietary treatment was commenced and the pre-diet and post-diet plasma tyrosine levels until age 5 years were recorded.
Results: Pre-diet plasma tyrosine concentrations were between 500 and 1500 μmol/l. Median age at commencement of dietary treatment was 12 weeks (range 5–112 weeks). Post-diet tyrosine concentrations ranged from 60–300 μmol/l. 75% of IQ scores were borderline (70–80) or extremely low (<70) with no significant differentiation between verbal and nonverbal scores. 2 patients had special educational needs and 2 had concentration difficulties. There was a weak negative correlation between the age at which patients started dietary treatment and full scale IQ ($r=-0.514$; $p=0.192$).
Conclusion: Early control of plasma tyrosine concentrations may be a significant factor in determining cognitive outcome in Tyrosinaemia type III.

O-004

LONG-TERM OUTCOMES OF PATIENTS WITH TREATED HOMOCYSTINURIA (CBS DEFICIENCY) IN IRELAND 1971–2009

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Objectives: to examine the longterm outcome of treated homocystinuria patients (HCU, CbS deficiency), and to examine ways to quantify patient exposure to free homocystine and total homocysteine.
Methods: A retrospective case note review of 46 HCU patients (92% pyridoxine nonresponsive), born between 1971–2009. Patients were divided into 4 groups: 23 New Born Screening (NBS) good/moderate control (Group I); 7 NBS poor control (Group II); 10 not detected on NBS (Group III); 6 born pre-NBS (Group IV). Median age of 28.23 years (0.4 - 54.6) included 950 treatment years. Free homocystine measured between 1971–2009, and total homocysteine from 1997.
Alternative statistical models were used to determine control: area under HCy curve; and expected homocystine level (free homocystine exposure/years of exposure); level of exposure to free homocystine to time of clinical event.
Results: complication rate for Group I 2%, Group II 17%, Group III 45% and Group IV 36%. 84% of NBS attained 3 rd level education, compared to 43% for those presenting clinically.
Conclusions: (based on analysis of free homocystine): children with expected free homocystine levels >12.3 μmol/L are likely to develop ectopia lentis, and children, whose cumulative exposure to homocystine <100 μmol/L up to 4 years, are less likely to develop learning disabilities.

O-005

TREATMENT OF ACUTE DECOMPENSATIONS OF MAPLE SYRUP URINE DISEASE IN ADULT PATIENTS WITH A NEW PARENTERAL AMINO ACIDS MIXTURE

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Background: Acute decompensations of maple syrup urine disease (MSUD) are usually treated by enteral feeding including amino-acids mixture without leucine, valine, isoleucine. However, it is difficult in case of gastric intolerance. Thus, we developed a new parenteral amino acids mixture.

Methods: 17 decompensations in 4 adult patients with MSUD treated by parenteral mixture (group P) were compared to 18 previous decompensations treated by enteral feeding in the same patients (group E). Total amount of leucine in blood was estimated as 0.65xbody weight xleucine concentration and clearance of leucine at day 3 (D3) was calculated by initial minus D3 amount of leucine/3.

Results: Mean leucine concentration at presentation was similar in the groups P and E (15.7 and 15.9 mg/dL). Mean clearance of leucine at D3 was significantly higher in the group P than in the group E (1759.4±1021.0 vs 982.2±958.5 mg/day, P=0.026), without side effect. Mean duration of hospitalisation was similar (4 vs 4.5 days, P=NS). No patient in the group P worsened and needed to be dialysed whereas one patient was dialysed in the group E.

Conclusion: This new parenteral amino acids mixture is safe and more efficient for the treatment of acute MSUD decompensation than classic enteral feeding.

P-001

ACUTE AND CHRONIC TYROSINE ADMINISTRATION INCREASED ACETYLCHOLINESTERASE ACTIVITY IN BRAIN AND SERUM OF RATS

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Background: Tyrosine levels are abnormally elevated in tissues and physiological fluids of patients with inborn errors of tyrosine catabolism, especially in tyrosinemia type II, which is caused by deficiency of tyrosine aminotransferase. Hypertyrosinemia is associated with neurologic and development difficulties in several patients and the mechanisms of brain damage in this disorder are poorly known.

Objectives: We investigated the effect of L-tyrosine administration on acetylcholinesterase activity in hippocampus, striatum, cerebral cortex and serum of rats.

Methods: For acute administration, 10- and 30-day-old Wistar rats were killed one hour after a single intraperitoneal injection of L-tyrosine (500 mg/kg) or saline. For chronic administration, two injections (at 12 h interval) of L-tyrosine (500 mg/kg) or saline were given starting at postnatal day 7 for 21 days; twelve hours after the last injection, the animals were killed. Brain was removed and serum was collected for acetylcholinesterase activity evaluation.

Results: We observed that acute (10- and 30-day-old) and chronic administration of L-tyrosine increased acetylcholinesterase activity in hippocampus, striatum, cerebral cortex and serum, as compared to control group.

Conclusions: These results suggest that L-tyrosine administration may alter cholinergic synapses, and this may be involved in the pathophysiology of brain damage found in patients affected with hypertyrosinemia.

P-002

EVALUATION OF BRAIN-DERIVED NEUROTROPHIC FACTOR IN BRAIN OF RATS SUBMITTED TO AN ANIMAL MODEL OF MAPLE SYRUP URINE DISEASE

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Background: Maple syrup urine disease (MSUD) is an inherited amino-acidopathy resulting from dysfunction of the branched-chain keto acid dehydrogenase complex, leading to accumulation of the branched-chain amino acids (BCAA) leucine, isoleucine and valine. Affected patients usually present CNS disturbances. Thus, we investigated BDNF levels in brain of rats submitted to an animal model of MSUD.

Methods: For acute administration, three injections (1 h interval) of BCAA pool or saline were given subcutaneously to rats on postnatal day (PD) 10 or 28. For chronic administration, two injections (12 h interval) of BCAA pool or saline were given subcutaneously starting at PD 7 for 21 days. One hour (acute) or twelve hours (chronic) after the last injection, the animals were killed by decapitation and the brain was removed; BDNF levels were measured using a sandwich enzyme-linked immunosorbent assay.

Results: We observed that acute administration of BCAA increased BDNF in striatum and hippocampus in 10-day-old rats. In contrast, in 30-day-old rats, BDNF were increased in striatum and cerebral cortex. Chronic administration increased BDNF in hippocampus and cerebral cortex.

Conclusion: Considering that an increase in BDNF in the brain may impair memory, we speculate that the present findings may be related to brain dysfunction in MSUD.

P-003

EVALUATION OF ACETYLCHOLINESTERASE ACTIVITY IN AN ANIMAL MODEL OF MAPLE SYRUP URINE DISEASE

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Background: Maple syrup urine disease (MSUD) is an autosomal recessive inborn error of metabolism caused by deficiency of the activity of the mitochondrial enzyme complex branched-chain ketoacid dehydrogenase, leading to accumulation of the leucine, isoleucine and valine, branched-chain amino acids (BCAA). Neurological sequelae are present in most patients, but the mechanisms underlying the neurotoxicity in this disorder are yet unclear.

Objectives: We studied the activity of acetylcholinesterase in hippocampus, striatum, cerebral cortex and serum of rats submitted to an animal model of MSUD.

Material and methods: For acute administration, three injections (1 h interval) of BCAA pool or saline were given subcutaneously to rats on postnatal day (PD) 10 or 28. For chronic administration, two injections (12 h interval) of BCAA pool or saline were given subcutaneously starting at PD 7 for 21 days. One hour (acute) or twelve hours (chronic) after the last injection, the animals were killed by decapitation, the brain was removed and serum was collected; acetylcholinesterase activity was measured.

Results: Acute and chronic administration of BCAA pool increased acetylcholinesterase activity in brain and serum of rats.

Conclusions: The administration of BCAA pool may induce cholinergic synapses dysfunction; this may be an important factor in the pathophysiology of MSUD.

P-004

EFFECT OF CHRONIC AND ACUTE ADMINISTRATION OF TYROSINE ON BDNF LEVELS IN RATS

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Background: Tyrosine (Tyr) levels are abnormally elevated in tissues and body fluids of patients with tyrosinemia type II, which is caused by tyrosine aminotransferase deficiency and provokes central nervous system disturbances in affected patients.
Objectives: We investigated the effect of acute administration Tyr of neurotrophic factor (BDNF) levels in brain of 10 and 30-day-old rats, and chronic administration during your development.
Material and methods: Ten and thirty-day-old Wistar rats were killed one hour after a single intraperitoneal injection of Tyr (500 mg/kg) or saline. Chronic administration of Tyr was injected on seventh to thirtieth-day-old Wistar rats with intraperitoneal injection of Tyr (500 mg/kg) or saline each 12 hours. Rats were killed 12 hours after last injection. BDNF levels were measured in both administrations.
Results: We observed acute administration Tyr decreased BDNF levels in striatum of ten-day-old rats, furthermore the same occurred in striatum and hippocampus of 30-day-old rats. Chronic administration Tyr increased BDNF levels in striatum rats.
Conclusions: Results suggest decreased BDNF levels by acute Tyr administration can induce neuronal damage, while increased BDNF levels Tyr after chronic administration may be a form of compensation due to L-tyrosine's toxicity. These findings may contribute to understanding the pathophysiology in hypertyrosinemic patients.

P-005

ENZYMATIC DIAGNOSIS OF MAPLE SYRUP URINE DISEASE IN JAPAN: APPLICATION OF HPLC-BASED RADIOISOTOPE-FREE METHOD MEASURING ISOVALERYL-COA PRODUCTION.

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Backgrounds: To confirm the diagnosis of cases showing elevated levels of leucine in blood, we developed a simple enzymatic diagnosis method for maple syrup urine disease which measures isovaleryl-CoA converted from 2-isoketocaproate by crude lysate of lymphocytes using HPLC (J Inherit Metab Dis 27, 633–9, 2004).
Patients: We applied the enzymatic diagnosis method to subjects with 4 mg/dl or higher concentration of leucine; in dried blood spots of (A) 9 symptomatic and (B) 12 asymptomatic newborns, and in plasma of (C) 5 symptomatic children.
Results: All subjects of group A, 5 of group B, and 1 of group C, were diagnosed with MSUD, respectively. Among the 11 subjects with normal results, marked elevation of blood leucine levels was observed in 2 sibling cases repetitively, suggesting thiamin-responsive MSUD; genetic analysis is under way.
Discussion: Our simple enzymatic assay is a quite useful tool for clinical practice of the disease. It can be a laborious task to detect gene mutations for MSUD because branched-chain α-ketoacid dehydrogenase complex consists of many subunits. Enzymatic diagnosis enables us to focus on cases where genetic analysis is strongly required.

P-006

PPM1K DEFECTIVE GENE IN A MILD VARIANT MAPLE SYRUP URINE DISEASE (MSUD) PATIENT WITH A PATERNAL UNIPARENTAL ISODISOMY OF CHROMOSOME 4

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We present the first case of a MSUD patient with a mild variant phenotype and bearing a null mutation in the PPM1K gene, which encodes for the regulatory enzyme, protein phosphatase 2Cm, of the branched-chain alpha keto acid-dehydrogenase complex. After completing the direct sequencing of the BCKDHA-E1alpha, BCKDHB-E1beta, DBT-E2 and DLD-E3 genes and considering that genomic rearrangements are the second most common cause of monogenic disorders, patient's DNA was analyzed using the single-nucleotide polymorphism genotype array technology. Results showed a copy neutral homozygous pattern for chromosome 4 compatible with a uniparental isodisomy (UPD) involving the entire chromosome. In other autosomal recessive disorders, homozygosity mapping has provided a hint about the chromosomal location of the probable disease-causing gene. Therefore, as the PPM1K gene is located in region 4q22.1 of chromosome 4, we sequenced the complete coding region of PPM1K (NM_ 152542.3) identifying a homozygous nucleotide change c. 417_418delTA (p.His139fs) in cDNA and genomic DNA from patient. The mutation was only inherited from the father, confirming a paternal UPD. Inmunoblotting assay of the expressed mutant PPM1K showed absence of PP2Cm protein. Our data are consistent with the previously assumed relationship between the loss of PP2Cm activity and an intermediate presentation of MSUD.

P-007

ELEVATED CSF/PLASMA RATIOS OF BRANCHED CHAIN AMINO ACIDS IN PATIENTS WITH MSUD AND METABOLIC COMA

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Introduction: In patients with maple syrup urine disease (MSUD) elevated concentrations of branched chain amino acids (isoleucine (Ile), leucine (Leu) and valine (Val)) are detectable in CSF, plasma and urine. Changes in CSF/plasma ratios have not been reported in this inborn disorder of metabolism.
Patients: Patient 1 (17 years, male) with MSUD during gastroenteritis developed metabolic decompensation with coma. CSF and plasma concentrations of branched chain amino acids were elevated (in μmol/L, CSF: Ile: 178, Val: 352, Leu: 832; plasma: Ile: 557, Val: 1262, Leu: 2341). Additionally, CSF/plasma ratios were elevated (Ile: 0.32, Val: 0.28, Leu: 0.36, (more than 2 SDS above "disease control"-values (1)).
Patient 2 (10 days, female) in her first crisis before diagnosis developed postnatal metabolic coma with massively elevated concentrations of branched chain amino acids (CSF: Ile: 159, Val: 216, Leu: 1127; plasma: Ile: 365, Val: 664, Leu: 3221) and elevated CSF/plasma ratios (Ile: 0.44, Leu: 0.35, Val: 0.32) as well.
Conclusion: Elevated CSF/plasma ratios of branched chain amino acids in MSUD patients can indicate intracerebral trapping of these amino acids.
Reference: (1) Scholl-Bürgi et al Pediatrics 2008

P-008

ASSESSMENT OF BIOCHEMICAL PROFILES DURING METABOLIC DECOMPENSATION IN 14 PATIENTS WITH MAPLE SYRUP URINE DISEASE (MSUD)

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Background: We investigated biochemical changes during metabolic decompensation in 14 individuals with MSUD, currently being treated at the National Centre for Inherited Metabolic Disorders, Dublin.
Objectives: Retrospective analysis of biochemical profiles in plasma associated with episodic metabolic decompensation, particularly branched-chain amino acids, alanine, proline, glucose and acid-base status.
Patients and Methods: Our cohort of MSUD patients comprises 8 females, 6 males; mean age 20.6 yrs (range 4–33); 11 classic, 3 variant forms; data collection period 1978–2011. Treatment during crises (leucine>800 μM) ranged from special diet, supplements, infusion of glucose, electrolytes, fat, insulin, occasionally thiamine, to intensive care treatment and rare use of dialysis.
Results: Median peak leucine concentration was 1125 μM (812–6022), leucine/alanine ratio ranged from 2.94 to 91.79. Leucine correlated positively with glucose ($r=+0.4360$, $p<0.001$) and negatively with bicarbonate ($r=-0.3870$, $p<0.005$). As a trend, there was a correlation between alloisoleucine and proline ($r=+0.2908$, $p<0.05$); alanine and proline correlated to each other ($r=+0.6537$, $p<0.0001$).
Conclusion/Discussion: Our findings suggest bioenergetic disturbances in patients with MSUD during crises despite the absence of severe metabolic acidosis. In view of this, additional cofactors, e.g. coenzyme Q10, may also be considered as further treatment options during crises.

P-009

SUCCESSFUL EARLY TREATMENT OF NEONATAL MAPLE SYRUP URINE DISEASE

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Background: The neonatal form is the most severe and most frequent type of maple syrup urine disease (MSUD). In Serbia, MSUD is not included in newborn screening programme.
Objectives: Our aim is to investigate outcomes in critically ill newborns with early recognition and treatment of MSUD.
Case report: Three newborns were diagnosed with MSUD in Neonatal Intensive Care Unit of our Institute during 2005–2010 period. All patients have been admitted for somnolence and poor suck beginning in the second week after birth. Diagnosis of MSUD was established within first 48 hours after admission with values of leucine ranging from 2351–2756 mcmol/L. Initial treatment included cessation of oral feeding and continuous hemodiafiltration for 24–60 hours. During the first 24 hours of treatment, we observed a 4–6 fold decrease in leucine concentration in these patients. Cognitive and physical development of all 3 patients is in normal range, with follow-up of 1–5 years.
Conclusion: Early clinical recognition and urgent treatment that includes hemodiafiltration could provide normal development for children with neonatal form of MSUD.

P-010

MAPLE SYRUP URINE DISEASE IN BRAZIL: A CROSS-SECTIONAL STUDY OF 48 PATIENTS

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Maple syrup urine disease(MSUD)is an IEM caused by the deficiency of the branched-chain α-keto acid dehydrogenase complex,leading to the buildup of leucine, isoleucine and valine and their toxic by-products, causing acute cerebral damage and chronic sequelae.There are no epidemiological data for MSUD in Brazil, a disease not included on the national newborn screening panel. To characterize a cohort of Brazilian MSUD cases, data were collected through interview with doctors these patients. 79% of the patients were retrieved, and complete data obtained on 48 cases. Results: 72% of the cases were from Southern Brazil, 70% presented symptoms in the first 10 days of life. Metabolic formula was promptly available in 22% of the cases, and 88% presented delayed neuropsychomotor development. 52% had seizures and 62% respiratory abnormalities. There was a family history in 19% of the cases. Some of the patients who had early diagnosis did not have a normal development due to treatment failure during metabolic crisis or incorrect monitoring and biochemical control. 21% of the cases died before the age of 10 months, confirming a largely severe disease presentation.These results show that a comprehensive management program is needed in order to provide a better outcome to Brazilian MSUD patients.

P-011

PREGNANCY IN MAPLE SYRUP URINE DISEASE (MSUD). TWO CASE REPORTS

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Objectives: There are few reports today about pregnancy in MSUD. We present two new cases with a special focus on the protocol to avoid mother metabolic decompensation (MD) at delivery.
Case reports: The two patienta presented with the severe form of neonatal MSUD and experienced multiple MD from neonatal period through adulthood. The first patient is mentally handicapped and had 4 MDs during the first trimester of pregnancy. Delivery occurred at 39 weeks of pregnancy and the baby presented with intra uterine growth retardation (weight and height <10th percentile, head circumference: 25th percentile). He showed moderate developmental delay and at 12 years old, he required special schooling. Brain MRI performed at 1 year showed periventricular leucomalacia. The second pregnancy was well controlled from the beginning but the birth occurred at 32 weeks by caesarian section because of aging of the placenta. The boy also presented IUGR but has a normal development until now. The first patient experienced five MD episodes in the post-partum period, while a specific per and post-partum management protocol was performed to the second patient who did not experienced any MD.
Conclusion: MSUD pregnancies must be carefully managed, mainly during the first trimester and delivery.

P-012

SINGLE DOSE NTBC-TREATMENT OF HEREDITARY TYROSINEMIA TYPE 1

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Background: NTBC (2-(2-nitro-4-trifluoromethylbenzoyl)-1,3cyclohexanedione) is the mainstay of treatment in tyrosinemia type 1 (HT 1). The recommended administration is to be carried out in two divided daily doses.
Patients and Methods: We monitored plasma NTBC concentrations in a series of seven patients who were changed from a multiple dose to a single NTBC dose. Two additional patients were directly started on single dose NTBC after diagnosis. In three patients, studies on NTBC kinetics were performed over 6 and 24 hours, respectively.
Results: Plasma NTBC concentrations did not significantly decrease in any of the patients after changing from multiple to single dose NTBC treatment. In fact, in one patient, a significantly lower NTBC dose was required after changing to a single dose indicating a better compliance. Kinetic studies as well demonstrated that plasma NTBC concentrations were completely stable over a period of 24 hours with a single dose application as expected with a known NTBC plasma half life of 54 hours.
Conclusion: NTBC administration in tyrosinemia type 1 can be carried out in one single dose improving patients' compliance with the drug treatment.

P-013

TREATING TYROSINEMIA TYPE 1: EXPERIENCE FROM KUWAIT

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Background: Hereditary tyrosinemia type1(HT1)is a potentially lethal disease if not diagnosed and treated properly.Combined treatment with nitisinone (NBTC) and low-tyrosine diet has resulted in greater than 90% survival rate.
Case report: This report describes clinical data of 6 Kuwaiti patients having HT1.The index case was the product of consanguineous marriage male presented at five months age with hepatic failure, died at age of 7 months. Based on this family history all following siblings were screened ,three children (two males and one female)were affected. The second index case (the cusion of the first index case) was a female died at age of 6 months with hepatic failure. Her sister was diagnosed on birth based on family history. Once diagnosis was confirmed, all patients were started on tyrosine free diet and NTBC 1 mg/kg/day twice daily. They were monitored regularly.
Results: Patients are leading normal life, 2 males aged 15 and 9 and 2 females aged 13 and 8. Although they have poor dietary control, their tyrosine level ranged between 800–1000. None of the patients had any complication of the disease or the drug.
Conclusion: Medical advances improved prognosis of patients with HTI. Screening of HTI is recommended in Kuwait.

P-014

A CASE OF THE TYROSINEMIA TYPE I IN UKRAINE: EXPERIENCE AND OUTCOME

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Introduction: Tyrosinemia type I (HT1) is a rare congenital metabolic disorder of tyrosine metabolism, resulting in the accumulation of toxic metabolites that damage the liver and kidneys.
Case Report: A 16 month old girl presented failure to thrive, rickets, significant visceromegaly (hepatomegaly with hepatic nodules, splenomegaly and nephromegaly) and anaemia. Her psycho-motor development was delayed. The height, weight were less than 2nd percentile. Fanconi syndrome was suspected.
TMS showed highly elevated levels of tyrosine (568 mcmol/L, normal< 200), and phenylalanine (205 mcmol/L, normal<125). Other biochemical investigations showed high concentration of urine succinylacetone (542 mmol/mol de creat, normal<20) and alphaphetoprotein 1750 ng/ml normal<13.4). The hepatic transaminases, alkaline phosphates were elevated too. Genotype is IVS12+5 g/aSer352Arg.
She has been treated since the age of 18 months with Orfadin (dose 1 mg/kg/day) and with the phenylalanine—and tyrosine- restricted diet with good response and tolerance and adequate metabolic status is maintained. Her mental and motor developments have normalized.
Conclusion: In our case, the clinical manifestation was typical HT1. The Orfadin treatment and protein restrict diet with free tyrosine formula have improved considerably the prognosis for our HT1 patient.

P-015

NEUROCOGNITIVE OUTCOME IN PATIENTS WITH HYPERTYROSINEMIA TYPE I AFTER LONG-TERM TREATMENT WITH NTBC

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Objectives: Implementation of NTBC into treatment of hypertyrosinemia type I (HT I) greatly improved survival by prevention of acute liver failure and hepatocellular carcinoma. However, there are first reports of cognitive impairment in patients with elevated plasma tyrosine concentrations.
Methods: Neurocognitive development in 9 patients with HT I under long-term NTBC therapy was assessed using standardized psychometric test batteries.
Results: High plasma tyrosine concentrations were frequently documented in 7 out of 9 patients. Psychometric testing revealed a total IQ score below the average and an inhomogenous test profile with significant differences between the different testing scales in 5 out of 7 patients, respectively. Motor abilities were subnormal in 4 out of 7 patients. Logopedic evaluation in children at school age documented dysfunction or retardation in language development in all but one of the tested patients, however, all but one patient had a migration background.
Conclusions: A high number of patients performed below normal in the assessment of development, motor function and speech. We propose intellectual impairment as a long-term complication in HT type I with elevated plasma tyrosine under NTBC treatment as observed in other hypertyrosinemias. These findings remain to be reproduced in greater patient numbers.

P-016

TYROSINEMIA TYPE 1 PRE AND POST NITISINONE: A PORTUGUESE CENTER EXPERIENCE

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Background: The pathological features of tyrosinemia type 1 are believed to be the consequence of the accumulation of succinylacetone and its precursors. Until 1992, diet and liver transplantation were the mainstay of therapy but the emergence of nitisinone has changed the natural history of this disease.

Patients and Methods: Retrospective analysis of eight patients with tyrosinemia type 1 followed in our centre since 1990.

Results: Three patients were pre-nitisinone introduction: two died at the time of diagnosis by liver failure; the third was submitted to a liver transplant. Three patients with chronic liver disease, diagnosed at 11, 18 and 8 months, received nitisinone for 5, 10 and 9 years, with persistently undetectable succinylacetone. They underwent liver transplantation for suspicion of hepatocarcinoma but this was only confirmed in two patients with α-fetoprotein raise. A 5-month old patient died four days after onset of nitisinone due to liver failure and a 4-year-old boy, identified by newborn screening program, remains on nitisinone treatment with a reasonable evolution.

Conclusions: Despite nitisinone therapy and normal values of succinylacetone, hepatocarcinoma developed in two patients. Neonatal screening and early treatment can be crucial. Until better understanding of the underlying causes, close surveillance of patients is need.

P-017

HEPATOSPLENOMEGALY REVEALING TYROSINEMIA TYPE 1

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Background: Main clinical features of tyrosinemia type 1 include acute liver failure in infancy or chronic liver dysfunction and renal Fanconi syndrome in late-presenting cases. Enlarged spleen is usually an indicator of lysosomal storage diseases and is not described in patients with tyrosinemia type 1.

Case report: A 17 months old girl was referred to the neurologist because of walking difficulties which were in fact due to abdominal distension. Hepatosplenomegaly was documented clinically and by abdominal ultrasound and CT. Diagnostic work-up revealed renal Fanconi syndrome, the clue symptom leading to the correct diagnosis by determination of urinary succinylacetone. Severe liver cirrhosis with oedemas and ascites, and acute respiratory distress syndrome necessitated resuscitation in the ICU until a favorable response to treatment by NTBC. After two months, regression of hepatomegaly and of the enlarged size of the spleen was observed, even if splenomegaly in this case was indicative of severe liver cirrhosis with consecutive portal hypertension.

Conclusion: Splenomegaly as a marker of hepatic cirrhosis may be a revealing symptom of tyrosinemia type 1.

P-018

HYPOPHENYLALANINEMIA IN A NEWBORN IDENTIFIED AFTER NEWBORN BLOODSPOT SCREENING (NBS) FOR TYROSINEMIA TYPE I (TYR1)

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Background: Treatment of Tyrosinemia type I consists of NTBC and dietary restriction of Phenylalanine (Phe) and Tyrosine (Tyr).

Case Report: Our patient was born after an uneventful pregnancy/delivery. At day 7 she was admitted because of positive NBS results for TYR1 (succinylacetone 4.7 μmol/l; threshold 1.2 μmol/l). Treatment was initiated with NTBC and restriction of Phe and Tyr (breastfeeding combined with Phe and Tyr free formula), based on blood Tyr concentrations.

Due to increased Tyr concentrations, natural protein intake was decreased to a minimum of 0.5 g/kg/day. During the subsequent months, she developed first eczema, while later decreased growth parameters followed, and thereafter cortical myoclonus appeared and decreased psychomotor development seemed to arise despite adjustments of the natural protein intake both ways, and Tyr concentrations varying from some 215 to 609 μmol/l . Because of hypophenylalaninemia (< 20 μmol/l), ~ 30 mg/kg/day Phe-supplementation was started at day 192. Subsequently, all parameters improved.

Conclusion: The need of Tyr and Phe varies among patients. Extra Phe usually is not considered as Phe is largely converted into tyrosine. However, this case shows that hypophenylalaninemia is an important complication, necessitating Phe-supplementation, even when this results in lower natural protein intake.

P-019

DETERMINATION OF HOMOGENTISTIC ACID IN URINE BY CAPILLARY ELECTROPHORESIS WITH UV DETECTION

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Homogentisic acid (HGA) is a diagnostic metabolite that accumulates in patients with alkaptonuria. The cause of the disease is deficiency of homogentisic acid oxidase. Patients with alkaptonuria excrete HGA in the urine. Therefore diagnosis of alkaptonuria can be confirmed by urine HGA analysis.

Capillary electrophoresis (CE),a highly effective analytical technique, has been employed for the clinical analysis. The advantage of CE method is high separation efficiency, excellent resolution, short analysis time, electrolyte and sample consumption. CE is particularly suitable in the analysis of complex natural matrices, owing to its higher resolving power. Therefore, biological fluids can be directly analyzed without any pretreatment step or with a simple process of sample pretreatment by CE. In this work CE method for the determination of HGA in urine samples has been developed and validated. Separation and determination were carried out with ultraviolet detector at 190 nm. The optimum conditions were achieved using phosphate buffer 47 mM at pH 7.0 and voltage of 22 kV. Under these conditions the presence of HGA is detected in less than 8 min. Good linearity ($r2>0.99$) and repeatability (%RSD<2%) was obtained.

The developed method was simple, easy, rapid and inexpensive for quantitative determination of urinary HGA.

P-020

IN VIVO EVIDENCE THAT RAT BRAIN EXPOSED TO HIGH GLYCINE CONCENTRATIONS IS SUSCEPTIBLE TO OXIDATIVE DAMAGE

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Background: Large amounts of glycine (Gly) occur in brain of patients affected by nonketotic hyperglycinemia (NKH), which is an inherited disorder characterized by brain abnormalities whose pathomechanisms are still poorly established.
Objectives: The effects of intrastriatal administration of Gly to rats on relevant oxidative stress parameters were investigated.
Methods: Thiobarbituric acid-reactive substances (TBA-RS), carbonyl formation, sulfhydryl content, reduced glutathione (GSH) levels, nitric oxide production and the activities of the antioxidant enzymes glutathione peroxidase (GPx), glutathione reductase (GR), catalase (CAT), superoxide dismutase (SOD) and glucose-6-phosphate dehydrogenase (G6PD) were measured in striatum from 30-day-old rats.
Results: Gly injection significantly increased TBA-RS (lipid peroxidation) and protein carbonyl content (protein oxidation). Furthermore, the activities of GPx, GR, SOD and CAT were altered by Gly. In contrast, Gly administration did not change sulfhydryl, GSH and G6PD activity.
Conclusions: Gly induces lipid and protein oxidative damage and also modulates the activity of antioxidant enzymes in striatum. In case these findings also occur in human NKH, it is feasible that oxidative stress may be involved in the pathophysiology of the brain injury observed in patients with this disease.
Financial support: Research grants from CNPq, PROPESq/UFRGS, FAPERGS, PRONEX, FINEP Rede Instituto Brasileiro de Neurociências (IBN-Net), INCT-EN and FIPE/HCPA.

P-021

CLINICAL AND GENETIC VARIABILITY IN NON-KETOTIC HYPERGLYCINEMIA (NKH)

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NKH is caused by deficiency of the glycine cleavage multi-enzyme system (GCS) with three specific components encoded by GLDC, AMT, and GCSH. It is characterized by accumulation of glycine in body fluids and various neurological symptoms. In this study, we analyzed the mutation spectrum of twenty-two NKH patients grouped by clinical and biochemical findings as three subgroups: sixteen patients with neonatal presentation of convulsions and displaying high levels of CSF-glycine (88–246 μM); two cases with infantile outcome (CSF-glycine 24–26 μM); four patients with infantile onset, CSF-glycine from 12–34 μM and progressive neurological disease leading to death. The genetic screening included the exon-sequencing analysis of GCS genes and a multiplex ligation-dependent probe amplification to detect GLDC deletions. GLDC mutations were identified in 15 of the 16 neonatal-onset NKH. The spectrum included six different types of GLDC deletions mostly affecting regions comprising exons 1–15 and 17 potential-pathogenic point mutations. Three different mutations in AMT gene were found in two patients; one neonatal (p.[K294fs+T87_Q113del]) and one infantile (p.[Arg22Cys+Arg222Cys]). For the third subgroup of patients no mutations were detected either in the GLDC, AMT, or GCSH gene suggesting a different primary gene lesion as responsible for the disease.

P-022

UMBILICAL CHOLINE AND RELATED METHYLAMINES BETAINE AND DIMETHYLGLYCINE IN RELATION TO BIRTH WEIGHT

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Background: Low birth weight (LBW) is associated with increased morbidity and mortality for the newborn and increased risk on chronic diseases in adulthood. Choline has an essential role in the integrity of cell membranes, methylation reactions and memory development.
Objective: We examined whether umbilical/maternal choline and related methylamines betaine and dimethylglycine (DMG) concentrations were associated with LBW in Dutch women.
Methods: Blood was sampled from umbilical cords at delivery (n=1126). Maternal blood was sampled at 30–34 weeks of gestational age (n=366). We calculated birth weights standardized for gestational age (SBW) and defined LBW as SBW <2500 grams.
Results: Maternal concentrations of all analytes were lower compared to umbilical cord concentrations. Plasma betaine and DMG between mothers and newborns were strongly correlated. Higher umbilical cord choline and betaine were associated with lower birth weight (β=−60[−89;-31] and β=−65[−94;-36]). Odds ratio for LBW was 4.12 [1.15;14.78] and 5.68 [1.24;25.91] for the highest umbilical choline and betaine quartile respectively compared to the lowest quartiles.
Conclusion: We observed an increased risk of lower birth weight with increased umbilical choline and betaine in venous umbilical cord blood. These results might reflect a change in choline consumption or metabolism or a disturbed placental function.

P-023

EFFICACY AND SAFETY OF CYCLIC PYRANOPTERIN MONOPHOSPHATE IN THE TREATMENT OF SIX NEWBORN PATIENTS WITH MOLYBDENUM COFACTOR DEFICIENCY TYPE A

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Background: Molybdenum cofactor deficiency (MoCD) is characterized by severe and rapidly progressive neurological damage caused by the loss of sulfite oxidase activity, one out of four molybdenum-dependent enzymes. Without effective therapy death in early infancy has been the usual outcome.

Objectives: MoCD type A patients carry mutations in MOCS1 causing a loss of cyclic pyranopterin monophosphate (cPMP), the first intermediate in the Moco pathway. An experimental substitution therapy with cPMP has been initiated in six patients.

Patients: Patients have been diagnosed in utero or at the age of 2–20 days and treatment was initiated on days 0–36 with daily i.v. administration of 80 μg/kg and stepwise increase to 240 μg/kg body weight. Results: Within days, all urinary markers of sulfite oxidase (sulfite, S-sulfocysteine, thiosulfate) and xanthine oxidase deficiency (xanthine, uric acid) returned to almost normal and stayed constant throughout treatment. Clinically, all infants became more alert, convulsions and twitching disappeared within the first two weeks and the EEG showed the return of rhythmic elements and markedly reduced epileptiform discharges.

Conclusion: Substitution of cPMP represents the first causative therapy available for MoCD patients. We demonstrate efficient uptake of cPMP and restoration of molybdenum cofactor-dependent enzyme activities. Further neurodegeneration was stopped.

Conflict of Interest declared.

P-024

FOLLOW-UP OF TWO INFANTS WITH MOLYBDENUM COFACTOR DEFICIENCY (MOCD) GROUP A, ON LONG-TERM TREATMENT WITH CYCLIC PYRANOPTERIN MONOPHOSPHATE (CPMP)

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We report on the efficacy of long-term cPMP substitution in two unrelated children with MOCD group A who both share the same homozygous mutation in the MOCS1 gene.

Diagnoses were made on day 1 of life in Baby 1 due to a previously affected sibling and on day 4 in Baby 2 who started having intractable seizures from day 1 of life.

Daily intravenous cPMP infusions were started on day 7 and 5 of life and continued for a period of 18 and 15 months in Baby 1 and 2, respectively, without interruption and using an identical protocol. Treatment efficacy and safety were carefully monitored.

Both infants have been tolerating cPMP without adverse effects, apart from intercurrent central venous line infections. We observed a rapid and sustained good clinical and biochemical response. Baby 1 has shown very satisfying developmental progress whereas Baby 2, despite earlier treatment, suffers from static, dystonic cerebral palsy and cystic encephalopathy due to early non-progressive necrotic changes, probably pre-dating cPMP substitution.

Infants with MOCD type A can be safely and effectively treated with long-term cPMP substitution. The extent of encephalopathy does not only depend on genotype and time delay between birth and start of cPMP substitution.

P-025

α-AMINOADIPIC SEMIALDEHYDE IS ELEVATED IN MOLYBDENUM COFACTOR DEFICIENCY

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Alpha- aminoadipic semialdehyde (α-AASA) is the hallmark accumulating metabolite in pyridoxine-dependent epilepsy due to Antiquitin deficiency (PDE). Elevated levels may be measured in urine by LC-MS for diagnosis of the disorder, according to age-dependent reference ranges. The typical ranges seen in Antiquitin deficiency are 7–138 mmol/mol Cr under 12 months and 1.3–40 mmol/mol Cr over 12 months.

Patients with molybdenum cofactor deficiency (MoCoF) and PDE may both present with a neonatal epileptic encephalopathy (NEE). Urine samples analysed by LC-MS in our laboratory to aid differential diagnosis of NEE have shown elevated α-AASA in all cases subsequently confirmed by molecular genetic analysis to have MoCoF deficiency. α-AASA ranged from 9.6–16.9 mmol/mol Cr in two patients under 6 months (reference range <5) and 2.9 mmol/mol Cr in a single patient over 12 months of age (reference range <2).

α-AASA may form a sulphonate with sulphite when present in excess. This may be excreted in urine and liberated during the alkalinisation step of sample analysis, providing a possible mechanism for the observation. Detection of elevated urinary sulphite or sulphocysteine in MoCoF deficiency will differentiate the two conditions guiding appropriate management.

P-026

MOLYBDENUM COFACTOR DEFICIENCY: A NEW HPLC METHOD FOR FAST QUANTIFICATION OF S-SULFOCYSTEINE IN URINE AND PLASMA

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Background: Molybdenum cofactor deficiency (MoCD) is a rare inherited metabolic disorder characterized by severe and progressive neurologic damage mainly caused by resulting sulfite oxidase deficiency (SOD). Elevated urinary levels of sulfite, thiosulfate and S-sulfocysteine (SSC) are hallmarks in the diagnosis of MoCD and SOD.
Objectives: Recently, a first human exposure and successful treatment of MoCD type A based on a substitution therapy with cyclic pyranopterin monophosphate (cPMP) has been reported. Knowing the rapid progression of the disease symptoms in untreated patients, an early diagnosis of MoCD will be crucial for treatment outcome.
Results: Here we describe a fast and sensitive method for the analysis of SSC in human urine samples using high-performance liquid chromatography (HPLC). The analysis is based on pre-column derivatization with o-phthaldialdehyde (OPA) and separation on a C18 reverse phase column coupled to UV detection. Total analysis time of the described method is three minutes and quantification is linear in the range of 0.5 to 500 μM SSC. SSC values from 45 control pediatric and 43 adult urine as well as 60 plasma samples are reported.
Conclusion: The described SSC quantification method is cost effective as well as useful for routine diagnosis.
Conflict of Interest declared.

P-027

LYSINURIC PROTEIN INTOLERANCE (LPI) :EXPERIENCE OF A GREEK CENTER

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Background: Lysinuric Protein Intolerance (LPI) is an AR kidney and intestinal transport disorder (OMIM222700) affecting lysine, ornithine and arginine (SLC7A gene). Symptoms include protein-induced hyperammonemia, growth retardation, hepatosplenomegaly, osteoporosis, alveolar proteinosis, hemaphagocytosis.
Objective: Six patients with hyperdibasic aminoaciduria are described to highlight LPI clinical heterogeneity and a diagnostic pitfall.
Case reports: Patients 1, 2: A soldier, 23y, with short stature, hepatosplenomegaly, osteoporosis, eye lesions, manifested psychotic episodes and seizures after high protein meals. Two SLC7A7 mutations identified in the patient and his 27y sibling ("hyperammonemia of unknown etiology", 1.80 m, centripetal fat, thin extremities, osteoporosis). Patient 3: Albanian 6y with failure to thrive (bone-age 3y), extreme aversion to protein, hepatosplenomegaly, lung lesions (HRCT), osteoporosis. Aminoacid profile indicated LPI. By 12, death from alveolar proteinosis and hemaphagocytosis. Patients 4,5: Eight-year old with hyperammonemic encephalopathy. LPI diagnosed (two SLC7A9 mutations), also present in his asymptomatic 11y sibling. Patient 6: Male 4y with bronchiolitis (Singulaire regimen) and liver dysfunction. Symptoms with hyperdibasic aminoaciduria indicated LPI. Extensive SLC7A7 analysis negative but SLC7A9, SLC3A1 mutations present.
Conclusions: Our experience highlights clinical heterogeneity of LPI, the need for clinical awareness and effective treatment and that liver and pulmonary symptoms in the presence of cystinuria can masquerade as LPI.

P-028

AN ADULT PATIENT WITH LATE DIAGNOSED LYSINURIC PROTEIN INTOLERANCE

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Background: Lysinuric protein intolerance (LPI) is an autosomal resessive aminoaciduria affecting the transport of cationic amino acids (CAA; arginine, lysine, ornithine) at the basolateral membrane of epithelial cells in the intestine and kidney. LPI manifests with vomiting, attacks of coma, mental retardation due to hyperammonia episodes, diarrhea, failure to thrive, hepatosplenomegaly, bone marrow abnormalities, osteoporosis, pulmonary alveolar proteinosis, altered immune response and chronic renal disease.
Case Report: 36 year old female patient followed with vomiting, failure to thrive during childhood consulted for glycogen storage disease type 7 with signs of mild haemolysis and glycogen storage at liver biopsy. Lysinuric protein intolerance was suspected with findings of vomiting attacks and failure to thrive during childhood, protein aversion, short stature, osteoporosis, hyperferritinemia, hemophagocytosis, mild hemolysis,glycogen storage at liver biopsy, elevated lactate dehydrogenase, hypercholesterolemia and hypertrigliceridemia. The patient was diagnosed as LPI by low plasma levels and high excretion to urine of CAA.
Conclusions: Presentation with variable clinical and laboratory findings because of the multisystemic involvement may cause missed or delayed diagnosis in patients with LPI. The diagnosis of lysinuric protein intolerance should be considered in patients presenting with hyperferritinemia, elevated lactate dehydrogenase and hemophagocytic lymphohistiocytosis in addition to protein intolerance or aversion.

P-029

PHYSICAL EXERCISE PREVENTS COGNITIVE IMPAIRMENT OF RATS SUBJECTED TO EXPERIMENTAL HYPERPROLINEMIA

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In the present study we investigated whether physical exercise would prevent proline-induced memory deficits in Morris water maze tasks, as well as its effects on brain-derived neurotrophic factor (BDNF) immunocontent and brain acetylcholinesterase (AChE) activity in cerebral cortex and hippocampus of Wistar rats. Animals were divided into 4 groups: (1) control; (2) proline; (3) exercise and (4) proline plus exercise. Rats were submitted to proline administration from the 6th to 29th day of life, when the treatment was discontinued and the treadmill exercise was performed from day 30th to 60th day. Twenty-four hours after the last exercise session, the rats were subjected to behavioral testing in the water maze and then sacrificed for determination of BDNF and AChE activity. Proline impairs memory on spatial reference and working memory tasks and exercise prevented such effects. BDNF was reduced in both cerebral structures and AChE activity was increased only in hippocampus. Our results suggest that memory deficit caused by hyperprolinemia may be associated, at least in part, to decrease in BDNF immunocontent and increased AChE activity. Taken together, these findings reinforce the potential neuroprotective of physical exercise as an experimental therapeutic strategy to minimize cognitive deficits. Support by CNPQ, FAPERGS.

P-030

COMPARTMENTALIZATION OF LYSINE METABOLISM AND ITS ROLE FOR GLUTARYL-COA DEHYDROGENASE DEFICIENCY

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Background: Glutaryl-CoA dehydrogenase deficiency is an inborn error of lysine metabolism. Patients are characterized by neurodegeneration affecting basal ganglia induced by glutaric and 3-OH-glutaric acid accumulation. The precise pathway of lysine degradation and its subcellular localization is unclear. It is thought that there is a main, mitochondrial and a minor, peroxisomal pathway. We studied the individual steps of both pathways in Gcdh-deficient mice and potential therapeutic aspects of their modification.
Methods: We tested activities and localization of saccharopine dehydrogenase, aminoadipate semialdehyde dehydrogenase, aminoadipate transaminase, 2-oxoadipate dehydrogenase, and pipecolate oxidase in liver, brain, and kidney. Further, we induced peroxisomal biogenesis by clofibrate and examined its effect on production of glutaric and 3-OH-glutaric acid.
Results: Saccharopine dehydrogenase, entry of lysine into the mitochondrial degradation pathway, was present in liver and kidney mitochondria but not detectable in brain. Aminoadipate semialdehyde dehydrogenase and aminoadipate transaminase activity were found in cytosolic and mitochondrial fractions of all tested tissue. Pipecolate oxidase, representing the peroxisomal pathway, was detected in kidney and brain. Strikingly, clofibrate treatment decreased instead of increasing glutaric acid levels.
Conclusions: In liver lysine is mainly oxidized by mitochondria, whereas in brain lysine oxidation occurs in peroxisomes. Renal lysine degradation is possible in both organelles.

P-031

NOVEL DELETION IN HYPOTONIA-CYSTINURIA SYNDROME

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Background: Hypotonia-cystinuria syndrome (HCS) that is caused by microdeletions of SLC3A1 and PREPL on chromosome 2p21 presents with generalized hypotonia at birth, failure to thrive, growth retardation and cystinuria type I. We here report a novel deletion in a Turkish patient with hypotonia-cystinuria syndrome.
Case report: A 8 year-old-male patient born of consanguineous marriage was diagnosed as lysinuric protein intolerance by hypotonia, feeding problems and urine amino acid analysis at the age of 2 months and protein restriction and L-citrulline therapy was started. The patient was reevaluated when 8 years old because of the absence of hyperammonemia attacks even on a high protein intake, normal blood ferritin, lactate dehydrogenase levels and hypotonia-cystinuria syndrome was suspected. There was no sign of nephrolithiasis or urolithiasis. The plasma and urine amino acid analysis revealed normal plasma and high urine levels of lysine, arginine, ornithine, cystine. The demonstration of a homozygous deletion in SLC3A1 and PREPL genes confirmed the the diagnosis of HCS.
Conclusions: This deletion has not been described before. The deletion extends from the intron between PPM1B and SLC3A1 to the intron between exon 2 and exon 1 of PREPL. All exons of SLC3A1 and all coding exons of PREPL are deleted.

P-032

CLINICAL AND MOLECULAR CHARACTERIZATION OF CYSTINURIA IN FRENCH PATIENTS

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Cystinuria is an autosomal recessive disorder of dibasic amino acid transport in in kidney and intestine leading to increased urinary cystine excretion and nephrolithiasis. Two genes, SLC3A1 and SLC7A9, coding for rbaT and bO,+AT, account for the genetic basis of cystinuria.
This study reports the clinical characterization and SLC3A1 (type A) and SLC7A9 (type B) mutations identified in a French cystinuria cohort.
A total of 106 patients from 97 families with cystinuria and 18 relatives were investigated. A questionnaire addressing, age at first symptoms, stone recurrence rates, metabolic evaluations, medical therapy and follow-up was completed.
Mutation screening using sequencing analysis was performed. Quantitative Multiplex PCR of Short fluorescent Fragments analysis was developed for the detection of genomic microdeletions and microduplications. Splicing reporter minigene assay was used to evaluate the splicing effect on unclassified variants.
63 and 27 mutations have been identified in SLC3A1 and SLC7A9 genes respectively, of which 58 were novel. These mutations included 56 missense/nonsense, 13 deletion/insertion, 8 splicing, 13 large-scale rearrangements (18% of mutated alleles). Thus, 163 and 56 mutated alleles have been reported in SLC3A1 and SLC7A9 genes respectively.
This report expands the spectrum of SLC3A1 and SLC7A9 mutations and supports that digenic inheritance is unlikely.

P-033

CLINICAL, LABORATORY FINDINGS AND OUTCOME OF 22 PATIENTS AFFECTED OF CYSTINOSIS IN IRAN (1994 TO 2010)

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Background: Cystinosis is caused by deficiency of cystinosin, the cystin transporter located in the lysosomal membrane. Late diagnosis results in accumulation in major organs (liver,kidneys, thyroid, pancreas,eyes,muscles, bone marrow). The diagnosis is confirmed by measurement of WBC cystine.
Objective: to study the clinical,laboratory findings and outcome in 22 Iranian patients affected with cystinosis.
Methods: A study on 22 patients. The diagnosis was confirmed by observing cystine crystals in cornea or in bone marrow aspiration.
Results: Total 22 , female;15, 15/22 had related parents,.Mean & Median age at onset:9.63 & 19 months. Age at diagnosis:3.54 years.Mean time between symptom onset & diagnosis:33 months.Failure to thrive 22/22., Polyuria & Polydipsia; 12/22,Hypothyroidism 9/18,Photophobia; 4/22Fair-skin; 4/13 ,Myopathy; 7/15, ,Fanconi Syndrom; 22/22anemia 20/22, Phosphaturia; 15/15,Hypercholestrolemia 5/15,Diabetes Mellitus 0/22, Hypokalemia,hypocalcemia,hyponatremia,each one;18/22.Creatinine level rising: 8/22,ESRD; 4/22;Two underwent kidney transplantation. Mean age at renal failure onset ; 9 years ,4/8 were poorly compliant and 4/8 were diagnosed at dialysis department.
Conclusion: Although cystinosis is a rare disease it should be suspected in any infant with FTT because of its complications,specially renal failure. The gap between diagnosis and treatment was 3.54 years, higher than other studies (1.6 years),but the outcome was better (63% had normal creatinine compared to 22.5% in Brazil).

P-034

LONG-TERM RESULTS OF A PHASE II CLINICAL STUDY OF CYSTADROPS. (CYSTEAMINE EYE DROPS)

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Background: Ophthalmic symptoms in cystinosis are not affected by oral cysteamine.
Methods: To determine the lowest effective dose of Cystadrops. (0.55% cysteamine hydrochloride carboxymethylcellulose eye drops gel formulation) and its safety profile, 8 cystinosis patients (8–21 years), receiving cysteamine hydrochloride 0.1% (reference product) during a 30-day run-in period were included. After switch to Cystadrops. at the same dosing frequency (3–5 daily instillations), corneal lesions were assessed periodically during 2 years using in vivo confocal microscopy (validated IVCM score), optical coherence tomography (OCT) and slit-lamp examination. Dosing frequency was individually adapted, based upon the ophthalmology results.
Results: The IVCM total score significantly decreased from baseline to D90 ($p<0.0001$). From D90 to M24, this score remained stable after reducing the dosing frequency (from 3–5 to 1–4 instillations). Epithelial layers were sensitive to treatment adaptations. Mean (SD) crystal thickness, measured by OCT, decreased by 17.5 % (10). Visual acuity remained stable; photophobia continuously decreased. The frequent adverse drug reactions (stinging and blurred vision) decreased all along the study period.
Conclusion: Based on the most robust and validated method (IVCM) for the assessment of crystal deposits, Cystadrops. (1 to 4 instillations) was effective and safe in treating the ophthalmic symptoms of cystinosis.
Conflict of Interest declared.

P-035

SUCCESSFUL USE OF TOPICAL CYSTEAMINE IN CYSTINOSIS

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Cystinosis is an AR lysosomal disorder. Free cystine accumulates continuously in lysosomes. Specific treatment with oral cysteamine results in long-term reduction of lylosomal cystine. However, corneal cystine crystals do not respond with oral cysteamine.
A 26 years old female patient with typical nefro-cystinosis, had severe photophobia. The diagnosis was made on the first year of live and needs kidney transplant at 7 years of age. She developed epilepsy when she was 12 years. She has long been treated with oral Cystagon, which is not available in eydrops formulation. The Pharmacy Department of our Hospital have prepared and tested stability of cysteamine eydrops with the following formulation: cysteamine cloridrate 0,1136 g, sodic EDTA 0,1 g, benzalconium chloride 0,01 g and isotonic soluction of NaCl for 100,0 ml. This formulation is stable for 2 months and doesn't need refrigeration. The patient has now been treated with cysteamine eyedrops for 6 years. It is well tolerated and adherence to therapy is good. The severe photophobia disappeared. Ophthalmologic evaluation with confocal microscopy shows reduction on density corneal cystine crystals density.
This cysteamine eyedrops formulation, used in this patient no needs refrigeration, is stable for a long period and most importantly, it is well tolerated and efficient.

P-036

MULTICENTRE AGE-RELATED AMINO ACID REFERENCE INTERVALS FOR CEREBROSPINAL FLUID, PLASMA AND CSF:PLASMA RATIOS

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Retrospective analysis of paired CSF and plasma amino acid results from 8 different UK laboratories was undertaken over a 2 year period. Samples from subjects with known metabolic disease were excluded. Amino acid analysis was by ion exchange chromatography (post column ninhydrin detection). Inter-laboratory analytical bias was assessed by comparing ERNDIM returns over 8 distributions during the period of data collection and analysis of the subject data between each laboratory with the Kruskall-Wallis test, which demonstrated no significant differences.
Paired samples were analysed from 271 subjects (163 male, 103 female). The mean age of the subjects was 2.9 years (median 1.44, SD 4.01, range 1 day to 24 years). The Kruskall-Wallis test demonstrated no significant difference between gender for the three data sets. Non parametric reference intervals were determined for 18 amino acids in CSF, plasma and CSF:plasma ratios. Age related reference intervals were determined for the following groups; < 6 months, 6 months—12 months, 1–5 years and 5–24 years. Spearman correlation demonstrated that 10/18 plasma amino acids, 16/18 CSF amino acids and 14/18 CSF:plasma ratios varied with age ($p<0.001$). Use of these age related reference intervals will aid interpretation of plasma and CSF amino acid results.

P-037

INBORN ERRORS OF AMINO ACID METABOLISM IN THAILAND : TWENTY—THREE YEARS EXPERIENCE

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Background: Inborn errors of amino acid metabolism can lead to an acute or progressive intoxication from the accumulation of toxic compounds proximal to the metabolic block. These disorders usually present clinical signs of 'intoxication' which may be acute or chronic. The diagnosis most commonly relies on plasma amino acid quantitative analysis. Most of these disorders are treatable and require the emergency removal of the toxin by special diets, extra-corporeal procedures, or "cleansing drugs.

Materials & Methods: Retrospective study of 42 families with confirmed diagnosis of amino acid disorders are herein reported.

Results: We had identified at least 42 families: hyperphenylalaninemia (3 classical PKU, 4 mild hyperphenylalaninemia and one tetrahydrobiopterin deficiency); 13 maple syrup urine disease (MSUD); 6 nonketotic hyperglycinemia; 2 siblings with homocystinuria; 12 urea cycle defects (2 CPS deficiency, 2 OTC deficiency, 6 citrullinemia type I; 2 argininosuccinic aciduria and one hyperornithinemia (OAT deficiency).

Conclusion: Prior to 1987, the inherited metabolic disorders in Thailand were diagnosed clinically and treatment was very limited. The establishment of 'Genetic Metabolic Center' in 2004 at Siriraj Hospital subsequently provided early diagnosis and treatment which led to better outcome in our patients.

P-038

CHALLENGES IN THE TREATMENT OF A PATIENT AFFECTED BY BOTH ARGININOSUCCINIC ACIDURIA AND METHYLMALONIC ACIDURIA

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Most of the inborn errors of metabolism (IEM) are autosomal recessively inherited. Rarely, more than one IEM are seen in the siblings of one family or in the same sibling. Here we report a patient with both argininosuccinic aciduria (ASA) and methylmalonic acidemia (MMA) and our therapeutic approach. Chronic management of both diseases consist of a low-protein diet. But the treatment of more than one disorders affecting aminoacid metabolism was challenging.

A 2 -day- old male patient was admitted to our hospital since a previous sibling had been diagnosed with ASA. The first child of the family had died due to MMA but the family refused prenatal diagnosis in this pregnancy. Laboratory investigations of the patient revealed elevated C3 propionyl carnitine, citrulline and argininosuccinic acid levels on serum tandem MS, elevated methylmalonic acid on urinary organic acid analysis and increased argininosuccinic acid levels on urine tandem MS. Both ASA and MMA were diagnosed. The dietary regimen of the patient was planned with MMA being the basis and all the replacement therapies were given both for ASA and MMA.

When 4 months old, his metabolic status was well, plasma ammonia level and blood gases were normal and he had no ketosis.

P-039

CITRIN DEFICIENCY; VARIATION IN PHENOTYPE WITH IDENTICAL GENOTYPE

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We describe 6 cases of Citrin deficiency, from 5 unrelated families, born to consanguineous parents of Pakistani origin, presenting to the Liver Unit in Leeds, UK.

Four were investigated for neonatal jaundice and had raised citrulline and galactosuria. Two had raised phenylalanine on newborn screening. Two had liver biopsies which showed cholestasis, portal fibrosis and micro and macrovesicular steatosis. Liver function tests and amino acid profiles normalised in all 4 over 10–18 months

A fifth child, initially investigated in 1996, at 6 months of age, for failure to thrive, poor tone and rickets, had hepatomegaly, generalised aminoaciduria and galactosuria. A liver biopsy at 1 year of age showed cirrhosis and focal mild macrovesicular steatosis. The diagnosis of citrin deficiency was only made in 2010 following an infant cousin being diagnosed elsewhere.

Four children are homozygous for the R588Q mutation in the SLC25A13 gene. The sixth case, a 10 year old asymptomatic sibling, has the same homozygous mutation.

All patients are well after 1–15 years.

Citrin deficiency should be considered with prolonged neonatal jaundice, especially if citrulline is raised or the liver biopsy shows steatosis. However this series suggests a wide variation in clinical phenotype even with the same mutation.

O-006

INFANTS WITH OAT DEFICIENCY MAY REMAIN AT RISK FROM HYPERAMMONAEMIA UP TO 1 YEAR OF AGE

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Male infant presented at 8 weeks with respiratory difficulties and encephalopathy, a respiratory alkalosis, hyperammonaemia (355 μmol/l). Investigations revealed raised plasma ornithine (632 μmol/l) but no orotic acid or homocitrulline in his urine. Treatment with arginine, benzoate and phenylbutyrate resulted in rapid recovery. OAT deficiency was confirmed by enzyme and molecular studies. On treatment his ornithine increased to over 1000 μmol/l. Arginine supplements were discontinued at 11 weeks of age. However after a further 3 weeks ornithine had fallen to 10 μmol/l, and glutamine increased to >1000 μmol/l with ammonia at 144 μmol/l. Arginine supplements were therefore restarted at 4 months. Within a short period there was again a marked increase in ornithine to 983 μmol/l and so supplements were stopped. By 7 months his glutamine had increased to >900 μmol/l and ornithine fell to 31 μmol/l so arginine was again restarted. At 12 months his ornithine showed a persistent increase to so that arginine was stopped. Since then there has been no fall in ornithine levels or increase in glutamine.

This case demonstrated that flux through the ornithine synthetic/catabolic pathway can reverse repeatedly in the first year of life so infants with OAT deficiency remain at risk from hyperammonaemia.

P-040

ANATOMICAL PATHOLOGICAL FINDINGS IN PATIENTS WITH UREA CYCLE DISORDERS

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Urea cycle disorders (UCD) are due to enzyme defects resulting in hyperammonaemia and variable clinical presentation. We conducted a retrospective review of post mortem (n=4; all OTC deficiency and aged <1 month), liver ex-plants (n=3; all OTC deficiency) and liver biopsy examinations (n=14) in UCD patients. A single pathologist reviewed all specimens.

Findings included:

CNS: soft, flabby but macroscopically normal brains. There was generalised congestion, swelling and vacuolation of astrocytic nuclei, neuronal loss, pyknosis and cytoplasmic shrinkage.

Liver: CPS-1: increase in lipid droplets and swollen mitochondria (n=3); OTC: mild to extensive fibrosis (n=3), portal-portal bridging fibrosis (n=2), prominent increase in glycogen particles on electron microscopy resembling glycogen storage disorder (n=1), mild portal triaditis, focal hepatitis (n=1), necrosis (n=2), cholestasis (n=1) and fatty change (n=1); ASS: slight widening of portal tracts, bile retention and feathery vacuolation of hepatocytes (n=1). ASL: prominent lipid droplets and swollen mitochondria (n=1), hepatic fibrosis at 3 and at 6 years and cirrhosis at 23 years of age (n=1).

These findings emphasise the importance of monitoring progressive hepatic changes (fibrosis; cirrhosis) in surviving patients with UCD.

P-041

HIGH INCIDENCE OF HYPERAMMONEMIA IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA RECEIVING PEGYLATED ASPARAGINASE

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Background: The outcome of childhood acute lymphoblastic leukemia has improved substantially after introduction of Asparaginase. Pegylated asparaginase (PEG-Asparaginase) has a longer half life and has been introduced recently to optimize therapeutic efficacy. We investigated whether introduction of PEG-Asparaginase affected the risk of hyperammonemia, an infrequent side effect of asparaginase treatment.

Patients and methods: We analyzed blood samples and clinical data of eight consecutive patients with acute lymphoblastic leukemia receiving PEG-asparaginase in our hospital.

Results: Maximum ammonia levels ranged from 89 to 400 μmol/L. Symptoms varied from mild anorexia and nausea to headache, vomiting, dizziness and lethargy and led to interruption of PEG-asparaginase in three patients. Analysis of plasma amino acid concentrations during PEG-asparaginase therapy revealed a depletion of asparagine (6/6) and a marked reduction in glutamine concentration (5/6), in conjunction with strongly increased concentrations of both aspartic acid and glutaminic acid. Urea cycle intermediates were normal.

Conclusions: Peg-asparaginase was associated with symptomatic hyperammonemia in all patients, unlike previous reports. We propose that the ammonia produced through hydrolysis of aspargine and glutamine is largely responsible for the hyperammonemia in patients receiving Peg-asparaginase.

P-042

HYPERAMMONEMIC ENCEPHALOPATHY AFTER ASPARAGINASE THERAPY

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Background: L-Asparaginase is commonly used in chemotherapy protocols for lymphoproliferative disorders. Asparagine is an essential amino acid for tumor cells and required for their rapid malignant growth. The mechanism of tumor lysis of l-Asparaginase is hydrolysis through of the amido group of asparagine to aspartate and ammonia. L-Asparaginase has been reported to cause hyperammonemia, which theoretically may be induced by increased ammonia production exceeding the capacity to detoxify and/or decreased detoxification capacity.

Case reports: A retrospective chart study was performed in 4 female patients, who were evaluated for encephalopathy caused by hyperammonemia after l-Asparaginase, PEG-Asparaginase or Erwinase (all incorporating l-Asparaginase). Extensive metabolic (blood amino acid profile, urine organic acids, and urine orotic acid) and molecular studies failed to identify a specific inborn error of metabolism.

Conclusions: 1. Because L-Asparaginase can cause hyperammonemia, close neurological observation after administration is recommended, including measurements of ammonia.

2. The metabolic profile is characteristic of peripheral ammonia overload. The individual tolerance of l-Asparaginase may involve l-asparaginase clearance instead of individual variations or mutations in the (regulation of) urea cycle enzymes.

3. The treatment of hyperammonemia in this situation is to emphasize hemodialysis rather than alternative ammonia conjugating agents.

P-043

AMINO ACID PROFILES IN PATIENTS WITH UREA CYCLE DISORDERS AT ADMISSION TO HOSPITAL DUE TO METABOLIC DECOMPENATION

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Urea cycle disorders (UCD) result from inherited defects in the ammonia detoxification pathway, leading to episodes of hyperammonaemia and encephalopathy during metabolic decompensation.

We analysed the results of plasma ammonia levels and amino acids profiles taken simultaneously or within 30 minutes of each other during acute admissions of all patients with UCD at our centre over 28 years. Samples from 96 admissions (79, 9 and 8 admissions for OTC, CPS and ASS deficiencies, respectively) from 14 patients fulfilled these criteria. Amino acids levels were measured by ion exchange chromatography with post column ninhydrin derivatisation and interpreted in relation to age-related reference ranges.

Plasma levels of all measured essential amino acids were low in 18 samples, and low/low-normal in almost all other samples. There was a particularly strong positive correlation between low plasma branched-chain amino acids and either Phenylalanine ($r=0.49;p<0.0001$) or Threonine ($r=0.49;p<0.0001$). Ammonia correlated with glutamine especially in the <6 yr age group and with glutamate at glutamate levels <150 μmol/L, particularly in the >6 yr age group ($r=0.68;p<0.00001$). There was a negative correlation between ammonia and Phenylalanine/Tyrosine ratio in the <6 yr age group ($r=-0.3;p=0.023$).

We conclude that protein deficiency is common at admission of patients with UCD with hyperammonaemia.

P-044

UNSUPERVISED PROFILING OF PLASMA AMINO ACIDS IN CITRULLINAEMIA TYPE I

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Introduction: We asked if multivariate profiles of plasma amino acids could help to improve the follow-up of patients with citrullinaemia type I (argininosuccinate synthetase deficiency, ASD).

Patients and Methods: We prospectively collected 318 plasma amino acid samples in a cohort of 18 patients with ASD with follow-up of 0.4–19.6 years. Principal component analysis (PCA) summarizes multiple normalized biomarkers into fewer unsupervised "multivariate biomarkers" (PC1-2, etc.).

Results: Amino acids routinely used as follow-up markers correlated significantly with PC2, which accounted for the greatest amount of variance of pathological relevance (PC1 was likely related to fasting time). Mean values of PC2 were increased in patients with mild or no disability, compared to those with moderate-severe neurodevelopmental disability (including psychomotor delay, ataxia, or seizures; $p=0.002$). This and regression analysis pointed to PC2 as an improved predictor of differential developmental outcome. PC2 values also showed negative correlation with age (-0.71, $p<<0.001$), suggesting that developmental disability might result, in part, from "accelerated aging".

Conclusions: PCA generated a highly informative multivariate biomarker for the follow-up of ASD patients. Unsupervised multivariate analyses of plasma amino acids can provide unanticipated disease-, age- and patient-related findings with possible implications for the personalized management of metabolic disorders.

P-045

UTILITY OF ARRAY CGH IN THE MOLECULAR DIAGNOSIS OF UREA CYCLE DISORDERS

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Background: Urea cycle disorders (UCD) are a group of devastating inborn errors of metabolism characterized by hyperammonemia. Prompt definitive diagnosis can help with proper treatment and carrier/prenatal diagnosis. Although Sanger sequence analysis is the current gold standard for mutation identification, it cannot detect large deletions.
Methods: A custom oligonucleotide array comparative genomic hybridization (oligo aCGH) with high-density coverage of UCD genes was used to evaluate deletions in UCD genes in DNA samples from patients with biochemical and clinical diagnoses of UCD. Deletions and breakpoints were confirmed and determined by PCR/sequencing.
Results: Oligo aCGH identified 17 deletions in the OTC gene, 4 in CPS1, and one each in the ASS1 and ARG1 genes. The size of the deletions ranged from 240 bp to 11 Mb. The OTC gene region is a hot spot for genome rearrangement, with deletions accounting for about 15% of mutations. The identification of deletions enabled prenatal diagnosis in 2 families with OTC mutations and one each in families with mutations in ASS1 and ARG1.
Conclusions: aCGH should be pursued for the detection of large deletions if sequence analysis failed to identify mutations in patients with biochemically confirmed UCD, especially in females negative by OTC gene sequencing.

P-046

CPS I DEFICIENCY: 10 YEARS AFTER LIVER TRANSPLANTATION

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Background: Congenital deficiencies of urea cycle enzymes are cause of hyperammonemia. Coma and cerebral edema are the major causes of death; survivors of coma have intellectual impairment. Plasma aminoacids and urinary orotate helps to localize the defect. CPS deficiency is a diagnosis of exclusion and should be confirmed by enzyme assay on a liver sample. There is a medical treatment for hyperammonemia. Liver transplantation appears to be excellent treatment for the urea cycle defects because correct hyperammonemia; the neurological outcome correlated with the condition prior to transplantation. Objective: To present successful outcome of CPS I deficiency after liver transplantation. Case report: normal male newborn started at 36 hours of age with irritability, refuse feeding, vomiting and hyperpnoea.
Respiratory alkalosis, ammonia 590 μg%, elevated glutamine, cero citrulline, normal orotate. Enzyme assay on liver tissue confirmed the diagnosis of CPS I deficiency. Medical treatment started. No coma episodes. At 20 months of age liver transplantation was performed.
Afterward, no hyperammonemia, normal plasma aminoacids profile. Patient quality of life improved. Normal development, some learn difficulties at school. He is 12 years. Conclusions: Liver transplantation can be an effective treatment for patients with CPS deficiency, it correct hyperammonemia and change patitent's quality of life.

P-047

FIBROLAMELLAR HEPATOCELLULAR CARCINOMA MASQUERADING AS ORNITHINE TRANSCARBAMOYLASE DEFICIENCY

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A 21 year old female with fibrolamellar hepatocellular carcinoma (FHCC) required several hospital admissions for acute episodes of confusion. The patient's cancer was initially treated several years previously by liver resection. She later relapsed with abdominal lymph node metastases but no hepatic recurrence. Her synthetic liver function tests were unremarkable but she was found to have raised ammonia with peak of 410 μmol/L. Further investigation revealed a grossly elevated urinary orotic acid, quantified at 420.3 μmol/mmol creatinine and increased uracil. Amino acid analysis showed increased glutamine and decreased ornithine, which, in combination with the other biochemical findings, suggested a diagnosis of ornithine transcarbamoylase (OTC) deficiency. DNA sequence analysis of the OTC gene, however, found no evidence of pathogenic sequence variants or exon deletions/duplications. Imaging studies reviewed and revealed no evidence of parenchymal liver disease, recurrent liver tumour or portosystemic shunting. The patient is currently on palliative chemotherapy (sorafenib), post-dating her confusion and her hyperammonaemia is being controlled with ammonia scavenging agents.
Similar cases have been reported in the literature of patients with FHCC, in some cases related to chemotherapy. It is tempting to speculate FHCC may release chemical substance which inhibit OTC and may represent a distinct syndrome.

P-049

TWO DE NOVO MUTATIONS AT THE SAME NUCLEOTIDE IN THE OTC GENE OF A MALE

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Background: Ornithine Transcarbamylase (OTC) deficiency is a heterogeneous X-linked disorder comprising severe neonatal-onset hyperammonaemic encephalopathy and variable later-onset disorders.
Case presentation: A developmentally normal 18 month boy presented with seizures, acute encephalopathy and elevated ammonia 115 μmol/L (10–50). CSF glutamine was 1085 μmol/L (319–742) and arginine was 7 μmol/L (12–30) with increased urinary orotate. His mother was asymptomatic.
Method: DNA was extracted from urine and two blood specimens from the proband as well as maternal blood. The coding region and the intron-exon boundaries of the OTC gene (NM_000531.5) were sequenced.
Results: The proband had a normal (46XY) karyotype excluding Klinefelters' syndrome. Sequence analysis in 3 samples consistently revealed two mutations at the same nucleotide in the proband: c.542A>T (p.Glu181Val) and c.542A>G (p.Glu181Gly); the former being 2–3 times more prevalent with neither being in the mother.
Discussion: Mutations at residue 181 are consistent with OTC deficiency. p.Glu181Gly has been reported in a neonate. p.Glu181Val is novel and is predicted by PolyPhen-2 to be benign though SIFT predicts it to affect protein function with low confidence. Possible causes include de-novo mutation in mother followed by somatic mutation in the fetus, or initial somatic mutation in the fetus and a subsequent second mutation.

P-050

PRESENTATION OF AN ACQUIRED INHERITED UREA CYCLE DISORDER LIVER TRANSPLANTATION FROM A DONOR WITH ORNITHINE TRANSCARBAMYLASE DEFICIENCY

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Background: Ornithine transcarbamylase deficiency (OTCD) is the most common inherited urea cycle disorder. OTCD is inherited in an X-linked manner. Clinical severity ranges between fatal neonatal presentations and asymptomatic adults.

Case Report: Our female patients with Alagille syndrome underwent orthotopic liver transplantation (LT) at the age of 19 months. The donor was a healthy appearing 2 year old boy who had a cerebral bleeding without a medical history or family history.

More than 16 years later she developed hyperammoniemic crisis after forced feeding after a surgical intervention, associated with increased urinary orotic acid excretion. In donor liver, the diagnosis OTCD was established both enzymatically (OTC enzyme activity 982 nmol/hr/mg protein; normal 18.500–64.800) and genetically (homozygous c.637 C> A mutation).

Conclusion: To our knowledge this is the third report of a liver transplant recipient presenting with acute hyperammonemia after LT from a donor with unrecognized OTCD. Determination of ammonia or urinary orotic acid excretion is a potential parameter to screen donor livers, in order to improve follow-up after transplantation.

P-051

RECURRENT STROKE EPISODES IN OTC DEFICIENCY: CLINICAL AND BIOCHEMICAL ASPECTS AND REPORT OF A NOVEL MUTATION

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Ornitine transcarbamylase deficiency (OTCD) is the most frequent urea cycle error and results from mutations in the OTC gene—that encodes a 354-residue polypeptide. The clinical picture in females is highly variable even within a family, depending on the X-inactivation pattern in liver. Mutations that lead to late-onset presentations may lead to life-threatening disease and may be unrecognized. We present a 14 months-old girl who manifested with recurrent stroke episodes after a respiratory infection that triggered a hyperammonemic crisis; investigations revealed hyperammonemia (384 μmol/L), coagulopathy, elevated liver enzymes, lactic acidosis and massive orotic aciduria (orotate/creatinine ratio 5000 mmol/mol creatinine, reference range <11), being highly suggestive of ornithine transcarbamylase deficiency.

DNA analysis revealed a novel mutation in the 6th exon of the OTC gene [c.628A>T (p.Lys210X)], leading to a premature stop of translation. Searching for OTCD mutations is laborious and expensive; if all OTCD causing mutations were known, mutation screening might be simpler. OTCD should be considered in the differential diagnosis of unexplained acute neurological presentation, even without a suggestive family history. This report expands the clinical, biochemical and molecular spectrum of OTCD with a female case heterozygous for the novel c.628A>T OTC mutation.

P-052

THE ROLE OF TRANSCRIPT VARIANTS OF ARGININOSUCCINATE LYASE FOR CLINICAL VARIABILITY

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Background: Argininosuccinate lyase (ASL) deficiency belongs to the classical urea cycle disorders but the disease can have a wide clinical variability. While some patients present as neonates with life-threatening hyperammonemia, others develop severe neurological disease in the absence of hyperammonemia or develop severe liver disease. The basis of this phenotypic variability is poorly understood. Beside other hypotheses, we speculate that ASL transcript variants might partly explain this phenomenon.

Methods: PCR amplification of cDNA from 17 different tissue sources was done both full-length and in fragments to study the occurrence of transcript variants and the tissue dependency of this phenomenon. Further, a bioinformatic model was exploited to predict on the effect of found transcript variants on the ASL homotetramer formation.

Results: ASL transcript variants occur at a high rate in all tissues studied. The most prevalent variants comprise in-frame deletions of exons 2 or 7. The predictive model suggests that deletion of exon 2 might neither affect the formation of the ASL homotetramer nor the active center.

Conclusion: ASL transcripts might contribute to the phenotypic variability of ASL deficiency. Ongoing expression studies of the most prevalent variants will elucidate whether the mutant ASL proteins still preserve some residual ASL activity.

P-053

ARGINASE DEFICIENCY A HEREDITARY SPASTIC DIPLESIA , REPORT OF 20 CASES

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Background: Hyperargininemia is an urea cycle disorders caused by arginase deficiency with clinical manifestations strikingly different from others,usually progressive,being discovered after two years of age, including psychomotor delay (PMD) ,specially walking and speech delay, growth retardation,seizures,hyperactivity and sometimes atethosis,but symptomatic hyperammonemia progressing to encephalopathy is rare.the major physical finding is spastic tetraplegia more prominant in lower limbs.Close inspection of the patients suggests that clinical abnormalities occur in early infancy .Diagnosis is made by hyper argininemia,hypo-ornithinemia,mild hyperammonaemia and orotic aciduria due to unavailability of ornithine ,so limited influx through the OTC reaction and confirmed by undetectable arginase in RBCs and WBCs.

Objective: To report clinical,laboratory findings and outcome of 20 patients affected of argininemia at two main metabolic center in Tehran(1993–2010). Cases: Age at diagnosis;48 D −10 yr,males;13,abnormal or delayed walking;10 cases,convulsion;8,delayed speech;4,hyperactivity;4,restlessness;3 ,attacks of vomiting terminating in coma;2. We found spastic paraparesis in 14 cases, normal blood ammonia;3, 2–3 times normal;15, >5 times;2 ,plasma arginine level; >5 times;8,2-3 times;9,1.5-2times;3 orotic aciduria;5 /7, deficient arginase(Western blot);20,Conventional treatment was started with well response with regard to growth and development(follow up of 7.5–17 years).

Conclusion: A progressive spastic diplegia in a previously normal child may suggest a neurodegenerative disease. Plasma aminoacid determination is mandatory,because effective therapy is available.

P-054

METABOLIC PROFILING OF CHEETAHS PRONE TO HYPERAMMONEMIA

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Background: Hyperammonemia is well known in the cat family on an arginine deficient diet. Several studies have been conducted to explain this phenomenon. Morris and Rogers described arginine as an essential amino acid for the cat family and Jones found that cats are unable to produce ornithine. Levillain found that hyperammonemia in cats can be prevented and treated with either arginine or citrulline supplementation but not with ornithine. One cheetah breeding population, on a well balanced diet, in South Africa experienced chronic problems with hyperammonemia. The urinary creatinine excretion in the cat family is extremely high. Since creatine is synthesized from arginine, the high creatinine values indicated an enormous flux through arginine. Therefore arginine cannot be essential and the low arginine and citrulline concentrations indicated the presence of a possible catabolic pathway for at least one of the intermediates of the urea cycle.
Results: All the cheetahs who were unaffected by hyperammonemia excreted high concentrations 3-aminopiperidine-2-one. This metabolite was almost absent in the urine of the cheetahs prone to hyperammonemia.
Conclusions: These results may indicate that cheetahs may use 3-aminopiperidine-2-one as a source for ornithine or that an increased catabolism of ornithine to glutamic acid may result in hyperammonemia.

P-055

EUROPEAN REGISTRY AND NETWORK FOR INTOXICATION TYPE METABOLIC DISEASES (E-IMD)

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Background: Patients with organic acidurias (OADs) and urea cycle defects (UCDs) have an enormous need for improved medical awareness, optimization of the diagnostic process and therapy, and improved networking between healthcare professionals and patients.
Methods: An initiative named "European registry and network for Intoxication type Metabolic Diseases (E-IMD)" funded by the European Commission through DG Sanco has been started in January 2011. E-IMD aims to promote health for patients with OADs and UCDs.
Results: E-IMD already has 40 partners from 17 countries linking healthcare professionals, patient's representatives, industry and government authorities within Europe, Canada and the US. E-IMD will continue to expand its network by inviting new members. The registry will be launched in July 2011 and is expected to collect data on 600 individuals with an OAD or UCD over the next 3 years.
Conclusion: The new network will improve access to rapid diagnosis and care for patients with OADs and UCDs. E-IMD will help make this happen by 1/ objectively evaluating management strategies and outcome of patients (patient registry, www.e-imd.ukhd.de), 2/ providing evidence-based diagnostic and management protocols, and 3/ empowering patients and patient organisations by providing up-to-date information in their own language (website, www.e-imd.net). Contact address: Stefan.Koelker@med.uni-heidelberg.de.

A-001

CLINICAL MANIFESTATIONS OF ORNITHINE TRANSCARBAMYLASE DEFICIENCY [OTC DEFICIENCY] ILLUSTRATED IN THE INDEX PATIENT

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OTC deficiency, first described in 1962, was recognised initially because the child's urine stank of ammonia and characteristically vomited repeatedly failing to gain weight when her diet was changed from breast milk to an artificial formula with a higher protein intake in the first few weeks of life. This leads to dehydration and a low c.s.f. pressure. Over a period of time brain size is limited with premature closure of the fontanelles and the child is irritable and screams. The urine contains fine needles of orotic acid. Hypertonia leads to opisthotonus as illustrated.
Because of high levels of blood ammonia and until the hepatic enzyme deficiency was demonstrated the disorder was termed hyperammonaemia. It is now the most frequent inborn error of the urea cycle.
Some of these features are illustrated in the attached DVD of the index patient, then twenty months old, being examined by one of the authors of the two papers first describing the syndrome.
OTC deficiency presents in different ways neurologically and with different degrees of severity. The index patient was severely affected and it is suggested that the very marked muscular tonus illustrated is caused by the effect of ammonia on spinal reflexes.

A-002

LARGE PEDIGREE OF ORNITHINE TRANSCARBAMYLASE (OTC) DEFICIENCY. CASE REPORT

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Ornithine transcarbamylase (OTC) deficiency is an X-linked urea cycle disorder affecting both sexes. We describe a large pedigree with OTC deficiency. A 15 months old girl was hospitalised with vomiting, poor appetite and lethargy. Elevated liver enzymes, lactic acid and ammonia (210 μmol/l) were found in blood. Glutamine in plasma was slightly elevated, otherwise aminoacids in plasma and urine were normal, orotic acid in urine also was normal. In anamnesis one of patient's sisters died suddenly at the age of 5 years with Rey syndrome, coma, encephalopathy. Another sister was strongly vegetarian. The mother's two brothers died in the neonatal period. The patient's niece died at the age of 11 months after viral respiratory disease with rapid coma and brain death and three cousins died in second and third day of life with respiratory problems, seizures, coma. Ammonia levels were not examined. OTC deficiency was suspected in our patient and the finding of the R142Q/N mutation in the OTC gene confirmed the diagnosis. The same mutation was found in the patient's mother, sister and in postmortem material in one of the deceased cousins. Our report shows the importance of early checking of ammonia in all patients with acute encephalopathy, coma.

A-003

HYPERAMMONIEMIA WITH NEONATE ONSET IN UKRAINE

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Background: Metabolic disorders with involving of ornitine cycle lead to increasing of blood's ammonia level. The neonate hyperammoniemia are present with weakness, vomiting, lethargy, convulsions, coma, temperature lability.

Case report: 3 weeks age child was consulted by geneticist in intensive unit care. The previous diagnosis was hypoxic-ischemic perinatal damade, CNS depression syndrome, febrile convulsions. There were detected: lethargy, hypodynamia, hypotonia, regurgitation, increased readiness for convulsione, pale skin and hypotrophy. Anamnesis: the child was hospitalized with regurgitation, lethargy, acrocyanosis, tonic convulsions, episodes of "fixing" look, dyspnea, oxygendependence, which were appeared with febrile temperature and stuffiness in nose at the 2 weeks age. There were signs of cerebral edema during neurosonography. We conducted additional observation, blood's ammonia and amino acids levels. Ammonia concentration was increased to 137.52 mmol/l; the levels of amino acids (mmol/l) were increased: ornithine 1.146, arginine 0.228, glutamate 1.329, aspartate 0.061. We diagnosed the ornitine cycle defect with hyperammoniemia, neonate onset. The child's condition was improved on base of therapy; blood's ammonia level decreased to normal.

Conclusion: It is necessary detect blood's ammonia and amino acids levels for children with symptoms of CNS depression, seizures, developmental delays, because early diagnosis are pointed for timely treatment and prevention complication.

O-007

COLOUR VISION AND CONTRAST SENSITIVITY IN PATIENTS WITH PHENYLKETONURIA—EVALUATION OF THE DOPAMINE AND LONG-CHAIN POLYUNSATURATED FATTY ACIDS (LCPUFA) DEPLETION HYPOTHESES BY ASSESSMENT OF RETINAL FUNCTION

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Contrast sensitivity, colour vision, electroretinography (ERG), Frequency Doubling Technology Perimetry (FDT) and their correlation with blood phenylalanine (phe) and docosahexaenic acid (DHA) levels were assessed in 36 patients with Phenylketonuria (PKU) and 18 age matched controls. Contrast sensitivity (Vistech test) was significantly lower ($p=0.001$) and total error scores in colour vision (28 HUE Farnsworth test) significantly higher in patients than controls ($p=0.011$). Comparison of ERG results between patients and controls showed a significant difference for scotopic ($p=0.014$) and photopic ($p=0.03$) stimuli. Patients showed greater mean deviation in FDT ($p=0.038$). Multivariate regression analysis of factor scores showed a trend between phe level and contrast sensitivity (spatial frequencies 3, 6, 12, 18 cpd; $p=0.08$) and correlation between phe level and colour vision/FDT ($p=0.04$). Patients' IQ was significantly correlated with colour vision/ FDT results ($p=0.01$), but not with phe levels. Level of DHA in erythrocytes was not associated with any of the dependent variables. Results show that retinal function in PKU patients differs from healthy controls. The correlation of phe levels and contrast sensitivity, colour vision and FDT suggests a possible imbalance between phe and tyrosine affecting retinal dopamine levels.

O-008

QUANTIFICATION OF PHENYLALANINE HYDROXYLASE ACTIVITY BY LC-MS/MS

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Residual phenylalanine hydroxylase (PAH) activity is the key determinant for the phenotype severity in phenylketonuria (PKU) patients and is reported to correlate with the genotype. Activity of in vitro expressed recombinant mutant PAH proteins may predict the patient's phenotype and pharmacological response to tetrahydrobiopterin (BH4). We developed a PAH assay using a LC-MS/MS method for quantitative determination of phenylalanine and tyrosine after derivatization with Phenomenex EZ:faast kit. Several frequent PAH mutations (R158Q, R261Q, R408W, I65T) were expressed in eukaryotic COS-1 cells and quantified for PAH activity. In addition, PAH activity was measured in several cell lines (Huh-7, Hep3B) as well as in liver, brain, and kidney tissue from wild-type and PKU mice. The PAH assay is linear for both amino acids, phenylalanine as well as tyrosine ($r2 \geq 0.99$) and exhibits a low limit of detection of less than 200 nmol/L. In addition the method allows differentiation of endogenous and enzymatically produced tyrosine in cell extracts. Intra-day coefficients of variance (CV) were less than 2.7% and inter-day CV less than 5.7% in lower tyrosine range. The accuracy determined through recovery was 105%. In conclusion, quantification of phenylalanine and tyrosine by LC-MS/MS is highly specific, reproducible, and faster than previously used methods.

O-009

VARIABILITY IN BLOOD PHENYLALANINE IN PATIENTS WITH PKU

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High variability in blood phenylalanine (phe) levels have been shown to be related to a poorer cognitive outcome in individuals with phenylketonuria (PKU) suggesting that intermittently high (or low) levels may have a deleterious effect in addition to that associated with high average blood phe concentrations.

We have analysed the standard deviation (SD) of blood phe and the dietary phe tolerance in 84 patients with PKU on dietary treatment during their first 4 years.

Phe SD ranged from 70 to 460 μmol/l and phe intake from 120 to 940 mg/day. There was a strong inverse correlation between phe SD and dietary phe tolerance ($p<0.0001$) confirming that those with 'severe' PKU showed a much higher variability in phe blood levels.

Some individuals with a low phe SD but who, on the basis of their absolute blood phe levels had been assessed to need a relatively strict diet, were able to markedly increase their natural protein intake while still maintaining satisfactory phe control.

We conclude that phe SD may be useful to provide additional information as to the severity of PKU and may suggest which patients might have a higher phe tolerance than previously thought.

O-010

NEUROLOGICAL AND NEUROCOGNITIVE DEFICITS IN ADULTS WITH EARLY TREATED PHENYLKETONURIA

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Background: The long-term prognosis of treated phenylketonuria (PKU) and the need for a life-long diet are still controversial issues.
Methods: We investigated 60 adult PKU patients patients with early treated classical PKU aged 20 to 45 years (mean age 33) and 60 healthy controls, matched for age and socioeconomic status. Patients and controls were assessed for their IQ and information processing abilities. MRI of cerebral white matter was performed in patients. Assessments were repeated at a 5 year-follow up.
Results: The full scale IQ was significantly lowered in patients compared to controls. Information processing was normal in young patients and in all controls. Patients older than mean age 33, however, showed a slowing in their information processing. Virtually all patients showed cerebral white matter abnormalities in MRI. Abnormalities were more severe in patients with distinct slowing in their information processing.
Conclusions: Neuropsychological assessment in adults with early treated PKU revealed neurocognitive impairment particularly in older patients. This seems to refer to extensive MRI abnormalities in adulthood. They may at last cause neurological impairment and a slow down of information processing speed. Results indicate dietary control during adulthood in PKU.

O-011

STRUCTURE-FUNCTION RELATIONSHIP OF BH4 AND ITS DERIVATIVES REVEALS DISTINCT AND GENOTYPE SPECIFIC PHARMACOLOGICAL CHAPERONE EFFECTS

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Missense mutations in the phenylalanine hydroxylase (PAH) gene can induce protein misfolding with loss of function of the PAH protein, the molecular basis of phenylketonuria. Sapropterin dihydrochloride, the synthetic form of tetrahydrobiopterin (BH4), was recently approved as the first pharmacological chaperone drug and shown to correct the loss-of-function phenotype by rescue of PAH misfolding. The aim of this study was to investigate the structure-function relationship of the BH4-molecule with respect to stabilization of PAH conformation. We compared the efficacy of BH4 and BH4-derivatives (BH2, Sepiapterin and 6-MPH4) with substitutions at the pterin ring system and/or the dihydroxypropyl side-chain to correct misfolding of wild-type and mutant PAH, respectively. BH4 and two dihydropterins (BH2, Sepiapterin) induced a compacted PAH conformation leading to mutation-specific increased thermal and thermodynamic stability. 6-MPH4, a cofactor without inhibitory potency lacking the dihydroxypropyl side-chain, led to protein destabilization. Interestingly, the presence of BH4 and BH2 but not Sepiapterin shifted the PAH unfolding model (2-state to 1-state). In conclusion, the structure-function relationship of these compounds is the molecular basis for structure aided drug design to improve the efficacy of pharmacological chaperones taking the patient's genotype into consideration.

O-012

L-PHE-TREATMENT OF ENU1/2 MICE LEADS TO STIMULATION OF TETRAHYDROBIOPTERIN SYNTHESIS, PROBABLY THROUGH GTPCH-GFRP MEDIATED ACTION, WITH CONCOMITANT REDUCTION IN UBIQUITINATION OF MUTANT PAH, AND INCREASE IN PAH ACTIVITY

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The Pahenu1/2 (ENU1/2) mouse is a heteroallelic orthologous model for moderate hyperphenylalaninemia. While untreated, our mice display blood phenylalanine (Phe) concentrations ranging between 200–800 μmol/L as compared with ~50–110 μmol/L in their normal C57BL/6 counterparts; they also display decreased hepatic phenylalanine hydroxylase (PAH) activity (2.8%) and PAH protein content (19.9%). Here we show that PAH of the ENU1/2 mouse is highly ubiquitinated. Administration of a single dose of Phe (1.1 mg/g body weight), which does not affect blood Phe in normal mice, leads to a 2–3 fold transient increase and a 1.6-fold increase of both non-ubiquitinated PAH protein and PAH activity in the ENU1/2 mouse, followed by an increase in tetrahydrobiopterin (BH4) in liver. Despite the fact that total GTPCH activity and its regulatory protein GFRP are not affected in liver extracts, BH4-increase is probably mediated through GTPCH-GFRP action in vivo. In contrast to that reported for normal mice, supplementation of ENU1/2 with BH4 reduces blood Phe without affecting total PAH protein content and activity. Thus, it appears that BH4-treatment of ENU1/2 is effective in increasing hydroxylation levels without exerting a chaperone effect on the mutant V106A/F263S-PAH. Our results reveal novel mechanisms in hyperphenylalaninemia and responsiveness to BH4 supplementation.
Conflict of Interest declared.

O-013

ORAL PEGYLATED PHENYALANINE AMMONIA LYASE, THE NEXT STEP IN ENZYME SUBSTITUTION THERAPY FOR THE HYPERPHENYLALANINEMIAS

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Background: Phenylketonuria (PKU) results from deficient phenylalanine hydroxylase enzyme (PAH, EC 1.14.16.1) activity. If unmanaged, it causes persistent increases in intracerebral phenylalanine (Phe), and irreversible postnatal brain damage. However, the long-proven dietary treatment is difficult, therefore interest is shifting to less socially-imposing therapies.
Objectives: We recently reported on injectable forms of pegylated phenylalanine ammonia lyase (PEG-PAL) (currently in clinical trials) which correct hyperphenylalaninemia (HPA). However given that treatment is lifelong, and that a benign and non-invasive formulation would be preferable, we have continued our investigation of therapeutically applicable oral formulations.
Methods: We used the Pahenu2/enu2 PKU mouse model to study the reversal of HPA with various oral formulations of PEG-PAL; this route targets the enterorecirculation of Phe in the intestine before its reabsorption.
Results: 5 kDa PEG-Av-p.C503S/p.C565S/p.F18A PAL, given orally, yielded a statistically significant and therapeutically relevant reduction (i.e. a 425± 66 μM decrease from 1052±74 μM pre-treatment values) in plasma Phe levels. The effect occurred in a dose- and loading-dependent manner.
Conclusion/Discussion: Oral PEG-PAL therapy, serving as an adjunct treatment, has the potential to safely correct HPA.
Conflict of Interest declared.

O-014

PROTEIN ENGINEERING TO OBTAIN PHENYLALANINE HYDROXYLASE PROTEINS WITH HIGHER STABILITY

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Phenylketonuria is caused by a deficient activity of human phenylalanine hydroxylase (hPAH). This protein has been regarded as a highly unstable enzyme, fact postulated to be responsible for the lack of the full-length hPAH 3D structure. We aimed to obtain hPAH mutant chimerical forms presenting a higher activity and stability. Firstly, the hPAH mutants C29D, C29S, C284S, C445S, D145K, D151K, E181K and E360K, were produced in a prokaryotic expression system. Due to their higher expression level the C29S and E360K proteins were selected for further characterization regarding the oligomeric profile, enzyme kinetics, substrate activation and thermal inactivation.

When compared to the hPAHwt, the E360K presented a higher degree of aggregates. The C29S showed an increase in catalytic activity (1.3-fold), Vmax (1.6-fold) and catalytic efficiency (1.3-fold), but a lower substrate affinity. A comparative analysis of the thermal stability profile, with the hPAHwt (Tm=50±0.16°C), showed a Tm of 53±2.4°C for the C29S.

Our data suggest that hPAH is very susceptible to changes in the superficial charge. Interestingly, although the N-terminal domain has been regarded as the major contributor for hPAH instability the Cys to Ser substitution in residue 29 is able to increase its stability.

Work supported by FCT (PTDC/EBB-BIO/101237/2008; SFRH/BD/47946/2008)

O-015

PHENYLKETONURIA: EVIDENCE TOWARDS INCREASED STRICTNESS IN TREATMENT DURING THE FIRST 12 YEARS OF LIFE

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Background: Debate on necessary strictness of treatment in Phenylketonuria (PKU) remains. Upper target phenylalanine (Phe) concentrations for the first 12 years of life vary between 240 and 360 μmol/l, the latter being used most frequently.

Objective: To examine whether 240 μmol/l as upper target lifetime Phe results in better executive functioning compared to 360 μmol/l.

Method: 64 PKU patients (mean age 11.1 years, SD 2.2) and 73 controls (mean age 11.0, SD 2.2) performed three tasks measuring inhibitory control and working memory. Lifetime Phe was determined by taking the mean of half-year median Phe levels.

Results: GLM analyses of variance showed that PKU patients with lifetime Phe<=240 μmol/l outperformed patients with Phe >240 μmol/l. When patients with Phe<=360 μmol/l were compared to those with Phe >360 μmol/l, fewer and less pronounced differences were observed. Compared to controls, patients with Phe<=240 μmol/l performed comparable on all tests, which was not true for patients with Phe<=360 μmol/l.

Discussion: When inhibitory control and working memory were required simultaneously, average Phe concentrations of<=240 μmol/l were advantageous compared to<=360 μmol/l, urging consideration of lowering the upper target Phe to 240 μmol/l during the first 12 years of life.

O-016

EXPERIMENTAL EVIDENCE THAT PHENYLALANINE IS STRONGLY ASSOCIATED TO OXIDATIVE STRESS IN ADOLESCENTS AND ADULTS WITH PHENYLKETONURIA

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Background: Few studies have looked at acceptable serum phenylalanine levels in later life in patients with PKU. We examined the oxidative stress status of adolescents and adults with PKU.

Methods: Forty PKU patients aged over fifteen years were enrolled, and were compared with thirty age-matched controls. Oxidative stress markers, anti-oxidant enzyme activities in erythrocytes, blood anti-oxidant levels and nitric oxide production were examined.

Results: Plasma thiobarbituric acid reactive species and serum malondialdehyde-modified LDL levels were significantly higher in PKU patients than control subjects, and correlated significantly with serum phenylalanine level. Plasma total anti-oxidant reactivity levels were significantly lower in the patient group, and correlated negatively with phenylalanine level. Erythrocyte superoxide dismutase and catalase activities were higher and correlated significantly with phenylalanine level. Glutathione peroxidase activity was lower and correlated negatively with phenylalanine level. The oxidative stress score calculated from these six parameters was significantly higher in patients with serum phenylalanine of 700–800 micro mol/l. Plasma anti-oxidant substances, beta-carotene, and coenzyme Q10 were lower. Serum nitrite/nitrate levels were higher together with low serum asymmetric dimethylarginine.

Conclusions: Oxidative stress status is closely linked with serum phenylalanine levels. Phenylalanine in PKU should be maintained at below 700–800 micro mol/l even in adult patients.

P-056

DIAGNOSIS AND MANAGEMENT OF PKU: AN INTERNATIONAL SURVEY

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There is a growing need for consensus building and evidence-based international guidelines regarding the determination of the response to BH4 and treatment initiation, as well as target blood Phe levels. In addition, treatment protocols for the management of PKU and HPA need to be optimized and clarified. For this reason we initiated an international online survey which included 34 questions on the classification of HPA and PKU, diagnostic procedures including BH4 challenge, molecular diagnostics, and management. A total of 95 professionals from 27 countries participated in the survey, with most responses from the USA (n=21) and Germany (n=13). About 86% of participants were physicians (MD or MD, PhD) about 10% dietitians and about 90% of them use either local or national guidelines for the management of patients. DNA analysis is ordered in about 54% of PKU patients and in about 63% of centers BH4 loading test is a part of the diagnostic program. In about 63% of PKU centers BH4 challenge is performed in all age groups, using 10 mg/kg (7%) or 20 mg/kg (93%) and a >30% decrease in Phe level as response criteria (90%). Increase in Phe tolerance is another criterion for BH4-responsiveness for 60% of survey participants.

P-057

INTERNATIONAL DEVELOPMENT OF PKU-QOL) QUESTIONNAIRES TO ASSESS THE IMPACT OF PHENYLKETONURIA (PKU) AND ITS TREATMENT ON DAILY LIFE: COMPREHENSION TESTS

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Objectives: The aim of this study is to develop and validate PKU-specific quality of life questionnaires assessing the impact of PKU and its treatment on daily life. The PKU-QOL) questionnaires were simultaneously developed in UK English, French, German, Italian, Spanish and Dutch for children (6–11 years), adolescents (12–17 years), and adults (18 years+) with PKU and their parents to assess health, feelings, diet and supplements, daily life and medication. Linguistic validation in Turkish was also performed.

Patients and methods: Comprehension tests with children (N=18), adolescents (N=23), adults (N=23) with PKU and parents (N=28) were conducted to assess questionnaire understanding, acceptability and content validity across countries and populations.

Results: In general, PKU-QOL) questionnaires were relevant, well-understood and acceptable to the majority of participants across countries. However, young children experienced difficulties completing the questionnaire alone, requiring a narrower age range for the child version. Items were added, deleted or reworded based on interview results and discussions with PKU experts. Formatting and answer choices were modified to aid patient understanding.

Conclusion: PKU-QOL) questionnaires were well-understood and comprehensively assessed the impact of PKU and its treatment on patients' and parents' daily lives. A validation study is underway to assess PKU-QOL) questionnaires' psychometric properties.

Conflict of Interest declared.

P-058

ROUTINE SCREENING YIELDS HIGH INCIDENCE OF PSYCHIATRIC DISTRESS IN PHENYLKETONURIA (PKU) CLINICS

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A Diversified Approach for PKU Treatment (ADAPT) was implemented at Children's Memorial Hospital and the University of Utah to integrate mental health screening into the management of phenylketonuria (PKU). All PKU patients, ≥5 years presenting for their regular clinic visits over a 10 month period in 2010, were offered screening for psychiatric distress. Screening offered by the clinic coordinators included the Pediatric Symptom Checklist (PSC) in children and the Brief Symptom Inventory (BSI) in adults (>18 years of age), and typically took 15–20 minutes, not impeding clinic flow. Almost all patients (95%) agreed to screening, and those who screened positive were referred to mental health care professionals. Of the 150 patients screened, 30% were positive for psychiatric distress and a greater proportion of adults (50%) screened positive than children (16%). Patients positive for psychiatric distress had higher (P=0.004) mean phenylalanine (Phe) values; mean Phe values were positively correlated with several BSI subscales. Phe values were also positively correlated with age (P<0.0001). The ADAPT model was successfully implemented in these two clinical practices, and yielded a high incidence of psychiatric distress suggesting routine mental health screening should become part of routine clinic appointments for PKU patients.

Conflict of Interest declared.

P-060

GROWTH AND NEUROPSYCHOLOGICAL FUNCTION IN PATIENTS WITH HYPERPHENYLALANINEMIA ON FREE DIET

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Background: Raised phenylalanine concentrations in the brain can impair neuropsychological function. Consequently there is a lack of consensus about the severity of hyperphenylalaninemia (defined as blood phenylalanine concentrations less than 120 μmol/L) at which treatment should be started. Most Clinical Centres use the phenylalanine concentration threshold greater than 360 micromol/L.

We assessed growth and neurological outcome in patients with hyperphenylalaninemia with no dietary natural protein restriction.

Methods: we studied 16 children (8 male-8 female; mean age 18.8 months, range 11.8-37.5 months) with hyperphenylalaninemia, detected by neonatal screening. Cofactor defects were excluded by investigation of pterins and dihydropteridine reductase activity in blood.

Phenylalanine and tyrosine levels were analyzed monthly, according with the changes in the diet. All the participants were monitored by physical (weight, height, BMI, head circumference) and neurological examination (clinical and electroencephalographic features); only 9 of them (6 female-3 male) were assessed by psychological tests (Griffith's, Brunet-Lezine).

Results: Growth was normal in all patients, comparing with their peers. No neurological abnormalities were found and all the children showed normal psychomotor development.

Conclusion: Despite phenylalanine levels higher than 120 micromol/l, patients with hyperphenylalaninemia have a lower risk of neuropsychological dysfunction than do those with phenylketonuria.

P-061

THE EVALUATION OF COGNITIVE DEVELOPMENT AND ADOLESCENT WITH PHENYLKETONURIA

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Some studies have shown an association between phenylketonuria (PKU) and cognitive problems in children and adolescents, despite the diet started early in life. We assessed the cognitive level in a group of patients affected by PKU, followed with dietary and psychological interventions.

We enrolled 52 patients (31 M+21 F) with PKU detected by neonatal screening, The IQ of 21 subjects aged 6–18 was assessed by Wisc-R. The mental development of 31 patients aged 0–5 was evaluated by Brunet Lezine, Stanford-Binet and Leiter-r scales.

Patients aged 6–18 had a mean total IQ of 101,1±17,1;verbal IQ was 101.3±15.2 and non-verbal IQ was 100.0±18.9. In this group, the total, verbal and non-verbal IQ was respectively 99.4±19.2, 101.7±18.5 and 96.9±20.1 for male, and 104.0±13.7, 100.8±10.1 and 104.3±17.6 for female. The mean cognitive developmental score for patients aged 0–6 was 102,3±10,4 for the whole group, 101,3±11,2 for male and 103,3±9,1 for female.

In our sample of children and adolescent with PKU, treated with a dietetic and psychological approach, the cognitive development was within the range of normality, with slightly lower scores in male.

Further studies are needed testing the efficacy of a dietary-psychological integrated approach to prevent neuropsychological impairment in patients with PKU.

P-062

COMPUTERIZED NEUROPSYCHOLOGICAL TESTS AND BRAIN MAGNETIC RESONANCE SPECTROSCOPY IN PATIENTS WITH PHENYLKETONURIA

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Adults with phenylketonuria can develop neuropsychological abnormalities as a result of hyperphenylalaninemia. These symptoms can be detected with computerized neuropsychological tests. Brain hyperphenylalaninemia can in turn be measured with use of magnetic resonance spectroscopy. The aim of the study was to assess the brain phenylalanine concentration in hyperphenylalaninemic adults without neuropsychological deficits.

Assessment of sustained attention, working memory and inhibitive control was performed in a group of 30 non-compliant adults with phenylketonuria by means of computerized CANTAB system. Brain/blood phenylalanine ratio was analyzed in patients without neuropsychological abnormalities (brain phenylalanine concentration was measured with use of magnetic resonance spectroscopy).

Worsening of neuropsychological efficiency correlated with high levels of plasma phenylalanine. Neuropsychological abnormalities were recorded in 28 patients. In the remaining two patients relatively low brain/blood phenylalanine ratios were observed. This finding supports the hypothesis on the presence of mechanisms limiting brain phenylalanine concentration in selected patients with phenylketonuria, which was postulated by other authors. These potential mechanisms could result in lower brain toxicity of hyperphenylalaninemia. It should be stressed, however, that such situation can probably be expected very rarely.

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P-063

NEUROLOGICAL COMPLICATIONS AND BEHAVIORAL PROBLEMS IN PATIENTS WITH PHENYLKETONURIA IN A FOLLOW-UP UNIT

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Objective: To investigate the relationship between neurological and behavioral complications of PKU patients with neonatal screening (NS) and late diagnosis (LD), and dietary control. Material and Methods: Retrospective study of the PKU patients diagnosed and controlled in our unit from 1985—January 2010.

Results: 121 patients were evaluated (median age: 16.0) (1 month-46 years). 71% diagnosed through the NS;. 12.4% have mild-PKU, 19% moderate-PKU and 68.6% classic-PKU. 88.4% were treated with protein-restricted diet, 11.6% with BH4. 95.3% of the NS patients have normal IQ, while 20% of LD patients have severe mental retardation (MR), 20% light MR and 7% normal IQ. In NS patients, there was a significantly negative correlation between IQ and the index of dietary control (IDC) of the last year and that of the first six years of life ($p<0.001$). The proportion of patients with LD and neurological and behavioral complications is significantly higher than those of NS ($p<0.001$).

Conclusions: These results showed the impact of the NS on the IQ of PKU patients as well as the relationship between the IDC of the 6 first years of life on the IQ and the neurological complications and that of the recent IDC on the behavioral complications.

P-064

DEPRESSION AND HEALTH RELATED QUALITY OF LIFE IN CAREGIVERS OF CHILDREN WITH PHENYLKETONURIA

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Background: Phenylketonuria is a treatable disease with strong perspective of normal cognitive and physical development and low frequency of hospitalisations. However, frequent blood tests, necessity of complex diet, possible psychiatric symptoms and stigma of hereditary disease could affect disease-related family burden.

Objectives: The aim of this study is to investigate health related quality of life (HRQoL) and depression in mothers who care for children with PKU in Serbia.

Patients and Methods: The cross-sectional study was conducted among 32 mothers giving care to children with PKU. Caregivers` HRQoL was assessed by using the SF-36 questionnaire. Beck Depression Inventory II (BDI -II) scale was used to measure mothers` depression.

Results: Values of mental and physical composite scores of SF-36 in PKU caregivers were significantly higher than standard values for general population. Also, all subdomains of SF-36 had higher than standard values, apart from vitality and role emotional scores which corresponded to average values of general population. Severe depression was noted in 2 caregivers (6,25%), while more than two thirds showed only minimal signs of depression.

Conclusion: Care giving to PKU children does not represent significant risk for depression and lower HRQoL in their mothers.

P-065

PHENYLALANINE CONCENTRATIONS VERSUS PHENYLALANINE:TYROSINE RATIOS IN PREDICTING COGNITIVE ABILITIES OF INDIVIDUALS WITH PHENYLKETONURIA

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Background: The value of the essential amino acid tyrosine (Tyr) in PKU treatment is unclear. Low tyrosine concentrations may interfere with adequate dopamine synthesis, which is important for cognitive functioning, and high Phe:Tyr ratios are suggested to interfere with neurocognitive outcome.
Objective: To examine the importance of Phe:Tyr ratios and absolute Phe concentrations in predicting neurocognitive outcome.
Method: 64 PKU patients (mean age 11.1 years, SD 2.2) performed three tasks measuring inhibitory control and working memory. Phe and Tyr concentrations were determined from blood samples taken on the day of testing.
Results: Simple linear regression analyses showed that both concurrent Phe levels and Phe:Tyr ratios were significant predictors of inhibitory control. Multiple linear regression analyses showed that, when Phe and Phe:Tyr ratio were introduced simultaneously, only Phe:Tyr ratio remained a significant predictor of speed and accuracy of performance in the inhibitory control task.
Discussion: Phe:Tyr ratio was a stronger predictor than concurrent blood Phe concentration of performance on tasks measuring specific (dopamine-mediated) cognitive abilities, emphasizing the importance of tyrosine monitoring in PKU studies. As tyrosine levels are highly variable between measurements, great care should be paid to correct conditions of tyrosine measurement.

P-066

THE EFFECT OF LNAA ON DIET INTAKE FOR PKU PATIENTS

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Background: Supplementation of large neutral amino acid (LNAA) and a semi-free (SF) diet has been shown to have a positive effect on well-being on adults with PKU. However, patients are used to low protein diet and find it often difficult to eat sufficient natural protein. This can result in malnutrition. The aim of this study was among others to determine the effect on diet intake from LNAA in different dosages and combinations.
Material (Patients) and methods: This was a prospective, double-blind, cross-over study consisting of four consecutive 3-week phases. Twelve subjects (6 males, 6 females) with PKU were recruited, 11 completed the study. Two different brands of LNAA (A and B) were tested. Each phase consisted of LNAA A or B, either in low or high dosage. Subjects were instructed to follow their usual SF diet and complete a 3-day food record at start, and at the end of each period.
Results: Protein intake varied from 76–102 grams/day (mean) and energy intake was 9341–10098 kilojoules/day (mean). There was no correlation between protein- and energy intake and the amount or brand of LNAA.
Conclusions: LNAA A & B in different dosages or combinations do not affect protein or energy intake.

P-067

DIETARY MANAGEMENT OF PHENYLKETONURIA (PKU) IN PRE-TERM INFANTS DIAGNOSED BY NEWBORN SCREENING (NBS): FOUR CASE REPORTS

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Background: The dietary management of infants with PKU diagnosed on NBS is well established. However, there is little published experience of the management of PKU in pre-term infants. We report on the management of 4 premature infants with PKU diagnosed by NBS.
Case reports: The infants were born between 32+4 and 33+6 weeks gestation. Initial Phenylalanine (Phe) levels were reduced from a maximum of 806–1580 μmol/L to 10–190 μmol/L within 4 to 6 days on restricted Phe feeds, all providing around 50 mg/kg. Despite increasing Phe intake to between 80 and 90 mg/kg, Phe levels were slow to rise in 3 of the 4 cases. All infants achieved Phe levels within an acceptable range (120–300 μmol/L) during the first months of life on a Phe intake of between 75 and 90 mg/kg.
Discussion/Conclusion: The Phe intake required to achieve acceptable Phe levels in comparable term babies with PKU in these centres ranges from 50 to 65 mg/kg. Premature babies have a higher tolerance to dietary phenylalanine than term babies in our experience, tolerating up to 2 g protein/kg. Blood Phe levels are much more fluctuant in the first months of life, perhaps reflecting the variable rates of growth in pre-term infants.

P-068

OVERWEIGHT AND OBESITY IN THREE COHORTS OF FEMALES WITH EARLY TREATED PKU

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Background: In all western countries an obesity epidemic in children has arisen during the last few decades.
Objectives: To study the development in BMI for girls with classic PKU on protein restricted diet.
Patients and Methods: Three cohorts of females with classic PKU: A) 17 patients born in the years 1961–1978, B) 12 patients born in the years 1981–1989, C) 20 patients born in 1990–2000. A comparison regarding BMI and overweight/obesity at different ages was made between the 3 groups and the population in general.
Results: The prevalence of overweight/obesity in 14-years-old girls increased from 17.6 %/0 % in group A to 35.7 %/20 % in group C. In 2007 the prevalence of overweight/obesity in 14–16-years-old Danish schoolgirls was 25.4%. The overweight was already obvious from 6 years of age and in most cases it ran in the family. Most of the teenage girls with overweight/obesity had a poor compliance.
Conclusion/Discussion: Our girls with PKU have strict dietetic restrictions, but their BMI increases even more than girls without PKU, maybe reflecting that fat and carbohydrates are not restricted in the diet. The increased risk of type 2 diabetes is worrying. More focus on the preschool overweight is needed.

P-069

RAISED BODY MASS INDEX (BMI) OF ADULT PATIENTS WITH PKU

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Background: There is little research on BMI of adults with PKU. While restricted protein diets are considered healthy due to the use of "free" fruits and vegetables and restrictions on higher fat products; low protein foods typically contain sugar and fat to increase palatability and may contribute to weight gain.
Methods: BMI was classified according to the WHO criteria for 185 non-pregnant adult patients with PKU (94 on protein restricted diet).
Results: 34% of patients were a healthy weight, 4% underweight, 39% overweight and 23% obese. Patients off-diet were heavier than those on-diet (p=0.003) with 68% of males and 70% of females off-diet having a BMI>= 25 kg/m2 compared to 58% men and 51% women on-diet and 66% of men and 57% women in the general population. While more patients on-diet (43%) were overweight versus those off-diet (35%) and in the general population (38%), only 12% were obese compared to 34% off-diet and 23% in the general population. A moderate, positive correlation was found between BMI and phenylalanine level.
Conclusions: Patients with PKU, either on- or off-diet are more likely to be overweight than the general population. Weight management should be a lifelong component of their treatment.

P-070

FREQUENCY OF OBESITY IN PATIENTS WITH HYPERPHENYLALANINEMIA

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Objective: to determine the frequency of obesity in patients with phenylketonuria (PKU)/hyperphenylalaninemia (HPA) and related factors with obesity.
Patient and Methods: The medical records of the 300 patients with PKU/HPA were reviewed retrospectively.
Results: Mean age of the patients with PKU/HPA was 123.3±74.2 months (2 months-33 years). Of 254 (84.7%) with PKU, 46 with HPA. Two hundred fifty-five patients (85%) were used Phe-restricted diet and Phe-free protein substitutes. Frequency of obesity and overweight was 13.7% (n=41). Twenty-three (7.6%) patients were obes. There were no differences between obes and non-obes patients (n=259) with HPA in age, sex and Phe-restricted diet features. Thirty-eight obes patients (92.7%) were used Phe-restricted diet. Mean blood Phe level was 654.5±345.4 mmol/L in overweight/obes patients. There was no any difference between obes and non-obes patients in mean blood Phe levels. Maternal and paternal obesity/overweight was observed 61.5%, 80.7% respectively in obesity/overweight patients.
Conclusion: The frequency of overweight and obesity in patients with HPA were higher than in general population of Turkey. It might be related to parental obesity and high energy intake with Phe-restricted diet. The content of the diet, daily energy intake, physical activity and body weight of the patients with HPA should be closely monitored.

P-071

PKU DIET RELAXATION INFLUENCES FATTY ACID INTAKE PATTERN

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Background: After diet relaxation due to BH4 therapy or previous overtreatment, PKU patients consume less fruits and vegetables, but considerable amounts of meat, milk, normal bread and pasta.
Objective: Investigation of the influence of emerging consumption patterns of patients on relaxed PKU diets on their fatty acid intake.
Methods: The intake of total fat, saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids of 16 PKU patients (7–22 years, 9 on BH4 therapy) with phe intakes from 570–2700 mg, was investigated by food protocol analysis (excluding protein supplements) using a nutrient calculation programme. Patients were assigned to group A (< 1500 mg phe intake, n=12) or group B (> 1500 mg phe intake, n=4).
Results: Patients of group A have statistically significant lower intake of total fat and all fatty acid groups compared to group B. Mean values: total fat 40 g/d vs. 71.9 g/d, p=0.031; SFA 13.8 g/d vs. 32.2 g/d, p=0.013; MUFA 8.6 g/d vs. 23.7 g/d, p<0.002; PUFA 4.6 g/d vs. 8.3 g/d, p=0.039. Total fat and SFA as %energy of group B are above recommendations and above healthy peer groups (DONALD study).
Conclusion: Diet relaxation leads to less favorable fatty acid patterns.
Conflict of Interest declared.

P-072

RISK OF MICRONUTRIENT DEFICIENCIES IN RELAXED PKU DIET

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Background: The introduction of sapropterin for BH4-responsive PKU led to the re-evaluation of the phe tolerance of many PKU patients. Some patients are able to relax their PKU diet due to BH4 therapy or higher than expected phe-tolerance and change their habitually limited consumption patterns; intake of protein supplements is usually reduced. Patients may though not eat a fully complete diet likely leading to deficiencies in essential micronutrients.
Objective: Investigation of the consumption patterns and micronutrient intakes of PKU patients on relaxed diet.
Methods: The micronutrient intake of 18 PKU patients (range 7–22 years, mean 13.7 years) with phe intakes ranging from 570–2700 mg phenylalanine (mean 1306 mg), was investigated by the analysis of food protocols (excluding protein supplements) using a nutrient calculation programme. 9 patients were on BH4 therapy.
Results: Patients on relaxed PKU diet start to eat considerable amounts of meat, milk, normal bread and pasta. They consume less fruits and vegetables. Intakes of essential micronutrients like calcium, zinc, vitamins B6 or B12 resulting from diet without supplements are far below the recommendations.
Conclusion: Close monitoring of the nutrient supply of PKU patients on relaxed diets is mandatory. BH4 therapy does not reduce the complexity of dietary treatment.
Conflict of Interest declared.

P-073

BONE MINERAL DENSITY (BMD) IN PATIENTS AFFECTED BY HYPERPHENYLALANYNEMIA (HPA): EFFECTS OF CALCIUM INTEGRATION AND PHYSICAL ACTIVITY EVALUATED WITH DUAL ENERGY X-RAY ABSORPTIOMETRY (DEXA)

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Several studies suggested a compromised BMD in HPA affected patients on diet-therapy. Our aim was to assess bone mineralization and evaluate effects of calcium integration and physical activity in HPA patients.
Patients and Methods: To analyze BMD we divided 117 patients basing on diet-therapy and diet-adherence; to analyze 12-months calcium integration we considered 29 patients on diet; to analyze physical activity we divided 117 patients basing on diet-therapy, calcium integration and physical activity. DEXA measured L1-L4 mineralization; we reported body mass index, Zscore-BMI, serum Calcium, Phosphate, Magnesium, Alkaline Phosphatase and Phenylalanine.
Results: Zscore-BMD was statistically different between patients on diet and on free-diet, higher in the group with good adherence to diet than in the group with poor adherence. Zscore-BMD and serum Phe did not correlate proportionally. Zscore-BMD and blood-parameters improved after calcium integration. There was no significant difference between patients on diet with both calcium integration and physical activity versus patients on free-diet and physical activity.
Conclusions: Current analysis verified bone density reduction in HPA patients on diet. Restricted diet seemed to be associated to worse BMD. Treating with both calcium integration and physical activity we could optimize bone formation in order to prevent osteopenia and/or osteoporosis risk.

P-074

WHEN WILL PKU INFANTSBLOOD PHENYLALANINE REACH THE DESIRED LEVEL? -A STUDY OF 207 PKU PATIENTS

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Background: The Kennedy Center has 371 patients listed with phenylketonuria (PKU) and mild hyperphenylalanemia (MHP). 354 patients are screened for mutations in the phenylalanine hydroxylase gene.
Objective: To study if there is a time difference as to when the infants' blood phenylalanine (phe) reached the desired level, depending on which phenylalanine free infant formula the infants drink.
Patients and methods: 207 PKU patients treated from birth at the Kennedy Center were included.
The PKU Infants were divided into three groups according to phenotype; Classical, Moderate and Mild. Five different formulas: four powdered and one liquid (ready-to-feed) were administered to the three groups. Number of days from start of treatment to blood phe reached the desired level below 300 μmol/L (until year 2000 420 μmol/L) was recorded.
Results: Median number of days to reach desired blood phe level:
Classical (N=115) Liquid: 6 days; Powdered: 8–18 days Moderate (N=11): Liquid: (−); Powdered: 5–12 days Mild (N=80): Liquid: 4 days; Powdered: 5–8 days
Conclusion: The liquid infant formula seems to be more efficient than the powdered formulas in reaching the desired blood phe level. Other studies have shown that the process of mixing the powdered formula gives rise to errors.

P-075

RECOGNIZING CATABOLIC STATES DURING DIETARY TREATMENT OF PHENYLKETONURIA. AN APPLICATION OF METABOLIC MODELLING

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In a non-intervention dietetic study of 20 patients (1–15 years, 12 f., 8 m.) with clinically classic phenylketonuria (PKU), food intake and phenylalanine (phe) blood levels were documented daily during 2 periods of 1 week each in 15 patients, and during 1 week only in 5 patients. The study periods were selected according to the current phe blood levels, with phe being within target range during one period, and above-target during the other one. By using a kinetic single-compartment model, the zero-order net protein synthesis was found diminished at elevated phe levels in 12 of the 15 patients, indicating sub-clinical catabolic states as a frequent cause of 'diet failure'. In contrast, the first-order constant of metabolic (plus renal) phe disposal was found almost identical during both periods (R=0.9782). Beside the interpretation of inverse diurnal variation of serum phe (Güttler et al, 1969), the question of catabolic states has been considered only very rarely in reports and guidelines on PKU management. Distinguishing such states from non-compliance may motivate compliant patients and their families to even stronger adhere to their dietetic schedule.

P-076

HEPATOCYTE TRANSPLANTATION FOR BH4 NON-RESPONSIVE PHENYLKETONURIA: A FIRST CLINICAL EXPERIENCE

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Background: PKU treatment is based on strict restriction of the phenylalanine intakes, allowing to maintain blood phenylalanine concentration into the therapeutic goal, but responsible for severe psychosocial burden. Most patients with severe phenotype are non-responders to BH4 and new therapeutic strategies are needed.
Case Report: In a 5 year-old boy with classical BH4 non-responsive PKU, despite intensive training and close medical follow-up, a good metabolic control was not achieved because socio-familial concerns. The patient, compound heterozygote for the P281L and IVS10-11 G>A mutations in the PAH gene, had a daily tolerance of 250 mg. After ethical committee approval and informed consent, the patient received two intraportal infusions of fresh hepatocytes (2x100x106/kg). Thereafter, blood phenylalanine concentrations decreased and oral tolerance increased, while the phenylalanine half-life assessed by oral loading tests, decreased form 43 h to 19 h and phenylalanine hydroxylase activity in liver biopsy reached 8%. However, three months later, the blood phenylalanine concentrations increased again and phenylalanine intakes had to be reduced, most probably due to insufficient liver repopulation and transient engraftment.
Conclusion: Liver cell transplantation is a new promising option emerging for selected cases of PKU, but requiring further evaluation and development of new strategies to improve high quality and permanent engraftment.

P-077

PARENTERAL NUTRITION IN MANAGEMENT OF PKU PATIENT DURING CHEMOTHERAPY FOR LYMPHOMA

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Background: Phenylketonuria is an inborn error of the phenylalanine metabolism. Currently, dietary long term control of phenylalanine levels is the management strategy, necessary to provide the best potential for optimum outcome. The metabolic control of phenylalanine levels is a challenge during illness. We present the management with parenteral nutrition of a 6 years old boy with stage III intraabdominal Burkit's lymphoma who underwent illeal resection and chemotherapy.
Material (Patients) and Methods: Metabolic control of PKU was established by using: intravenous amino acid solutions, intravenous lipids, and a combination of enteral and oral feedings. Regular amino acid and custom made amino acid solution that was devoid of phenylalanine and had 1 g/L of tyrosine added were used. Phenylalanine levels were monitored closely with 94 blood dot card levels taken during the 109 day course of hospitalization.
Results: Monitored levels were: 18.4 % above , 52.7% below and 29% within therapeutic range of 120 to 360 mmol/L.
Discussion: Custom made amino acids solution and intralipids were essential in preventing high phenylalanine levels during the course of chemotherapy in our patient. Phenylalanine control in PKU patient can be maintained during a course of chemotherapy.

P-078

MUNCHAUSEN BY PROXY IN PHENYLKETONURIA

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Objectives: We report the first case of Munchausen by proxy in phenylketonuria (PKU).
Case report: We report the case of a PKU boy whose metabolic follow up was difficult in the first year of life. He was referred to our centre for the management of very high Phe level (3300 μmol/L) which were non responsive to classical treatment. Despite an emergency regimen (PKU aminoacids substitutes+high caloric intake and 0 Phe intake) and a weight gain was 1.7 kg, the Phe level remained increased for 3 weeks. As we were suspicious of Munchausen by proxy, we asked to the mother to leave the hospital for a week and Phe level normalized within 3 days. Accidentally, we found in the child's bag, a bottle of a high caloric, high protein formula which could be the source of the extra protein given to the child. After the child was retired from his family, the Phe level never rise up again.
Conclusion: Munchausen by proxy must be evoked when there is a discrepancy between the administered diet and the metabolic status and after elimination of all the other causes of metabolic decompensation.

P-079

BENEFICIAL EFFECT OF L-CARNITINE AND SELENIUM SUPPLEMENTATION ON OXIDATIVE STRESS IN PHENYLKETONURIC PATIENTS

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Background: The involvement of reactive species in pathophysiology of phenylketonuria (PKU) is well established. In previous studies it was verified that PKU patients (treated with a protein-restricted diet supplemented with a special formula not containing L-carnitine and selenium) presented high lipid and protein oxidative damage as well as reduction in antioxidants. Our goal in this study was to evaluate the effect of a supplementation with L-carnitine and selenium, two well-known antioxidant compounds, on oxidative stress in PKU patients.

Methods: We investigated various oxidative stress parameters in blood of 18 treated PKU patients before and after 6 months of supplementation with a special formula containing L-carnitine and selenium.

Results: It was verified that the treatment with the antioxidants was capable to revert the lipid and protein oxidative damage. Additionally, the supplementation normalized the glutathione peroxidase activity. It was verified a negative correlation between lipid peroxidation and L-carnitine levels as well as a positive correlation between glutathione peroxidase activity and selenium concentration.

Conclusions: Our results suggest that supplementation with L-carnitine and selenium is important for PKU patients, since it could help to correct the oxidative stress process which contributes, at least in part, for neurological symptoms in PKU.

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P-080

STUDY OF GLUTATHIONE PEROXIDASE (GPX)AND GLUTATHIONE REDUCTASE (GRX) IN PKU IN ANDALUCIA

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Background: In recent years, evidence has emerged indicating that oxidative stress is possibly involved in the pathology of phenylketonuric patients (PKU). PKU enzymatic and non-enzymatic antioxidant defences are decreased in plasma and erythrocytes of PKU patients, which may be due to increased free radical generation or secondary to the deprivation of micronutrients which are essential for these defenses

Objectives: To compare the levels of the antioxidant enzyme activity of glutathione pathway: Glutathione peroxidase (GPx), and glutathione reductase (GRx) in PKU in relation to the healthy control group.

Material and methods: We studied 42 PKU pateints compared with 30 healthy controls. Activity measurement was performed by enzymatic spectrometry.

Results: The GPx activity is decreased significantly in PKU ($p=0.01$). The GRx activity is significantly higher in the PKU group ($p=0.006$).

Conclusion: GPx activity correlated with levels of selenium in plasma (cofactor of the enzyme GPx). Our patients take selenium-supplemented formulas, so we should consider whether supplementation may not be effective.

The increased activity of GRx can be explained as occurring to avoid the toxic effect caused by increased levels of GSSG (oxidized glutathione) in PKU patients.

P-081

TOTAL ANTIOXIDANT CAPACITY (TAC), ACTIVITY OF CATALASE (CAT) AND SUPEROXIDE DISMUTASE (SOD) IN PATIENTS PKU

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Background: Alterations in antioxidant systems (AOS) have been related to multiple inborn errors of metabolism. Since many of the antioxidants come from the diet, patients with phenylketonuria (PKU) are susceptible to different dietary deficiencies of antioxidant vitamins and trace elements. It is important to identify alterations of this system in order that they can be corrected with supplements.

Objectives: Comparing the levels of total antioxidant capacity (TAC) and activity of antioxidant enzymes superoxide dismutase (SOD), and catalase (CAT) in PKU compared to the controls.

Material and methods: We studied 42 PKU under dietary treatment and compared with 30 controls. TAC levels and SOD activity were determined by enzymatic spectrometry and CAT activity by spectrophotometry.

Results: The activity of CAT and SOD is decreased significantly in PKU (CAT: $p=0.02$, SOD: $p=0.001$). We found no significant differences in TAC levels between PKU and controls.

Conclusion: The antioxidant enzymes CAT, SOD are considered the main enzymatic defences against free radical production, and are decreased significantly in PKU in our area, which reinforces the hypothesis that oxidative stress is increased in PKU, and that pathogenic processes also occur in patients under protein dietary.

P-082

REGULAR EXERCISE PREVENTS DECREASED ANTIOXIDANT SYSTEM IN THE BRAIN OF HYPERPHENYLALANINEMIC RATS

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Background: Phenylketonuria (PKU) is caused by deficiency of the phenylalanine hydroxylase, leading to accumulation of phenylalanine. Clinical features of PKU include mental retardation, microcephaly, and seizures. Mechanisms of brain damage still not clear, but increased oxidative stress is associated.
Objective: Verify the effects of exercise on oxidative stress parameters in the brain of hyperphenylalaninemic rats.
Methods: Sedentary (Sed) and exercise (Exe) rats groups were subdivided into saline (SAL) and PKU. PKU groups were induced hyperphenylalaninemia through administration of alpha-methylphenylalanine and phenylalanine for 17 days, while SAL groups received saline. Exe groups conducted 2-weeks of aerobic exercise lasting for 20 min per day. At the 18th day, the animals were killed and the brain was homogenized to determine tiobarbituric acid reactives substances (TBA-RS) content, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities.
Results: PKU model caused an increase in TBA-RS and SOD, and reduces CAT and GPx. Exercise was able to prevent all changes seen in the PKU group, except for the SOD activity.
Conclusion: Hyperphenylalaninemic rats were more responsive to the benefits provided by regular exercise. Physical training can be an interesting strategy for the restoration of the antioxidant system in PKU. (CNPq, CAPES, IBNnet, FAPERGS, and PROPESQ/UFRGS)

P-083

ACUTE PHENYLALANINE ADMINISTRATION INCREASES CYTOKINE LEVELS IN BRAIN OF YOUNG RATS

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Background: Phenylketonuria (PKU) is a rare genetic disease caused by a deficiency in phenylalanine hydroxilase activity, leading to an accumulation of phenylalanine (Phe). Clinically, patients present severe intellectual disability, whose pathophysiology is still uncertain.
Objective: In the present work we investigated the levels of different cytokines in cerebral cortex of rats submitted to an experimental model of PKU.
Methods: Male 30-day-old Wistar rats received a single subcutaneous Phe injection (5.2 μmol/g) and/or p-chlorophenylalanine (p-Cl-Phe; 0.9 μmol/g), an inhibitor of phenylalanine hydroxilase. Control group received saline solution at the same volume. One hour after Phe administration, cerebral cortex was isolated and the levels of the cytokines interleukine-1B (IL-1B), interleukine 10 (IL-10) and α-tumoral necrosis factor (TNFα) were evaluated.
Results: IL-1B and IL-10 levels were increased by the simultaneous administration of Phe and p-Cl-Phe, but not by isolated administration. On the other hand, TNFα levels increased in animals receiving Phe, p-Cl-Phe or Phe plus p-Cl-Phe, as compared to control group.
Conclusion: Taken together, these data suggest that Phe administration alters cytokine homeostasis in brain of young rats. Our results may help to explain, at least in part, the characteristic brain impairment observed in PKU patients.
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P-084

GLUTATHIONE NEUROMETABOLISM EVALUATION IN A MODEL OF HYPERPHENYLALANINEMIA IN RATS

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Background: Glutathione (GSH) is an important antioxidant that eliminates reactive species and acts as cofactor for many antioxidant enzymes. Several studies have reported the involvement of oxidative stress in PKU patients and changes in GSH/GSSG ratio (an important index of oxidative stress) were found in tissues from a hyperphenylalaninemia (HPA) animal model.
Objectives: The activity of some enzymes involved in GSH metabolism was evaluated in brain of rats subjected to a chemically-induced HPA.
Material and methods: Six-day-old Wistar rats received daily injections of α-methyl-phenylalanine (1,6 μmol/g), phenylalanine hydroxylase inhibitor, and Phe (2,1 μmol/g) for 7 days. Controls received saline instead. Animals were killed and the brain removed and homogenated to measure the activity of glucose-6-phosphate dehydrogenase (G6PD), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST) and glutamylcysteine ligase (GCL).
Results: The activities of GPx, GR and G6PD were decreased in HPA group while GCL activity was increased. On the other hand, HPA did not alter GST activity.
Conclusion/discussion: We demonstrated that alterations in the activity of enzymes involved in brain GSH metabolism is probably underlying the changes in GSH/GSSG ratio in HPA while reactions of phase II detoxification, represented by GST, seems to be not involved. (CNPq, CAPES, IBNnet, FAPERGS, and PROPESQ/UFRGS)

P-085

THE SPECTRUM OF PHENYLKETONURIA IN ARMENIAN POPULATION: IDENTIFICATION OF 3 NOVEL PAH MUTANT ALLELES

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We present the spectrum of PAH mutations upon investigating the 35 patients identified with hyperphenylalaninemia in Armenia, and, where possible, their families as well. Whereas one case was diagnosed with dihydropteridine reductase (DHPR) deficiency, all others showed mild or classical phenylketonuria (PKU). By analyzing all 13 exons plus exon-intron boundaries of the PAH gene, we identified the expected two mutant alleles in 23 PKU patients, three mutations in 1 subject, one mutation in 5 subjects, and no mutation in 5 PKU patients. The most prevalent mutation was the well defined splicing error in intron 10, c.1066-11 G>A (17/68 alleles). The three alterations, c.836 C>T (p.P279L) in exon 7, c.1129 T>G (p.Y377D) in exon 11, and c.1244A>T (p.D415V) in exon 12, have not been reported in the PAH locus database (www.pahdb.mcgill.ca) and might thus be specific for the nearly-homogenous Armenian population.

P-086

THE DIAGNOSIS OF AUTOSOMAL DOMINANT GUANOSINE TRIPHOSPHATE-CYCLOHYDROLASE 1 DEFICIENCY (SEGAWA DISEASE): THE COMBINED ROLE OF URINE PTERINS AND PHENYLALANINE LOADING TEST.

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Background: The diagnosis of autosomal dominant Dopa-responsive-dystonia (DYT-5a) relies on GCH1 gene examination and the Neopterin level in cerebral spinal fluid, while pterins are considered normal in urine.
Methods: We studied urinary Neopterin and Biopterin and Phenylalanine/Tyrosine ratio under oral Phenylalanine loading test in two DYT-5a pedigrees and in 71 age-matched patients affected by movement disorders not due to alterations of biogenic amine metabolism.
Results: Two different CGH1 gene alterations segregated alternatively in Family 1 (c.631-632 del AT and c.671A>G), while exon 6 deletion involved three generations in Family 2. Urine Neopterin was lower than reference values in 4 patients and 3 out of 4 carriers and significantly lower in GCH1 mutated subjects than in controls. Under Phenylalanine loading Phenylalanine/Tyrosine ratio at the 2nd hour was altered in all the patients and in 1 out of 2 asymptomatic carriers.
Conclusions: The combined assessment of urinary pterins and Phenylalanine/Tyrosine ratio under Phenylalanine loading is a reliable and non-invasive tool for the biochemical diagnosis of Segawa disease.

P-087

PTPS DEFICIENCY AND PSYCHIATRIC DISORDERS

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Deficiency of 6-pyrovoyl-tetrahydropterin synthase is the most common cause of tetrahydrobiopterin metabolism disorders.
Typical features of the disease are neuromotor signs, which are due to monoamine depletion. Isolated psychiatric symptoms are not commonly described.
We report a 17-year-old female, detected by neonatal screening for hyperphenylalaninemia and diagnosed at the age of 18 months as affected by PTPS deficiency (genotype T67M/K129E). In cerebro-spinal fluid (CSF), homovanillic acid (HVA) and 5 hydroxy-indolacetic acid (5HIAA) were reduced (253 nmol/L and 64 nmol/L respectively).
Despite scarce evidence of characteristic extrapyramidal symptoms, our patient presented a stable cognitive deficiency (IQ 52), childhood-onset of anxiety disorders as separation anxiety and social phobia and pubertal onset of obsessive thoughts, hallucinations, odd behaviour and compulsiveness. These psychiatric features followed a fluctuant trend.
On two different occasions of psychiatric symptom recrudescence during adolescence, CSF examination showed a normal level of 5HIAA and HVA and very high concentrations of 3-O-methyl-dopa and 5-hydroxy-tryptophan.
Sertraline was introduced in augmentation of traditional therapy, with significant regression of symptoms.
These findings suggest that normal levels of biogenic amines in CSF may not correspond to symptomatology abatement: normal concentrations of amine metabolites may not reflect neurotransmitter availability and activity at the receptor level.

P-088

ANTISENSE THERAPY CORRECTS ABERRANT PTS-SPLICING

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We report the effect of cellular antisense therapy using a new transporter structure to suppress pseudoexon activation in primary dermal fibroblasts from patients with mutations in the PTS gene encoding 6-pyruvoyltetrahydropterin synthase (PTPS), and also a deeper functional analysis of the mutant change c.164-712A>T that activates the exonization of an AluSq sequence previously reported. The results obtained using ex vivo minigenes showed that the change c.164-712A>T at position +6, but also at position +3 of the pseudoexon (c.164-715 T>A), seems to strengthen the pseudoexon 5′ splice site thus improving complementarity to U1 snRNA. We have demonstrated that even though two different 3′ss could be used to insert a 70-bp or 45-bp pseudoexons, these changes predominantly activate the most upstream one. Regarding the use of a new vehicle effective for in vivo delivery into a wide variety of tissues, we have used a conjugates of an octa-guanidine dendrimer covalently linked to the morpholino oligos to perform a parallel set of experiments. The RT-PCR and also the pterin profile showed that the AMO transporter-conjugates successfully corrected the mis-splicing recovering the metabolic pathway and confirm the therapeutical option for this type of mutations.

P-089

TETRAHYDROBIOPTERIN REDUCES PLASMA PROLACTIN CONCENTRATIONS IN PKU PATIENTS

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Background: Reduced cerebral neurotransmitter concentrations may contribute to cognitive dysfunction and mood disturbances in PKU. Some patients report improved executive functioning and mood during BH4 treatment at comparable plasma Phe concencentrations. We hypothesized that BH4 increases cerebral neurotransmitter synthesis in PKU patients.
Methods: BH4 treatment effects were studied in 18 several-week BH4-responsive subjects (age 17.5±9.6 years, 9 male). Plasma concentrations of prolactin (a marker of cerebral dopamine availability), monoaminergic neurotransmitters, and neurotransmitter metabolites prior to BH4 treatment were compared to long-term stabilization concentrations.
Results: BH4 significantly reduced prolactin in male patients (270±168 vs. 195±132 mE/L , p=0.008), but not in female patients (295±192 vs. 249±99 mE/L, p=0.329). Unexpectedly, adrenalin and metanephrine were significantly reduced after BH4 treatment (p=0.034 and p<0.001). A similar trend was observed for noradrenalin (p=0.091). Serotonin concentrations were unaffected by BH4 (p=0.251). Dopamine was undetectable.
Conclusions: BH4 treatment reduces plasma prolactin concentrations in male patients. This reduction is consistent with increased cerebral dopamine availability, possibly caused by BH4 treatment. Follow-up studies should investigate executive function and mood prior to and during BH4 treatment, as well as the cerebral effects of several-week BH4 treatment in non-responsive PKU patients.
Conflict of Interest declared.

P-090

BH4-LOADING TESTS IN PHENYLKETONURIA (PKU): COMPARISON OF THE RESULTS OF 24-HOUR NEONATAL PERIOD TEST WITH 72-HOUR BH4-PRETREATMENT TEST IN THE SAME PATIENT

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Background: For the decision of BH4-responsiveness many uncertainties about the BH4 loading test exist.
Objectives: To test if BH4-pretreatment loading test extended to 72-hr and 24-hr neonatal BH4-loading test performed in the same patient are relevant.
Methods: In 43 patients with PKU, two BH4 loading tests, first at the neonatal and second at 10,4±4,4 years of age were performed with a single dose of oral synthetic 6R-BH4 (20 mg/kg) and sapropterin (20 mg/kg twice), respectively. Patients with ≥30% reduction in phenylalanine (phe) levels were regarded as responsive.
Results: Comparison of 24-hr neonatal BH4-loading test with the 72-hr (after protein loading of 102±48 mg/kg) revealed that basal phe levels were 1192±680 Mmol/L and 620±240 Mmol/L, respectively. BH4 reduced the phe concentration with ≥30% in 28% in the neonate and 44% in the 72-hr test starting at 24 hrs. In addition 72-hr BH4-loading test diagnosed eight patients (19%) as 'slow-responders'.
Conclusion: The higher responsiveness in the 72-hr test compared to neonatal 24-hr test might because of the difficulties of performing the test in the neonate and the use of divided doses of sapropterin in the second test. 24-hr BH4 loading is sufficient to decide for responsiveness, prolonged test picks slow-responders.

P-091

THE 24- AND 48-HOUR BH4-LOADING TEST, WHAT DOES IT TELL US? A DUTCH MULTICENTER STUDY

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Background: There is no consensus how to assess BH4 responsiveness in PKU patients, including the duration of the BH4-loading test (BLT). Our goal was to test if a 24 or 48 hr BLT can reliably predict long-term responsiveness.
Methods: Data on 169 HPA-deficient patients from six Dutch UMCs were collected. Phase I: BLT (20 mg/kg BH4 at T0 and T24, blood samples at T= 0, 8, 16, 24 and 48 hrs). Patients with ≥30% reduction in Phe at ≥1 moment entered phase II: the trial of BH4 treatment, establishing increase in natural protein, BH4 dose and amino acid supplement intake. Long-term response was defined as ≥30% reduction in mean Phe, increase of natural protein intake of >4 gram and/or 50%.
Results: During 24 hrs 66 (39%) patients were responsive. The 48 hr sample added another 13 patients (total 47%). At present, 34/66 and 8/13 patients completed phase II. Insufficient response was seen in 5 and 4 patients, respectively, whereas long-term responsiveness was confirmed in 28 and 4 patients. Median Phe intake increased by 21.8 mg/kg/day (p=0.000).
Conclusion: Predictive value of the 24 hrs BLT is the largest, but BLT should at least last 48 hrs. The relevance of a prolongation to a 72 hrs BLT needs to be studied.
Conflict of Interest declared.

P-092

NEW EVIDENCE FOR ASSESSING TETRAHYDROBIOPTERIN-RESPONSIVENESS IN PHENYLKETONURIA DELIMITING THE SCOPE OF THE TETRAHYDROBIOPTERIN LOADING TEST

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Background: Our study aimed at designing a simple and standardised test for assessing tetrahydrobiopterin (BH4) responsiveness, focusing on improving PKU patients' care during its performance.
Methods: 102 phenylketonuric patients were recruited for the study. The BH4-loading test was based upon the 50% criterion and entailed a Phe overload prior to BH4 administration. Blood samples were drawn at 0, 7, 12 and 24 h after BH4 ingestion. We also performed a therapeutic test combining BH4 administration and a daily protein intake in accordance with RDAs for one week, to examine whether the 24-h test had detected all responders.
Results: the test detected all responders to BH4 treatment. Sensitivity, specificity and likelihood ratios for the 24-h test were 0.94, 1.00, 94 and 0.06, respectively. 95% of patients presenting a Phe level at diagnosis below 720 μmol/L responded to the test and patients presenting a Phe concentration above 1440 μmol/L were non-responders.
Conclusion: The novelty of our protocol lies in its validation via enrolling a large cohort and improving PKU patients' management by reducing the number of blood samples to be drawn and avoiding them being admitted to hospital. Besides, we demonstrated that this 24-h test is clinically useful for screening PKU patients.

P-093

TETRAHYDROBIOPTERIN-RESPONSIVE PHENYLKETONURIA: THE CZECH EXPERIENCE

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Background: Phenylketonuria (OMIM 261600) is an inherited metabolic disease due to a deficiecy of hepatic phenylalaninhydroxylase (PAH;12q24.1). Tetrahydrobiopterin (BH4) responsiveness in patients with specific mutations in the PAH gene is a subtype of hyperfenylalaninaemia (HPA) charecterised by a positive BH4 loading test.
Methods: We tested 23 patients with HPA/PKU, 4–39 years of age, selected (based on genotype) as a potentially BH4 responsive and loaded with BH4 (20 mg/kg).
Results: Overall 10/23 showed positive response of more than 30% decrease in blood Phe levels 8 h after BH4 challenge, and 7/23 showed this decrease after 24 h. The majority of the responsive patients belong to mild HPA (13/17). In p.E390G, p.A300S, p. A403V, p. Y414C, p.I306V, p. G272X and p.Y387H mutations were 100% associated with BH4 responsiveness. The p.R158Q mutation was inconstantly responsive. The EX5del-4 kb mutation was responsive regardless of the second allele (p.A403V, p.R408W). In patient with mild PKU and novel mutation p. K396R (genotype p.R408W/p.K396R) no responsiveness was noticed.
Conclusion: The best responders were patients with mild HPA. Very interesting group are patients with the novel mutation in the PAH gene.

P-094

EFFICACYAND SAFETY OF SAPROPTERIN DIHYDROCHLORIDE IN LONG-TERM FOLLOW-UP OF PATIENTS WITH TETRAHYDROBIOPTERIN-RESPONSIVE MILD PHENYLKETONURIA IN JAPAN.

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Background: Sapropterin dihydrochloride (Biopten.) is first synthesized in Japan as a 6R-isomer of tetrahydrobiopterin (BH4), a natural cofactor for phenylalanine hydroxylase (PAH) in 1982. In Japan, Biopten. is first approved for the treatment of BH4 deficiency in 1992, and then for BH4-responsive PAH deficiency (BRPD) in 2008.

Objectives: To evaluate efficacy and safety of BH4 treatment in patients with BRPD, we followed up development and examined side effects.

Patients and Methods: We examined serum phenylalanine levels, EEG, MRI, and complications in 33 BRPD yearly at 22 medical centers in Japan.

Results: Among 33 BRPD 14 were treated with BH4 only, and 19 were treated with BH4 plus low phenylalanine diet. An initial age of BH4 treatment was 4.9 years (15 patients were less than 4 years old), and their mean age at end of follow-up was 7.8 years. Average duration of treatment with BH4 (mean, 8.5 mg/kg/day) was 7 years (range, 1–14 years). No abnormalities of height and weight were observed in all patients. No unwarranted side effects were reported throughout the long-term course of treatment.

Conclusion: Biopten. therapy in BRPD is highly efficacious for reducing serum phenylalanine levels and provides excellent safety with no unwarranted side effects.

P-095

THE KUVAN. ADULT MATERNAL PAEDIATRIC EUROPEAN REGISTRY (KAMPER): BASELINE DEMOGRAPHICS

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Objectives: KAMPER aims at providing information on the long-term outcomes of approximately 625 Kuvan treated patients with hyperphenylalaninemia (HPA) due to phenylketonuria (PKU) or BH4 deficiency, over the course of 15 years.

Methods: Observational, multi-centre, drug registry, including a maternal subregistry.

Results: At first year interim analysis four countries contributed a total of 73 patients [80% (n=53) PKU and 20% (n=15) BH4-deficiency]. Until now, patients were recruited in France (19 PKU-10 BH4-deficiency); Germany (24–1), Spain (13–0), Italy (2–4). More countries plan to join the registry with recruitment starting soon in Austria and Greece. The median age (Q1-Q3) of recruited PKU patients is 9.7 years (6.4–14.9): 4–8 years (n=24); 8–12 years (n=13); 12–18 years (n=12); 18–65 years (n=9). For BH4-deficiency patients the median age is (Q1-Q3) 12.7 years: (3.9-16.2): <4 years (n=4); 4–8 years (n=3); 8–12 years (n=0); 12–18 years (n=5); 18–65 years (n=3). The male/female ratios are 0.53 among PKU and 0.47 among BH4-deficiency patients. Genotyping was reported in 62% of PKU and 0% of BH4-deficiency patients.

Conclusions: KAMPER provides a unique opportunity to gather a large collection of long-term follow up data related to BH4-responsive HPA in about 10 European countries.

Conflict of Interest declared.

P-096

BRAIN MRI FEATURES IN PATIENTS WITH PHENYLKETONURIA (PKU) IN LONG-TERM TREATMENT WITH TETRAHYDROBIOPERIN

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Aim: To examine the presence of brain white matter involvement in tetrahydrobiopterin (BH4) responsive PKU patients.
Material and Methods: Brain MRIs (T2, FLAIR and DWI sequences) were assessed in 7 PKU BH4-responsive patients (age range 7–23 years; plasma phenylalanine levels 500–1200 μmol/L, and phenylalanine tolerance 350–700 mg/day before starting BH4), receiving BH4 (Schircks Inc. and Kuvan., 10 mg/kg) for a period of 5–8 years. Four patients were on unrestricted diet and 3 were on a mild phenylalanine-restricted diet at the moment of the study.
Results: We detected normal MRI in 3 out of 7 patients (age range 7–9 yrs, treatment period range 6–8 yrs, mean blood phenylalanine levels 295± 58 μmol/l, phenylalanine tolerance 800–2700 mg/day). In the remaining 4 patients (age range 8–23 yrs, treatment period range 5–8 yrs, mean blood phenylalanine levels 292±44 μmol/l, phenylalanine tolerance 1000–1600 mg/day) minimal white matter abnormalities (in posterior areas in 3 patients, in frontal area and centrum semiovale in one patient) were detected.
Conclusions The lower blood phenylalanine levels and the increasing dietary phenylalanine intake achieved by means of long-term BH4 treatment, might protect the brain from the white matter lesions we reported previously in classic-PKU patients (Manara R, 2009). Further research is needed to reach definitive conclusions.

P-097

THE KUVAN. ADULT MATERNAL PAEDIATRIC EUROPEAN REGISTRY (KAMPER): PATIENT CHARACTERISTICS

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Objectives: KAMPER aims at providing information on the long-term outcomes of approximately 625 Kuvan treated patients with hyperphenylalaninemia (HPA) due to phenylketonuria (PKU) or BH4 deficiency, over the course of 15 years.
Methods: Observational, multi-centre, drug registry, including a maternal subregistry.
Results: First year interim analysis included data from 73 patients (PKU n=58, BH4-deficiency n=15). All results are presented as median (Q1–Q3). Baseline mean Phenylalanine concentration (μmol/L): 550 (288–641) (n=39) and 232 (54–1493) (n=11); in PKU and BH4-deficiency patients, respectively. Identified by newborn screening: 93% of PKU patients; Phenylalanine concentrations (μmol/L) 483 (371–727) and at confirmatory test 793 (478–1150). Identified by newborn screening: 87% of BH4-deficiency patients; Phenylalanine concentration (μmol/L) 467 (336–727) and at confirmatory test 888 (466–1574). Mean Kuvan doses are 15 (10–20) mg/kg/day and 3.6 (1.5–9.6) mg/kg/day in PKU and BH4-deficiency patients, respectively. The majority of patients were tested for BH4 responsiveness following a 24-hr loading test. Phenylalanine concentrations decreased≥30% in 51/55 of PKU and 9/9 of BH4-deficiency patients. Mild/moderate adverse events were reported in 9% of PKU patients (not drug related).
Conclusion: KAMPER will increase knowledge on current treatment practises of HPA patients with either PKU or BH4 deficiency across Europe.
Conflict of Interest declared.

P-098

BASELINE DEMOGRAPHICS OF THE FIRST 26 PATIENTS INCLUDED IN ENDURE: A PHASE IV, PROSPECTIVE, OPEN-LABEL, UNCONTROLLED, TRIAL TO ASSESS THE RESPONSIVENESS OF SUBJECTS WITH PHENYLKETONURIA TO TREATMENT WITH KUVAN. 20 MG/KG/DAY FOR 28 DAYS

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Background: A subset of Phenlyketonuria (PKU) patients may reduce blood Phenylalanine (Phe) concentrations following treatment with Kuvan. (sapropterin dihydrochloride) in conjunction with diet. Response to Kuvan. depends on geno- and phenotype and patients should be tested during a 28 days period. Current European clinical practice utilizes the 24–48 hours response tests which might not be able to detect late responders.
Objectives: To evaluate the proportion of responders (>30% reduction in blood Phe concentration from baseline) to Kuvan. 20 mg/kg/day measured at several time points during 28 days.
Methods: Open-label, single-arm, cohort study. Patients with known, suspected, or unknown mutations in the phenylalanine hydroxylase gene have been offered to participate in this trial. In Norway all PKU patients are centralized to one clinic and tested for PAH gene mutations.
Baseline demographics: Gender (M/F): 10/16; age [mean (SD, range)] 26.3 (11.6, 9–51) years; blood Phe concentration: 582.4 (210.8, 208–987) μmol/L; blood tyrosine concentration: 47.6 (19.2, 15.0–85.4) μmol/L; Phe intake: 1811.1 (1422.9, 400–5254) mg/day
Conclusion: Patients included in the ENDURE trial so far are mainly adolescents/young adults with milder forms of PKU. Upon completion, ENDURE will provide an estimation of the proportion of patients that respond to Kuvan. beyond 48 hours of testing.
Conflict of Interest declared.

P-099

SAPROPTERIN TREATMENT IN PHENYLKETONURIA: EXPERIENCES IN AN OUTPATIENT SETTING

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Sapropterin is approved for PKU treatment in Germany. We report on our experience with Sapropterin in 79 PKU outpatients (age 4 to 42 years). After having attained stable plasma phenylalanine (Phe) levels >6 mg/dl, Sapropterin response was tested for 2 weeks (1st week: 10 mg/kg/d, 2nd week: 20 mg/kg/d). In case of a decrease in plasma Phe levels >30%, we continued the medication while gradually increasing dietary natural protein intake and determined the increase of Phe tolerance over a 6 weeks period. 30 patients (37,97%) responded to Sapropterin. Treatment was discontinued in 8 patients because of non-compliance (n=3) or missing increases in Phe tolerance (n=5). The 22 patients treated with Sapropterin showed a mean increase in Phe-tolerance of 191,1% (40,0-483,3%). On average we could reduce amino acid supplements by 59,2% (11,8-100%). Non-response to sapropterin was associated most frequently with the presence of PAH mutations R408W (n=20) and IVS12+1 (n=8) in our cohort. However, non-response could not be predicted based on these specific mutations if the mutation was combined with another mutation. Sapropterin response is associated with an increase of Phe-tolerance in most cases. The presence of the R408W or the IVS12+1 mutation in PAH does not predict non-response when present on one allele.
Conflict of Interest declared.

P-100

DECREASED FLUCTUATION OF BLOOD PHENYLALNINE UNDER BH4 TREATMENT IN PKU PATIENTS

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Background: Recently fluctuation of blood phenylalnine (phe) was described as important variable for intellectual outcome in early treated phenylketonuria (PKU). New treatment options in PKU like BH4 supplementation may influence fluctuation of blood phe.
Objectives: Investigation of BH4 effect on fluctuation of blood phe expressed as standard error of estimation (SEE) in early treated PKU patients.
Methods: In 16 patients with PKU treated with a phenylalanine restricted diet and/or BH4 (observation time 24 to 110 months) mean blood phe/year and mean SEE were calculated using a total of 2286 measurements. 8 of 16 patients were treated only with BH4, 8 with a phenylalanine restricted diet and BH4.
Results: Range of mean blood phe was 242 to 777 μmol/l, mean SEE was from 86 to 239 μmol/l. In only 3 patients mean blood phe was above 360 μmol/l and a SEE above 200 μmol/l. Long term observation revealed that two of these 3 patients were not responsive to BH4 treatment.
Discussion: There is evidence that BH4 may reduce phe fluctuation in BH4 responsive patients. More studies are necessary to prove a positive effect of decreased phe fluctuation on intellectual outcome under BH4 treatment.
Conflict of Interest declared.

P-101

USE OF TETRAHYDROBIOPTERIN (BH4) IN PATIENTS WITH PHENYLKETONURIA: IMPACT ON METABOLIC CONTROL, NUTRITION HABITS AND QUALITY OF LIFE

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Background: We investigated metabolic control, nutrition habits and health-related quality of life (HRQoL) in potentially BH4 sensitive phenylketonuria (PKU) under BH4 treatment.
Subjects and Methods: Of 41 patients screened, 19 were potentially BH4 sensitive (neonatal BH4 test, mutation analysis; 9 females, 4–18 yrs). We analysed phenylalanine concentrations in dried blood (phe), nutrition protocols and HRQoL (KINDL®) beginning one year before, during the first six weeks and after three months of BH4 therapy.
Results: 8/19 patients could increase phe tolerance (629+/−476 mg vs. 2131+/−1084 mg, p=0.006) while maintaining good metabolic control (phe concentration 283+/−145 μM vs. 304+/−136 μM, p=1.0). Intake of vitamine D (110%+/−22 vs 30%+/−19, p=0.001), iron (140%+/−26 vs 71%+/−31, p=0.01), iodine (118%+/−23 vs 37%+/−24, p=0.006) and calcium (136%+/−19 vs 62%+/−38, p=0.042; % of German recommendations) was significantly lower during BH4 treatment. BH4 sensitive patients had HRQoL scores comparable to age-matched healthy children; no change of HRQoL under BH4 treatment, although available questionnaires appear inappropriate to detect aspects relevant to PKU.
Conclusion: The unexpected deficiency in micronutrient intake should be verified prospectively. Substitution seems necessary independent of the substitution of phe-free amino acid mixtures. Specific HRQoL questionnaires should be developed for PKU.
Conflict of Interest declared.

P-102

HYPERPHENYLALANINEMIA DUE TO PHENYLALANINE HYDROXYLASE DEFICIENCY (HPA-PAH): EVALUATION OF TETRAHYDROBIOPTERIN (BH4) RESPONSIVENESS IN BRAZILIAN PATIENTS

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Objective: To determine BH4 responsiveness in 18 patients with HPA-PAH who present the median Phe≤10 mg/dL when following dietary treatment.
Methods: Participants received a simple Phe (Test 1) and a combined Phe/BH4 loading test (Test 2), using 100 mg/kg L-Phe and 20 mg/kg BH4. Blood samples were collected at baseline, 3, 11, and 27 h after Phe ingestion (time Points 0, 1, 2 and 3). Responsiveness was defined according to three criteria which took in account, for Tests 1 and 2, the values of Phe at time Points 1 and 2 (criterion 1) and at time Points 1 and 3 (criterion 2), and the difference in percentage of the areas under the Phe curve (criterion 3). The cut-off point for all criteria was a reduction of≥30%.
Results: Six patients were responsive according to at least one of the criteria used. Responsiveness was concordant for criteria 1 and 2 when compared with criterion 3 (kappa=0.557;p=0.017). BH4 plasma levels in Test 2 increased in association with L-Phe and BH4 loading.
Conclusion: The comparison of simple Phe and combined Phe/BH4 loading seems to be an adequate criterion to evaluate responsiveness to BH4 in patients with HPA-PAH and good metabolic control when following a dietary treatment.

P-103

SAPROPTERIN TREATMENT IN HYPERPHENYLALANINEMIC PATIENTS BELOW FOUR YEARS OF AGE

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Background: The main treatment for PKU is lifelong dietary phenylalanine restriction. Early dietary treatment is effective in hyperphenylalaninemia, but this Phe restricted diet has negative aspects. A subset of patients shows a clinically significant reduction in blood phenylalanine when treated with pharmacological doses of Sapropterin. Sapropterin has been approved for the treatment of hyperphenylalaninaemia in patients>or= 4 years of age.
Objective: Assessing the treatment with sapropterin in children less than four years.
Patients: PAH deficiency patients younger than 4 years treated with sapropterin.
Results: Six children less than 4 years have been treated with sapropterin. All of them but one were responsiveness. Two of them began the treatment from the newborn period and the other three began it above one year. Considering all together, the Phe (nmol/ml) mean and SD was 215,4+ 108,49; the Phe intake (mg) mean and SD was 1025+745. Children who began when they were more than one year old, the Phe intake (mg) mean and SD before sapropterin treatment was 570,14+324,83 and after treatment was 1340,96+978,86. There has been no side effects.
Comments. Sapropterin treatment is a valid alternative to the treatment with a diet limited in phenylalanine in hyperphenylalaninemia in patients less than 4 years.

A-004

A CASE OF DIHYDROPTERIDINE REDUCTASE DEFICIENCY

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Tetrahydrobiopterin (BH4) is the essential cofactor of Phenylalanine- (Phe), Tyrosine- (Tyr), and Tryptophan- (Trp) hydroxylases. BH4 deficiency, a rare but severe type of hyperphenylalaninemia, is characterized by progressive neurologic symptoms despite early detection and treatment with a Phe-restricted formula. It is caused by enzyme defects in the biosynthesis or regeneration of BH4. We present a boy with hyperphenylalaninemia due to dihydropteridine reductase (DHPR) deficiency, DHPR is the enzyme necessary for recycling of the cofacter BH4. Some DHPR deficient patients could be misdiagnosed, because their serum Phe levels were not lowered after BH4 loading. An expensive dose of BH4 is needed to maintain the Phe, Tyr, Trp hydroxylation reaction. Severe BH4 deficiency is a naturally occurring model of cerebral catecholamine and serotonin shortage. Our patient improved on BH4 therapy, but the concentration of homovanillic acid (HVA) and 5-hydroxyindole acetic acid (5HIAA) in cerebrospinal fluid (CSF) remained low. Administration of the neurotransmitter precursors L-dopa/carbidopa and 5-hydroxytryptophan appeared to be the most effective treatment and may prevent neurological damage if started early in life. The role of dopamine as a growth hormone releasing factor at the hypothalamic level was established. Growth rate is nearly normal in our patient.

A-005

HELICOBACTER PYLORI INFECTION IN CHILDREN WITH PHENYLKETONURIA

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Objectives: to determine the frequency of Helicobacter pylori infection and investigate the effects of the infection on blood phenylalanine concentrations in children with phenylketonuria.

Patients and Methods: In this cross-sectional study, 66 children with phenylketonuria and 32 healthy controls were recruited for this study. The two groups were compared for frequency of Helicobacter pylori infection. Blood phenylalanine concentrations were measured simultaneously with tests measurement for the detection of Helicobacter pylori infection in phenylketonuria patients.

Results: Frequency of the Helicobacter pylori infection was higher in phenylketonuria patients (27.3%) than in the healthy controls (9.4%). Among all the phenylketonuria patients, the Helicobacter pylori-positive group had higher blood phenylalanine concentration (884.8±424.2 μmol/L) than in the Helicobacter pylori negative (575.7±430.3 μmol/L) group ($p<0.05$).

Conclusion: The frequency of Helicobacter pylori infection was found higher in children with phenylketonuria. This can be related to the lack of personal hygiene due to neurological damages caused by poor metabolic control. High blood phenylalanine concentrations in H. pylori-positive children with phenylketonuria might be related to the increase of blood phenylalanine concentrations through possible catabolic effects of the H. pylori infection. The data presented in this study needs to be reproduced by advanced and standardized clinical trials.

A-006

A NOVEL MUTATION IN A TURKISH PATIENT WITH DIHYDROPTERIDINE REDUCTASE (DHPR) DEFICIENCY

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Background: Dihydropteridine reductase (DHPR) deficiency caused by mutations in the QDPR (quinoid dihydropteridine reductase) gene is a defect in the regeneration of tetrahydrobiopterin(BH4) which is an obligatory cofactor for the aromatic amino acid hydroxylases. DHPR deficiency results in hyperphenylalaninemia and deficiency of various neurotransmitters in the central nervous system, with progressive severe neurological symptoms. We here report a novel mutation described in a patient with severe DHPR deficiency.

Case Report: Psychomotor retardation, and seizure at 6 months of age was noticed. The evaluation for severe psychomotor retardation, dystonia, involuntary movements, hypersalivation, and convulsions at the age of 18 months revelated hyperphenylalaninemia (560 micromol/L). Undetectable DHPR activity in dried blood sample (<0.05 mU/mg Hb, reference activities: 1.8–3.8) led to the diagnosis of severe DHPR deficiency. Gene sequencing subsequently revealed a novel mutation (homozygous c.295+5 G>A (IVS5+5 G>A) in the QDPR gene.

Conclusions: To date, more than 34 different mutations have been identified in affected patients. Presence of very similar phenotypes in identical QDPR genotypes supports that genotype is a major determinant of phenotype in DHPR deficiency. The novel mutation we described in our patient causes severe phenotype but no enzyme expression analysis has been performed in that mutation yet.

O-017

MATERNAL HOMOCYSTEINE IN ASSOCIATION WITH BIRTH WEIGHT: SYSTEMATIC REVIEW AND META-ANALYSIS

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Background: Low birth weight is associated with increased neonatal morbidity and mortality but also with life-long consequences such as cardiovascular diseases. Maternal homocysteine concentrations (tHcy) have been linked to a wide range of adverse pregnancy outcomes and could influence birth weight. We performed a systematic review and meta-analysis on the association of maternal tHcy and birth weight.

Design: A literature search using Pubmed revealed 78 abstracts. Studies were eligible if information on maternal tHcy, birth weight and the association between maternal tHcy and birth weight was available. Effect estimates were converted to odds ratios with a cut-off level of birth weight <p10 for gestational age (SGA) and maternal tHcy>p90.

Results: This search yielded 19 studies for analysis, consisting of 21,326 individuals. Pooled analysis resulted in a crude OR of 1.31[1.15;1.51] to get a SGA infant when maternal tHcy is>p90. If this estimate is expressed as a linear effect, it corresponds to −38[−20;-58] g for 1 sd increase in maternal tHcy. Adjustment for known confounders was not possible but a tendency to decreased strength of association was observed in studies after adjustment for strong determinants.

Conclusion: Higher maternal tHcy concentrations are associated with an increased risk for being SGA.

O-018

OXIDATIVE STRESS INDUCES CONSTITUTIVE INDUCTION OF MULTIPLE PRO-INFLAMMATORY AND CHEMOTACTIC CYTOKINES IN CBS DEFICIENT HOMOCYSTINURIA

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Cystathionine beta-synthase (CBS) deficient homocystinuria (HCU) if untreated, typically results in cognitive impairment, connective tissue disturbances, and atherosclerosis and thromboembolic disease. We investigated the expression levels of 25 separate cytokines/chemokines in both a transgenic mouse model of HCU and human subjects with the disease. HCU mice exhibited highly significant inductions of the pro-inflammatory cytokines Il-1alpha, Il-1beta and TNF-alpha which were normalized or significantly lowered by treatment with either betaine or the antioxidant amino sulfonic acid taurine. In untreated/poorly compliant human subjects with HCU, we observed constitutive induction of multiple pro-inflammatory cytokines (IL-1alpha, IL-6, TNF-alpha, Il-17 and IL-12(p70)) and chemotactic chemokines (fractalkine, MIP-1alpha and MIP-1beta) compared to normal controls. The expression levels of anti-inflammatory cytokines were normal in both HCU mice and humans with the disease. In human subjects, conventional therapy lead to either normalization or significant reduction of all of the pro-inflammatory cytokines and chemokines investigated.We conclude that HCU is a disease of oxidative stress induced chronic inflammation and that aberrant cytokine expression has the potential to contribute to multiple aspects of pathogenesis. Our findings indicate that antioxidant and anti-inflammatory strategies could serve as useful adjuvant therapies for this disease.

O-019

EXPRESSION OF METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) WILDTYPE (WT) AND MUTANT ALLELES IN IMMORTALIZED FIBROBLASTS.

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Background: MTHFR deficiency causes one type of homocystinuria with a severe, but widely variable clinical presentation. About half of patients, usually with less severe presentation, show residual enzyme activity from 2.6% to 68%. This activity is often associated with reduced affinity for NADPH and disturbed inhibition by the allosteric regulator S-adenosylmethionine, sometimes with altered responsiveness for the cofactor FAD and always with normal affinity for the substrate methylenetetrahydrofolate. In 43 patients we found 40 different (26 missense) mutations showing that majority are private.
Objectives: To study functional consequences of MTHFR mutations in a mammalian expression system.
Methods: Transient transfection of immortalized MTHFR deficient fibroblasts with mutant and wt MTHFR-cDNA constructs by electroporation followed by determination of MTHFR activity and kinetics using our physiological assay.
Results: Transfection with MTHFR-wt resulted in >100 times control cell enzyme activity. Similar over-expression was found with 5 representative mutant alleles from patients with residual enzyme activity and also the same in vitro FAD-responsiveness and abnormalities in enzyme kinetics were found as in patient fibroblasts.
Conclusion: In contrast to previous studies in bacterial or yeast expression systems our approach allows reliable evaluation of the sometimes unclear functional consequences of nucleotide changes in the MTHFR gene.

O-020

LESSONS FROM PHENYLKETONURIA: MISFOLDING OF CYSTATHIONINE BETA-SYNTHASE MUTANTS AND EFFECT OF CHAPERONES

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Misfolding of mutant proteins is a common pathogenic mechanism in many genetic diseases including phenylketonuria. Since misfolding of mutant phenylalanine hydroxylase enzymes is successfully targeted by sapropterin administration we explored whether misfolding plays a role in cystathionine beta-synthase (CBS) deficiency and whether it can be modulated by chaperones. We have studied a series of 27 disease-causing mutations located in different domains of the CBS molecule representing ~70% of patient-derived mutant alleles. Expression in prokaryotic and eukaryotic cells showed a propensity of mutants to misfold/misassemble, which was accompanied by their impaired catalytic activity. Co-expressional addition of chaperones to E.coli cultures increased activity and formation of tetramers for about one half of mutants while experiments using eukaryotic CHO-K1 cells increased formation of tetramers and activity of only selected mutants; the most significant effect was observed for mutation p.R125Q both in CHO-K1 cells and in cultured fibroblasts carrying the p.R125Q mutation.
All these studies indicate that about one half of all known patient CBS alleles can possibly produce enzymes prone to misfolding/misassembly and that correction of misfolding by chaperones may become an important therapeutic target similarly to phenylketonuria.
Supported by Charles University Research Project MSM0021620806, the fibroblast line was kindly provided by Dr.B.Wilcken.

P-105

HYPERHOMOCYSTEINEMIA MODULATES CYTOCHROME C OXIDASE ACTIVITY IN PARIETAL CORTEX OF RATS: NEUROPROTECTIVE ROLE OF FOLIC ACID

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Homocystinuria is an inborn error of metabolism biochemically characterized by cystathionine beta-synthase deficiency, leading to homocysteine (Hcy) tissue accumulation. Clinically, patients present mental retardation, seizures, and vascular complications. Folic acid therapy has been used, since it is a methyl donor in Hcy metabolism, as well as its own antioxidant properties. In the present study we evaluated the effect of Hcy on the activities of creatine kinase and respiratory chain enzymes (complex II, succinate dehydrogenase and cytochrome c oxidase) in parietal cortex of rats. We also evaluated the neuroprotector effect of folic acid on biochemical alterations elicited by Hcy. Acute treatment: 22 day-old rats received folic acid daily administrations for one week, 12 hours later the animals were subjected to a single Hcy administration. Chronic treatment: 6 day-old rats received daily folic acid and/or Hcy injections up to 28th day-of-life. Results showed that Hcy acute administration reduced cytochrome c oxidase activity, while chronic Hcy administration increased it. Hcy did not alter other enzymes evaluated. Folic acid prevented Hcy effects, probably by acting as an antioxidant. In conclusion, folic acid prevented the effects of Hcy administration on respiratory chain activity, suggesting a relevant role of this vitamin preventing neurotoxic effects of hyperhomocysteinemia.

P-106

HYPERHOMOCYSTEINEMIA INCREASES CYTOKINES AND PROSTAGLANDIN E2 IN RAT HIPPOCAMPUS

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In the present study we evaluate the effect of chronic homocysteine administration on some parameters of inflammation, such as cytokines (TNF-α, IL-1β and IL-6), chemokine CCL2 (MCP-1), nitrite levels, prostaglandin E2, as well as acetylcholinesterase activity in rat hippocampus. 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 day-of-age. Rats were sacrificed 1 or 12 h after the last injection. Results showed that chronic hyperhomocysteinemia significantly increased proinflammatory cytokines (TNF-α, IL-1β and IL-6), chemokine CCL2 (MCP-1), prostaglandin E2 and nitrite levels in hippocampus.of rats. Acetylcholinesterase activity also was increased after homocysteine administration. Our findings show that chronic hyperhomocysteinemia increases inflammatory parameters, suggesting that this process might be associated, at least in part, to the brain dysfunctions characteristic of some homocystinuric patients. Supported by CNPq and FAPERGS.

P-107

CHRONIC MILD HYPERHOMOCYSTEINEMIA INDUCES OXIDATIVE DAMAGE IN CEREBRAL CORTEX OF RATS

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The purpose of this study was to develop a chronic chemically-induced model of mild hyperhomocysteinemia in adult rats. We produced levels of Hcy in the blood (30 μM), comparable to those considered a risk factor for the development of neurological and cardiovascular diseases, by injecting subcutaneously homocysteine (0.03 μmol/g of body weight) twice a day from the 30th to the 60th postpartum day. Controls received saline in the same volumes. Using this model, we evaluate the effect of chronic administration of homocysteine on redox status in blood and cerebral cortex of adult rats. Reactive oxygen species and thiobarbituric acid reactive substances were significantly increased in plasma and cerebral cortex, while nitrite levels were reduced in cerebral cortex, but not in plasma of rats subjected to chronic hyperhomocysteinemia. We also observed that homocysteine disrupted enzymatic and non-enzymatic antioxidant defenses in the blood and cerebral cortex of rats. Considering that experimental animal models are useful to understand the pathophysiology of human diseases, the present model of mild hyperhomocysteinemia may be useful in the investigation of additional mechanisms involved in tissue alterations caused by homocysteine. Supported by CNPq and FAPERGS.

P-108

EFFECT OF CHRONIC HYPERHOMOCYSTEINEMIA ON NA+, K+−ATPASE ACTIVITY AND GLUTAMATE UPTAKE IN HIPPOCAMPUS OF RATS: PREVENTION BY GUANOSINE

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Homocystinuria is an inborn error of metabolism caused by cystathionine-β-synthase deficiency, leading to tissue accumulation of homocysteine (Hcy). Affected patients may present with seizures and mental retardation. It has been demonstrated that homocysteine might promote glutamatergic excitotoxicity due to overstimulation of NMDA receptors. Studies show that guanosine (Guo) prevents seizures induced by changes in glutamatergic system in rats. In the present study we evaluated the influence of Guo on the effects elicited by severe hyperhomocysteinemia on Na+, K± ATPase activity and glutamate uptake. Wistar rats received daily twice subcutaneous injection of D,L-Hcy (0.3–0.6 μmol/g body weight), and/or Guo (7.5 mg/kg) once a day from 6th to their 21th day of life. Twelve hours after the last injection the rats were sacrificed and hippocampus was dissected. Results showed that hyperhomocysteinemia reduced activity of Na+, K±ATPase and glutamate uptake in rat hippocampus. Guo prevents only the decrease Na+, K±ATPase activity. Our findings suggest that Hcy might alter neuronal excitability and increase levels of glutamate in the synaptic cleft which could lead to excitotoxicity. The mechanisms of prevention of Guo on the activity of Na+, K±ATPase is still unknown and need further studies to be elucidated. Supported by CNPq, FAPERGS.

P-109

STUDY OF THE ROLE OF HOMOCYSTEINE IN CELLULAR OXIDATIVE STRESS

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An increased reactive oxygen species (ROS) production and apoptosis rate have been associated with several disorders of cobalamin metabolism, particularly with the cblC type of methylmalonic aciduria combined with homocystinuria . In order to analyze the role of homocysteine in stress response, we have evaluated several parameters related to oxidative stress and apoptosis in fibroblasts from patients with defects of MTHFR, MTRR and MTR genes causing homocystinuria. All cell lines showed a significant increase in ROS content and in MnSOD expression level, and also a higher rate of apoptosis with similar levels to the ones found in cblC fibroblasts. The amount of phosphorylated forms of p38 and JNK stress-kinases was also increased. ROS content and apoptosis rate were increased in control fibroblasts and glioblastome cell line by shRNA-mediated silencing of MTRR gene expression, indicating the possible function of homocysteine in these processes. The toxic built-up of homocysteine in patients with homocystinuria might be partially responsible for the deleterious effects in stress response, and support the potential of using antioxidants as a novel therapeutic strategy to improve the severe neurological outcome of these rare diseases.

P-110

S-ADENOSYLHOMOCYSTEINE ACCUMULATION INCREASES DDAH ACTIVITY IN CULTURED HUMAN ENDOTHELIAL CELLS, VIA A NON-DNA DEPENDENT METHYLATION PATHWAY

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Intracellular homocysteine exclusively results from S-adenosylhomocysteine (SAH) reversible hydrolysis, catalyzed by SAHhydrolase. Moreover, homocysteine inhibits global DNA methylation, via accumulation of SAH. Asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase, may play a role in hyperhomocysteinemia-induced endothelial dysfunction. Dimethylarginine dimethylaminohydrolase (DDAH) is the major route of ADMA degradation.

We investigate the effect of intracellular SAH accumulation and global DNA hypomethylation, on DDAH activity.

Human umbilical vein endothelial cells were cultured with or without adenosine-2,3-dialdehyde (ADA), a SAHhydrolase inhibitor. Global DNA hypomethylation was assured with 5-aza-2-deoxycytidine (AZA). SAH levels and global DNA methylation were quantified by LC-MS/MS. ADMA levels were determined by HPLC. DDAH mRNA and protein levels were quantified by qRT-PCR and Western blot, respectively. DDAH activity in cell homogenates was assessed by measuring the citrulline produced during 6 hours.

ADA increased SAH intracellular levels, resulting in global DNA hypomethylation. DDAH-1 mRNA levels (but not DDAH-2) were up-regulated, and correlated with the decreased ADMA. ADA increased DDAH activity, without changes in protein expression, implicating possible post-translational events involved in DDAH protein regulation. AZA treatment, despite evident global DNA hypomethylation, affected neither DDAH activity nor ADMA production, suggesting that SAH influences DDAH activity by a non-DNA dependent methylation pathway.

P-111

MILD ISOLATED HYPERMETHIONINEMIA SECONDARY TO METHIONINE ADENOSYLTRANSFERASE (MAT) I/III DEFICIENCY WITH ASYMPTOMATIC ELEVATION OF CREATINE KINASE

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Background: Methionine adenosyltransferase (MAT) I/III deficiency, caused by mutations in the MAT1A gene, is characterized by persistent isolated hypermethioninemia. MAT catalyzes the biosynthesis of S-adenosylmethionine (AdoMet) from methionine and ATP. AdoMet is a major methyl donor for a large number of important biological reactions. Clinical manifestations are not universal; observations suggest the risk of neurological complications, increase with higher plasma methionine concentrations.

Case Report: We describe a 22 month old Burmese child with mild hypermethioninemia ascertained by newborn screening. Serum methionine was persistently higher than those of controls, ranging between 60–170 μmol/l (normal<40). Interestingly, serum creatine kinase (CK) was significantly raised at 1028 U/l (normal 25–200). There was no clinical evidence of myopathy; electromyography and brain MRI have been organized.

Results: Serum tyrosine, total homocysteine, folate, B12, plasma methylmalonic acid, liver function tests and urine organic acids were normal. Plasma AdoMet and S-adenosylhomocysteine (AdoHcy) quantitative assays were normal, suggesting MAT I/III deficiency.

Conclusions: Diagnosis of MAT deficiency should be suspected in all persons with unexplained hypermethioninaemia. Associated neurological complications should be monitored for, regardless of the severity of enzymatic deficiency. Clinical manifestations are highly variable, thus further studies that assess possible genotype-phenotype correlations are warranted.

P-112

A HETEROLOGOUS EXPRESSION SYSTEM TO STUDY CBS MUTANT PROTEINS PRESENTING A DISTURBED SAM REGULATION

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The key regulatory point of transsulfuration pathway is catalysed by cystathionine beta-synthase (CBS), a homotetrameric enzyme activated by S-adenosyl methionine (SAM), which binds to CBS C-terminal domain. However, the activation mechanism remains unclear.

To study this mechanism we developed an expression system to produce recombinant CBS mutant proteins identified in a group of 18 CBS deficient patients presenting a disturbed SAM regulation, and localized away from the C-terminal domain, namely in the N-terminal domain (P49L) and in the catalytic core (G151R, G153R, K269del, I278T, R336H).

CBS wild-type cDNA was cloned into pET28b expression vector. Mutations were obtained by site-directed mutagenesis. Enzyme activity and SAM activation ratio were determined according to standard procedures. Expression levels and purity grades were evaluated by SDS-PAGE and oligomeric profile by native-gel electrophoresis.

Functional CBS wild-type protein was obtained in high yield and purity grade. From the studied CBS mutants only P49L presented a high residual activity (81% ofWT) but a lower SAM activation (20.3% of WT). The remaining mutants displayed lower residual activities (0.70–3.2% of WT) and SAM activation (1.9–32.8% of WT). The developed expression system is a valuable tool to characterize CBS mutants and elucidate SAM activation mechanism.

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P-113

REMETHYLATION OF HOMOCYSTEINE: INFLUENCE OF TCN2 AND MTR POLYMORPHISMS ON PLASMA HOMOCYSTEINE CONCENTRATIONS

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Homocysteine remethylation, catalysed by methionine synthase (encoded by MTR gene) needs cobalamin as cofactor; moreover, transcobalamin, encoded by TCN2 gene, is its main transporter into cells.

This study aimed at evaluating the prevalence of MTR-2756A>G, TCN2-776 C>G and TCN2-67A>G polymorphisms, and their association with plasma total homocysteine (tHcy) concentrations, in two healthy populations (9-year-old, n=188; 17-year-old, n=117) from Madeira Island, Portugal.

Concerning TCN2 polymorphisms, the whole population (n=305) displayed mutant allele frequencies of 9.1% (67 G) and 47.4% (776 G), while wild-type genotypes were present in 83.7% (67AA) and 28.4% (776CC). Regarding MTR-2756A>G polymorphism, frequency of mutant allele was 22.5% and the wild-type genotype was present in 59.1% of sample set (n=171).

Mean plasma tHcy concentrations increased with age (4.59±0.15 μM; 8.72±0.60 μM) and only differed significantly within gender, in adolescents. Additionally, tHcy did not differ significantly between TCN2 and MTR genotypes in both age-groups, but adolescents harbouring MTR-2756GG genotype showed a trend to higher tHcy values, which reached statistical significance with stratification according to gender.

In summary, our results showed a lower prevalence of TCN2-67 G allele, while the relative frequencies of TCN2-776 G and MTR-2756 G alleles are similar to other European populations. Finally, our results suggest that MTR-2756 G allele can significantly influence tHcy within gender.

P-114

DISTURBED CELLULAR METHYLATION CAPACITY LOWERS NITRIC OXIDE BIOAVAILABILITY IN HUMAN VASCULAR ENDOTHELIAL CELLS

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Inhibition of cellular methylation reactions by S-adenosyl homocysteine (AdoHcy), which accumulates in the setting of hyperhomocysteinemia, has been suggested to contribute to endothelial dysfunction in homocysteine (Hcy)-related vascular disease. We aimed at determining whether intracellular AdoHcy accumulation affects NO production by human endothelial cells.

Human vascular endothelial cells were incubated with adenosine-2,3-dialdehyde (ADA) (0, 5, 10 and 20 μmol/L), an inhibitor of AdoHcy hydrolase, for 12 to 24 hours. Extracellular Hcy and asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase (eNOS), were measured by HPLC. Intracellular AdoHcy was determined by LC-MS/MS. Nitrite was quantified by Griess reaction to evaluate NO production. Co-incubations with L-NG-nitroarginine (L-NNA) (1 mmol/L), an eNOS inhibitor, were also performed. eNOS transcriptional and translational levels were studied by qRT-PCR and Western blotting, respectively. eNOS function was evaluated by assessing the effectiveness of L-arginine to L-citrulline conversion in cell lysates.

ADA elicited intracellular accumulation AdoHcy, while extracellular Hcy, ADMA and nitrite levels decreased. Co-incubation with L-NNA suppressed this effect. For ADA at 20 μmol/L, eNOS expression and activity decreased by 40% and 20%, respectively. Intriguingly, transcription of NOS3 increased. Thus, ADMA-independent postranscriptional mechanisms modulated by cellular methylation capacity may lower NO production by vascular endothelial cells.

P-115

A CASE OF SEVERE METHYLENETETRAHYDROFOLATE REDUCTASE DEFICIENCY PRESENTING AS NEONATAL ENCEPHALOPATHY, SEIZURES, MICROCEPHALY AND CENTRAL HYPOVENTILATION

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Background: Methylenetetrahydrofolate reductase (MTHFR) deficiency is an autosomal recessive inborn error of folate metabolism. MTHFR is a key regulatory enzyme in folate-dependent remethylation of homocysteine. S-adenosylmethionine produced is a major methyl donor which catalyzes crucial reactions for brain development. The disorder is characterised by hyperhomocysteinaemia and hypomethioninaemia. A broad clinical spectrum includes psychomotor retardation, microcephaly, seizures, central respiratory failure and death. Premature vascular disease or psychiatric manifestations occur in the late onset forms.
Case Report: We report a female neonate with severe MTHFR deficiency presenting with epileptic encephalopathy, congenital microcephaly, central hypoventilation with gross cerebral atrophy, hypomyelination and pontine hypoplasia on brain MRI. She remained ventilator dependent and succumbed at 10 weeks of age despite treatment with oral S-adenosylmethionine, betaine, folinic acid, riboflavin and hydroxycobalamin. Her non-consanguineous parents had moderately elevated homocysteine levels.
Results: Homocysteinaemia (190 μmol/L), undetectable methionine in plasma and cerebrospinal fluid. MTHFR activity in cultured fibroblasts was virtually absent; 0.1 nmol/h.mg protein (normal 11.5–30.7). Homozygous pathogenic mutations c.1408 G>T; p.Glu470X were detected and were inherited from her parents.
Conclusion: Severe MTHFR deficiency may present with congenital microcephaly, possibly indicating antenatal insults from hypomethioninaemia and associated methylation defects. Carrier parents may have moderately elevated homocysteine levels warranting folate supplementation.

P-116

GENETIC POLYMORPHISMS OF MTHFR (677 T AND 1298 C) AND HOMOCYSTEINE METABOLISM AS MATERNAL RISK FACTOR FOR DOWN S SYNDROME PATIENTS IN NORTH INDIAN POPULATION.

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Background &Aim: Down syndrome (DS) is caused by the presence of three copies of chromosome 21, due to the failure of chromosomal segregation during maternal meiosis. The study was designed to investigate the MTHFR C677T and A1298C polymorphisms, with homocysteine as a maternal risk factor for DS.
Materials and Methods: Eighty mothers of individuals with confirmed full trisomy 21, and 100 control mothers of north Indian population were evaluated. Molecular analysis of MTHFR C677T and A1298C polymorphisms was performed by PCR-RFLP method. Biochemical correlation of total serum homocysteine was evaluated.
Results: The frequency of genotypes of MTHFR were 677CC (82.5%), 677CT (15%) and 677TT (2.5%) in the patients with DS, and among the 100 control group, genotypes 677CC (91.0%), 677 CT (6.0%) and 677TT (3.0%) were found. As for polymorphism 1298 AC, the patients with DS presented genotypes with frequencies 1298AA (15.0%), 1298 AC (52.5%) and 1298CC (32.5%) respectively and in the control group the frequencies of genotypes were 1298AA (61.0%), 1298 AC (22.0%) and 1298CC (17.0%) respectively. Homocysteine concentrations were significantly high in MTHFR 1298CC genotype in DS mothers than in control mothers.
Conclusion: MTHFR 1298CC genotype reduces the enzyme efficiency in the group of DS suggesting its involvement in chromosomal imbalance.

P-117

TWO PATIENTS WITH CBLG DEFICIENCY COMPLICATED WITH ATYPICAL HEMOLYTIC URAEMIC SYNDROME

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Mutations in the gene encoding methionine synthase result in methylcobalamin deficiency G (CblG) disorder leading to hyperhomocysteinemia and low methionine. The most frequent clinical findings reported are megaloblastic anemia and neurological symptoms.
Patient 1 was hypotonic at birth and anaemia was observed at one month. She was weak, had feeding difficulties and a gastrostomy was installed at 7 months. Delayed development led to diagnosis and treatment start at one year. Almost 6 years old she presented with kidney failure due to an atypical hemolytic uraemic syndrome (HUS) from which she recovered after 6 months. Now at age eleven she has short stature and attends a special school. Patient 2 presented neonatally with apnoeas, hypotonia, seizures and poor weight gain. After initiation of treatment he recovered but not to complete normality. At two years he also developed an atypical HUS and a tracheal stenosis was diagnosed. Now, 3 years old, kidney function has normalized but he still suffers from hypertension, needs both a gastrostomy and a tracheostomy tube and has a short stature. His cognitive function is almost normal.
In conclusion these patients raise questions about treatment strategies and the mechanism behind the development of atypical HUS in CblG deficiency.

P-118

COBALAMIN C DISORDER ASSOCIATED WITH PRENATAL INTRAVENTRICULAR HEMORRHAGE AND HYDROCEPHALUS: A CASE REPORT

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Inborn errors of cellular cobalamin (Cbl) metabolism are disorders of decreased production of adenosylcobalamin or methylcobalamin either both or alone, cofactors for methylmalonyl CoA-mutase and methionine synthase, respectively. cblC deficiency is due to a defect in Cbl(III) to Cbl(II) conversion resulting in accumulation of methylmalonic acid and homocysteine. Neonatal presentation is typical but milder forms may present later with neurological deterioration. Features uncommon in inborn errors of metabolism such as dysmorphisms, ocular abnormalities, and congenital malformations are seen in cblC. Hydrocephalus, a recognised complication has been previously noted postnatally only and not secondary to cerebral haemorrhage.

We describe a case with prenatal presentation of right sided ventriculomegaly and grade three intraventricular hemorrhage (IVH) diagnosed at 28 weeks gestation by ultrasound and MRI. Newborn screening showed elevated C3 acylcarnitine and confirmatory investigations led to the diagnosis of cblC disorder. A congenital bleeding disorder was excluded. At present his development is normal on hydroxycobalamin and betaine therapy despite marked right ventriculomegaly.

To our knowledge, this is the first description of prenatal IVH and hydrocephalus associated with the cblC disorder and only the second report of prenatal abnormality. cblC disorder remains one of the few inborn errors of metabolism associated with prenatal malformations.

A-007

NEONATAL THROMBOTIC MYCROANGIOPATHY (TMA) IN FOUR PATIENTS WITH CBLC DEFICIENCY

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ClbC is the most common inborn error of cobalamin metabolism. Neonatal TMA is extremely rare but if it occurs, CblC deficiency should be suspected. We report 4 cases admitted in our hospital over 5 months. Symptoms started early in life (19–30 days): feeding difficulties, failure to thrive and severe hypotonia, in 1 case left ventricular dilatation detected antenatally.

The clinical characteristics at onset were:

PLT (103/mmc) 142 76 120 32
Hb (g/dL) 6.5 7.3 7.8 9
LDH (U/l) 768 911 1101 818
Hapto (mg/dL) <20 <20 <20 <20
sCr (mg/dl) 0.7 0.4 0.6 0.3
uPr/uCr 2.0 10.3 3.8 na
uHb over ++ ++ over

The finding of hypomethioninemia, homocystinuria and methylmalonic aciduria led to the diagnosis of MMA with homocystinuria. Intravenous hydroxocobalamin, oral betaine and folic acid were started. Remission of TMA and kidney failure took place in 10 days. Currently the patients (3–7 months of age) are associated with developmental delay and nystagmus. Mutation analysis identified homozygosity for c.271-272dupA (p.Arg91LysfsX14) of the gene MMACHC in 2 patients.

Our "cluster" of TMA due to CblC deficiency, points out that this disease might be a lot more common than diagnosed. Whenever neonatal TMA is detected homocysteinemia should be determined

O-021

DIHYDROFOLATE REDUCTASE (DHFR) DEFICIENCY: A NOVEL INBORN ERROR OF METABOLISM RESULTING IN HAEMATOLOGICAL AND NEUROLOGICAL DEFECTS IN THREE CHILDREN FROM TWO FAMILIES

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We describe an infant with folinic acid responsive megaloblastic anaemia, cerebral folate deficiency and seizures. Additionally, he has severe developmental delay, cerebral tetrahydrobiopterin deficiency, cerebellar and cerebral atrophy. Because his serum folate and homocysteine levels were normal, we hypothesized that the defect was in the folate metabolism but did not affect tetrahydrofolate regeneration from 5-methyltetrahydrofolate.

Due to the history of consanguinity in his family, we performed autozygosity mapping that defined multiple regions of homozygosity, including a 3 Mb region encompassing the gene coding for dihydrofolate reductase. DHFR sequence analysis identified a homozygous c.238 C>T (p.Leu80Phe) mutation in our proband, his affected deceased sibling and in an unrelated child with similar clinical features. The mutation alters a highly conserved residue, was absent in 146 ethnically matched controls and results in significantly reduced enzyme levels and activity. Protein modelling suggested that the Phe residue could disrupt cofactor binding and/or destabilize the mutant protein.

The affected children had normal development until three months, indicating the possibility of better outcome with earlier diagnosis and treatment. Our findings suggest that the persisting developmental delay could be partially due to cerebral tetrahydrobiopterin deficiency, thus supporting further evaluation of the role of DHFR in pterin metabolism in the brain.

O-022

DIHYDROFOLATE REDUCTASE DEFICIENCY DUE TO A HOMOZYGOUS DHFR MUTATION LEADS TO CONGENITAL MEGALOBLASTIC ANEMIA AND CEREBRAL FOLATE DEFICIENCY

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The importance of intracellular folate homeostasis is illustrated by the severity of complications due to inborn errors of folate metabolism or folate deficiency.

We examined three children of distantly related parents presenting with megaloblastic anemia and neurological symptoms. Erythrocyte folate was decreased. 5-Methyl-tetrahydrofolate (5MTHF) in the cerebrospinal fluid (CSF) was very low in one and absent in two siblings. Treatment with folinic acid (5FTHF) increased erythrocyte folate, count and indices, and led to CSF 5MTHF normalization.

Homozygosity mapping identified a common region on chromosome 5 including the DHFR gene encoding dihydrofolate reductase (DHFR). Genomic sequencing of DHFR revealed a homozygous mutation c.458A>T (p.D153V).

The erythrocyte and plasma folate profile by LC-MS/MS was compatible with DHFR deficiency. DHFR activity and MTX binding capacity in EBV-immortalized lymphoblastoid cells were severely reduced in all patients as compared to controls. Heterozygous cells presented an intermediate DHFR activity and MTX binding capacity.

RT-PCR of DHFR mRNA revealed no significant differences between DHFR wildtype, heterozygous, or homozygous cells. In contrast, DHFR protein expression was reduced in mutated cells.

In conclusion, DHFR deficiency leads to a complex hematological and neurological disease, accompanied by cerebral folate deficiency, which can successfully be treated with 5FTHF.

O-023

DELINEATING THE MITOCHONDRIAL VITAMIN B12 PATHWAY THROUGH STRUCTURAL AND INTERACTION STUDIES

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Methylmalonyl-CoA mutase (MUT), an enzyme responsible for the catabolism of branch-chain amino acids, fatty acids and cholesterol, requires adenosylcobalamin (AdoCbl) to convert L-methylmalonyl-CoA into L-succinyl-CoA. In humans, MUT function depends on the accessory protein MMAA for proper AdoCbl utilization and the racemase enzyme MCEE for substrate provision. To better understand the role of all three proteins, we have solved their crystal structures in various ligand-bound and unbound forms. We show that MCEE adopts a conserved crotonase fold with a cobalt-bound active site but does not feature a narrow binding channel expected for CoA binding. Deleterious mutations found in MCEE-deficient patients are mapped to the surface of the protein, suggesting a potential role in protein-protein interaction. The structures of both MMAA and MUT reveal highly conserved monomeric subunits compared to their bacterial homologs, yet show substantially different dimeric assembly. We demonstrate that MMAA exhibits GTPase activity that is modulated by MUT and that the two proteins interact in vitro and in vivo, the physiological importance of which is highlighted by the MMAA patient mutation G188R, which retains basal GTPase activity but has abrogated interaction. Together our data provide a basis for understanding protein function and disease mutations in this important pathway.

O-024

CRUCIAL ROLE OF HOMOCYSTEINE MEASUREMENT FOR EARLY DIAGNOSIS OF VITAMIN B12 DEFICIENCY IN THE FIRST DAYS OF LIFE

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Background: Vitamin B12 deficiency causes decreased methionine synthase and L-methylmalonyl-CoA mutase activity resulting in accumulation of homocysteine, methylmalonic acid and propionylcarnitine.
Methods and Results: We report 65 asymptomatic newborns born in the state of Qatar in whom newborn screening detected slightly elevated homocysteine (mean: 18.4 μmol/l; range: 10.2 μmol/l to 38.0 μmol/l) at 2 days of age and were not found to be affected by classical homocystinuria. Twenty mothers were found to have vitamin B12 deficiency. Both infants and their mothers with documented vitamin B12 deficiency received hydroxylcobalamin 1 mg IM injection. This has led to complete normalization of both homocysteine and B12 levels.
Retrospectively the combinations of the ratios C3/C0, C3/C2 and C16:1OH/C2 in the detection of nutritional vitamin B12 deficiency in the Qatari population were tested with a reference data set (n=73994) analysed within the last 4.5 years.
Conclusions: Our finding suggests that elevated tHcy levels can in addition be indicative of nutritional vitamin B12 deficiency in addition to the diagnosis of inherited defects of homocysteine and B12 metabolism already in the first days of life.

P-119

THE EXTENDED CLINICAL SPECTRUM OF CEREBRAL FOLATE TRANSPORT DEFICIENCY

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Cerebral folate transport deficiency is an inherited brain-specific folate transport defect that is caused by mutations in the folate receptor 1 (FOLR1) gene coding for folate receptor alpha (FRalpha). We have recently described patients with pathogenic mutations in the FOLR1 gene that experienced the onset of a epileptic leukencephalopathy with frequent cerebral convulsions, severe ataxia, dystonic movements and profound developmental regression in the third year of life.
We have screened more than 50 DNA samples from paediatric patients with reduced methyl tetrahydrofolate (MTHF) concentration in the CSF. The pathogenic effect of four novel FOLR1 mutations was demonstrated by Western blotting and by reduced folic acid binding to FRalpha mutants. Mistargeting of mutant FRalpha to intracellular compartments was revealed by immunofluorescence microscopy.
Two patients presented with a more protracted course comprising developmental delay, autistic behavior and ataxia in early childhood but developed movement disorder and epileptic seizures at school age. The cranial MRI showed cerebellar and cerebral atrophy, hypomyelination and patchy white matter lesions.
We conclude that cerebral folate transport deficiency should be considered for any patient with developmental retardation and atactic movement disorder. Since patients benefit from folinic acid treatment the diagnosis should be made as early as possible.

P-120

FOLATE RECEPTOR AUTOANTIBODIES AND CEREBRAL FOLATE DEFICIENCY IN SCHIZOPHRENIA

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Background: Cerebral Folate Deficiency (CFD) defines any condition with lowered CSF folate and normal blood folate levels. Serum autoantibodies directed against Folate Receptor alpha (FR) at the choroid plexus block folate transport to the brain. CFD due to FR auto-immunity was recently identified in one case with catatonic schizophrenia.
Methods: Among eight schizophrenic patients (age range: 13–30 years) CSF was analyzed for N5-methyltetrahydrofolate, monoamines and pterins. Serum was investigated for blocking FR autoantibodies.
Results: FR autoantibodies were detected in 7 of these patients with fluctuations in antibody titer at various time points. Among 5 patients, CSF folate and serotonin metabolites were lowered. Folinic acid (0.5–1 mg/kg/day) administration to 4 patients with low CSF folate led to noticeable clinical improvement.
Conclusions: Brain folate is essential for more than 100 metabolic processes in the nervous system and influences neurotransmitter turnover. The presence of FR autoantibodies over time predisposes to episodes associated with CNS folate depletion. Our findings may explain the alternating negative and positive symptoms characteristic of schizophrenia and may be due to fluctuations in antibody titer observed. Evaluation for neuro-immunologic causes, including FR autoantibodies is recommended for patients with intractable schizophrenia, who can benefit from folinic acid supplementation.

P-121

PSYCHOMOTOR REGRESSION, MYOCLONIC EPILEPSY AND HYPOMYELINATION IN TWO SISTERS WITH A CEREBRAL FOLATE TRANSPORT DEFICIENCY SECONDARY TO A MUTATION IN THE FOLR1 GENE

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A previously healthy infant developed an ataxic gait in the 2nd year of life, followed by choreic movements. At the age of 3, brain MRI showed a diffuse abnormal signal of the white matter suggesting hypomyelination and a reduced peak of choline in spectroscopy. EEG, NCV and ophthalmologic exams were normal. During the next year, clinical evolution was characterized by severe psychomotor regression and myoclonic epilepsy. Repeated brain MRI showed the same picture. Extensive metabolic investigations remained normal. Her young sister developed the same clinical picture. At this time a puncture revealed a profound deficiency of 5MTHF in CSF (<1 nmol/ ; normal : 53–182), highly suggestive of defective folate-specific transport across the blood-CSF barrier. Molecular investigations confirmed the presence of two mutations c.332 G>T (p.Glu108X) and c.373 C>T (p.Arg125Cys) in the coding region of the FOLR1 gene. Both parents were heterozygous carriers. Treatment with folinic acid was started with some clinical improvement, but with the persistence of severe epilepsy. Folate receptor defect is a newly identified neurometabolic disorder. Very few patients are described but psychomotor regression with ataxia, myoclonic epilepsy and disturbances of myelination, are clinical hallmarks of the disease. This disorder is potentially treatable, but long-term outcome remains unknown.

P-122

FORMIMINOGLUTAMIC ACIDURIA DUE TO GLUTAMATE FORMIMINOTRANSFERASE-CYCLODEAMINASE DEFICIENCY CASE REPORT

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Glutamate formiminotransferase—cyclodeaminase deficiency (FIGLU-ria, FTCD deficiency—OMIM:229100) is an autosomal recessive disorder. It is a very rare inborn error of histidine catabolism. Very few cases have been reported until now. Clinical presentations may vary from mild development delay to severe mental retardation and seizures.
We report a 5 years old girl with FIGLU-ria. She is the first child to healthy non-consanguineous parents. The neonatal period was uneventful. Global development delay was diagnosed at the age of 3 years and worsened during the 5th year, accompanied by dyscoordination, attention deficits, hyperactivity, autistic features, severe delay of speech development and behavioral disorders. EEG, routine laboratory investigations were normal. Analysis of urinary organic acids revealed a huge peak of hydantoin-5-propionic acid. Serum folic acid, vitamin B12 and plasma homocysteine concentrations were within normal range, hematological investigations did not reveal megaloblastic changes. Increased concentrations of plasma and urinary formimino-L-glutamate were found—23 μmol/l [ref. < 10] and 427 μmol/mmol creat [ref.—0] respectively. Mutation analysis of the FTCD gene revealed the patient being hemizygous for c.447 C>T (R135C) with RT-PCR indicating that the other allele contained a deletion. Both mutations are located in transferase domain part of the FTCD gene.

P-123

LUMINAL EXPRESSION OF CUBILIN IS IMPAIRED IN IMERSLUND-GRDSBECK SYNDROME WITH COMPOUND AMN MUTATIONS IN INTRON 3 AND EXON 7

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Juvenile Megaloblastic Anaemia 1 (OMIM # 261100) is a rare autosomic disorder characterized by selective cobalamin (vitamin B12) malabsorption and inconstant proteinuria produced by mutations in either CUBN or AMN genes. Amnionless, the gene product of AMN, is a transmembrane protein that binds tightly to the N-terminal end of cubilin, the gene product of CUBN that binds to intrinsic factor-cobalamin complex, in the distal intestine and the proximal renal tubule. We report in two siblings a compound AMN heterozygosity with c.742 C>T, p.Gln248X and c.208-2A>G mutations, that led to premature termination codon in exon 7 and exon 6, respectively and produced a dramatic decrease of receptor activity in urines, despite absence of CUBN mutation and normal affinity of the receptor for intrinsic factor binding. Heterozygous carriers for c.742 T and c.208-2 G had no pathological signs. These results indicate that amnionless is indispensable for the proper luminal expression of cubilin, in humans.

P-124

PROSPECTIVE EVALUATION OF THE INFLUENCE OF GASTRIC INTRINSIC FACTOR (GIF) GENE VARIANTS WITH COBALAMIN DEFICIT

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Severe mutations in the gastric intrinsic factor (GIF) gene lead to congenital megaloblastic anemia. Little is known on the frequency and influence of GIF variants on the cobalamin (vitamin B12) absorption. We therefore aimed at identifying GIF variants in subjects with biological evidence of cobalamin deficit, in 2200 subjects prospectively recruited in our University hospital (North East of France) during 2 years. The selection criteria included decreased cobalamin, increased homocysteine and/or methylmalonic acid and/or macrocytosis. Among the 2200 subjects, 176 cases presented with hyperhomocysteinemia and/or increased methylmalonic acid, including 45 cases with combined criteria of cobalamin deficit. These 45 cases were compared to 70 controls. Five GIF variants were identified in 14 cases. One case was compound heterozygous for c.-48—1434 C>T and c.246 C>T, three were heterozygous for c.-48–1434 C>T, one for c.-48–672 T>C in the 5'upstream region, 2 for c.A68G in exon 1 and 6 for c.246 C>T in exon 2. A new mutation was found in exon 7 in one case. Allelic frequencies in controls were respectively of 0.09, 0.00, 0.06, 0.05 and 0.00. In conclusion, our study evidenced an influence of GIF variants in cobalamin deficit, including a new mutation. All were heterozygous, suggesting the influence of other determinants.

P-125

QUANTIFICATION OF VITAMIN B6 VITAMERS IN HUMAN CEREBROSPINAL FLUID BY ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY—TANDEM MASS SPECTROMETRY

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Background: Since defects in vitamin B6 metabolism are associated with human diseases, there is growing need for sensitive analysis of B6 vitamers in cerebrospinal fluid (CSF).
Methods: We developed a UPLC-MS/MS method using stable isotope labeled internal standards. B6 vitamers were separated with a buffer containing acetic acid, heptafluorobutyric acid and acetonitrile. Positive electrospray ionization was used to monitor transitions. B6 vitamer concentrations were established in CSF samples from 20 subjects (age 0.7 to 16.3 years) and in two patients on pyridoxine (PN) supplementation.
Results: Run time was 3.5 min, intra-assay variation ranged between 2.7% and 23.5%. We observed that a clear difference between B6 vitamer profiles in CSF and plasma exists. CSF concentrations were 14.8–42.5 (PL), 0.1–0.5 (PM), 0.09–4.1 (PA) and 8.8–42.0 (PLP) nmol/L. B6 vitamer concentrations were not affected by a rostrocaudal gradient but were sensitive to light and freezing-thawing. PMP was undetectable in all samples and PN was only detectable in patients on pyridoxine supplementation, who showed elevated CSF concentrations of all unphosphorylated B6 vitamers.
Conclusion: This innovative UPLC-MS/MS method for quantification of B6 vitamers in CSF will enable diagnostics and follow-up of disorders associated with disturbed vitamin B6 metabolism.

P-126

APPLICABILITY OF URINARY MEASUREMENT OF Δ1-PIPERIDINE-6-CARBOXYLATE, THE ALTER EGO OF α-AMINOADIPIC SEMIALDEHYDE, IN ANTIQUITIN DEFICIENCY

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α-AASA is in spontaneous equilibrium with its cyclic form Δ1-piperideine-6-carboxylate (P6C). Ongoing diagnostic screening revealed that for some individuals with milder ALDH7A1 variants, and patients co-treated with a lysine restricted diet, α-AASA was modest increased. This prompted us to investigate the diagnostic power and added value of urinary P6C compared to α-AASA.
Urine samples were diluted to a creatinine content of 0.1 mmol/L, followed by the addition of 0.01 nmol [2H9]pipecolic acid as internal standard,5 μL was injected onto a Waters C18 T3 HPLC column. Chromatography was performed using water/methanol 97/3 (v/v) including 0.03 % formic acid with a flow rate of 150 μL/min and detection was accomplished in the MRM mode: P6C: m/z 128.1>82.1; [2H9]pipecolic acid m/z 139.1>93.1 . The intra CV (n=10) for a urine sample of a proven PDS patient was 5.3% the inter CVs (n=5) for two urine samples from proven PDS patients were 8.4% and 13.4%. In all 40 urine samples from 35 individuals with proven PDS, we detected increased levels of P6C. The diagnostic power of the assessment of urinary P6C and α-AASA were comparable, with P6C being somewhat more discriminative then α-AASA in children<1 year.

P-127

VITAMIN B6 VITAMER CONCENTRATIONS IN CEREBROSPINAL FLUID OF (PRE-)TERM NEWBORNS SHOW A STRONG INVERSE CORRELATION WITH AGE

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Background: Since defects in vitamin B6 metabolism may be missed by currently used biomarkers, direct analysis of B6 vitamers in cerebrospinal fluid (CSF) is valuable. CSF concentrations of B6 vitamers have not been reported before (except for pyridoxal 5'-phosphate (PLP)).
Methods: B6 vitamer concentrations were determined in 66 unique CSF samples of 35 newborn infants (25 preterm and 10 term) by UPLC-MS/MS. Samples were bedside frozen and shed from light.
Results: Concentrations of pyridoxal (PL), pyridoxamine (PM), pyridoxic acid (PA) and PLP were strongly age-dependent with concentrations in preterm newborns (corrected age (c.a.) 30–37 weeks of gestation) being at least twice as high as in term newborns (c.a. ≥42 weeks). Pyridoxine (PN) and pyridoxamine 5'-phosphate concentrations were below limits of quantification. Interestingly, in CSF of two very preterm newborns (c.a. <30 weeks), significant amounts of PN were present while PLP concentrations were relatively low.
Conclusion: In CSF of newborn infants PL, PM, PA and PLP are present and inversely correlated with age. Results indicate that vitamin B6 metabolism may differ between preterm and term newborns. Age-dependent B6 vitamer reference values, taking gestational age into account, are indispensable not to overlook deficiencies in newborns.

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PYRIDOXAL 5-PHOSPHATE (PLP) AND TRYPTOPHAN DEFICIENCY ASSOCIATED WITH A SYNDROME OF EPILEPSY, ATAXIA AND ASPERGER SYNDROME WHICH IS RESPONSIVE TO PLP THERAPY

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We report a 13-year old female with Asperger syndrome, epilepsy, sleep disorder, episodic ataxia and tremor. Investigations showed a reduced CSF pyridoxal 5'-phosphate (PLP) (6 nmol/L, range 10–37 nmol/L) with normal CSF monoamine neurotransmitters and low-normal plasma PLP (22 nmol/L, range 15–73 nmol/L). She had reduced whole-blood serotonin (539 nmol/L, range 600–1600 nmol/L), low aromatic amino-acid decarboxylase activity (24.4pmol/min/ml, range 36–129pmol/min/ml) and isolated reduction in plasma tryptophan (14,17,27 μmol/L, range 30–87 μmol/L). Urinary alpha-AASA excretion was normal and sequencing of the pyridox(am)ine 5'-phosphate oxidase gene revealed no mutations.
She has shown good improvement on PLP therapy with cessation of seizures, tremor, ataxia and improvement in social functioning.
Tryptophan depletion is associated with autistic symptomatology and disturbance of circadian rhythm. PLP deficiency may result in seizures. Plasma tryptophan levels are regulated by the inducible enzyme tryptophan-2, 3-dioxygenase (TDO2) which diverts metabolism of tryptophan into the kynurenine pathway away from the methoxyindole pathway for serotonin and melatonin synthesis. Increased TDO2 activity (via secondary induction/gain-of- function mutation) may lead to the biochemical phenotype described. The mechanism of reduced PLP levels is difficult to explain but, if kynurenine levels are elevated, it may be due to the formation of a Schiff base between PLP and kynurenine.

P-129

B6-DEPENDENT SEIZURES: DONT NEED TO STOP PYRIDOXINE TO CONFIRM DIAGNOSIS

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Background: Pyridoxine-dependent epilepsy (PDE) is a rare autosomal recessive, treatable disorder. Diagnosis has classically relied on clinical response to pyridoxine in neonatal and childhood refractory seizures. Recently, biomarkers and molecular studies allow confirmation of some clinically suspected patients.
Objectives: To confirm the clinical diagnosis of four of our patients with neonatal PDE.
Patients and Methods: Medical records of four male patients (two siblings), aged from 5 to 22 years-old, born of nonconsanguineous' caucasian parents, were reviewed. All are on pyridoxine treatment and free of seizures. Three have normal neurodevelopment and the oldest of the siblings had the most severe manifestations and has mild psychomotor delay. Measurement of urinary alpha-aminoadipic semialdehyde (α-AASA) and sequence analysis of the antiquitin (ALDH7A1) gene were performed.
Results: All have elevated urinary α-AASA. Sequence analysis identified a large homozygous deletion in one patient and two heterozygous mutations in each of the others.
Conclusion: Pyridoxine should be tried in all cases of infantile and childhood refractory epilepsy. Urinary α-AASA is a reliable biomarker of PDE, even under pyridoxine treatment. Detection of mutations in the ALDH7A1 gene, encoding α-AASA dehydrogenase, establishes the diagnosis and allows for an adequate genetic counseling.

P-130

HYPOGLYCORRHACHIA IN BIOTINIDASE DEFICIENCY

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We present two children with early infantile seizures, hypoglycorrhachia and increased CSF lactate. The clinical picture and routine biochemical findings raised the possibility of GLUT1 deficiency (CSF:plasma glucose <0.4) or mitochondrial disease. However urine organic acid analysis was suggestive of biotinidase deficiency, subsequently confirmed by low plasma biotinidase activity and clinical response to biotin.
Both infants presented at 2 months with seizures. The CSF:plasma glucose ratios were 0.35 and 0.33 (reference range >0.60) and CSF lactates 5.1 and 6.4 mmol/L (reference range 1.1–2.2 mmol/L).
There is an extensive differential diagnosis for infants with early infantile seizures. In the absence of infection, increased CSF lactate concentrations may raise the suspicion of a mitochondrial disorder, while low CSF glucose may be attributed to GLUT1 deficiency. However a low CSF lactate is expected in GLUT1 and low CSF glucose is not a typical feature of mitochondrial disease. Although hypoglycorrhachia is not generally reported in biotinidase deficiency, we suggest this diagnosis should be borne in mind where it occurs with seizures and raised CSF lactate. We speculate that the pyruvate carboxylase deficiency that arises in biotinidase deficiency may be responsible for these biochemical findings, perhaps due to lack of oxaloacetate.

P-131

HIGH SINGLE MUTATION FREQUENCY FOR BIOTINIDASE DEFICIENCY IN RUSSIA

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Background: Biotinidase deficiency (BDD) is an autosomal recessive disorder of biotin metabolism. Early diagnosis and administration of biotin can prevent irreversible brain damage.
Objectives: We investigated the clinical and molecular features of this disorder in Russian patients.
Patients and Methods: During 5 years we have diagnosed 17 patients from 15 unrelated families . Age of clinical manifestation varied from 3–5,5 months, age of diagnosis from 3 month- 1,5 year. All patients received treatment with biotine (dose 10–30 mg/day) with good response. Brain MRI showed cortical atrophy with white matter hypomyelination in all patients and two patients had subdural hematoma. All patients improved rapidly if biotin therapy was initiated early. The abnormal findings of MRI markedly improved in 3 patients during 1 year. Patients with delayed diagnosis have variable degree of developmental delay, hearing and visual loss. Mutation screening revealed only 2 different mutations in our cohort.
Results and conclusion: Genetic testing in 15 families showed c.98–104del7ins3 as the most common mutation in Russian patients (83% of affected chromosomes); the second common mutation is R538C (17%). BDD is a treatable disorder with a high single mutation frequency in the Russian patients.

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FORGOTTEN NUTRITIONAL DISEASE MAY MIMIC METABOLIC DISORDERS: SCURVY

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Background: Vitamin C is the cofactor in biosynthesis of collagen, catecholamines and iron metabolism.
Objectives: The clinical features of scurvy may resemble metabolic disorders and hamper early diagnosis.
Case report: A three-year old male child was admitted to hospital with complaints of growth retardation, irritability, joint deformities, limited mobility, wheezing and frequent illness. Severe malnutrition, coarse facial appearance, gingival hypertrophy, organomegaly, significant osteoporosis, and anemia that did not respond to iron treatment were detected and differential diagnosis for metabolic disorders were considered.
Screening for inborn errors revealed generalized aminoaciduria and elevated blood free carnitine. Enzyme analyses excluded lysosomal storage disorders. Dietary history of the patient revealed very poor consumption of vitamin C which was about 13% of RDA and had continued for a long period. Vitamin C level was 0.05 mg/L (n: 4–21) in plasma, 2.85 mg/24 hour (n:10–30) in urine, and 1.03 mg/6 hours in urine after intravenous loading. The patient was given 200 mg/day vitamin C orally. Irritability and bone sensitivity decreased after the first two days and clinical improvement ensued.
Conclusion: Clinical features in scurvy resemble symptoms observed in some metabolic disorders. Careful clinical evaluation, dietary history and vitamin C assay may exclude uneccessary metabolic work-up.

P-133

LONG TERM TREATMENT WITH COPPER HISTIDINE IN MENKES DISEASE: REPORT OF TWO PATIENTS WITH DIFFERENT CLINICAL COURSE

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Background: Menkes disease (MD) is an X-linked disorder of copper transport caused by ATP7A gene mutations. Treatment with parenteral copper-histidine can change prognosis when initiated in early stages of neurological disease.
Case Reports: Patient 1 was diagnosed of MD (15 bp del.exon1) at the age of 2 months, showing hypotonia, joint hyperlaxity and femoral fracture. No seizures were reported. Parenteral copper-histidine was started at the age of 5 months and continued for 9 years. At 10 years of age attends normal school and has no seizures. Current MRI shows marked tortuosity of intracranial vessels without brain atrophy. Connective tissue abnormalities include joint hyperlaxity, bone deformities and asymptomatic urinary bladder diverticula. Mild renal tubulointerstitial damage is due to chronic copper supplementation. Patient 2 was diagnosed of MD (c1971t>A mutation) at the age of 3 months after admission for refractory epilepsy. Treatment started immediately but neurological status did not improve. He developed severe mental retardation and intractable epilepsy. He was under treatment until the age of 5 years when he died.
Discussion: Early treatment in Menkes disease is critical for the prevention of neurological damage although diagnosis is difficult in presymptomatic stage. Long term effects of treatment include bone deformities and tubulointerstitial damage.

P-134

EUROWILSON: A EUROPEAN NETWORK FOR WILSONS DISEASE

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Background: Wilson's disease (WD) is a rare inherited disease. Accumulation of copper is mainly observed in the liver and the brain. EuroWilsoN is a European network including 67 scientific country coordinators from 19 countries to improve quality health care.
Objective: Main Objectives are to improve knowledge of the disease, information and services to patients and health professionals, access to diagnosis and high-quality health care.
Methods: Meetings were organized with all members of the network, including patient representatives. Eurowilson members participated to the elaboration of European guidelines. We registered in a database all new patients seen in Europe since 2005.
Results: Collaboration of physicians and patient representatives allow us to share experiences to improve quality health care. Most of European laboratories participate to the European molecular genetics network to increase quality of genetics analysis. European guidelines are now accessible on website. Data from registry, confronted to European guidelines, realize a good picture of current European practice.
Discussion: Diagnosis of WD is still based on clinical, radiological and biological arguments. In order to improve the rapidity of the diagnosis and the quality health care, we need to have a coordination of all health professionals and patient representatives with access to multidisciplinary expertise.

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VARIABLE CLINICAL PICTURE OF WILSON DISEASE IN SIBLINGS—CASE REPORT

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Background: Wilson disease is an autosomal recessive disorder in which copper accumulates in tissues due to deficit of hepatic ATPase. Clinical picture is extremely variable. Hepatic manifestation is the most common feature in childhood. Neuropsychiatric disorder appears in adulthood.
Case Report: We present a case of a family with variable clinical picture in 2 siblings. 14 years old boy was sent with 3 years history of unexplained hepatopathy first revealed during acute gastritis. Detailed examinations were performed including ceruloplasmin, which was normal during first year.
Accidently 17 years old brother was present at a first visit. He presented with hypertonic-hypokinetic syndrome which developed during 3 months and was explained by psychiatric treatment for anxiety and depression with progressive cognitive deterioration and rigidity. Suspected Wilson disease in both was confirmed by patognomic laboratory findings (low ceruloplasmin, high urine copper excretion), MRI findings in older one and definitely by DNA analysis. Because of persistent hyperbilirubinemia and otherwise negative findings in their sister all the siblings were examined and Gilbert syndrome was confirmed.
Conclusion: Clinical and laboratory variability during course of the disease and even in the same genotype may cause confusion in diagnostics. Therefore repeated laboratory investigation is strongly recommended.

P-136

TEN CHINESE PAEDIATRIC PATIENTS WITH WILSONS DISEASE

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Background: Wilson's disease (WD, MIM #277900) is an autosomal recessive disorder leading to systemic copper accumulation and multi-organ damage. For pediatric patients, hepatic manifestations predominate. Untreated WD causes progressive liver and neurological deterioration. Early treatment is the most effective way of preventing these serious outcome.
Objective & Methods: We presented our experience of managing 10 paediatric WD patients. Data on clinical symptoms, laboratory findings, ultrasound findings, liver biopsies, genetic studies, treatment and outcome were studied.
Results: Our patients were between 2 to 18 years of age at diagnosis. 1 patient presented in liver failure. 7 had abnormal liver functions detected incidentally. 2 were diagnosed through sibling screening. The most consistent abnormal liver function was an elevated alanine transaminase ranging from 60 to 419 IU/l (<58). Ceruloplasmin levels were all <0.1 g/l (0.21–0.59). Mean urinary copper excretion was 4 μmol/day (<1.0). 1 patient was treated initially with penicillamine and another trientine. 7 patients were started on zinc therapy. All remained well on follow up.
Conclusion: Our patients represented a group of asymptomatic WD patients diagnosed and treated very early. With good treatment compliance, favourable outcome can be anticipated. One way of diagnosing these presymptomatic patients is through following up abnormal liver functions.

P-137

A ZINC SULPHATE-RESISTANT ACRODERMATITIS ENTEROPATHICA PATIENT WITH A NOVEL MUTATION IN SLC39A4 GENE

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Acrodermatitis enteropathica (AE) is a rare autosomal recessive disorder of zinc deficiency due to an abnormal intestinal zinc transporter. It is characterized by the triad of acral dermatitis, alopecia, and diarrhea. Once AE is correctly diagnosed, patients are treated with orally administered zinc sulphate. In some patients, relapses occur during adolescence, despite the regular treatment. Here we discuss the clinical and molecular features of a 13-year-old adolescent girl with acrodermatitis enteropathica who was resistant to high dose zinc sulphate therapy. We successfully treated the patient with zinc gluconate and vitamin C, and we detected a novel homozygous c.541_551dup (p.Leu186fsX38) mutation in the exon 3 of her SLC39A4 gene.

A-008

NEURO-PSYCHIATRIC MAINFESTATIONS OF CEREBRAL FOLATE DEFICIENCY ASSOCIATED WITH FOLATE RECEPTOR AUTOIMMUNITY

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Cerebral Folate Deficiency Syndrome(CFD) has been adopted for a group of neuro-psychiatric disorders associated with low spinal fluid 5 N-methyltetrahydrofolate (MTHF) concentrations in the presence of normal folate metabolism outside the CNS. One important mechanism underlying CFD is the presence of serum autoantibodies of the blocking type directed against the folate receptor (FR) attached to membranes at the plasma-side of choroid plexus epithelial cells, which normally mediates the MTHF transport to the CNS.

Two clinically recognizable syndromes of FR-antibody mediated CFD have been detected in young children: the infantile-onset CFD syndrome presenting 4 to 6 months after birth and a spastic-ataxic syndrome manifesting after one year. In addition, serum FR autoantibodies and CFD have also been reported in Rett, Aicardi-Goutières syndrome and infantile autism. During late adolescence CFD associated with fluctuating autoantibodies has been identified in intractable schizophrenia.

This clinical heterogeneity associated with FR-autoantibody mediated CFD might be explained by differences in the time of onset and period during which these FR autoantibodies are generated leading to folate deficiency during various critical stages of early brain development. More awareness of CFD in association with FR autoimmunity should lead to earlier detection and diagnosis of these potentially treatable neuro-psychiatric disorders.

O-025

LONG-TERM FOLLOW-UP AND TREATMENT IN NINE BOYS WITH X-LINKED CREATINE TRANSPORTER DEFECT

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The creatine transporter (CRTR) defect is a recently discovered cause of X-linked intellectual disability which is associated with cerebral creatine deficiency. Treatment with creatine monohydrate has not proved effective. Supplementation with creatine precursors L-arginine and glycine might increase endogenous cerebral creatine synthesis. The effect of this treatment is still controversial.

We followed nine boys aged between 8 months and 10 years with molecularly confirmed CRTR defect with repeated 1H-MRS and neuropsychological assessments during 4–6 years of combination treatment with creatine monohydrate, L-arginine and glycine. Treatment did not lead to a significant increase in cerebral creatine content as observed with H1-MRS. After an initial improvement of locomotor and personal-social IQ subscales, no lasting clinical improvement was recorded. Additionaly, we noticed an age-related decline of IQ subscales in boys affected with the CRTR defect.

O-026

ANATOMICAL PHENOTYPING IN MOUSE MODELS OF AGAT AND GAMT DEFICIENCY WITH MAGNETIC RESONANCE IMAGING

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Background: It is not known whether mouse models of creatine synthesis disorders AGAT and GAMT deficiency express any anatomical phenotype in the brain.

Methods: Applying high-resolution magnetic resonance imaging combined with advanced image analysis techniques and statistics, the study aimed to characterize anatomical changes between AGAT and GAMT mutant mouse and there corresponding heterozygote and wild type genotype.

Results: When analyzing absolute volumes of brain structures for the AGAT group, we found that 15 different brain structures had significant size differences among genotypes, including the corpus callosum, fimbria and internal capsule. These structures also had significant size differences when normalized for total brain size. Significant peaks (local areas of difference in relative size) were found for a number of areas in the brain, including the corpus callosum, hypothalamus and cerebral cortex. For the GAMT group, we found that the corpus callosum and internal capsule are significantly different in size among genotypes. These differences were found for both absolute and normalized volumes. Significant peaks were found in several regions of the brain, including the cerebellar cortex and hippocampus.

Conclusion: AGAT and GAMT knockouts express a similar neuro-anatomical phenotype that, interestingly and in contrast to patients, is more pronounced in AGAT deficiency.

O-027

A MOUSE MODEL OF CREATINE DEFICIENCY DUE TO TARGETED DISRUPTION OF ALANINE:GLYCINE AMIDINOTRANSFERASE EXHIBITS MUSCLE HYPOPLASIA, MITOCHONDRIAL DYSFUNCTION, AND WEAKNESS

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Phosphocreatine serves as an important reservoir of high-energy phosphate for ATP synthesis in tissues that have fluctuating demands for energy. Human disorders of creatine biosynthesis and transport exist, leading to intellectual disabilities, epilepsy, and poor growth. Creatine biosynthesis requires two enzymes, the first, L-arginine:glycine amidinotransferase, the product of the Gatm gene, catalyzes the rate-limiting transfer of the amidino group from arginine to glycine, yielding guanidinoacetate and ornithine. We generated a mouse strain lacking Gatm. Homozygous Gatm deficient mice have an almost complete absence of creatine and guanidinoacetate, and exhibit poor growth and muscle weakness. There is a reduced amount of glycogen in the liver in conjunction with reduced glycogen synthase activity, lipid accumulation in liver, skeletal and cardiac muscle, and a marked hypoplasia of skeletal muscle. A consequence of creatine deficiency is the global reduction in skeletal muscle mitochondrial respiratory chain activities, and a compensatory increase in mtDNA content and citrate synthase activity. Muscle mitochondria accumulate striking paracrystalline arrays of cristae. Cardiac function as assessed by stress echocardiography is normal. Treatment with creatine leads to muscle proliferation and improves strength. This animal model of creatine deficiency demonstrates the central role creatine metabolism plays in growth and muscle homeostasis.

P-138

CREATINE DEFICIENCY SYNDROME: CASE REPORT

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Creatine metabolism disorders result in severe neurologic disease characterized by developmental arrest, neurologic deterioration, movement disorders, mental retardation, autistic-like behavior, and epilepsy.

Objectives: report clinical, biochemical, imaging, and treatment data of two cousins (3y and 1y) with GAMT deficiency using stable isotope dilution gas chromatography-mass spectrometry.

Results: The older case had delayed milestones & at age 15 months showed global development delay with therapy-resistant myoclonic seizures. At this time we performed measurements of guanidinoacetate (GAA) and creatine (Cr) in urine, plasma, and cerebrospinal fluid (CSF) & creatine/creatinine ratio in urine and started treatment.

From age 3 he showed marked hyperactivity and impulsive behavior & moderate to severe learning. After treatment with creatine, arginine restriction and ornithine-supplemented diet, seizure severity and learning disorder were reduced but cognition did not improve.

The second case was investigated because of delayed milestones at age 5 months. Because of the positive family historyhe was controlled and showed a positive result. He is now 16 months old with normal development.

Conclusion: This report confirms that GAMT deficiency, a heterogeneous, treatable disorder, detected by increased levels of guanidinoacetate in body fluids should be considered in patients of any age with unexplained developmental delay and epilepsy /or autistic symptoms.

Conflict of Interest declared.

P-139

MOLECULAR SPECTRUM OF CREATINE DEFICIENCY

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Background: The creatine/creatine-phosphate system plays an essential role in keeping energy homeostasis in human tissues with high energy demand, such as brain and muscle. Deficiencies of creatine can cause developmental delay, intellectual disability and behavioral disorders. Three genes are involved in inborn errors of creatine deficiency syndromes: autosomal recessive GAMT and AGAT, as well as an X-linked SLC6A8 (CT1) encoding a creatine transporter. Deficiency of these enzymes can be distinguished by the concentrations of guanidinoacetate (GAA), creatine, and creatine/creatinine ratio in plasma and urine. However, biochemical analyses do not provide definitive diagnosis.

Method: Coding regions of AGAT, GAMT, and SLC6A8 (CT1) genes were sequenced on 25, 55, and 105 individuals, respectively.

Results: Mutations in 3, 10, and 13 patients, respectively, were identified. The majorities (>70%) were novel, null mutations with an overall mutation detection rate of 14.2%, suggesting that biochemical and clinical diagnosis requires molecular confirmation.

Conclusion: Molecular analysis should be pursued on patients with suspicion of creatine deficiency. Since these diseases are treatable, early diagnosis assures early treatment, proper genetic counseling and patient management. Furthermore, identification of mutations aids in carrier and prenatal diagnosis.

P-140

RESPONSE TO CREATINE SUPPLEMENTATION IN 2 BOYS WITH X-LINKED CREATINE TRANSPORTER DEFICIENCY WITH RESIDUAL CREATINE PEAK ON MR SPECTROSCOPY (MRS)

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Background: X-linked creatine transporter (SLC6A8) deficiency presents with developmental delay, seizures and behavioral disturbance in boys due to cerebral creatine deficiency. Oral creatine supplementation generally has not resulted in an improvement in neurological symptoms.

Case reports: The first boy presented at age 12 years with autism, developmental delay and seizures. A hemizygous c.1222_1224del TTC (p.408del) mutation was detected. The second boy presented at age 3 with mild global developmental delay with significant speech impairment. A novel hemizygous c.238 T>C (p.Y80H) variant was found in the SLC6A8 gene. MRS for both boys revealed a significantly decreased but not absent creatine peak. In view of the small residual creatine peak on MRS, treatment with oral creatine monohydrate supplementation was initiated.

Results: Both boys demonstrated an improvement in developmental abilities, particularly speech, on creatine supplementation. In addition, the first boy became seizure-free and had a resolution of abnormal involuntary movements. Despite a clinical response to creatine supplementation in both boys, the size of the MRS peak remained unchanged on serial MRS studies.

Conclusion: Boys with X-linked creatine transporter deficiency with a residual creatine peak on MRS may benefit from supplementation with oral creatine monohydrate.

P-141

DEVELOPMENT OF A MODEL OF CREATINE DEFICIENCY SYNDROMES BY RNAI TARGETING ARGININE:GLYCINE AMIDINOTRANSFERASE (AGAT)

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Creatine deficiency syndromes, due to defects in AGAT and GAMT (creatine synthesis pathway) or in SLC6A8 (creatine transporter) are inborn errors of metabolism essentially affecting the brain. Autosomal recessive AGAT deficiency is the least common of the three creatine deficiencies, having been described in only 3 families worldwide. In these patients, AGAT deficiency may be due to nonsense-mediated decay of mRNA due to stop codon mutations. Symptoms include speech delay, mental retardation, epileptic seizures, autism and brain atrophy.

To better understand the consequences of creatine deficiency in the brain, short-hairpin RNAs (shRNAs) were used to silence the expression of AGAT in the rat oligodendroglia-glioma hybrid cell line (ROC). Our results show an average of 88% decrease in AGAT enzyme expression in normal conditions. We show that in ROC cells, cell survival was not affected by AGAT silencing, both in normal conditions and under creatine deficiency (serum deprivation).

Adeno-Associated Viruses (AAV) transducing shRNAs targeting AGAT mRNA are currently produced to test the effect of creatine deficiency in 3D organotypic primary cultures of brain cells.

P-142

EFFECT OF CREATINE SUPPLEMENTATION ON CELLULAR METABOLIC STRESS IN CREATINE TRANSPORTER DEFICIENT (CRTR) FIBROBLASTS

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As creatine (Cr) plays an essential role for the maintenance of ATP levels in tissues with high demand, such as brain, creatine deficiency syndromes could result in energy depletion. This depletion appears as a plausible mechanism for causing overproduction of reactive oxygen species (ROS) that can lead to loss of the cell redox control resulting in cell aberrant proliferation even apoptosis.

We selected two CRTR fibroblast cell lines (genotypes: p.F360del and p. W154X) with low intracellular Cr levels, overproduction of ROS, increased apoptosis and aberrant proliferation. The aim of this study was to explore the effect of Cr supplementation (500 μM 24 h) on intracellular Cr levels, and stress parameters.

Results showed that intracellular Cr levels, measured by MS-MS, increased in both cell lines (7.9 and 4.6 fold) without reaching control levels. In addition, significant reduction of ROS levels, decrease of the apoptotic population (G0 phase) and progression of cells to S-G2/M stages were also detected by flow cytometry.

In conclusion, Cr at high concentrations is able to penetrate cells probably through passive transport, and may act normalizing cell cycle progression and ROS levels, outstanding the contribution of energy depletion toward metabolic stress at least in selected CRTR cells.

P-143

CLINICAL AND NEUROPSYCHOLOGICAL FOLLOW-UP OF AGAT-D PATIENTS AFTER TEN YEARS FROM THE DIAGNOSIS

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Background: Arginine:glycine amidinotransferase (OMIM 602360) deficiency (AGAT-d) is an autosomal recessive disease characterized by specific biochemical, brain spectroscopy and genetic findings. So far only six AGAT patients were described in the literature (Bianchi MC. et al.2000; Battini R. et al.2002; Edvardson S.2010) and supplementation of Cr has been shown to improve clinical symptoms in affected cases. Pre-symptomatic treatment has been reported in only one subject (Battini R. et al.2006).

Patients: We report the neuropsychological follow up of 4 AGAT-d cases, all members of the same family, two sisters (aged 20 and 18 yrs), one male cousin (15 yrs) and one presymptomatic child (7 yrs) during Cr supplementation. Three patients were firstly supplied with 400 mg/kg bw/day of Cr; in the following years we gradually decreased the dose up to 100 mg/kg bw/day. The first newborn affected by AGAT-d was early treated with 100 mg/ bw/day of Cr.

Results and Conclusions: We confirm the efficacy of Cr treatment in AGAT-d patients demonstrated by clinical improvement especially in adaptive behaviour and communicative skills. The different severity of mental retardation in the symptomatic patients (mild to moderate) seems to correlate with the age of the diagnosis. Early diagnosis prevents the disease.

P-144

CLINICAL IMPROVEMENT ON TREATMENT IN A PATIENT WITH GUANIDINOACETATE METHYLTRANSFERASE (GAMT) DEFICIENCY

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Guanidinoacetate methyltransferase (GAMT) deficiency is an inborn error of creatine biosynthesis. The enzyme catalyzes the conversion of guanidinoacetate to creatine. The clinical phenotype includes developmental delay/ intellectual disability, speech delay, seizures behavioral difficulties, and neurologic symptoms. Treatment with diet (arginine restriction/ ornithine supplementation) and creatine supplementation has been tried to decrease guanidinoacetate in the body. Treatment, both pre-symtpomatic and symptomatic, has shown significant improvement in clinical outcome. We evaluate a patient with GAMT deficiency. The patient is now 8.5 years old. He presented at 3.5 years with hypotonia, global developmental delay, and difficult to treat seizures. Testing revealed GAMT deficiency. After starting treatment, the patient no longer has clinical seizures, taking anti-epileptic medication and has an improved EEG. Developmentally, he has made great strides. He has progressed from being unable to engage independently in classroom routines and activities, to reading at an approximately kindergarten to early first grade level, writing his name, counting objects and doing single digit addition. He has progressed form needing a PECS system for communication at the age of two and a half years to now consistently using 4–6 word phrases and descriptive speech. Our patient demonstrates the significant clinical improvement with treatment of GAMT deficiency.

P-145

A NEW EXPERIMENTAL MODEL OF GAMT DEFICIENCY BY RNAI IN 3D PRIMARY BRAIN CELL CULTURES SHOWS IMPAIRMENT OF NERVE CELL DEVELOPMENT AND INCREASE OF APOPTOSIS

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Creatine deficiency syndromes (due to AGAT, GAMT or SLC6A8 deficiencies) are inborn errors of metabolism characterized by an absence or a severe decrease of Cr in central nervous system, which is the main tissue affected. To investigate the effects of creatine deficiency on developing CNS, we developed a new experimental model of GAMT deficiency by gene knock-down, using RNA interference in 3D organotypic rat brain cell cultures in aggregates.

A specific shRNA for the rat GAMT gene was transduced in brain cell aggregates by an adeno-associated virus (AAV2) under the control of the CMV promoter. The AAV2-transduced shRNA was able to efficiently knock down GAMT expression, as shown by the strong decrease of GAMT protein by western blotting.

We show that GAMT knock-down strongly affected axonal growth (neurons, immunohistochemistry for pNF-M) and astrocytes (immunohistochemistry for GFAP), as well as increased cell death and apoptotic pathways in developing brain cells (TUNEL experiments and immunohistochemistry for activated caspase 3).

Our results may contribute to understand some of the alterations of CNS development affecting GAMT-deficient patients.

P-146

EFFECT OF GUANIDINOACETATE ON ENERGY METABOLISM IN RAT STRIATUM:NEUROPROTECTOR ROLE OF CREATINE

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Guanidinoacetate methyltransferase (GAMT) deficiency is a neurometabolic disorder characterized by tissue accumulation of guanidinoacetate. Affected patients present with epilepsy and mental retardation whose etiopathogeny is unclear. Since reports have shown that guanidinoacetate alters brain energy metabolism and that creatine, which is depleted in patients with GAMT deficiency, can act as a neuroprotector, in the present study we investigated whether creatine could prevent the alterations of respiratory chain complex II, Na+,K± ATPase and creatine kinase caused by intrastriatal administration of guanidinoacetate in adult rats. Animals were pretreated during 7 days with daily intraperitonial administration of creatine. After, these animals were divided into two groups: Group 1 (sham), rats that suffered surgery and received saline; and group 2 (guanidinoacetate - treated) and they were sacrificed 30 min later. Results showed that the administration of creatine was able to reverse the altered activities of complex II, Na+,K±ATPase and creatine kinase. These findings indicate that the energy metabolism deficit caused by guanidinoacetate can be prevented by creatine that probably acts as an antioxidant. These data may contribute, at least in part, to a better understanding of the mechanisms related to the energy deficit and oxidative stress found in the GAMT deficiency. Supported by CNPq and FAPERGS.

P-147

DETERMINATION OF NEW REFERENCE VALUES FOR GAA AND CREATINE FROM A LARGE COHORT OF CONTROLS SUBJECTS AND DESCRIPTION OF THE FRENCH PATIENTS AFFECTED OF CREATINE DEFICIENCY DISORDERS (CDS)

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Background: Clinical symptoms of CDS are non specific and measurement of GAA and creatine is an important step for the diagnosis of these diseases.

Objectives: To determine sex and age matched reference values for plasma and urine GAA and creatine over a large cohort of control subjects and describe all known patients affected with CDS in France.

Patients and Methods: The controls cohort included 6417 subjects without CDS recruited in 6 French public hospitals during 28 months. Creatine and GAA were measured in plasma and urine and data were exploited by different statistical tools. Clinical, biological and genetic data from patients affected with CDS since 2003 were collected.

Results: Analyses of control data highlighted new age reference range for plasma and urine GAA and creatine and a sex distinction for urine values. 51 patients affected with CDS (14 with GAMT deficiency and 37 with CRTR deficiency) were described.

Conclusion: These new references values as a function of sex and age should improve the diagnosis of CDS, in particular to reduce the false positive rate. To conclude, we propose a diagnostic algorithm including biochemical investigations, gene studies and brain 1H-MRS, to improve the screening and the follow-up of the CDS patients.

P-148

REFERENCE LIMITS FOR U-CREATINE/CREATININE RATIO IS STRONGLY DEPENDENT UPON U-GUANIDINOACETATE/ CREATININE RATIO AND AGE

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Elevated urinary excretion of creatine (CRE) is an important marker of creatine transporter (SLC6A8) deficiency. In addition to the alimentary sources of creatine (e.g. meat) the endogenous production comes from methylation of guanidinoacetate (GUA). The level of a biological component may be dependent upon its nearest metabolic precursor or other closely related substance, as has been shown for many other analytes where two dimensional reference areas do not have a quadratic form.

Lg(U-creatinine) is fairly proportional to lg(age) in the interval 1–18 years of age, but deviates from that outside this interval. To investigate the functional relationship between the variables we performed a linear regression analysis from our laboratory production during the last 3 years, excluding 4 patients with documented SLC6A8 deficiency, one with muscular dystrophy and one with creatine supplementation. The model lg (U-CRE/creatinine)i=a+b.sexi+c.lg(age)i+d.lg(creatinine)i+e.lg(GUA)i+ fi where fi represents a residual, gave the following significant estimates: a**=1.11; c**=−0.548 ; d*=− 0.220 ; e**=1.009. (n=1255; **P<10–30; *P=7.10-8). Thus there is a one-to-one relationship between CRE and GUA, which requires the use of multidimensional reference ranges.

P-149

DETERMINATION OF URINARY CREATINE AND GUANIDINOACETATE BY HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY AND TANDEM MASS SPECTROMETRY

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Background: Creatine is an important intermediate in energy metabolism and its deficiency causes several neurological problems. Urine determination of creatine (Cr) and guanidinoacetate (GAA) is a cumbersome technique usually done by CG/MS with derivatization.

Objetive: To present a sensitive and robust method for routine measurement of urinary Cr and GAA, combining solid-phase extraction (SPE), hydrophilic interaction liquid chromatography (HILIC) separation, and detection by tandem mass spectrometry.

Methods: Sample preparation: addition of deuterated internal standards, phosphate buffer and water in 96-well plates. Extraction: in-house assembled 96-well SPE using a strong cation-exchange resin. Separation of analytes was obtained on a Luna Silic column. Detection was done by positive-mode electrospray ionization using selected reaction monitoring with a Waters Quattro Micro TMS. Clinical validation was performed with NCCLS approved guidelines.

Results and conclusion: Combination of cation exchange SPE with HILIC provided an efficient and specific method for simultaneous detection of Cr and GAA without derivatization, in less than 4 minutes. Achieved linearity was up to 10.4 mmol/L for Cr and 13.0 mmol/L for GAA. Total assay imprecision was bellow 16%. Limit of quantification were 5.9 μmol/L for Cr and 12.6 μmol/L for GAA. No ion suppression or enhancement was observed.

O-028

MYO-INOSITOL TREATMENT REDUCES SEIZURES AND IMPROVES CLINICAL OUTCOME IN A NEW PATIENT WITH THE ULTRARARE PHOSPHOLIPASE C BETA 1 DEFICIENCY

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The phospholipase C beta 1 (PLCbeta1) enzyme is essential for neurotransmitter-mediated signal transduction. Following receptor occupancy, PLCbeta1 catalyzes the production of myo-inositol 1,4,5-trisphosphate (IP3) and diacylglycerol from phosphatidylinositol 4,5-bisphosphate (PIP2). PLCbeta1 gene defects may disrupt CNS development and function. One male infant with early-onset epileptic encephalopathy and a homozygous loss-of-function 0.5 Mb deletion in the PLCbeta1 gene has been reported (Kurian et al, Brain 2010). We report a second male with malignant migrating epilepsy of infancy, and a homozygous deletion of the 5' non-coding region and exons 1–3 of PLCbeta1. Seizures began at 6 months, occurred 50-60/day, and were refractory to antiepileptic drugs and ketogenic diet. Baseline MRI showed diffuse parenchymal volume loss. Mega-dose of myo-inositol (Ins) was administered gradually in an attempt to raise Ins in neurons and PIP2 levels at receptor sites and elicit a recruitment of other PLC isoforms. After 10 days of 500 mg Ins/kg/day, electroencephalographic seizures decreased to 5/day, and meaningful movements and alertness increased. TE MRS revealed a 2–4-fold Ins increase in the left basal ganglia and parietooccipital white matter regions. After 10-weeks of Ins treatment, there has been a significant decrease in seizure activity that may be related to increased brain Ins levels.

O-029

AUTISM SPECTRUM DISORDER ASSOCIATED WITH LOW SEROTONIN IN CSF, FUNCTIONAL MUTATIONS IN SLC29A4 AND CO-OCCURRENCE OF MUTATIONS IN SEROTONIN RELATED GENES

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Using candidate gene approach and complete exome sequencing, we identified in two unrelated patients with autism spectrum disorders (ASD) and isolated low serotonin in brain—as reflected by the serotonin end-metabolite 5-hydroxindolacetic acid in CSF—a combination of heterozygous non-synonymous mutations in serotonin-related and/or autism-associated genes, including SLC29A4, SLC6A4, ITGB3, and others. Besides the previously reported codon-alterations from association studies, we newly identified in the brain monoamine transporter gene SLC29A4, encoding a putative serotonin-reuptake transporter protein PMAT, the alterations p.A138T or p.D326E with reduced activity. DNA sequencing of SLC29A4/PMAT in 125 individuals with ASD and 300 unaffected (control) subjects revealed the non-synonymous heterozygous alteration p.M24L or p.D29G in 7 additional subjects with ASD (and not in 300 control subjects). According to a hypothetical "genetic accumulation-model" where the affected subjects must differ from non-affected family members (parents and siblings) in the sum of all mutations, the two ASD patients showed additional alterations in mainly serotonin homeostasis, but also other candidate genes that have previously been linked to ASD and/or intellectual disability. Our findings link a combination of mutations in several serotonin-related genes to ASD and mental retardation concomitant to low serotonin in CNS.

O-030

LC-MS BASED ANALYSIS OF THE CSF METABOLOME FOR THE STUDY OF INBORN ERRORS OF METABOLISM

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Metabolomics refers to the comprehensive analysis of small organic molecules from biological fluids or tissues. The two main techniques used in metabolomics are (i) proton NMR spectroscopy (H-NMRS) and (ii) mass spectrometry (MS) coupled to liquid chromatography (LC) or gas chromatography.

We have developed a LC-MS-based analysis of the cerebrospinal fluid (CSF) metabolome and applied this approach to a cohort of 100 patients with various neurological disorders including patients with suspected neurometabolic disorders and patients with known metabolic disorders. Among these patients, 60 were previously studied by in vitro H-NMRS spectroscopy (Mochel et al., 2009).

From the 36 metabolites previously identified by H-RMNS only 6 could not be found with LC-MS, whereas the latter method allowed the detection of 106 additional characterized metabolites. All patients with abnormal metabolite profiles on H-RMNS were also highlighted with LC-MS but this technique allowed further identification of abnormal profiles in some patients considered normal with regards to H-NMRS.

In conclusion, LC-MS arises as a complementary tool to H-NMRS for metabolic profiling of the CSF. This technique appears promising for the identification of novel IEM involving the nervous system.

P-150

NEUROMETABOLIC DISEASES DIAGNOSED BY CEREBRAL SPINAL FLUID (CSF) ANALYSIS: POSITIVE YIELD AND RETROSPECTIVE EVALUATION FROM A TERTIARY CENTER

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Background: Neurometabolic diseases diagnosed by CSF examination are, GLUT1 deficiency, serine-deficiency syndromes, glycine encephalopaty, cerebral folate deficiency, disorders of monoamine metabolism and GABA metabolism.

Materials and Methods: We retrospectively analysed and compared demographic, clinical, laboratory and neuroimaging features of 62 patients in whom CSF examination were performed.

Results: Among 62 patients, 16 (25.8%) had a final diagnosis, including AADC deficiency (n=4), SSADH deficiency (n=4), dopa-responsive dystonia (n=3), serine biosynthesis defect (n=1), cerebral folate deficiency (n=1), glycine encephalopathy (n=2), and pyridoxal phosphate dependent seizures (n=1). There was consanguinity in all, except one. Positive yield of a diagnostic LP for the diagnosis of inherited neurotransmitter metabolism disorder was overall 25.8%. Oculogyric crisis (50%) and diurnal variation (81.8%) were the only statistically significant variables, in patients with and without a specific diagnosis.

Conclusions: It is challenging to diagnose neurotransmitter defects, since there is not an ideal set of clinical symptoms. Consanguinity, diurnal variation and abnormal ocular movements are the most significant findings associated with a diagnosis of a specific neurometabolic disorder by CSF examination in our cohort. Early diagnosis is of great importance not only for specific treatments, but also for genetic counseling and prenatal diagnosis.

P-151

HYPOGLYCAEMIA AS A COMPLICATION OF AROMATIC L-AMINO ACID DECARBOXYLASE DEFICIENCY

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Background: AADC-deficiency is a rare autosomal recessive inborn error of metabolism characterized by severe developmental delay, prominent motor abnormalities, oculogyric crises and autonomic features. Prognosis is poor and available treatment options only have marginal therapeutic effect.

Objective and hypotheses: We describe a five year old boy with AADC-deficiency. Severe, unpredictable, episodes of hypglycaemia were documented when he was switched from bromocryptine to pramipexol, a more potent dopamine agonist, in order to try to improve his motoric disabilities. Episodes of hypoglycaemia are documented in other patients with this disease. The pathogenesis of hypoglycaemia in these patients however is unknown. I hypothezise that a potent dopamine agonist in these patients can give rise to hypoglycaemia based on inhibition of growth hormone secretion through activation of dopanine D2 receptors and/or by the autonomic dysfunction in these patients with virtually no sympathetic activity left.

Methods: During episodes of hypoglycaemia, serum growth hormone, serum insuline and serum cortisol and urinary free cortisol and catecholamines were measured.

Results: No overt hormonal abnormalities were found. The episodes of hypoglycaemia disappeared when the patient was switched back to bromocryptine.

Conclusions: Dopamine agnonists can give rise to episodes of hypglycaemia in patients with AADC-deficiency.

P-152

AROMATIC L-AMINO ACID DECARBOXYLASE (AADC) DEFICIENCY: TREATMENT WITH LIORESAL

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Background: AADC deficiency is a rare autosomal recessive metabolic disorder, characterized by the lack of decarboxylation of the aromatic amino acids, L-dopa and 5-hydroxytryptophan, causing a dopamine, epinephrine, norepinephrine, and serotonine (metabolites) deficiency. Less than 100 cases have been diagnosed throughout the world.

Methods: We report the case of a 7-month-old white male who was diagnosed with Aromatic L-Amino Acid Decarboxylase (AADC) Deficiency. This diagnosis was based on CSF monamine neurotransmitter analysis and corroborated by enzymatic and genetic testing.

Results: The proband was breech with neonatal hypotonia and has not met any developmental milestones. He presented for evaluation paroxysmal events which had been occurring for three months. The events are characterized by repetitive dyskinetic chewing motions without loss of consciousness. In addition to these events, the patient also had intermittent dystonic opisthotonic posturing, diaphoresis, occulogyric crises and sleep difficulties. He was tried on multiple antiepileptic medications which exacerbated his symptoms. Treatment with COMT inhibitor and Bromocriptine were not tolerated. The dystonic movements and sleep difficulties improved greatly with Lioresal and pyridoxine. Other symptoms such as diaphoresis are not

Conclusion: Paroxysmal dyskinesia with stereotypic chewing and sleep difficulties due to AADC improved with Lioresal.

P-153

NOVEL MUTATIONS IN THE TYROSINE HYDROXYLASE GENE IN CZECH PATIENT WITH TYROSINE HYDROXYLASE DEFICIENCY

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The diagnostics of neurotransmitter disorders is almost exclusively based on the quantitative determination of the neurotransmitters or their metabolites in cerebrospinal fluid (CSF). The implementation of neurotransmitter analysis in clinical praxis is necessary for early diagnosis and treatment. Material and **Methods:** Neurotransmitter metabolites in CSF were analyzed in 82 children (at the age 1 months to 17 years) with clinical suspicion for neurometabolic disorders using HPLC with electrochemical detection. Results: The CSF level of homovanillic acid (HVA) was markedly decreased in three children (64, 79 and 94 nmol/l) in comparison to age related controls (lower limit 218–450). Neurological finding including severe psychomotor retardation, quadruspasticity and microcephaly accompanied with marked dystonia, excessive sweating in the first patient was compatible with the diagnosis of tyrosine hydroxylase (TH) deficiency (type B phenotype) and subsequent molecular analysis revealed two novel heterozygous mutations c.636A>C and c.1124 G>C in the TH gene. The treatment with L-DOPA/carbidopa resulted in the improvement of dystonia. MRI studies in two other patients with microcephaly revealed postischemic brain damage, therefore secondary HVA deficit was considered in these children.

Conclusion: Diagnostic work-up in patients with neurometabolic disorders should include analysis of neurotransmitter metabolites in CSF. Supported by IGA MZ NS 10561-3/2009 and MZ0VFN2005.

P-154

LONG TERM OUTCOME IN TYROSINE HYDROXYLASE DEFICIENCY- TYPE B: A FIFTEEN YEARS FOLLOW UP IN A MALE PATIENT

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Introduction: Tyrosine hydroxylase deficiency includes two clinical phenotypes: an infantile progressive hypokinetic-rigid syndrome with dystonia (Type A) and a neonatal complex encephalopathy (Type B).

Case Report We have revised video recording, that were obtained in a 15 years follow-up, of a male patient with a THD-type B. In the first months of life he had a truncal hypotonia, a severe hypokinesia and a reduced facial mimicry. Oculogyric crises and episodes of stupor were reported. THD was diagnosed when he was 18 months old according to CSF biogenic amine alterations and pathogenic mutation on TH gene. In the following years he suffered from generalized choreoathetosis and daily on-off phenomena. He walked unsupported at 11 years old while mental and language functions remained severely impaired.

Different dopamine-mimetic drugs (including L-dopa-carbidopa, pramipexole, selegiline, entacapone, tolcapone, pyridoxine and rotigotine) were used. At the beginning he was a slow responder to pharmacological treatments. After the school age his response improved, despite fluctuations of symptoms and drugs-induced side effects (i.e. severe dyskinesia under pramipexole).

Conclusions: Our experience demonstrates that the course of motor disorders in Type B variant requires a very slow increase of dopaminergic therapy. Mental retardation remains the main problems in these subjects.

P-155

STUDY OF SYNAPTIC PROTEINS IN PATIENTS WITH TYROSINE HYDROXYLASE DEFICIENCY

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Background: Tyrosine hydroxylase (TH) deficiency is an IEM of neurotransmission with variable clinical presentation and L-dopa response, in which mechanisms of synaptic communication have not been described.

Objectives: To study clue synaptic dopaminergic and gabaergic proteins in CSF of patients with TH deficiency and their possible relation with phenotype and L-dopa response.

Methods: The following proteins: DAT, D2-receptor, VMAT2 and GABAVT were studied in the CSF of 10 subjects with TH deficiency (A phenotype: 4 patients; B phenotype: 6 patients; diverse mutations) by Western blot analysis. In 3 patients, these studies were pre and post-treatment. Results were compared to a control population.

Results: D2R pre-treatment was higher in patients than in controls. No significant differences were found between pre-treatment A and B phenotypes. D2R was higher post-treatment (paradoxical response) in a B patient (L-dopa induced dyskinesias, poor outcome), whereas it rapidly decreased in two A patients with an excellent L-dopa response. Coordinated regulation of DAT and D2R, as well as VMAT and GABAVT after L-dopa doses increase was observed.

Conclusions: Post-treatment D2R appears to have a paradoxical behaviour in B phenotype. Post-treatment GABAVT increase suggests GABA-dopa co-release. Study of CSF synaptic proteins may help understanding pathophysiological mechanisms in these disorders.

P-156

ASSOCIATION OF CEREBROSPINAL FLUID HOMOVANILLIC ACID AND NEUROLOGICAL DISEASES

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Background: Homovanillic acid (HVA) is the main catabolic product of dopamine. Decreased cerebrospinal fluid (CSF) HVA values may be related to dopamine deficiency, but also in secondary HVA alterations. We have analysed biogenic amines and other biomarkers in CSF samples collected from neuropediatric patients. We aimed to study the association between CSF HVA and other biogenic amines and different neurological diseases.

Methods: Samples from 1,386 subjects were analysed by HPLC-electrochemical detection. Subjects were classified in three groups according to HVA values compared to age-related reference value: low, normal or high levels.

Results: 16% (n=225) presented low values of HVA. Among them we detected patients with different primary deficiencies: tyrosine hydroxylase (n=10), aromatic L-amino acid descaboxylase (n=2), guanosine triphosphate cyclohydrolase 1 (n=4), pyridox(am)ine-5'-phosphate oxidase (n=1) and sepiapterin reductase deficiencies (n=1). As secondary deficiencies, we found hypoxic ischemic encephalopathy and mitochondrial cytopathies, as disorders associated with low HVA values. Regarding high HVA values, this finding was consistently associated with mtDNA deletion syndromes (Kearns-Sayre)

Conclusions: Analysis of HVA and others metabolites biomarkers in CSF is useful for the differential diagnosis of primary neurotransmitters diseases. However, concentrations of HVA may be altered by several diseases of the central nervous system as a secondary event.

P-157

DECREASE OF NEOPTERIN AND BIOPTERIN IN PLASMA AND CEREBROSPINAL FLUIDS OF PATIENTS WITH DOPA-RESPONSIVE DYSTONIA

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Background: Dopa-responsive dystonia (DRD) is caused by partial defects of guanosine 5'-triphosphate cyclohydrolase I (GTPCH). In this study, we measured neopterin and biopterin level in DRD patients to confirm biochemical diagnosis.
Methods: Eleven Japanese patients from 8 families were diagnosed as DRD patients due to heterozygous mutations in GCH1 gene, which codes for GTPCH (DRD (+/−)). Eleven non-dystonic controls were also measured. Their neopterin and biopterin concentrations in plasma and CSF were measured by using HPLC apparatus.
Results: The DRD patients had two missense mutations (A190V, T106I) and two frameshift mutations (K107fs, M211fs), one nonsense mutation (K239X), and a deletion of exons 2 and 3. Student's t test was used to compare values. It indicated that plasma neopterin level of DRD (+/−) was lower than those of controls ($p=0.0002$). Plasma biopterin level of DRD (+/−) and controls showed no significant difference ($p=0.2866$). Significant differences between DRD (+/−) and controls were also shown in CSF neopterin level ($p=0.0014$) and CSF biopterin level ($p<0.0001$). Data were expressed as average±SD.
Conclusions: The decrease in plasma neopterin concentration was related to DRD with GTPCH deficiency. It may be a useful diagnostic classification of DRD.

P-158

MONOAMINE OXIDASE A DEFICIENCY REVISITED: UNEXPECTED FINDINGS IN A SECOND FAMILY

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Background: Monoamine oxidase A (MAOA) deficiency, a defect in biogenic amine neurotransmitter catabolism, was first described in 1993 in a large Dutch family with many borderline mentally retarded males with prominent aggressive behaviour disturbance. So far no new patients with MAOA deficiency were found.
Methods: SNP-array analysis for genetic diagnostics and HPLC analysis for urinary metabolite analyses.
Results: SNP-array analysis was performed on a young Swedish boy with ADHD-like behaviour, borderline mental retardation and gross obesity.
A 1 MB deletion on the X-chromosome was found, encompassing the first three exons of the MAOA gene and no other known genes.
In urine samples of the boy, his older brother with the same clinical phenotype and their mother neurotransmitter metabolites were analysed for determination of the metabolic phenotype.
As expected the neurotransmitter metabolite profile in the index patient was characteristically abnormal and confirmed the functional deficiency. The mother showed quantitatively intermediate abnormalities, confirming her carrier status.
Surprisingly however the patient's brother had a completely normal metabolite profile, strongly suggesting that he did not carry the MAOA deficiency.
Conclusions: These findings raise questions about the true clinical phenotype of the MAOA deficiency in this new family.

O-031

PROPIONIC ACIDEMIA: DIAGNOSTIC, CLINICAL AND THERAPEUTIC ASPECTS- NEONATAL VERSUS SELECTIVE METABOLIC SCREENING

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Background: In propionic acidemia (PA) little information is available on the potential benefit of newborn screening (NBS). Systematic studies comparing the outcome with that of patients diagnosed via selective metabolic screening (SMS, prompted by clinical findings, family history or results of routine laboratory tests) are sparse.
Study design: 20 living PA patients diagnosed through NBS and 35 identified via SMS were evaluated retrospectively at 16 metabolic centres in Germany, Austria and Switzerland.
Results: Patients diagnosed through NBS had neither a milder clinical course regarding the number of metabolic crises nor a better neurological outcome. Among NBS patients, 63% were already symptomatic at the time of diagnosis and less than 10% of all patients have remained asymptomatic. Among all PA patients, 76% were found to be at least mildly mentally retarded with an IQ<69. IQ was negatively correlated with the number of metabolic decompensations, but not with the patients' age. Physical development was also impaired in most patients. Additional data collection revealed that mortality was significantly lower in NBS patients compared to patients diagnosed by SMS.
Conclusion: Early diagnosis of PA by NBS is associated with lower mortality, but not with a better outcome in surviving patients.
Conflict of Interest declared.

O-032

AMINOGLYCOSIDE MEDIATED CORRECTION OF NONSENSE MUTATIONS IN PROPIONIC ACIDEMIA

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Aminoglycosides and other compounds can promote premature stop codon (PTC) readthrough constituting a potential therapy for patients with nonsense mutations. Using an in vitro transcription-translation system we have established the proof of principle that nonsense mutations in the PCCA and PCCB genes causing propionic acidemia can be partially suppressed by aminoglycosides, with different efficiencies depending on the sequence context. To correct the metabolic defect, the amino acid incorporated at the PTC (usually Gln or Trp), should support protein function and this has been evaluated for 5 PCCA and 7 PCCB nonsense mutations in silico and by in vitro expression analysis of the predicted missense changes. Most missense changes retain partial activity confirming the feasibility of the approach. In selected patients' fibroblasts cultured for 5 days with different amounts of G418 and gentamycin we observe a significant increase (4–5 fold) in propionylCoa carboxylase activity, reaching up to 7–9% of treated control cells. The ability to partially correct nonsense PCCA and PCCB alleles represents a potential treatment o supplemental therapy for a number of propionic acidemia patients encouraging further clinical trials with the readthrough drug Ataluren as for other organic acidemias.

O-033

GLUTATHIONE STATUS IS DEPLETED IN COBALAMIN C DEFECT: A POSSIBLE CONTRIBUTION TO DISEASE PROGRESSION DESPITE STANDARD PHARMACOLOGICAL TREATMENT?

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Background: Cobalamin C (Cbl-C) defect is the most common inborn error of cobalamin metabolism causing methylmalonic aciduria and homocystinuria. Despite pharmacological treatment with OH-cbl, betaine, folate, and carnitine, the long-term outcome is unsatisfactory with progression of neurological and visual impairment. The pathophysiological mechanism(s) causing brain and ocular damage still remains to be elucidated. Recently, the contribution of oxidative stress has been hypothesized based on in vitro studies showing in Cbl-C fibroblasts a significant ROS increase that can be corrected by adding OH-cbl to cell culture (Richard, Hum Mutat 2009).
Objective: Since reduced glutathione plays an important role in ROS detoxification, we assessed in vivo by reverse-phase HPLC the glutathione status in blood cells obtained from 13 treated Cbl-C patients.
Results: The results showed a relevant impairment of the glutathione homeostasis, as indicated by decrease of total- ($p<0.005$), reduced- ($p<0.0001$), and free- ($p<0.001$) glutathione in Cbl-C patients when compared to controls. Accordingly, the oxidized/reduced glutathione ratio was also significantly increased ($p<0.03$).
Conclusions: Our findings for the first time demonstrate in vivo a significant redox imbalance in Cbl-C defect that may contribute to the disease progression and indicating that antioxidant drugs should be used in combination with standard therapy.

P-160

SUBACUTE BILATERAL OPTIC NEUROPATHY WITH PARTIAL NEUROSENSORY HEARING LOSS IN AN ADULT FEMALE WITH MUT0 METHYLMALONIC ACIDEMIA

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Case report: Bilateral visual loss occurred within five days in a 23-year old women with methylmalonic acidemia (MMA). The disease was metabolically well controlled by strict diet and carnitine supplementation since early childhood and the visual loss was not associated with metabolic decompensation. Ophtalmologic exams showed the typical signs of optic neuropathy, and common etiologies of this pathology were ruled out. Moderate enhancement of both optic nerves was present on MRI. Treatment attempts with high dose corticosteroids, and coenzyme Q10 combined with vitamin E were ineffective. Subacute partial bilateral neurosensory hearing loss occurred three months later, further complicating patient care.
Discussion: Very few cases of late onset optic neuropathy and neurosensory hearing loss associated with MMA have been reported, and a morphological correlate in MRI scans has not been documented before. Impaired mitochondrial function is suspected to be the underlying cause of bilateral optic atrophy and neurosensorial hearing loss in MMA, although the pathophysiology is not well understood. Managing late-onset or long term complications of metabolic disease is a central issue in the care of adult patients with inborn errors of metabolism.

P-161

A CASE OF ACRODERMATITIS DYSMETABOLICA CAUSED BY METHYL MALONIC ACIDURIA

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Methyl malonic aciduria (MMA) is a disease caused by a deficiency in the enzyme, methyl malonyl CoA mutase, which is responsible for the metabolism of branched-chain amino acids. MMA is generally characterised by attacks of ketosis, acidosis, nausea, vomiting, dehydration, lethargy and increased concentrations of methyl malonic acid in blood and urine. Patients with MMA are initially treated with vitamin B12, with those who do not respond placed on diets containing limited amounts of protein and branched-chain amino acids. Patients with uncontrolled MMA or infections due to catabolic stress may have skin lesions, specifically generalized exfoliative eruptions, a condition called acrodermatitis dysmetabolitica. These cutaneous eruptions are characterized by a deficiency in isoleucine, one of the eseential amino acids. We encountered a patient on an amino acid restricted diet who experienced skin lesions caused by a lack of isoleucine (plasma concentration, 4.71 μmol/L). Within days of liberalizing his restricted diet and after supplementation with 100 mg/kg/day isoleucine, the eruption resolved completely.

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A NEW CASE OF MALONIC ACIDURIA: EARLY DIAGNOSIS AND TREATMENT.

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Background: Malonyl-CoA decarboxylase deficiency (MLYCD) is a rare autosomal recessive inborn error of metabolism caused by a dysfunction in malonyl-CoA decarboxylase (EC 4.1.1.9), encoded by a five exon gene on chromosome 16q24.3, presenting with a variable clinical phenotype characterized by developmental delay, seizure, ketoacidosis, hypoglicemia and cardiomyopathy.
Case Report: We report an Italian male detected by newborn screening and treated since the age of eight days (high carbohydrate, low long chain fatty acid and medium chain triglyceride supplemented diet with L-carnitine addition). The boy was born at term and presented normal heart functioning, except for a tricuspid Ebstein-like dysplasia, and neuro-developmental status.
Methods: Genomic sequencing of MLYCD gene revealed two point mutations (c.672 G>A, c.869 C>T), not listed in the Human MLYCD Allelic Variant Database, responsible for a deleterious effect on protein structure and function at computational analysis (MuPro, SIFT, ConSEQ v1.1). At the age of 2 years he only suffered a mild language and psychomotor delay, while heart functioning became normal. Brain MRI examination was normal.
Conclusions: Thirty-five cases, including our patient, have been described to date. The relevance of an early diagnosis and treatment in order to prevent metabolic crises, cardiac decompensation and poor clinical outcomes remain to be confirmed.

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THE EFFECT OF HAEMODIALYSIS ON METHYLMALONIC ACID LEVELS AT TIMES OF METABOLIC DECOMPENSATION

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We present two patients aged four years and fourteen years with methylmalonic acidaemia who were admitted to intensive care with severe metabolic decompensation. Both patients have end stage renal failure and are on the renal transplant list. The children's renal function had significantly deteriorated with the decompensation and both went on to receive haemodialysis while on intensive care. We present data to demonstrate the effect of dialysis on the MMA levels and correlate the levels with the progression of renal recovery. This data is of interest because it is assumed that MMA has a direct nephrotoxic effect, as patients with propionic acidaemia do not have renal disease as a complication while sharing other similarities with MMA. These cases raise the questions: (i) Does haemodialysis aid renal recovery at times of decompensation by off-loading the effect of MMA on the kidneys (ii) Should we utilise MMA levels more in the management of MMA patients both when well and decompensated? (iii) Are there other biochemical parameters that can be linked with MMA levels, e.g. acylcarnitine profiles?

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METHYLMALONIC ACIDAEMIA (MMA) RATING SCALE AS A POTENTIAL TOOL FOR THE PREDICTION OF INTELLECTUAL OUTCOMES

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Background: Medical and intellectual outcome in MMA patients is diverse. Reliable prediction of cognitive outcome in MMA would assist parental counselling and health care planning.
Objectives: To develop a rating scale for MMA associated complications.
Patients and Methods: An objective rating scale of the severity of metabolic disease and associated medical and neurological complications was developed and used to assess 21 MMA cases (11 months—15 years). A comprehensive neuropsychological assessment was completed with 17 patients (6 Vit B12 responsive). Spearman's Rank correlations and Mann-Whitney U Tests were completed, using SPSS version 16.0.
Results: Expressive language and attention deficits were commonly seen in Vit B12 and Vit B12 non-responsive MMA cases. Total rating scale score was negatively correlated to Full Scale IQ Score ($r=-0.732$, p 0.004). Severity of medical presentation at diagnosis did not differ according to vitamin B12 responsiveness, nor was it correlated to medical or intellectual outcomes.
Conclusion/Discussion: Higher MMA Rating Scale scores were significantly correlated to lower IQ scores. Younger age at diagnosis was also significantly correlated to poorer medical and intellectual outcomes. Severity of presentation at diagnosis was not indicative of prognosis. Neuropsychological impairment of vitamin B12 responsive MMA cases continues to emerge.

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GROWTH HORMONE (GH) DEFICIENCY IN A CHILD WITH METHYLMALONIC ACIDURIA: IS ARGININE IMPORTANT?

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Background: Short stature and low GH levels have previously been reported in patients with methylmalonic aciduria. Diagnosis of GH deficiency is usually based on GH response to a provocative stimulus like glucagon.
Case Report: We describe a boy with vitamin B12 non-responsive methylmalonic aciduria. He presented in the neonatal period with intrauterine growth retardation and metabolic acidosis and following diagnosis was treated with carnitine and a low protein diet. He had mildly delayed developmental milestones. His renal function was deceased but stable with a glomerular filtration rate of 60 ml/min/1.73 m2.
Despite satisfactory progress with infrequent metabolic decompensation, growth remained poor in the face of adequate nutrition.
Coeliac screen, thyroid function, prolactin were normal but plasma arginine and Insulin-like growth factor (IGF1) were low. His bone age was normal for his chronological age.
Glucagon stimulation test showed a sub-optimal response of GH in contrast to the arginine stimulation test which produced a good response with a sufficient peak of GH (21.4mcg/L). Treatment with arginine was initiated. Early follow up showed an improved height velocity and normalisation of his IGF1.
Conclusion: In patients on protein restricted diets with faltering growth arginine levels should be measured and supplementation considered if low.

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RENAL CLEARANCE OF CARNITINE SPECIES IN METHYLMALONIC ACIDURIA (MMA)

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Objectives: Correlation of acylcarnitines and methylmalonate in urine and blood.

Material/patients and methods: Methylmalonate and underivatised acylcarnitines were analysed in bloodspots and 31 urine specimens from 22 MMA patients by tandem mass spectrometry.

Results: Urinary methylmalonate ranged from 121 to 9300 and propionylcarnitine 37 to 971 micromol/mmol creatinine with positive correlation. Bloodspot propionylcarnitine correlated positively with urine propionylcarnitine and methylmalonate. Bloodspot methylmalonylcarnitine ranged from 0.17 to 1.23 micromol/L and correlated with both urine methylmalonylcarnitine ranging from 0.47 to 4.07 micromol/mmol creatinine and methylmalonate. Propionylcarnitine excretions ranged from 0.3% to 40.2 % of the sum of methylmalonate and methylcitrate excretion. Lower urine methylmalonate excretions showed higher percentage excretion. Methylmalonylcarnitine excretion accounted for 0.11% to 0.90% of the sum of the methylmalonate and methylcitrate excretion. Blood free carnitine ranged from 35 to 293 micromol/L. Despite carnitine supplementation, fractional renal clearance compared to creatinine clearance ranged from 0.02 to 0.21, demonstrating effective reabsorption. Fractional renal clearance compared to creatinine clearance for propionylcarnitine ranged from 0.16 to 1.38 and for methylmalonylcarnitine from 0.06 to 1.06, with positive correlation.

Conclusion: Propionylcarnitine and methylmalonylcarnitine are subjected to renal reabsorption and represent a small percentage of the methylmalonate and methylcitrate excretion particularly at higher methylmalonate excretion values.

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DIFFERENT PHENOTYPIC MANIFESTATIONS IN TWO SIBLINGS WITH METHYLMALONIC ACIDURIA TYPE B

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Methylmalonic aciduria cblB type is due to mutations in the MMAB gene which encodes the ATP:cobalamin adenosyltransferase enzyme. We describe two siblings with identical genotype and different biochemical phenotype one detected through expanded newborn screening and classified as cblB type by somatic cell complementation. He presented deficient propionate uptake rescued after cellular B12 treatment. He was compound heterozygous for two novel variant changes p.H183L (c.287 T>C) and p.R190dup (c.568-570dup). The subsequent familiar genetic analysis revealed that his clinically asymptomatic sibling, nine years old, presented also both variant changes. She exhibited only mild excretion of MMA and close to normal propionate uptake. Functional analysis revealed that p.H183L mutant protein had specific activity and affinity parameters similar to wild-type. It is noticeable that the efficiency of expression and purification was nearly 70-fold lower that wild-type ATR and also had a high reduced stability compared to wild-type indicating that it is likely a new destabilizing mutation in the MMAB gene. Mutant protein p.R190dup was highly unstable and the specific activity was undetectable. The structural and functional analyses suggest that both mutations are likely disease-causing, but there must be an additional genetic defect in MMAB or phenotypic modifiers genes to explain the different phenotype.

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COBALAMIN B DISORDER IN PREGNANCY

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Women with methylmalonic acidemia may be at risk of metabolic acidosis, hyperammonemia or renal failure in the postpartum period. We describe the course and outcome of a pregnancy in a patient with methylmalonic acidemia due to Cobalamin-B-deficiency and a chronic renal failure caused by the underlying disease.

Methylmalonic acidemia was diagnosed at 5 months of age; Cobalamin-B-deficiency was genetically confirmed at the age of 26 years. Pregnancy occurred at the age of 27 years. Her long-term treatment consisted of the protein restricted diet, supplementation of isoleucine-methionine-threonine-valine-free amino-acid mixture, L-Carnitine and intramuscular application of Hydroxycobalamin (10 mg/every other week). During pregnancy the treatment was continued and control visits at the centre intensified. Her renal function was stable and the urinary excretion of methylmalonic acid decreased from 1600 to 477 mmol/mol Creatinine in the third trimenon. At term a healthy male infant was born spontaneously. Postpartal the urinary excretion of methylmalonic acid increased markedly accompanied by a decline of the renal function, but improved partially in the following months.

This is the first report of pregnancy in a woman with Cobalamin-B-deficiency. A close observation is necessary to ensure an optimal care during and after pregnancy in patients with inborn metabolic diseases.

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SIMULTANEOUS DETECTION OF PLASMA METHYLMALONIC ACID, TOTAL HOMOCYSTEINE, METHIONINE AND 2-METHYLCITRIC ACID USING LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY (LC/MS/MS)

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Background: Elevations of Methylmalonic acid (MMA), Total homocysteine (tHcy), Methionine (Met) and 2-methylcitric acid (2MCA) can indicate disorders in related pathways, such as Methylmalonic acidemia, Homocystinuria, Hypermethioninemia, Propionic acidemia and Cobalamin deficiency and Cobalamin disorders.

Objectives: To develop a very simple, fast and sensitive Liquid Chromatography Mass Spectrometry (LC/MS/MS) method for simultaneous detection of plasma MMA, tHcy, Met and 2MCA.

Methods: 100uL of plasma or serum was used. 10uL was injected into the LC/MS/MS after a very simple liquid-liquid extraction. Instrument run time was 5 minutes. tHcy and Met were measured and quantified in positive ion mode. MMA and 2MCA were measured and quantified in negative ion mode. Chromatography is carried out using gradient elution through a Kinetex- C18 column.

Results: The assays are linear up to 100 uMol/L for MMA, up to 200 uMol/L for tHcy. Recovery was 107% for MMA, 106% for tHcy, respectively. Intraday imprecision (CV) was 2%, 6% and 7% respectively and interday imprecision (CV) was 3%, 11% and 9% for MMA, tHcy and Met, respectively. Reference intervals were established.

Conclusion/Discussion: fifteen patients in total with variable disorders were successfully diagnosed. This test is also useful as a second-tier or confirmatory test for positive newborn screening.

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AN UNDERIVATISED METHOD FOR THE RAPID QUANTIFICATION OF METHYLMALONIC ACID IN PLASMA BY UPLC-TANDEM MASS SPECTROMETRY

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Introduction: Methylmalonic acid (MMA) quantitation is useful for diagnosis and monitoring methylmalonicaciduria (MMAU) patients and assessing IEM patients on restricted diet at risk of B12 deficiency. We report an improved method without derivatisation using UPLC-MS/MS with significantly reduced sample preparation time, use of toxic reagents and instrument run time compared with the previous butylation method on conventional HPLC-MS/MS.

Method: Plasma ultrafiltrate is combined with deuterated internal standard and acidified with formate. MMA is separated from isomeric succinate by UPLC and detected by electrospray ionisation tandem mass spectrometry (Acquity TQD Waters Corp.). A standard curve was prepared by adding in known concentrations of MMA to plasma from a healthy subject.

Result: This method was verified by parallel runs of patients and ERNDIM samples with the previous method. Excellent agreement between the two methods was found: r2=0.99, y=0.9787x+0.0374 (n=109), paired t-test showed no significant difference (p=0.016). Between run precision is acceptable. The method is linear up to 200 μmol/L. Analysis of stored ERNDIM samples found results within 2 SD of consensus means across a wide range of values

Conclusion: This improved method has shown to be rapid and reliable for the quantification of methylmalonic acid in plasma.

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NOVEL APPROACH FOR THE DETERMINATION OF METHYLCITRATE IN DRIED BLOOD SPOTS (DBS) BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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Newborn screening for propionic (PA) and methylmalonic acidemias (MMA) based on measuring propionylcarnitine (C3) is neither sensitive nor specific. Due to a significant overlap in C3 concentration between affected and unaffected individuals, ratios such as C3/C2 were introduced to improve the newborn screening algorithms. However, the false positive rate of C3 disorders remains significant.

While methylcitrate (MCA) is a specific marker for C3 disorders, it is not detectable by the current MS/MS screening method. To overcome this, we developed a simple and specific LC-MS/MS method for MCA in DBS samples. The method is based on derivatization with DAABD-AE which was undertaken to improve the poor MS properties of MCA.

No separate extraction step was required and derivatization was achieved by incubating a 3.2 mm disc with DAABD-AE at 60:C for 45 min. MCA peak eluted at 2 min and the injection to injection time was 10 min. The method was successfully applied for the analysis of DBS samples from established PA and MMA patients (n=10) as well as controls (n=310).

We anticipate that the implementation of this method will improve the screening process for PA and MMA by reducing the false positive rate and increasing the positive predictive value.

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A STABLE ISOTOPE DILUTION GC/MS METHOD FOR THE NANOMOLAR RANGE DETERMINATION OF METHYLMALONIC ACID IN BIOLOGICAL FLUIDS

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Methylmalonic acid (MMA) has been suggested as a potential predictive biomarker for vitamin B12 deficiency. Moreover, this parameter may be crucial in the characterization of different subsets of Methylmalonic Acidurias (MMAUs). To answer this matter, we developed a sensitive and accurate stable-isotope-dilution GC/MS assay for measurement of MMA in the nanomolar range.

A two-step sample treatment was performed, consisting of solvent extraction by ethyl acetate and derivatization with N-methyl-N-(tert-butyldimethylsylil)-tri-fluroacetamide (MTBSTFA). The selected ions for MMA and methyl-2H3-MMA quantification were m/z 289 and m/z 292 respectively.

Linearity was observed in the concentration range of 0.1 to 1.6 μM in plasma with r2=0.999. Variabilities were: 0.4±0.02 μM (CV: 5.7%) and 1.63±0.12 μM (CV: 7.4%) in the intraday assays (n=6); 0.39±0.03 μM (CV: 7.2%) and 1.56±0.13 μM (CV: 8.2%) in the interday assays (n=6). The mean overall coefficient of MMA recovery from plasma spiked with 0.1, 0.2, 0.4, 0.8 and 1.6 μM was 101.4%. The LOD and LOQ were 12.5 and 50 nM respectively.

The above results demonstrate the sensitivity and accuracy of the method with unequivocal advantages in the analysis of plasma, providing crucial information to the differential diagnosis and follow-up of MMAUs.

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CONVERGING EVIDENCE OF MITOCHONDRIAL DYSFUNCTION IN CELL MODELS OF METHYLMALONIC ACIDURIAS

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Methylmalonic acidurias (MMA) constitute an important group of inherited metabolic disorders involving neurological deficits. Recent studies have shown an increased reactive oxygen species (ROS) production and apoptotic cell rate in fibroblasts especially from cblB patients with isolated MMA and cblC with combined MMA and homocystinuria. To gain insight into the pathophysiology of these disorders, the goal of this study was to examine mitochondrial dysfunction as a putative disease mechanism. An altered mitochondrial morphology of a grain-like structure was presented mainly in those fibroblasts with an increased ROS content (cblB and cblC), suggesting that ROS might lead to fission and a grain-like structure of the mitochondrial reticulum. Decreased oxygen consumption rate (OCR) was found in patients′ fibroblasts. When cells were respiring in glucose free-medium supplemented with galactose, basal and maximal OCR increased in patients and control fibroblasts. Permeabilized fibroblasts from cblB patients compared to patients from other groups and controls, revealed that in vitro mitochondria display an enhanced Ca2+ uptake velocity and premature opening of the permeability transition pore which has been described to provoke the release of mitochondrial proapoptotic factors. These studies using cell models establish that mitochondrial dysfunction is directly or indirectly involved in these cobalamin metabolism defects.

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DNA DAMAGE INDUCED BY PROPIONIC AND L-METHYLMALONIC ACIDS IN HUMAN PERIPHERAL LEUKOCYTES IN VITRO IS PREVENTED BY L-CARNITINE.

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Background: Patients with propionic (PAemia) and methylmalonic (MMAemia) acidemias present severe crises of metabolic decompensation in the neonatal period, in which the levels of propionic (PA) and L-methylmalonic (MMA) acids, respectively, can be as high as 2.5–5 mM. Treatment is constituted of a low-protein diet supplemented with L-carnitine. Recently, some works have suggested that lipid and protein oxidative damage may be involved in the pathophysiology of these diseases, but DNA damage has not been fully investigated.
Objectives: In this work, we aimed to investigate the in vitro effect of L-carnitine on DNA damage induced in vitro by PA and MMA.
Material and Methods: The alkaline comet assay was used to evaluate the DNA damage index induced in human leukocytes after incubation for 6 hours at 37:C with PA (2–5 mM) and MMA (0.5–5 mM) in the presence or absence of L-carnitine (30–150 μM).
Results: Our results showed that both PA and MMA induced a DNA damage index significantly higher than the control group. In vitro treatment with L-carnitine reduced PA- and MMA-induced DNA damage in a concentration-dependent manner.
Conclusions: Our present in vitro findings indicate that PA and MMA induce DNA damage and L-carnitine is able to prevent this effect.

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ISOLATION AND CHARACTERIZATION OF PROXIMAL TUBULE EPITHELIAL CELLS FROM URINE OF METHYLMALONIC ACIDURIA PATIENTS

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Background: Chronic tubulointerstitial nephritis is a frequent finding in patients with isolated methylmalonic aciduria. Among them, patients with mut0 and CblB disease have the highest risk of developing kidney disease. The underlying renal pathology has yet not been elucidated. Therefore, we aimed to establish an in vitro model for the human disease.
Methods: We prepared cells from urine of healthy donors and patients with mut0, mut- , CblA, and CblB disease and purified proximal tubule epithelial cells (hPTEC). For long-term cultivation we used a method to immortalize these primary cells with pRSVneo vector containing SV40 (pRNS1) using electroporation. Afterwards, hPTEC were characterized regarding morphology and marker protein expression.
Results: By RT-PCR we showed expression of organic anion transporters 1 and 3, P-glycoprotein, ATP-binding cassette transporters 4 and 6. Expression of proximal tubule marker aquaporin 1 was demonstrated by western blotting. In light microscopy, hPTEC displayed characteristic cobblestone shapes. Using immunostaining, we demonstrated expression of the tight junction protein ZO-1. We found a maximum increase in methylmalonic acid and propionyl-carnitine concentrations in hPTEC of mut0 patients, and less pronounced elevations in mut- patients.
Conclusions: In summary, we present a new in vitro model to study the renal pathology of methylmalonic aciduria.

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GENETIC AND OXIDATIVE STRESS CELLULAR STUDIES IN PROPIONIC ACIDEMIA

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We have investigated the cellular oxidative stress and apoptosis processes in a cohort of propionic acidemia (PA) patients harbouring different missense, frameshift mutations and large deletions. The novel missense changes were functionally expressed in a eukaryotic system to determine whether misfolding variants are involved in the cellular stress response and to examine the genotype-phenotype correlations. Eight patients were PCCA deficient and two PCCB deficient. PCCA mutations p.E148G, p. R288G and p.K298R had <4% residual PCC activity while PCCB mutations p.G188R and p.G255S were mild, with 27% and 64% PCC activity, respectively. Late onset patients carried mutations with partial residual activity, while neonatal patients were carriers of severe/null mutations. Most of the patients´ fibroblasts presented a significant increase of intracellular reactive oxygen species (ROS) content, and also a high rate of apoptosis, especially patient cells homozygous for p.E148G. P38 and JNK stress-kinases were found activated. The effect of several antioxidants, such as Tiron and Trolox, was tested in patients' fibroblasts revealing a significant decrease of ROS content (40–50%), indicating this could represent a therapeutical approach to prevent/alleviate the cellular damage in this disease.

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CYTOTOXIC NOT VASOGENIC EDEMA IS THE CAUSE FOR STROKE-LIKE EPISODES IN PROPIONIC ACIDEMIA

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Introduction: Stroke like episodes are a feature of inherited metabolic disorders, including propionic acidemia (PA) (1). So far, it was unclear, whether these episodes are caused by cytotoxic or vasogenic edema (2).
Patient: At one week, PA was diagnosed in parallel through appearing symptoms and positive newborn screening. The boy is treated with a protein restricted diet, l-carnitine, ß-blockers and diuretics. At 4 years he had a prolonged gastroenteritis. Two weeks later he showed noticeable behavioural changes with regression, especially in speech, confusion and abnormal reaction to people and objects well known to him. EEG showed bilateral diffuse slowing of background activity with right-sided accentuation, and with sharp wave foci bilaterally over the temporo-occipital regions. Evaluation of T2-weighted images, T2 relaxation maps, diffusion-weighted imaging (ADC maps) and T2-shine-through indicated the presence of cytotoxic edema in putamen and the cerebellum. After general anesthesia for imaging, EEG changes resolved. He recovered within some days.
Conclusion: This case illustrates that pathogenesis in propionic acidemia includes cytotoxic damage to the CNS, probably caused both by trapping of organic acids (3) and lack of anaplerotic substances for the Krebs cycle (4).
Reference: (1) Scholl-Bürgi, 2009, (2) Broomfield, 2010, (3) Sauer, 2010 (4), Brunengraber, 2006

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ACUTE RESPIRATORY DISTRESS SYNDROME IN TWO PATIENTS WITH ORGANIC ACIDEMIA INVOLVING PROPIONATE METABOLISM

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Acute respiratory distress syndrome (ARDS) is an acute condition characterized by bilateral pulmonary infiltrates and severe hypoxemia in the absence of evidence for cardiogenic pulmonary edema. Major risk factors associated with the development of ARDS include bacteremia, sepsis, trauma, fractures, burns, massive transfusion, pneumonia, aspiration, drug overdose, near drowning, postperfusion injury after cardiopulmonary bypass, pancreatitis, and fat embolism. Organic acidemias have not been previously reported as a risk factor for ARDS.

We present herein two patients with organic acidemia who were followed up with ARDS. One of them was mut0 methylmalonic acidemia (MMA) and the other was propionic acidemia (PA). They were admitted with vomiting and subsequently developed neutropenia, trombocytopenia and lactic acidosis. In within several days on emergency care, they exhibited hypoxia and respiratory insufficiency, the chest X-ray were suggestive of ARDS. Despite intensive care and ventilatory support, the patient with MMA died, the other survived.

The patients with organic acidemia involving propionate metabolism might have tendenceny to ARDS due to the possible role of toxic metabolites in the ARDS pathogenesis.

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HEMOPHAGOCYTOSIS IN THREE PATIENTS WITH ORGANIC ACIDEMIA INVOLVING PROPIONATE METABOLISM

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Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening condition due to hyperinflammation caused by aberrant proliferation of activated lymphocytes and macrophages secreting high levels of cytokines. Hemophagocytic lymphohistiocytosis (HLH) may develop secondary to infections, malignancies, immune deficiency syndromes, rheumatologic and metabolic disorders. Associations between hemophagocytosis and inborn errors of metabolism including lysinuric protein intolerance, multiple sulfatase deficieny, galactosemia, Gaucher Disease, Pearson Syndrome, galactosialidosis have previously been reported in the literature. We report three cases with disorders of propionate metabolism one with methylmalonic acidemia and two with propionic acidemia developing secondary hemophagocytosis during the course of their metabolic disease. This is the first report of such an association.

In the patients, no infectious agent was isolated from the cultures of various specimens. All the patients presented with metabolic acidosis and ketosis, but the fact that whether HLH was triggered by the toxic metabolites or vice versa is unclear. Whether the molecular basis of the diseases in the presented three cases serve as a determining factor is not known. Considering all these issues the mechanism playing a role in the development of hemophagocytosis in our three patients with organic acidemia is not clear yet.

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CARDIOMYOPATHY AS THE INITIAL PRESENTATION OF PROPIONIC ACIDAEMIA IN A TEENAGER

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Background: Propionic acidaemia due to propionyl-CoA carboxylase deficiency causes recurrent attacks of ketosis usually precipitated by excessive protein intake, constipation and intercurrent infection. The majority of patients present in the neonatal period with the remainder presenting in early childhood. Long term complications include cardiomyopathy.

Case: A 15 year old patient presented collapsed following a 3 day history of gastroenteritis. She had mild metabolic acidosis and severe heart failure. She was put on the transplant list with presumed diagnosis of viral myocarditis. Urine organic acids unexpectedly showed mildly elevated 3-hydroxypropionate, methylcitrate, and 3-hydroxybutyrate. Subsequently, plasma acylcarnitines showed moderately elevated propionylcarnitine. Treatment with IV carnitine, protein restriction and high calorie diet lead to marked improvement and she was taken off the transplant list. Further history revealed that she had avoided meat in her diet all her life. She had also consumed 18 beers just prior to her presentation.

Conclusion: This is one of only three reported cases of patients presenting with cardiomyopathy as the initial presentation with no previous history of acidosis. Alcohol was the precipitating factor for her presentation. This case emphasized the importance of a thorough metabolic work-up for any patients with cardiomyopathy.

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PROPIONIC ACIDAEMIA ASSOSIATED WITH VISUAL HALLUCINATION

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Background: Propionic acidaemia (PA), an autosomal recessive disorder, resulting from deficiency of propionyl-CoA carboxylase.

Brain abnormalities has been reported in patients with PA.

Methods: Out of 67 patients with PA, two were identified with a history of VHs.Both underwent biochemical, and neurological evaluation. Electroencephalogram (EEG), brain MRI and F-fluorodeoxyglucose positron emission tomography (FDG PET) scanning studies were obtained.

Results: Patient 1, a 12-year-old boy with PA. At 10 years he had VHs. Investigations revealed ketonuria, and hyperglcinemia. EEG was normal. Brain MRI revealed diffuse atrophy. Brain FDG PET was nonspecific.

Patient 2, a 7-years-old boy, had PA. At 6 years, he developed VHs.Labs revealed metabolic acidosis, hyperammonemia, and ketonuria.

VHs reoccurred,with seizure. Brain MRI revealed diffuse signal hyperintensity of the basal ganglia. EEG with focal epileptic spikes. Brain FDG PET was normal.

Conclusions: This is the first report of VHs in PA patients. Our patients have mild cognitive impairment. The VH was self-limiting in all patients. In patient 2 VH recurred once, with seizure, abnormal EEG, and brain MRI.

Acute and chronic brain abnormalities are well-documented in PA. Acute or chronic focal cerebral metabolic or toxic insult as part of the PA might etiologically explain the VHs in our patients.

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ANALYSIS OF 3-HYDROXYGLUTARIC ACID IN DRIED URINE SPOTS BY LC-MS/MS; APPLICATION FOR THE DIAGNOSIS OF GLUTARIC ACIDURIA TYPE 1

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Background: Accumulation of 3-hydroxyglutaric acid (3HGA) in body fluids is pathognomonic for type 1 Glutaric Aciduria (GA1). To date, methods for quantification of 3HGA mainly involve complex analyses using stable isotope dilution gas chromatography mass spectrometry (GC-MS). Here, we describe a simple liquid chromatography tandem mass spectrometry (LC-MS/MS) method to quantify 3HGA in dried urine spots (DUS) using DAABD-AE, a benzoxadiazole-type reagent designed for improving chromatographic and mass spectrometric properties of carboxylic acids.

Material and Methods: No extraction was required and derivatization was performed directly on a 3.2-mm disc of DUS. Sample pretreatment involved heating the reaction mixture at 60°C for 45 min and 5 μl portion was analyzed by LC-MS/MS.

Results: 3HGA measured in control samples (n=100) ranged between 0–7.1 mmol/mol creatinine. DUS samples from established low- and high-excretor GA1 patients revealed satisfactory results compared to those obtained previously by GC-MS (n=14).

Conclusion: This approach may be a useful primary screening method for low- or high-excretor variants in populations with high risk of GA1. Furthermore, for babies with elevated dried blood spot C5DC, follow-up testing of DUS GA and 3HGA will be a useful diagnostic test that may reduce the number of cases requiring enzymatic and molecular analyses.

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LYSINE ADMINISTRATION PROMOTES OXIDATIVE STRESS IN BRAIN OF GLUTARYL-COA DEHYDROGENASE-DEFICIENT (GCDH−/−) MICE

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Background: The pathogenesis of the brain damage in glutaric acidemia type I (GA I) is not well understood. Objectives: Relevant oxidative stress parameters were investigated in cerebral cortex, striatum, hippocampus, liver and heart of 30-day-old knockout mice with glutaryl-CoA dehydrogenase deficiency (Gcdh −/−) and in wild-type animals (Gcdh +/+).

Methods: Tissues were dissected, homogenized and assayed biochemically. Some Gcdh −/− and Gcdh +/+ mice received 8 μmol/g L-lysine i.p. 24 hours before the assays were performed.

Results: Significant changes were observed only in the brain of Gcdh −/− mice that had received lysine, which is converted to glutaric and 3-hydroxyglutaric acids. Cerebral cortex in these animals showed significantly higher thiobarbituric reactive acid substances (TBA-RS) and superoxide dismutase (SOD) activity and a diminution of reduced glutathione (GSH). In the striatum, TBA-RS, SOD and glutathione reductase were increased, while GSH levels and glutathione peroxidase activities were decreased. SOD activity was increased in the hippocampus. No changes were observed in liver and heart.

Conclusions: Brain, and especially striatum, in Gcdh −/− mice is more vulnerable to oxidative stress following lysine administration than liver and heart. Increased vulnerability to oxidative stress may contribute to the pathogenesis of brain damage in human GA I.

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THE CLINICAL, BIOCHEMICAL AND MOLECULAR FEATURES OF THREE IRANIAN PATIENTS WITH GLUTARYL—COA DEHYDROGENASE DEFICIENCY

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Background: GA1 is an autosomal recessive disease caused by the deficiency of mitochondrial glutaryl COA dehydrogenase. Patients usually have symptoms of hypotonia, seizure, loss of motor skills and progressive macrocephaly. Diagnosis is ascertained by the elevated C5DC, glutaric acid and hydroxy glutaric acid.
Case Report: We report three Iranian patients with GA1. All of them were from first cousin Iranian parents; had prolonged physiological Icter and macrocephaly. All three had highly elevated C5DC, glutaric acid and 3hydroxy glutaric acid. Two sibling were found to be homozygous for the GCDH gene mutation in the exon10 position390 (P.Gly390Ala). Surprisingly, in spite of similar mutation the 4 years old girl showed normal development without treatment whereas the development of her 15 months brother was severely impaired. The third patient, a 3 years old girl, was found to be homozygous for the GCDH gene mutation in the exon6 position 179 (C179Leu>Pro).
Conclusion: If not diagnosed early and treated preventively before recurrent encephalopathic crisis occur, GA type1 is usually associated with a severe irreversible neurologic syndrome. This report indicates examination of urinary organic acid and acyl carnitine profile in children with symptoms of developmental delay and macrocephaly.

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GLUTARIC ACIDURIA TYPE II (GA II) PRESENTING AS A LEUKODYSTROPHY

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Background: GA II is a disorder of fatty and amino acids metabolism due to defects of the Electron Transfer Flavoprotein (ETF) or ETF-CoQ oxidoreductase (ETF-QO). The disease is widely variable in its symptoms, severity, age of onset and rate of progression. Brain MRI typically shows T2-weighted hyperintensity of basal ganglia.
Objectives: To present a case in which leukodystrophy was a distinct and prominent imaging finding.
Case Report: The index case presented at 22 months of age for investigation of developmental delay, anemia, hypothyroidism and slightly dysmorphic features. MRI disclosed supratentorial leukoencephalopathy, sparing intense capsule, basal ganglia and thalami. Brainstem was also affected with signal intensity in T2 –weighted images. In the differential diagnosis mitochondrial and "vanishing white matter" disease were considered. On subsequent follow-up the patient manifested an episode of acute encephalopathy with hypoketotic hypoglycemia, metabolic acidosis and hyperammonemia.
Urinary organic acids and acylcarnitine profile were strongly abnormal and suggestive of GAII.
Conclusion/Discussion: The unusual phenotype of this patient, characterized by progressive encephalopathy, along with the MRI findings were misleading and suggesting a leukodystrophic process rather than an organic aciduria.
The presence of leukodystrophy should always prompt the investigation for an organic aciduria, even in the absence of acute decompensation.

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REDUCED RISK OF WHITE MATTER INJURY ON CEREBRAL MRI AND NEUROPATHOLOGY IN GLUTARIC ACIDEMIA TYPE I PATIENTS HOMOZYGOUS FOR THE GLUTARYL-COA DEHYDROGENASE IVS-1+5 G>T MUTATION

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Glutaric acidemia type I (GA-1) is an autosomal recessive disease caused by glutaryl-CoA dehydrogenase (GCDH) deficiency. Irreversible striatal damage occurs during acute encephalopathic crises in infancy causing severe movement disorder and other neurologic deficits. Magnetic resonance imaging (MRI) and neuropathology frequently demonstrate striatal injury and concomitant diffuse subcortical white matter abnormalities. We report thirteen patients homozygous for the IVS-1+5 G>T glutaryl-CoA dehydrogenase mutation: ten who underwent a total of 13 brain MRIs at various stages of their disease and three who underwent brain autopsy. MRI of six patients had striatal damage but only two demonstrated subcortical white matter abnormalities which are limited to the frontal lobes. Neuropathology of all three patients demonstrated neuronal loss in the striatum but only one patient had white matter vacuolation. In contrast, we report one patient with GA-1 who is a compound heterozygote for the IVS-1+5 G>T and 1209delG mutations. Her brain MRI and neuropathology demonstrated striatal damage and diffuse subcortical white matter abnormalities. The GCDH IVS-1+5 G>T mutation, whether as a homozygous or heterozygous genotype, may confer a severe clinical phenotype. However, the white matter changes seem to be less common in individuals homozygous for the IVS-1+5 G>T mutation.

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LYSINE AND GLUTARIC ACID AFFECTS CULTURED ASTROCYTES FROM THE KNOCKOUT MOUSE MODEL OF GLUTARIC ACIDEMIA TYPE I

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Background: The role of glial cells in glutaric acidemia I (GA-I) pathogenesis is mostly unknown.
Objectives: We evaluated the effects of pathophysiological concentrations of glutaric acid (GA) and lysine on glial cells from the glutaryl-CoA dehydrogenase (GCDH) knockout (KO) mouse model.
Methods: Functional and morphological parameters were analyzed in cortical and striatal cultures of astrocytes and oligodendrocytes from newborn C57 GCDH KO and wild type mice submitted to 5 and 10 mM GA or 10 mM lysine during 24 hours.
Results: Lysine caused mitochondrial dysfunction, as verified by decreased mitochondrial potential in KO cultured astrocytes, measured by ratiometric fluorescence of the JC1 probe and MTT activity. Cellular glutathione levels decreased 25%, whereas carboxy-H2DFFDA signals augmented 35%. Astrocytic proliferation was increased by 30% related to untreated cells. GA causes similar effects without significant differences among the concentrations employed. No changes in glutamate transporter GLT1 and glutamine synthase expressions were found in KO astrocytes even upon GA or lysine exposure. In addition, 80% less of oligodendrocyte progenitors were obtained from the KO cultures.
Conclusions: Mitochondrial dysfunction together with increased oxidative stress and exacerbated astrocytic proliferation in GCDH KO mice could actively contribute to the neuronal loss and myelin defects in GA-I.

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CO-EXPRESSION OF GCDH AND OAT1 IN NEURONS AND PROXIMAL TUBULE CELLS

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Tissue-specific expression studies of Glutaryl-CoA dehydrogenase (Gcdh) in adult rats revealed expression in the whole rat brain, almost exclusively in neurons, and surprisingly high expression in the juxtamedullar cortex of the kidney. The organic anion transporter 1 (OAT1) mediates basolateral uptake of glutarate derivatives from proximal tubule cells and contributes to their renal clearance. In brain, OAT1 is expressed at the choroid plexus, in neurons of cortex and hippocampus. We hypothesized that Gcdh and Oat1 are co-expressed in the same cells in kidney and brain and analyzed their mRNA expression by in situ hybridization on cryosections of adult rat brain, kidney and liver. In brain, Gcdh and Oat1 were found co-expressed in most neurons. Only the Purkinje neurons of the cerebellum were found to be Oat1 negative. In the kidney Gcdh and Oat1 are widely co-expressed with a specific high expression in proximal tubule cells. In conclusion there seems to be a functional coupling of Gcdh and Oat1 on a renal and neuronal level. Further studies are ongoing to confirm these findings in human tissues.

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SUCCINATE-SUPPORTED OXYGEN CONSUMPTION IS COMPROMISED BY ETHYLMALONIC ACID PROBABLY BY INTERFERING WITH INTRA MITOCHONDRIAL SUCCINATE TRANSPORT

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Background: Ethylmalonic acid (EMA) accumulates in short chain acyl-CoA dehydrogenase (SCAD) deficiency and ethylmalonic encephalopathy, disorders in which the pathophysiological mechanisms of brain damage are poorly unknown.
Objectives: It was investigated the in vitro effects of EMA on respiratory parameters measured by oxygen consumption supported by succinate or glutamate/malate in mitochondria from rat brain.
Material and methods: Mitochondrial fractions were prepared from brain of 30-day-old Wistar rats and incubated in a medium containing succinate or glutamate/malate. Oxygen consumption was then measured under various conditions.
Results: EMA increased state 4 (25%), decreased state 3 respiration (45%) and respiratory control ratio (RCR; 55%) with succinate- but not with glutamate/malate-supported oxygen consumption. EMA-induced decrease of state 3 in succinate-supported respiring mitochondria was significantly minimized by nonselective permeabilization of mitochondrial membranes induced by alamethicin. Malonic acid (MA) also diminished state 3 (40%) and RCR (40%), but did not affect state 4 respiration with succinate as substrate. However, mitochondrial permeabilization did not affect the MA-induced state 3 inhibition. Furthermore, the respiratory chain activities were not altered by EMA under these experimental conditions.
Conclusion/Discussion: It is concluded that EMA inhibits succinate uptake into brain mitochondria.
Financial support: CNPq, PROPESq/UFRGS, FAPERGS, PRONEX, FINEP IBN-Net and INCT-EN.

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INDUCTION OF OXIDATIVE STRESS BY 2-METHYLBUTYRYLGLYCINE IN RAT BRAIN

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Background: Short/branched chain acyl-CoA dehydrogenase deficiency (SBCADD) is a neurometabolic disorder biochemically characterized by tissue accumulation and high urinary excretion of 2-methylbutyric acid (MB) and 2-methylbutyrylglycine (MBG). Although affected patients present neurologic symptoms, the pathophysiology of the brain damage in SBCADD is not yet established.
Objectives: We studied the in vitro effects of MB and MBG on important parameters of oxidative stress in cerebral cortex of young rats.
Material and methods: Brain from 30-day-old Wistar rats was dissected, the cerebral cortex dissected, homogenized and utilized in the biochemical determinations.
Results: MBG, but not MB, increased thiobarbituric acid-reactive species (TBA-RS) (lipid oxidation). MBG also induced sulfhydryl oxidation and decreased glutathione (GSH) levels, reflecting a reduction of antioxidant defenses. In contrast, MB and MBG did not alter carbonyl formation. We also found that MBG-induced increase of TBA-RS levels and decrease of GSH were prevented by free radical scavengers, implying that reactive species were involved in these effects.
Conclusions: We therefore presume that lipid and protein oxidative damage induced by MBG may be involved, at least in part, in the pathophysiology of the neurological dysfunction found in SBCADD-affected patients.
Financial support: CNPq, FAPERGS, PRONEX and the FINEP research grant Rede IBN-Net and INCT-EN.

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METHOD FOR THE QUANTIFICATION OF URINARY (S)-2-(CARBOXYPROPYL)-CYSTEAMINE IN PATIENTS WITH 3-HYDROXYISOBUTYRYL-COA HYDROLASE DEFICIENCY USING LIQUID CHROMATOGRAPHY WITH TANDEM MASS SPECTROMETRY.

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Mutations in the HIBCH gene lead to 3-hydroxyisobutyryl-CoA hydrolase deficiency, an inborn error of valine catabolism that leads to the build up of a highly reactive intermediate, methacrylyl-CoA. Methacrylyl-CoA undergoes addition reactions with thiol compounds leading to the formation of S-2-carboxypropyl-cysteine (S2CP-cysteine) and S-2-carboxypropyl-cysteamine (S2CP-cysteamine).
We have developed a method to quantitate S2CP-cysteamine in urine using liquid chromatography with tandem mass spectrometry in positive ion mode using a synthesised deuterated analogue, d5- S2CP-cysteamine, as an internal standard.
Chromatographic separation was achieved using a Discovery HS F5 column (5 cm x 2.1 mm, 5 μm) with a mobile phase consisting of 4 mM ammonium acetate, 4 mM heptafluorobutyric acid buffer (pH 3.25) and acetonitrile.
For S2CP-cysteamine and d5-s2CP-cysteamine parent ions of mass 164 and 169 were detected respectively. Quantitation was carried out using fragmented daughter ions 119 for our analyte and 152 for the deuterated internal standard.
The method was validated in spiked urine over the linearity range of 0.2–100 μmol/L.
Elevated urinary levels of S2CP-cysteamine were demonstrated in two patients initially picked up because of increased hydroxy-C4-carnitine then diagnosed by enzymology and HIBCH gene analysis. The method may prove useful in monitoring treatment e.g. by valine restriction.

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ESTABLISHMENT OF MLPA METHOD FOR ACAT1 GENE AND IDENTIFICATION OF INTRAGENE DELETIONS AND DUPLICATION CAUSED BY ALU SEQUENCE-MEDIATED NON-EQUAL HOMOLOGOUS RECOMBINATION IN BETA-KETOTHIOLASE DEFICIENCY

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Beta-ketothiolase deficiency is an inborn error of isoleucine and ketone body metabolism, caused by mutations in mitochondrial acetoacetyl-CoA thiolase gene (ACAT1). We have so far analyzed gene mutations in more than 80 ACAT1 deficient patients. We previously identified a homozygous deletion including exons 2–4 in one patient and a homozygous duplication including exons 8 and 9 in another patient. However, such gene alterations are harder to identify in the heterozygous state than in the homozygous state through cDNA analysis. Since there are patients in whom one mutation cannot be detected by our routine genomic and cDNA analyses, we hypothesized that they would have heterozygous intragenic deletion or insertion. Hence, we established an MLPA method to detect copy numbers in each exon in the ACAT1 gene and, mixing patients' and control cDNA samples, successfully detected a heterozygous deletion including exons 2–4 and a heterozygous duplication including exons 8 and 9. We then screened 3 additional patients and found a heterozygous deletion of exons 3 and 4 in one patient, previously found to have a heterozygous A210V mutation. This deletion was likely caused by Alu sequence mediated non-homologous recombination.

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HMG-COA LYASE DEFICIENCY IN JAPAN: QUESTIONNAIRE-BASED FOLLOW-UP STUDY

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HMG-CoA lyase deficiency is an inborn error of metabolism affecting leucine catabolism and ketone body synthesis. Patients usually develop non-ketotic hypoglycemia in their infancy and childhood. In Japan, this disorder is rare and only 8 patients have been identified. Five of eight patients have been briefly reported in 2000 with their mutations (Muroi et al. Hum Genet). Our aim of this study is to collect follow-up data for frequencies of hypoglycemic attacks and prognosis. We could not collect the data for 1 patient. Four patients developed first attack within 4 days and onset was distributed from 1 day to 1 year and 3 months. One patient experienced only one hypoglycemic attack at 7 months and no further attack until 15 years old, whereas another patient developed more than 20 episodes until 6 years of age. The last hypoglycemic attack among 7 patients occurred at age of 15 years in a patient with developmental delay. Three patients have epilepsy, two of them also have developmental delay. This delay was due to severe hypoglycemic attacks during infancy. Five of 7 patients developed normally. HMG-CoA lyase deficiency in Japan has clinical heterogeneity and results in neurologic sequelae due to severe hypoglycemia in infancy.

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6 NEW CASES OF MALONIC ACIDURIA DETECTED BY NEWBORN SCREENING: CLINICAL PRESENTATION, MOLECULAR ANALYSIS AND TREATMENT OPTIONS

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Malonic aciduria is rare autosomal recessive disorder caused by deficiency of the enzyme Malonyl-CoA decarboxylase. Since the newborn screening (NBS) panel included malonic aciduria as a primary target a number of new cases has been recognized. We describe 6 cases of malonic aciduria detected by NBS. The disease severity is variable ranging from severe neonatal crisis with hypoglycemia and metabolic stroke to nearly normal development. One repeating feature observed in 4/6 patients is cardiomyopathy. One subject died from cardio-vascular complications. The rest five subjects are stable from the metabolic standpoint with various degrees of developmental delay or normal development. Molecular analysis revealed disease causing alterations in all 6 patients. Three had homozygous deletion of an entire exon: 1 and 5 (two) respectively. Two of the patients had homozygous frameshift deletions in exons 2 and 5 respectively, leading to premature stop codon. One patient had 2 missence mutations: 122 L>P/L and 345 W>W/C. After initial diagnosis treatment options included fasting avoidance, carnitine supplementation (if needed) and low fat diet enriched with medium chain triglycerides. Frequent monitoring especially in the first years of life (including echocardiograms) is recommended. On this regimen at least one patient improved his cardiac function and is currently disease free.

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MEVALONIC ACIDURIA AND CONGENITAL CMV INFECTION CLINICAL CHALLENGE IN DIAGNOSIS AND MANAGEMENT

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Background: Mevalonic aciduria (OMIM 610377) represents the severe phenotypical end of mevalonate kinase deficiency.
Case Report: Female neonate born after uneventfull pregnancy, presenting bicytopenia at birth. On D2 became severely ill, with abdominal distension, vomiting, suspicion of bowel obstruction, not confirmed. Developed cholestasis and during second week of life, presented a fluctuant rash, hepatosplenomegaly and minor dysmorphic signs were noticed. Brain MRI depicted cortical / subcortical signal abnormalities, evoking congenital CMV infection, which was confirmed by PCR on the neonatal Guthrie card; treatment with ganciclovir was started. Meanwhile organic acid profile showed an increased peak of mevalonic acid (13616 μmol/mmol Creat) and the diagnosis of mevalonic aciduria was confirmed by enzymatic and molecular studies. The initial prednisolone course had favourable response, but not sustained in subsequent cycles. The evolution has been marked by failure to thrive, needing parenteral nutrition, bicytopenia, with regular transfusions and reactivation of CMV infection with interstitial pneumonia that impose non invasive ventilatory support until the death at the fourth month of life.
Discussion: The manifestations of mevalonic aciduria are similar to congenital CMV infection and differential diagnosis is often an issue. In this patient, both disorders were confirmed with problematic therapeutic options and a poor prognosis.

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3-METHYLCROTONYLGLYCINE INDUCES LIPID AND PROTEIN OXIDATIVE DAMAGE IN BRAIN OF YOUNG RATS

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Background: Deficiency of 3-methylcrotonyl-CoA carboxylase (3-MCCD) activity is an autosomal recessive disorder biochemically characterized by accumulation and predominant high urinary excretion of 3-methylcrotonylglycine (3-MCG). Affected patients usually have neurologic dysfunction and brain abnormalities, whose pathogenesis is practically unknown.
Objectives: We investigated the in vitro effects of 3-MCG (0.1–5 mM) on important parameters of oxidative stress in cerebral cortex of 30-day-old rats.
Methods: Wistar rats were sacrificed by decapitation, the cerebral cortex dissected, homogenized and used for the biochemical assays.
Results: 3-MCG significantly increased thiobarbituric acid-reactive substances (TBA-RS) levels (30%) and carbonyl formation (40%), indicating that this compound causes lipid and protein oxidative damage in the brain. In contrast, glutathione (GSH) levels, sulfhydryl oxidation and total reactive antioxidant potential (TRAP), that reflect non-enzymatic antioxidant defenses, were not significantly altered by 3-MCG. Finally, 3-MCG did not alter nitric oxide production, implying that reactive oxygen species were involved in the oxidative damage induced by this compound.
Conclusions: It is presumed that brain is susceptible to oxidative damage provoked by 3-MCG and that this pathomechanism may contribute to the neurological dysfunction characteristic of the patients affected by 3-MCCD.
Financial support: Research grants from FIPE/HCPA, CNPq, PROPESq/ UFRGS, PRONEX/ FAPERGS, FINEP IBN-Net AND INCT-EN.

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COINCIDENCE OF 3-METHYLCROTONYL-COA CARBOXYLASE DEFICIENCY AND DIABETES MELLITUS TYPE 1—DIFFICULTIES IN MANAGEMENT

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The girl now 4.5-year-old, at age of 3 yrs was admitted to our Dept, because glycaemia 500 mg/dl. Diabetes mellitus type 1 was diagnosed. The girl was typically treated with insulin in constant iv infusion for 37 hrs. After 8 hrs she started to eat carbohydrate exchanges and take sc insulin injections. At the 4th day of hospitalization unexplained recurrent vomiting appeared. Only then the patient's mother mentioned about 3MCC deficiency identified by NBS, with no further specific recommendations. Urinary organic acid analysis by GC/MS confirmed the diagnosis. So additionally to carbohydrate, fat/protein counting, low-protein diet based on special formula and L-carnitine were introduced. Until now three hospitalizations occurred: 1st for implementation of personal insulin pomp, 2nd due to ketotic acidosis caused by technical problem with pomp/ catheter, 3 rd metabolic decompensation in the course of rotaviral infection, which required 6-day-long 10% glucose and insulin iv infusions. Current therapeutic recommendations are: low-protein (33 g/d) diet based on carbohydrate, fat/protein counting and personal insulin pomp (insulin requirement 19U/d). Conclusion: Coincidence of 3MCC deficiency and DM1 is a challenge for physician and dietetician, who have to create a proper diet, particularly difficult in emergency conditions, when hyperglycaemia should be treated avoiding insufficient calorie intake and protein overload, as well.

P-198

MITOCHONDRIAL BIOENERGETICS, CELLULAR ENERGY TRANSFER AND NA+,K+−ATPASE ACTIVITYARE COMPROMISED BY 3-METHYLCROTONYLGLYCINE IN BRAIN OF YOUNG RATS

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Background: 3- Methylcrotonyl-CoA carboxylase deficiency (3-MCCD) is an inherited metabolic disorder biochemically characterized by high tissue accumulation and urinary excretion of 3-hydroxyisovaleriate, 3-methylcrotonylglycine (3-MCG) and 3-hydroxyisovaleryl-carnitine. Clinically, patients present severe brain abnormalities and neurologic dysfunction, whose pathomechanisms are still unclear.
Objectives: We investigated the in vitro effects of 3-MCG (0.1–5 mM) on important parameters of bioenergetics and Na+, K±ATPase in cerebral cortex of 30-day-old Wistar rats.
Methods: Rats were sacrificed by decapitation, the cerebral cortex isolated, homogenized and used for the biochemical assays.
Results: 3MCG significantly reduced CO2 production from acetate (30%) and the activity of complex II-III (35%). 3MCG also inhibited the activities of mitochondrial creatine kinase (mCK) (65%) and Na+, K±ATPase (45%). Furthermore, antioxidants attenuated or fully prevented the effect of 3MCG on the activities of mCK and Na+, K±ATPase, suggesting the involvement of reactive species on these inhibitory effects.
Conclusions: 3-MCG impairs brain bioenergetics at the level of energy formation, transfer and utilization. It is presumed that these mechanisms may be involved in the pathophysiology of the neurological dysfunction that occurs in patients affected by 3-MCCD.
Financial support: research grants from CNPq, PROPESq/UFRGS, FAPERGS, PRONEX, FINEP Rede Instituto Brasileiro de Neurociência (IBN-Net), INCT-EN.

P-199

CONFIRMING CANAVAN DISEASE USING EPSTEIN-BARR VIRUS (EBV)-TRANSFORMED LYMPHOCYTES

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An increased level of N-acetylaspartate found in the pattern of urinary organic acids is a diagnostic hallmark of neurodegenerative Canavan disease (CD). Established confirmatory tests are the determination of aspartoacylase (aminoacylase 2) activity in cultured fibroblasts and mutation analysis by sequencing the ASPA gene. The enzyme activity test is of special importance, if mutation analysis yields no sequence variation with a clear pathologic significance. So far, a skin biopsy has been a prerequisite for testing aspartoacylase activity.
We have now studied by real-time PCR whether the ASPA gene is also transcribed in EBV-transformed lymphocytes and have determined enzyme activities in cell homogenates.
Our investigations demonstrate that the ASPA gene is transcribed in the lymphocytes to a similar extent if compared with fibroblasts. Based on the protein content, aspartoacylase activity is lower in EBV-transformed lymphocytes than in fibroblasts (< 20%), but even transformed lymphocytes revealed a difference in activities between patient cells and negative controls.
This opens a new diagnostic perspective, and may also help with the systematic study and characterization of patients with CD which is currently conducted by our metabolic center. More comprehensive information on CD is a prerequisite for better counseling on the clinical outcome.

P-200

CANAVAN DISEASE EXPERIENCE FROM INDIA

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Background: Canavan Disease(CD) caused by deficiency of aspartoacylase and increased excretion of N acetyl aspartate (NAA)is common in Ashkenazi Jews and has also been reported in other populations.Mutations causing Y231X (exon5) and E285A (exon6) are prevalent amongst Jews while A305E (exon6) accounts for 60% of non Jewish patients. Indian data is scarce which may have been due to non availability of NAA analysis by GCMS till recent past.Study design:We report a study of 35 clinically suspected CD cases (mean age 27 months) referred from across the country for NAA analysis. Clinically they presented with macrocephaly, developmental delay, poor head control and hypotonia. Results: A 20–200 fold increase in NAA was obtained in around 8 patients (23% of total) of which only 50% were born to consanguineous couples suggesting prevalence of CD amongst several communities in India. As an initial molecular work up we screened 6 CD patients for prevalent mutations Y231X, E285A and A305E by sequencing exons 5 and 6. These mutations were not detected in our patients, suggesting a varied set of mutations amongst them. **Conclusion:** A complete ASPA gene workup needs to be done to elucidate the mutation profile in our population.

P-201

MOLECULAR CHARACTERISATION OF CANAVAN DISEASE IN THE INDIAN SUBCONTINENT

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Canavan disease is characterized by macrocephaly, lack of head control, developmental delay, severe hypotonia –>spasticity at later stages, seizures, and leukodystrophy. Most cases reported are Ashkenazi Jews where the carrier frequency is 1/40. Prevalence of Canavan disease in the Indian Subcontinent is unknown as published data is lacking. Although Megalencephalic Leukodystrophy with subcortical cysts (MLC1) is the most common leukodystrophy in India, occurring mainly in the Agrawal community, Canavan disease is important in view of its devastating course, recessive nature with 25% risk of recurrence in family, and relative ease in diagnosis with increasing availability of MR Spectroscopy in India. A huge peak of N-acetyl aspartate on MRS or elevated urinary NAA is diagnostic. Gene studies are essential for families desiring prenatal diagnosis. We report 3 families from the Indian Subcontinent with ASPA mutations identified by sequencing in probands, and where two prenatal diagnoses were subsequently performed. All three children had typical clinical presentation and MRI findings, with elevated NAA either on MRS or urine. Mutations detected in the ASPA gene were: Novel homozygous mutation c.162 C>A in family 1(consanguineous, from Pakistan), homozygous c.859 G>A in family 2 (from India), and c.728 T>G / c.902 T>C in family 3 (non-consanguineous, from India).

P-202

NOVEL IDH2-R140 GAIN-OF-FUNCTION ENZYME ANALYSIS: A MODEL FOR D-2-HYDROXYGLUTARIC ACIDURIA TYPE II FACILITATING EVALUATION OF THERAPEUTIC INHIBITORS

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The recent discovery of IDH2-R140 mutations in D-2-hydroxyglutaric aciduria (D-2-HGA) has defined the primary genetic lesion in 50% of D-2-HGA patients, now denoted type II [Kranendijk_2010_Science]. Dang and Ward et al demonstrated gain-of-function in overexpression studies of IDH1-R132/IDH2-R172 cancer mutations [Dang_2009_Nature, Ward_2010_Cancer Cell]. The normal ability of IDH to reversibly convert isocitrate to 2-ketoglutarate (2-KG) is thereby altered to a new function that converts 2-KG to D-2-hydroxyglutarate (D-2-HG). To further characterize the enzyme mechanism, and to evaluate potential therapeutic interventions, we have developed a specific and sensitive IDH2-R140 enzyme assay.

The new assay determines the newly acquired gain-of-function of IDH2-R140 converting 2-KG to D-2-HG in homogenates of D-2-HGA type II lymphoblasts employing stable-isotope-labeled 2-keto[3,3,4,4-2H4]glutarate and UPLC-tandem-mass-spectrometry. Potential inhibitors were evaluated in an attempt to decrease IDH2-R140 enzyme activity, characterize enzyme kinetics and identify potential therapeutic interventions.

The mean reaction rate in D-2-HGA type II lymphoblasts was 8-fold higher than that of controls and D-2-HGA type I cells (14.4 nmol/h/mg protein vs. 1.9), with a corresponding 8 to 140-fold increase in intracellular D-2-HG. Oxaloacetate was the best competitive inhibitor of IDH2-R140 activity. Lymphoblast IDH2-R140 showed long-term cell culture stability, highlighting the utility of the lymphoblast model for future therapeutic studies.

P-203

PANCREATITIS IN ORGANIC ACIDAEMIA: DIAGNOSTIC AND MANAGEMENT CHALLENGES

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We present a series of patients with organic acidaemia complicated by pancreatitis. Patients A and B had propionic and methylmalonic acidaemias respectively. They presented with vomiting and developed severe, persistent abdominal pain requiring opiates, ketamine and intensive care support. Patient A had a marginally raised amylase but radiological findings were consistent with pancreatitis. She died after a prolonged PICU admission. Patient B had a 48 hour rise in amylase. He had a prolonged PICU stay and was discharged home on opiates. Patient C had propionic acidaemia and presented with vomiting. This resolved but he had an unexpected cardiac arrest, which he did not survive. Post-mortem showed pneumonia and pancreatitis. Patient D had methylmalonic acidaemia and died after a short illness. Post-mortem revealed acute on chronic pancreatitis, despite a normal amylase. These cases highlight: (i) Pancreatitis may be more common than thought (ii) Amylase is an unreliable marker. None of the patients had a significantly raised amylase. Diagnosis was based on clinical and radiological grounds (iii) Severe pancreatitis poses management difficulties in terms of analgesia and nutrition. Current best practice is to continue with enteral feeds and aggressive pain control, if necessary with intubation and sedation for a prolonged period.

P-204

CARGLUMIC ACID (NCG) AS TREATMENT OF HYPERAMMONEMIA EPISODES IN ORGANIC ACIDEMIAS/ACIDURIAS (OAS): A RETROSPECTIVE OBSERVATIONAL STUDY IN PATIENTS FROM EUROPE AND TURKEY

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Background: Due to its property to enhance CPS-1, NCG could be used to treat OA-associated hyperammonemia.

Design: Retrospective study in OAs patients with hyperammonemia episodes receiving NCG between 1995 and 2009. End-points were plasma ammonia (NH3), symptoms (neurological, psychiatric, psychomotor, hepatic) and safety

Results: Overall, 57 patients were included. 48 episodes of hyperammonemia (60.4% of neonatal onset) in 41 patients (4 Isovaleric, 21 Methylmalonic, 16 Propionic Acidemias) were analyzed for efficacy. Median (range) episode duration was 6 days (2–43).

Median (range) starting dose and duration of NCG was 75.5 mg/kg (13.3-303.0) and 4 days (1–15), respectively. The maintenance dose was slowly reduced; 43.8% of hyperammonemia episodes were treated with concomitant scavengers.

Median (range) NH3 decreased from baseline of 215 μmol/L (76–1633) to 52.0 μmol/L (15–158) (last value under NCG). Consistent NH3 decrease was observed in all OAs, irrespective of concomitant scavengers. Median time to achieve NH3<=60 μmol/L under NCG was 36.5 hours. The clinical symptoms improved following NCG initiation.

Among 74 adverse events observed, 22 were serious, mostly not drug-related, except for 1 death (neurological damage).

Conclusions: Carglumic acid rapidly and effectively reduces NH3 in OA-associated hyperammonemia, leading to an overall clinical improvement, with an acceptable safety profile.

P-205

ORGANIC ACID ANALYSIS AN INDIAN PERSPECTIVE

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Over a period of 3 years 2498 patients with suspected metabolic disorder were evaluated. The patients showed 2:1 male:female ratio. The study group included asymptomatic screen positive neonates(n=25), symptomatic neonates and young children with acidosis, hypoglycaemia, hyperammonemia etc. Inborn error of metabolism was detected in ~19.2% patients, prevalent being MMA(11.4%) and GA1(9.1%). Disorders such as PA, IVA,MSUD, tyrosinemia type1, canavan disease, biotinidase deficiency and others ranged from 0.5–2.4% of the affected subjects. In addition a few cases of rare disorders such as SSADH, 2 hydroxy glutaric aciduria etc were also diagnosed. Lactic acidosis and/or ketosis have been a consistent finding in several samples. Around 3.3% showed dicarboxylic aciduria either due to a metabolic defect / MCT administration or sickness. Early diagnosis has helped to initiate management in 7 MMA, 4 GA1, 2 IVA and 3 PA patients. Prenatal diagnosis in subsequent pregnancies was facilitated in referral laboratories for 2 MMA, 1 GA1 and 1 SSADH family. Thus it is observed that offering OA analysis within the country has helped to reveal inherited metabolic disorder profile amongst Indians and to initiate management and offer prenatal diagnosis to reduce the burden of recurrence in affected families.

P-206

TRICARBALLYLIC ACIDURIA IN A BREAST-FED NEWBORN DETECTED WITH URINARY ORGANIC ACID ANALYSIS BY GC-MS: MATERNAL CONSUMPTION OF MYCOTOXIN CONTAMINATED MAIZE

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Human exposure of the mycotoxin fumonisin B (FB) is greatest in regions where maize products are the dietary staple. The tricarballylic acid moieties of FB are structurally similar to sphingolipids which can cause neural tube defects due to altered sphingolipid metabolism by inhibition of ceramide synthase. The toxic effects of FB can also lead to acute mycotoxicosis, esophageal cancer in humans and cancer, pulmonary edema, leukoencephalomalacia in animals.

Case. A 5 days old female patient was admitted with elevated propionylcarnitine (5.5 μmol/L; cut-off 4.9 μmol/L) in expanded newborn screening. During second-tier tests, urinary organic acid analysis by GC-MS revealed excretion of "tricarballylic acid" (58 μmol/mol creatinine). The mother had vitamin B12 deficiency. Breast-feeding was withheld as a potential source of the baby's fumonisin exposure because her family traditionally consumed high amounts of maize and maize products. Tricarballylic aciduria ceased within three weeks. Samples of ground corn taken from the family revealed a high content of FB (4164 ppb; N< 100 ppb).

This is the youngest baby reported to be exposed to FB. Early diagnosis of this baby with urinary organic acid analysis led to initiation of the regional investigation of contaminated maize products by the Turkish Ministry of Health.

A-009

A PATIENT WITH METHYLMALONIC ACIDAEMIA (MMA) WHO UNDERWENT MAJOR SPINAL SURGERY FOR SCOLIOSIS

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Case Report

Our case is a 16-year-old girl with methylmalonic acidaemia. She has progressive scoliosis which caused restrictive lung function impairment and therefore spinal surgery was contemplated. There was limited experience in operating these patients who are extremely vulnerabe in encountering stress.

Anterior spinal fusion with instrumentation T11 to T14 was performed. Pre-operatively, sufficient glucose load was given during fasting. We closely monitored her blood gas, glucose, ammonia level, lactate, electrolytes and urine ketones. Intraoperatively, we avoided lactate containing intravenous fluid, N2O, NSAIDs and relaxants that are metabolized by ester hydrolysis. We used fresh blood for transfusions as old blood would give higher protein and acid load. TPN and carnitine infusions were started intraoperatively. Postoperatively, patient was transferred to PICU. Excellent pain control was given. Oral feeding was resumed as fast as patient could tolerate. We ensured that she has good bowel motion as gut bacteria can produce large amounts of propionic acid which is the direct precursor of MMA. Carbaglu and haemodialysis machine were arranged in case patient went into decompensation with hyperammonaemia.

Conclusion: Patients with organic acidaemia can undergo major surgeries under general anaesthesia. However, detailed planning and cooperation among different disciplines are necessary for the success.

A-010

A CASE OF CANDIDA ESOPHAGITIS IN MSUD DERANGEMENT

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A 37 years old female patient with classical MSUD was admitted to our hospital with a sore throat, anorexia, and decreased responsiveness. Her medical history contained psychomotor retardation, spastic tetraplegia and epilepsy as a result of dietary noncompliance in early childhood. A Candida stomatitis and dehydration were diagnosed. She was successfully rehydrated and treated with Fluconazole 200 mg orally for 10 days. After 4 days she was discharged in good condition. Metabolic laboratory results showed derangement of branched amino acids which was likely to be caused by an accidental switch of her amino acid mixtures to a urea cycle disorder preparation 21 days before admission. Although often thought of as a common complication, there is no peer reviewed publication regarding fungal infections during metabolic derangement in MSUD. Experiments in healthy rats show that an excess of dietary leucine can result in immune impairment as does a decreased BCKDH-activity in leucocytes and an increase of oxidative stress as seen in MSUD. Thus, immune impairment in MSUD derangement is comprehensible. Why fungal and not bacterial infections are prevalent in MSUD derangement, still remains to be elucidated.

A-011

ESTABLISHMENT OF METHYLMALONYL COA MUTASE ACTIVITY MEASUREMENT WITH LYMPHOCYTE BY HPLC

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Background: An assay of the enzyme activity is very important for diagnosis of metabolic disorder. The activity of methylmalonyl-CoA mutase has been measured using fibroblast. However, the collections of the fibroblast from the patient and cell culture are troublesome task. We examined the simple and easy method using lymphocyte.

Method: A mixture of methylmalonyl-CoA, lymphocyte and cobalamin in Tris-sulfate buffer (pH 7.5) was incubated for 15 min at 37°C. After the suspension was centrifuged (12,000 rpm, 10 min), the supernatant was analyzed by HPLC. STR-ODS 2 (6×150 mm, Shinwakagaku, Japan) was used as the HPLC column and detection of methylmalonyl-CoA and succinyl-CoA was performed by UV absorption (260 nm).

Results: Although succinyl-CoA was detected in lymphocytes from a healthy volunteer, none or a small amount of succinyl-CoA was generated in the lymphocytes of a patient with methylmalonic acidemia. The amount of generated succinyl-CoA was proportinal to methylmalonyl-CoA mutase activity.

Discussion: The measurement of methylmalonyl-CoA mutase activity with lymphocyte was possible. However, as succinyl-CoA decomposed with time by heat, analysis has to be performed carefully.

A-012

CANAVAN DISEASE: CASE REPORT

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Canavan disease (CD) is caused by elevated levels of N-acetylaspartic acid (NAA). The triad of hypotonia, macrocephaly and head lag in infant after the age of three to five months should raise the suspicion of Canavan disease. The diagnosis of CD relies upon measurement of the concentration of NAA in the urine using gas chromatography-mass spectrometry (GC-MS). For certain diagnosis should be performed aspartoacylase enzyme activity in cultured fibroblast or molecular analyse of ASPA gene.

Here presented a six months old girl patient applied with seizures to our clinic. In her physical examination lack of head control without macrocephaly and optic atrophy were found. We suspected neurometabolic disorder and performed metabolic screening tests. Urine GC-MS showed high level of NAA and magnetic resonance spectroscopy showed a peak of aspartic acid. After these results molecular analyse for CD were performed and confirmed the diagnose by the identified homozygous mutation (c.244dupA) in the ASPA gene. The first presentation of our patient were nonspesific neurological findings like seizures and hypotonia. Additionally there were no sign for macrocephaly which is generally seemed in CD.

Here emphasized the importance of metabolic screening test to arrive the diagnosis especially in non specific clinical manifestations.

A-013

QUANTITATIVE DETERMINATION OF METHYLMALONIC ACID IN CLINICAL SAMPLES BY USING A CERTIFIED COMMERCIAL AVAILABLE LC-MS/MS KIT

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Background: Methylmalonic acid (MMA) is a biochemical marker for inherited disorders of propionate metabolism and acquired vitamin B12 deficiency.
Methods: The objective of this study was to evaluate a validated commercial LC-MS/MS kit, ClinMass® Complete Kit, Recipe GmbH, Germany. The method includes an isotope-labeled internal standard for the quantification of MMA in serum/plasma, and in urine. In our study, we analyzed 21 urine samples including specimens from 4 known patients with propionic (PA) and 2 from patients with methylmalonic acidemia. In addition, we analyzed 14 plasma samples including 2 from patients with PA and one with methylmalonic academia.
Results: All analyzed samples from known patients showed elevated MMA concentrations and where identified accurately. The concentration of MMA in urine sample was in the range from 81 to 8.400 μmol/L, the concentration in plasma from one patient with methylmalonic acidemia was 617 μmol/L. The intra-assay coefficient of variation for the LC-MS/MS methods was below 10%.
Conclusion: The MMA kit provides a simplified and fast sample work-up protocol using an one-step precipitation, centrifugation and direct infusion without derivatisation or solid-phase extraction. The Kit is CE labeled and supplies certified reagents and can be used in a daily routine laboratory for metabolic disorders.
Conflict of Interest declared.

A-014

THE PRESENCE OF NEGATIVE CORRELATION BETWEEN BLOOD HEMOGLOBIN AND PROPIONYLCARNITINE LEVELS IN METHYLMALONIC AND PROPIONIC ACIDEMIAS

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Background: Methylmalonic acidemia (MMA) and propionic acidemias (PA) are the most common organic acidemias affecting the propionate pathway. Anemia, leukopenia and thrombocytopenia due to bone marrow supression are common findings in both disorders, specially in acute metabolic decompensation. It is suggested neurological injury in each disorder and renal dysfunction in MMA is related to mitochondrial dysfunction due to toxicity of methylmalonic acid, propionyl-CoA and 2-methylcitrate, but the pathogenesis of bone marrow supression is unknown.
Objectives: To evaluate whether there is any relation between blood hemoglobin, white blood cell (WBC), thrombocyte levels and urine organic acid excretions, blood free carnitin, acylcarnitine levels.
Material and Methods:
The study group consisted of 37 children (MMA=30, PA=7). Blood and urine samples were obtained at the same time.
Results: There was no correlation between thrombocyte, WBC levels and urine methylmalonic acid, methyl citrate, propionic acid excretion and blood free carnitine, methylmalonylcarnitine, propionylcarnitine levels. There was a significant negative correlation between hemoglobin and propionylcarnitine levels in both diseases (MMA:$r=-0.827$, $p<0.001$ and PA:$r=-0.692$, $p<0.001$)
Conclusions: The presence of negative correlation only between blood hemoglobin levels and blood propionylcarnitine levels in both diseases suggests that propionylcarnitin may have a role at the pathophysiology of anemia in these disorders.

A-015

CHOICE OF KIDNEY TRANSPLANTATION IN A 26-YEAR-OLD JAPANESE MALE WITH METHYLMALONIC ACIDURIA PRESENTING END STAGE RENAL FAILURE

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Methylmalonic aciduria (MMA) is an inborn error of organic acid metabolism. Patients with severe disease develop many complications despite treatment; often, the disease progresses to tubulointerstitial nephritis with progressive renal failure or to severe damage of the central nervous system. Liver, kidney, or combined liver and kidney transplantation is advocated when medical treatment is ineffective. Liver or combined liver and kidney transplantation is effective to improve the metabolic decompensation; however neurological deterioration has occurred in some individuals after the transplantation. Some individuals have received only renal allografts; Lubrano et al reported a 27-year-old woman with MMA MUT0, who received a kidney transplant at age 17 years, and presented no episodes of metabolic decompensation and normal renal function after the transplantation. We report a 26-year-old Japanese male with MMA MUT0 (R93H, IVS2+5 G>A), presenting end stage renal failure. The clinical symptoms appeared at age 7 months. He progressed into renal dysfunction at age 6 years. Recently serum levels of creatinine have reached above 5 mg/dl. Kidney transplantation is scheduled in June 2011.

O-034

VARIANT MUTATIONS IN MEDIUM-CHAIN ACYL-COA DEHYDROGENASE DEFICIENCY (MCADD): ARE THERE CONSEQUENCES FOR FOLLOW-UP?

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Background: Before extended neonatal bloodspot screening (NBS), MCADD patients presented with life-threatening hypoketotic hypoglycemia. Nowadays, asymptomatic newborns are identified by NBS with novel genotypes, which have not been found in clinically ascertained patients (i.e. ACADM variants). These children are treated as classical MCADD patients, while the necessity hereof is unknown.
Objective: To determine the effect of ACADM variants on enzyme activity and fasting tolerance.
Methods: MCAD enzyme activities were measured in cultured skin fibroblasts from 8 subjects with ACADM variants, using different substrates. In 5 subjects, a fasting study and phenylpropionic acid loading test were performed.
Results: Using phenylpropionyl-CoA, MCAD activity in fibroblasts was significantly higher in variants (mean±SD 8.1%±7.5) compared to classical patients (<1%).
Phenylpropionic acid loading caused urinary hippuric acid excretion in subjects with ACADM variants, indicating in vivo residual MCAD enzyme activity. Upon fasting, blood glucose, free fatty acid/ketone body (KB), and KB*glucose remained normal for 15–20 hours.
Conclusion: ACADM variants show residual MCAD enzyme activity in vitro and in vivo, as opposed to classical patients, correlating with normal fasting tolerance. The effect of fever on in vivo residual enzyme activity remains unknown, and follow-up studies are warranted to determine the clinical relevance of these variants.

O-035

VISCERAL OBESITY AND DISRUPTED FAT COMPOSITION DUE TO MEDIUM-CHAIN TRIGLYCERIDES IN MICE WITH A β-OXIDATION DEFECT

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Asymptomatic very long-chain acyl-CoA dehydrogenase (VLCAD)-deficient patients are often supplemented with medium-chain triglycerides (MCT) to prevent the development of cardiomyopathy and myopathy. MCT is considered a safe dietary intervention, however, long-term observations into later adulthood are still missing.

In the present study, we investigated the consequences of a long term MCT supplementation in VLCAD deficient (VLCAD−/−) mice. In vivo studies based on 1H and 13 C magnetic resonance (MR) techniques were applied to analyze noninvasively the MCT effects on abdominal fat distribution and composition as well as on liver fat. These data were subsequently correlated with serum transaminases and liver histology. In addition, hepatic biochemical markers of oxidative stress were assessed.

The replacement of LCT by MCT, results in a dramatic accumulation of visceral fat and serum free fatty acids in VLCAD−/− mice followed by a profound shift in body triglyceride composition when applied over one year. Liver histology 1H MRS and biochemical analysis revealed pronounced steatosis and marked oxidative stress.

MCT diet has been effective in prevention of cardiomyopathy and skeletal myopathy in FAO disorders. However, our data demonstrate that long-term MCT supplementation in the mouse model results in a severe hepatic phenotype similar to non-alcoholic steatohepatitis and metabolic syndrome.

O-036

ROLE OF THE LIPIN FAMILY IN METABOLIC MUSCULAR DISEASES: AN UPDATE

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Background: LPIN1 mutations were identified as a cause of severe early-onset rhabdomyolysis. The lipin family includes two other related members, lipin-2 and 3, which share strong homology and activity.

Objectives: To determine the involvement of lipins in patients presenting with various symptoms ranging from rhabdomyolysis to myalgia at any age.

Patients and Methods: LPIN1, LPIN2, LPIN3 coding regions were sequenced for 200 patients presented with rhabdomyolysis (n=170) of variable onset ranging from severe to mild (CK>or<10 000 UI/L) or myalgia without myoglobinuria (n=30).

Results: 35 patients presenting with severe rhabdomyolysis, occurring before age 10 years but one, had 2 LPIN1 mutations. 86% Caucasian families shared the same intragenic deletion. 40% heterozygous relatives presented mild muscular symptoms. Muscle biopsies showed excessive lipid droplets. Rhabdomyolysis were mostly precipitated by fever. Incubation of lipin-1-deficient myoblasts with pro-inflammatory cytokines leaded to significant increase of lipid droplets. Nine variants identified in milder phenotypes in either gene were non-functional in a yeast complementation assay except one.

Conclusion: LPIN1-related disease, a new subtype of lipid storage myopathy, constitute a major cause of severe rhabdomyolysis in childhood and occasionally in adults. Relatives can be symptomatic. No major LPIN2/LPIN3 defects are associated with muscular diseases.

O-037

CHARACTERIZATION OF MULTIPLE ACYL-COA DEHYDROGENATION DEFICIENCY THROUGH MITOCHONDRIAL PROTEOMICS

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Multiple Acyl-CoA dehydrogenation deficiency (MADD) is a metabolic disorder affecting fatty acid β-oxidation, resulting in a decrease of electron transference to respiratory chain. Pathophysiological mechanisms leading to clinical phenotype are believed to rely mainly on the resulting energy deficiency, although its consequences on mitochondrial function are still poorly understood. In order to bring new insights, a proteome approach was adopted. We isolated mitochondria from cultured fibroblasts, from a patient with a severe MADD presentation due to ETF-QO deficiency, characterize its mitochondrial proteome and compare it with normal controls. The used approach (2-DE-MS/MS) allowed the positive identification of 287 proteins in both patient and controls, presenting 35 of then significant differences in their relative abundance. Among the differentially expressed are proteins associated to binding/folding functions, mitochondrial antioxidant enzymes as well as proteins associated to apoptotic events. The overexpression of chaperones like Hsp60 or mitochondrial Grp75, antioxidant enzymes and apoptotic proteins reflect the mitochondrial response to a complete absence of ETF-QO. Our study provides a global perspective of the mitochondrial proteome plasticity in a severe case of MADD and highlights the main molecular pathways involved in its pathogenesis. An integrated perspective of mitochondrial plasticity face to MADD is given.

O-038

LONG CHAIN ACYL-COA DEHYDROGENASE DEFICIENCY: A NEW INBORN ERROR OF METABOLISM MANIFESTING AS CONGENITAL SURFACTANT DEFICIENCY

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Background: The physiologic role of long chain acyl-CoA dehydrogenase (LCAD) has long remained elusive. LCAD is expressed in type II pneumocytes, leading us to hypothesize that a patient with LCAD deficiency would present with congenital surfactant deficiency.

Design/Methods: Term infants with unexplained respiratory distress were evaluated for known causes of congenital surfactant deficiency and had a normal genotype. Lung tissue from patient and controls were evaluated with electron microscopy for lamellar body structure as well as LCAD antigen expression using immunostaining and confocal microscopy. Genetic studies were performed.

Results: One patient was identified. EM of lung tissue demonstrated eccentric deposits within the lamellar bodies. LCAD antigen was absent in patient lung tissue. Genomic DNA sequencing of patient DNA revealed a homozygous base pair change involving intron 6, causing a splicing error. At six months of age, a liver biopsy demonstrated accumulation of cis-3,4-methylene-heptanoylcarnitine, likely derivative from a LCAD substrate.

Conclusions: This is the first report of LCAD deficiency, presenting with a unique phenotype of congenital surfactant deficiency.

O-039

A NOVEL ETFDH C.158A>G MISSENSE MUTATION CAUSES EXON 2 SKIPPING AND MULTIPLE ACYL-COA DEHYDROGENATION DEFICIENCY (MADD)

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MADD is an inherited disorder of fatty acid, amino acid and choline metabolism. There is good correlation between MADD disease severity and mutations in ETFA, ETFB or ETFDH. A neonate was diagnosed with severe MADD based on increased C5-C18 acylcarnitines and low (2–5% of control mean) fatty acid oxidation flux in fibroblasts. Mutation analysis revealed homozygosity for a novel c.158A>G (p.Lys53Arg) mutation in ETFDH exon 2. Surprisingly, PolyPhen analysis predicts the mutation to be benign. However, in silico analyses suggested that the mutation creates an exonic splicing silencer (ESS) motif (TAGGGA) for the splicing inhibitory protein hnRNPA1. Consistent with this, patient ETFDH cDNA showed complete exon 2 skipping, which results in removal of most of the mitochondrial targeting signal. A mutation in the APC gene also creates a TAGGGA motif and causes missplicing. This illustrates that splicing regulatory elements are general. We are currently analyzing if the TAGGGA motif functions as an hnRNPA1 dependent splicing silencer using RNA affinity purification experiments and a heterologous splicing reporter minigene.

In conclusion, our findings are consistent with the severe MADD phenotype of the patient and underscore the importance of testing mRNA splicing consequences when predicting the effect of exonic sequence changes.

P−208

CARNITINE ACYLCARNITINE TRANSLOCASE (CACT) MUTATIONS IN INFANTS WITH SUSPICION OF CPTII DEFICIENCY

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Background: Carnitine-acylcarnitine translocase (CACT) deficiency is a life-threatening, rare autosomal recessive disease often presenting in early infancy with seizures, hypoketotic hypoglycemia, hyperammonemia, cardiomyopathy, liver failure, muscle weakness, and apnea, similar to other defects of long chain fatty acid beta-oxidation, with indistinguishable acylcarnitine profile form infantile CPTII deficiency. Previously, diagnosis of CACT deficiency relied on in vitro enzymatic assay that is tedious and not practical.

Objective: To develop Molecular methods for definitive diagnosis of CACT deficiency.

Method: Sanger sequencing and oligonucleotide aCGH were used for the analysis of the SLC25A20 gene. Literatures were reviewed.

Results: Among 100 individuals suspected of having CACT deficiency, mutations were identified in 7 unrelated families. Eight novel mutations were found, including a large 25.9 kb deletion encompassing exons 5 to 9 of the gene detected by aCGH. All patients presented clinical features and acylcarnitine profiles at early life consistent with CACT or CPTII deficiency. However, sequence analysis of CPT2 gene did not find mutations.

Conclusion: CACT deficiency is pan-ethnic. It may not be as rare as originally thought. Patients with infantile form of CPTII deficiency should be analyzed for mutations, including large deletions, in the SLC25A20 gene.

P-209

LONG TERM FOLLOW-UP OF THREE PATIENTS WITH CARNITINE-ACYLCARNITINE TRANSLOCASE DEFICIENCY PRESENTING IN THE NEONATAL PERIOD

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We report our experience in managing three surviving children (7 years, 5 years and 22 months) with carnitine-acylcarnitine translocase deficiency, confirmed by mutation analysis of the SLC25A20 gene.

Patient A, born prematurely at 32 weeks, developed ST elevation and ventricular tachycardia on cardiac monitor on day 17 when feeding was reintroduced after recovery from necrotizing enterocolitis. Patient B, who was a term infant already discharged from hospital, presented on day 3 with cardiorespiratory failure requiring cardiopulmonary resuscitation (CPR) in the Accident and Emergency Department. Patient C presented on day 1 with cardiorespiratory arrest successfully revived with CPR.

All 3 children were put on frequent feeding with a high carbohydrate / low fat diet, mainly with medium-chain triglycerides, supplemented with walnut oil rich in essential fatty acids. Patients A & C had significant feeding problems with food refusal requiring gastrostomy feeding and central venous line to maintain adequate glucose infusion, whilst Patient B was on a less meticulous diet with infrequent decompensations and no feeding difficulty. Patient B (aged 5 years) suffered from hypoxic brain damage resulting in mild grade mental retardation. The other 2 children have normal development despite frequent episodes of decompensation with hyperammonaemia and rhabdomyolysis.

P-210

THE BLOOD ACYLCARNITINE PROFILES AND CLINICAL PICTURE IN TWO PATIENTS WITH LATE-ONSET CARNITINE PALMITOYLTRANSFERASE TYPE II DEFICIENCY

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Background: To evaluate the relationship between the blood acylcarnitine profiles and clinical picture in patients with late-onset muscular phenotype of carnitine palmitoyltransferase (CPT II) deficiency (MIM 255110). CPT II deficiency is an autosomal recessive disorder of carnitine dependent transport of long-chain fatty acids into mitochondrial matrix.

Methods: We analysed clinical data and the blood acylcarnitine profiles [C16, C18:1, the (C16+C18:1)/C2 ratios] in 32 samples of two adult male patients with the muscular phenotype of CPT II deficiency.

Results: Patients presented with myalgia/weakness in 12/32 examinations. The elevation of the (C16+C18:1)/C2 ratios was found in 10/12 symptomatic and 9/20 asymptomatic cases. The levels of C16 acylcarnitine were elevated in 3/12 symptomatic and 2/20 asymptomatic cases. The elevation of C18:1 acylcarnitine concentrations was observed in 5/12 symptomatic and 5/20 asymptomatic cases. Higher levels of C18:1 acylcarnitine concentrations were accompanied by the elevation of C16 acylcarnitine concentrations in 3/5 symptomatic and 2/5 asymptomatic cases.

Conclusions: Our patients had no correlation between the absolute values of the (C16+C18:1)/C2 ratios, C16 and C18:1 acylcarnitine concentrations and clinical symptoms. The (C16+C18:1)/C2 ratio was the most sensitive marker for the diagnosis of CPT II deficiency.

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P-211

LIVER ACYLCARNITINES IN RATS UNDER ACUTE AND SUBCHRONIC TREATMENT WITH SODIUM VALPROATE

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Since valproic acid interferes with mitochondrial energy metabolism, carnitine imbalance may occur in liver, the major target of VPA-toxicity.

Aim: To investigate the acylcarnitine profile in animal liver tissues.

Methods: Wistar rats were treated with one single injection of sodium valproate (100, 500 mg/kg) or subjected to a subchronic regimen (100 mg/kg/day for two weeks). Carnitine and acylcarnitines were quantified in liver tissues using ESI-MS/MS.

Results: There was a significant dose-related increase of free carnitine and total acylcarnitine levels in the liver of rats treated with a single injection of valproate as compared with controls. Acylcarnitine levels were normal in livers of rats submitted to subchronic treatment except for malonyl-, isovaleryl-, 3-hydroxyisovaleryl-, adipoyl-, nonanoyl-, cis-4-decenoyl- and decanoylcarnitine which remained significantly increased.

Discussion: The results clearly show an accumulation of acylcarnitines in liver at the onset of valproate administration. However, the disturbances in carnitine homeostasis appeared to recover after a subchronic regimen, except for specific individual acylcarnitines involved in the metabolism of branched-chain amino acids and medium-chain fatty acids. Endogenous biosynthesis of carnitine or its redistribution from peripheral organs may play an important role in the rescue of free carnitine levels in liver after a drug induced stress on mitochondrial metabolism.

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THE CARDIAC MANIFESTATION AND RESPONSE TO L-CARNITINE TREATMENT IN 14 CASES WITH PRIMARY SYSTEMIC CARNITINE DEFICIENCY: CORRELATION WITH GENOTYPE

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Background: Primary systemic carnitine deficiency (PSCD) is caused by mutation in the SLC22A5 gene encoding carnitine transporter OCTN2. The disorder is characterized by cardiomyopathy, weakness, hypoglycemic hypoketotic encephalopathy and highly responsive to L-carnitine therapy.

Objectives: The aim was to investigate identification of clinical phenotype and genotypes with emphasis to cardiac manifestation and response to L-carnitine therapy in 14 patients in order to provide genotype-phenotype correlations.

Material and Methods: Mutations for SLC22A5 gene in 14 patients with PSCD followed at Istanbul Medical Faculty was confirmed by direct sequence analysis. Clinical phenotype; ECG, echocardiography, 24-hours holter-ECG; genotype; dosage, duration of L-carnitine therapy was evaluated.

Results: Age at diagnosis was 5.7+10.7 years (range:0.2-41.0). Follow-up duration was 4.4+2.2 years (range:2–9). L-carnitine 178.8+63.7 mg/day (range:75–300) was recommended. Symptoms at referral were Reye-like syndrome(4), cardiac insufficiency(4), convulsions(1), psychomotor retardation(1) and family history(4). At diagnosis, 8 patients (57.2%) had cardiomyopathy (2 dilated, 4 hypertrophic, 2 hypertrophic and dilated). Mutation analysis has harbored for five novel mutations including p.G152R, p.R169P, p.C236R, p.L363P, p. H48P(fs)89X and two known mutations p. R83L.and R471H. Homozygous amino acid changes for L363P, G152R, C236R and H48P(fs)89X mutations correlate with cardiomyopathy.

Conclusion: Cardiomyopathy resolved in all patients complaint to treatment. Patients with same mutation may have different clinical phenotypes.

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STUDIES ON THE MITOCHONDRIAL SYNTHESIS AND CELLULAR EXPORT OF ACYLCARNITINES IN MITOCHONDRIAL FATTY ACID BETA-OXIDATION DISORDERS

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Plasma acylcarnitines are important biomarkers in the diagnosis of mitochondrial fatty acid beta-oxidation disorders (mFAOD). Although regarded as a reflection of the acyl-CoAs accumulating intramitochondrially in mFAOD patients, the true etiology of plasma acylcarnitines still requires clarification. It is generally assumed that these intermediates are formed within mitochondria via the activity of carnitine palmitoyltransferase 2 (CPT2). However, their transport across the mitochondrial and plasma membrane into the extracellular space remains undefined. Herein we aimed to resolve the mechanism by which intramitochondrial acylcarnitines are synthesized and further transported from the mitochondrial matrix to the extracellular space. Using lentiviral shRNA, medium-chain acyl-CoA dehydrogenase (MCAD) gene was silenced in control, CPT2 and OCTN2 deficient human fibroblasts. Transfected cell lines were incubated with 120 μM of n-decanoic acid plus L-carnitine. In shMCAD transfected cell lines beta-oxidation proceeds until the formation of C8-CoA which will accumulate intramitochondrially. Extracellular acylcarnitine profiles were analyzed by ESI-MS/MS. Preliminary results show the absence of C8-carnitine in the medium of CTP2 deficient cells, pointing towards the involvement of this enzyme in the production of medium-chain acylcarnitines. In OCTN2 deficient cell lines however, extracellular accumulation of C8-carnitine is observed suggesting that the cellular export of acylcarnitines does not depend on OCTN2.

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SHORT/BRANCHED-CHAIN ACYL-COA DEHYDROGENASE DEFICIENCY (SBCAD) PRESENTING WITH BILATERAL BASAL GANGLIA INVOLVEMENT

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Background: Short/branched-chain acyl-CoA dehydrogenase deficiency (SBCAD or 2-methylbutyryl dehydrogenase) is a very uncommon autosomic recessive disorder of the isoleucine catabolic pathway, with variable and not well defined phenotype. We describe a case with clinical features not reported previously.

Case Report: A 14 months old boy was referred for evaluation on arrival to our country. He is the first son of healthy consanguineous parents from Pakistan, with a normal psychomotor development until the age of 8 months. During pneumonia he suffered an acute neurological regression with seizures and feeding difficulties. On admission, the patient showed visual contact, severe generalized hypotonia, hyperreflexia, oral and upper limb dyskinesias. Metabolic screening revealed hyperlactacidemia and increase in 2-methylbutyrylglycine, 2-ethylhydracrylic, ethylmalonic and methylsuccinic acids, all consistent with a diagnosis of SBCAD deficiency. Genetic testing is ongoing. Brain MRI shows bilateral striatal lesions. The patient is on a low-protein (low-isoleucin) diet.

Discussion: The clinical spectrum of SBCAD deficiency is not well defined because of the small number of reported cases and their different phenotype. While it may be asymptomatic in some patients, in others it manifests as a severe neurological disorder. To our knowledge our patient is the first presenting with bilateral striatal involvement.

P-215

MACRO-AST: MISLEADING FINDING IN AN ADOLESCENT WITH MCAD-DEFICIENCY

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The patient presented with brief hypoglycaemic seizures at the age of 3 and 4 days. Further diagnostic work-up revealed medium chain acylCoA dehydrogenase (MCAD)-deficiency.
In the following years, she developed normally, liver and muscle function tests were normal. At the age of 11 years, isolated AST elevation was first noticed. AST values remained elevated ever since at 600–800 U/l (normal <35 U/l), all other liver enzymes, coagulation, CK and carnitine were normal. An infection with hepatotropic viruses could be excluded.
Impaired fatty acid oxidation in MCAD-deficiency can compromise skeletal muscle, heart and liver function, especially in adolescents and adults. AST-elevation was considered to be related to MCAD-deficiency, further tests like liver ultrasound, echocardiography and ECG were unrevealing.
At the age of 15 years, the girl presented first in our metabolic clinic. As organ functions were normal and there was isolated elevation of AST we considered macro-AST. The presence of macro-AST was confirmed by polyethylene glycol precipitation.
Macro-AST is usually a harmless finding due to complex formation with immunoglobulins which cannot be excreted via urine.
Conclusion: AST-elevation in this patient was unrelated to MCAD-deficiency but due to macro-AST. IgE-elevation associated with pollen-allergy may play a role.

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VOMITING—FREQUENT SYMPTOM PRECEEDING SUDDEN INFANT DEATH IN MCAD DEFICIENCY

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It has been estimated that 15–20% of the children with MCAD deficiency (MCADD) died suddenly during a first metabolic decompensation. Data from analysis of clinical symptoms preceeding sudden death are still rare. Case report: A 9 months old gypsy boy was found dead at home in the morning. A postmortal acylcarnitine profile from a dry blood spot raised the suspicon of MCADD. Mutation analysis revealed homozygosity for the 985A>G mutation in the ACADM gene. Retrospective analysis of the clinical course revealed that on the day preceeding his death the patient had developed vomiting and diarrhea without fever and any signs of respiratory infection. A consulted primary care paediatrician did not recommend hospitalization and suggested only oral rehydratation after vomiting had reiterated. Autopsy revealed fatty liver as well as severe cerebral edema. Microscopic examination of lungs revealed signs of acute viral infection. Virologic tests did not detect virus of seasonal influenza or pandemic influenza type A (H1N1). Sudden death in four children with MCADD have been described by Yusupov et al. despite newborn screening and in all vomiting was the dominant symptom preceeding sudden death (Molecular Genetics and Metabolism, 101, 2010, p. 33–39). Clinical analysis of our case confirmed this observation.

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MEDIUM CHAIN ACYLCOA DEHYDROGENASE DEFICIENCY (MCADD) STATE IN FRANCE BEFORE NEWBORN SCREENING IMPLEMENTATION

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Introduction: MCADD is the most prevalent deficiency of mitochondrial fatty acid Beta-oxidation defects (FAOD) and is included in newborn screening program (NSP) of various occidental countries. France would implement MCADD in its NSP in future.
Methods: through a retrospective study, we analysed 66 paediatric cases affected with MCADD, confirmed by enzymatic or molecular analysis by 3 French laboratories.
Results: since the 1980s, 66 French cases have been collecte. According to these results, French MCADD's prevalence would be 1.1/million illustrating a probably large number of undiagnosed cases. Diagnosis was performed during acute decompensation in 86%, as sibling of a newly diagnosed case in 14%. Median age at diagnosis was 14 months. Clinical presentation was mostly hypoketotic hypoglycemia, liver involvement, and Reye syndrome. After 165-months mean follow-up, 72% present full recovery from attack, 10% have neurological impairment, 18% died. Death rate was 63% before 3 months, 6% between 3 months and 3 years and 0% after 3 years. Death was always observed during the first metabolic attack. All patients screened after a diagnosis in sibship remained asymptomatic after 118-months mean follow-up.
Conclusion: Ay present in France, MCADD is a severe under diagnosed disease, with high mortality and neurological sequelae.

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THE HUMAN MEDIUM-CHAIN ACYL-COA DEHYDROGENASE P.G377V PROTEIN: A NOVEL DISEASE-CAUSING MUTANT AFFECTING MITOCHONDRIAL FATTY ACID BETA-OXIDATION

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Medium-chain acyl-CoA dehydrogenase deficiency (MCADD, OMIM 201450) is the commonest fatty acid β-oxidation disorder and the most prevalent inborn error of metabolism in Portugal (1:8,804). It results from the compromise of the homotetrameric flavoprotein MCAD (EC 1.3.99.3). More than 60% of clinically manifested MCADD patients are homozygous for a single mutation the p. K304E (c. 985A>G).
We have recently genotyped a cohort of 60 MCADD patients. During this study we found in heterozygosity with the p.K304E mutation a previously unreported variant p.G377V (c.1205 G>T). In order to infer the disease-causing effect of this novel mutant we developed an E.coli expression system to produce a recombinant form of the p.G377V protein. This novel mutant showed 27% residual activity when compared with the hMCADwt recombinant protein. These results are consistent with the close proximity of residue 377 to the enzyme's substrate (Glu376) and FAD binding sites, the latter essential for dimerisation, and thus prove the disease-causing effect of the novel mutation. The developed expression system constitutes a powerful tool for the characterization of novel hMCAD mutants and open the way to the study of potential activity modulators of hMCAD variants aiming at the development of effective therapeutic approaches for this inherited metabolic disease.

P-219

LCHADD VERSUS MCADD IN RUSSIA

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Background: MCADD deficiency is the most common disorder of fatty acid oxidation (1 in 15,000 live births in Western Europe). A single gene change, K304E, accounts for 90% of MCADD cases. The frequency of LCHADD is 1 in 70,000 live births in Western Europe. 60–86% of reported patients with isolated LCHAD deficiency have a prevalent E474Q mutation.

Objectives: Following the recent diagnosis of 25 patients with b-oxidation defects (17 among them with LCHADD and 4 with MCADD) within 2 years of selective screening in our laboratory, we investigated the molecular features of LCHADD and MCADD in Russian patients.

Patients and Methods: DNA extracted from dried blood spots of 21 patients and 792 randomized derived from newborns from Moscow city were studied.

Results: All patients with LCHADD except one were homozygous for the mutation E474Q and all patients with MCADD were homozygous for the K304E mutation. 5/792 blood spots from newborns were heterozygous for the E474Q mutation , 2/722 blood spot heterozygous for the K304E and gives estimated newborn incidence of 1/84000 for LCHADD and 1/640000 for MCADD.

Conclusion: LCHAD deficiency is prevalent among other disorders of mitochondrial b-oxidation and MCADD has unexpectedly low incidence in the Russian population.

P-220

ESSENTIAL FATTY ACIDS IN PATIENTS WITH VERY LONG CHAIN ACYL-COA DEHYDROGENASE DEFICIENCY

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Background: Very long chain acyl-CoA dehydrogenase deficiency (VLCADD) is a disorder of long chain fatty acid beta-oxidation, that compromises energy homeostasis and leads to accumulation of long-chain fatty acids and derivatives. Patients may develop hypoglycemia, rhabdomyolysis, hepatomegaly and cardiomyopathy. The main goal of treatment is to avoid catabolism. In addition, many patients use a long chain fatty acid restricted diet. Because essential fatty acids (EFA) are long chain fatty acids, those patient may be at risk for developing EFA deficiency.

Methods: We retrospectively studied EFA measurements performed in erythrocytes of Dutch VLCADD patients on long chain fatty acid restricted diet, via gas-chromatography.

Results: 25 blood samples of 11 patients were available for analysis. Eight patients (18/25 samples) had decreased levels of linoleic acid (C18:2n-6). Alpha-linoleic acid (C18:3n-3) was not decreased in any of the patients (0/25). Elevated mead acid (C20:3n-9), a putative marker for EFA deficiency, was found in only one sample.

Conclusion: VLCADD patients are more prone to become EFA deficient, especially for linoleic acid. Metabolic consultants should be aware of this possibility and supplement EFA's. Furthermore, although patients were EFA deficient, mead acid did not increase. Mead acid might therefore not be a good marker for EFA deficiency.

P-221

PREIMPLANTATION DIAGNOSIS OF LONG-CHAIN 3HYDROXYACYL-COA DEHYDROGENASE DEFICIENCY (LCHAD)

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In our centre we have been providing preimplantation genetic diagnosis (PGD) since 2007. Up to now we have accomplished 117 IVF cycles followed by PGD in 40 genetic diseases. The success rate of the procedure (calculated on the fetal heart beat pregnancy) is around 43%.

The case report descibes PGD in a 39 year old woman. Her son from a consanguineous marriage (r=1/8) has LCHAD deficiency. Molecular analysis in the child showed homozygosity for the 1528 G-C mutation in the HADHA gene which maps to 2p23 (MIM ID 609016). Both parents are heterozygous for the 1528 G-C mutation and the risk of recurrence for their children is 25%.

We used genetic haplotyping technique by multiplex PCR on products of MDA (multiple displacement amplification) from 1 blastomere biopsied from the embryo. 9 embryos were biopsied, 4 of them were wild homozygotes, 4 of them were heterozygotes and in 1 embryo monosomy X was confirmed. 8 of the embryos were recommended to transfer, only 1 embryo was actually transferred. The pregnancy was confirmed by ultrasound investigation. The karyotype of the foetus from the chorionic biopsy was 46,XX. Molecular analysis of HADHA gene did not confirm 1528 G-C mutation.

P-222

RIBOFLAVIN RESPONSIVE DEFECTS AFFECTING FATTY ACID OXIDATION FROM NEWBORN TO ADULTHOOD

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Background: A wide variety of patients with abnormal fatty acid oxidation have responded clinically and biochemically to riboflavin.
Case reports/results: Case 1. Day 3 female with hypothermia, hypoglycaemia, hyperkalaemia, acidosis and seizures. Plasma, CSF lactates and ammonia increased. Urinary organic acids showed increased ethylmalonate, 2-hydroxyglutarate, glutarate, adipate & suberylglycine. Hexanoylgycine was 39 μmol/mmol creat (ref. <1.1) and dimethylglycine was 760 μmol/mmol creat (ref. <16) suggesting MADD. Plasma acylcarnitines showed a marked generalised increase in C6 to C18. [Mother's biochemistry was normal]. Five days of riboflavin (100 mg/day) resulted in normal acylcarnitines, organic acids (hexanoyglycine: 1.0) and clinical improvement. Dimethylglycine decreased to 67 after 3 months. Normal fibroblast fat oxidation in riboflavin replete medium.
Patient was well at 7 months of age.
Case 2. 37 year female with adult onset fibromyalgia, wheelchair bound with seizures and acidosis. With initially low plasma carnitine, after supplementation increased C6 to C18 acylcarnitines were noted. Increased urinary 2-hydroxyglutarate, isobutyrylglycine, isovalerylglycine and hexanoylglycine prompted riboflavin therapy. After 3 days on riboflavin organic acids normalised with hexanoylglycine reduced from 7.4 to 1.3 μmol/mmol creat. Acylcarnitines showed significant but temporary reduction. Some clinical improvement has been evident.
Conclusions: These cases demonstrate the variety of riboflavin responsive disorders.

P-223

EMETIC TOXIN OF BACILLUS CEREUS THAT ASSOCIATES WITH REYE-LIKE SYNDROME SEVERELY INHIBITS MITOCHONDRIAL FATTY ACID OXIDATION

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Background: Bacillus cereus occasionally causes diarrheic and emetic food poisoning. It was reported that cereulide, emetic toxin of Bacillus cereus, is associated with encephalopathy and Reye-like syndrome that resemble fatty acid oxidation (FAO) disorders. However, the underlying mechanism for Reye-like syndrome associated with Bacillus cereus is not known. Herein, the effect of cereulide on FAO was determined.
Materials and Methods: Control fibroblasts were cultured in the presence or absence of cereulide along with octanoic or palmitic acid as substrate. FAO capacity was determined by in vitro probe assay and tandem-mass-spectrometry.
Results: Octanoic or palmitic acid led to an increase of acetylcarnitine (C2) without causing accumulation of medium (C4, C6, C8 and C10) and long (C12, C14 and C16) chain acylcarnitines in the cells cultured without cereulide. In contrast, cereulide decreased C2 and markedly increased C4, C6, C8 or C16 compared to cells treated with octanoic or palmitic acid alone.
Conclusion: The acylcarnitine profile in the presence of cereulide was similar to neonatal multiple acyl-CoA dehydrogenase deficiency. The results indicate that cereulide severely inhibits mitochondrial FAO toward a broad range of acyl-CoAs. Malfunction of FAO resulting from intoxication of cereulide may be responsible for Reye-like syndrome associated with Bacillus cereus.

P-224

THREE CASES OF FAT OXIDATION DISORDERS CAUSING SUDDEN UNEXPECTED DEATH IN INFANCY THAT DEMONSTRATE THE IMPORTANCE OF SAMPLE TIMING POST MORTEM

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Investigation of a fat oxidation disorder by acylcarnitine analysis is common practice in the differential diagnosis of sudden unexpected death in infancy (SUDI). Taking samples as soon as possible after death minimises post mortem artefactual changes. We describe three recent cases that illustrate the benefit of a clear and well implemented SUDI policy by contrasting findings with samples taken at autopsy:
Case-1: A male infant collapsed and died on day 2. Bloodspots taken immediately after death showed increased long chain acylcarnitines; those at autopsy were far less suggestive of a fat oxidation defect. Carnitine acylcarnitine translocase deficiency was later confirmed by fibroblast studies.
Case-2: A 16 month old girl, initially found to have an isolated increase of urinary suberylglycine, but with normal bloodspot acylcarnitines, died unexpectedly of respiratory failure. Bloodspots taken at the time of death show increases in short and medium chain acylcarnitines but samples collected at autopsy were difficult to interpret. Investigations into a possible riboflavin transporter defect are ongoing.
Case-3: A one month old male infant died suddenly. Bloodspot acylcarnitine analysis showed a deficiency of long chain acylcarnitines (notably C16, C18:1 and C18) and normal free carnitine. Subsequent fibroblast studies confirmed carnitine palmitoyl transferase type 1 deficiency.

P-225

EVALUATION OF FATTY ACID OXIDATION DEFECTS USING LYMPHOCYTES LOADED WITH STABLE-ISOTOPE LABELED FATTY ACID

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Background: Acylcarnitine analysis using serum samples or dried blood spots does not always provide diagnostic results to evaluate fatty acid oxidation defect. Instead, we analyzed acylcarnitines in lymphocytes loaded with stable-isotope labeled fatty acid for the diagnosis of long-chain fatty acid oxidation defects.
Methods: [2H31] palmitic acid was added to glucose-free PBS solution of lymphocytes, which were collected from 3 ml of heparinized blood. The mixture was incubated for 2 hours, and washed lymphocytes were homogenized and centrifuged. Acylcarnitines in an aliquot of the supernatant, spiked with stable-isotope labeled acylcarnitines as internal standards, were analyzed by tandem mass spectrometry.
Results: In lymphocytes loaded with [2H31]palmitic acid, a series of labeled acylcarnitines, from 2H31C16- to 2H1C2-acylcarnitine (d1C2AC), were detected. The ratios of d1C2AC/d31C16AC in patients with long-chain fatty acid oxidation defects, except for CPT-1 deficiency, were decreased as compared with those in controls. Decreased ratios of d27C14AC/d31C16AC were observed in patients with CPT-2 deficiency, decreased ratios of d23C12AC/d27C14AC in patients with VLCAD deficiency, and increased ratios of d29C16OHAC/d31C16AC in patients with TFP deficiency, respectively.
Conclusion: This simple test gives us diagnostic information for long-chain fatty acid oxidation defects before mutation analysis, and is useful in newborn screening by tandem mass spectrometry.

P-226

RELEVANCE OF ENZYMATIC CONFIRMATION IN FATTY ACID OXIDATION DEFECTS IDENTIFIED BY NEWBORN SCREENING

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Background: Fatty acid oxidation defects are part of newborn screening programs worldwide. Since then disease incidences have significantly increased. New genotypes are identified with unknown clinical relevance.
Methods: We performed octanoyl-CoA and palmitoyl-CoA oxidation studies in lymphocytes of newborns with acylcarnitine profiles suggestive for medium-chain acyl-CoA dehydrogenase (MCAD) or very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency, respectively. In all individuals with residual activities <50%, molecular analysis of the respective gene was performed.
Results: For MCADD, two mutations were found in patients with residual activities of 0–49%. Patients with the prevalent c.985A>G mutation presented with residual activities of 0–8%, compound heterozygotes with c.199 T>C on one allele had activities of 28–49%. Heterozygotes carrying the c.199 T>C mutation presented with residual activities of 70–100 % as controls carrying no mutation.
In VLCADD, we identified two mutations in patients with activities of 0–22%. The majority presented with residual activities <10%. The mild c.848 T>C mutation resulted in activities of 10–13%, the c.339 C>A mutation was associated with a residual activity >20%.
Conclusion: Our functional studies identify patients with mutations that are clinically not relevant, such as the known c199T>C mutation in the ACADM gene and allow prognostic predictions in these disorders known for their molecular heterogeneity.

P-227

CARDIAC INVOLVEMENT IN CHILDREN WITH FATTY ACID OXIDATION DISORDERS

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Background: Fatty acid oxidation disorders (FAOD) are group of inborn errors of metabolism. The most common cardiac abnormality are cardiomyopathy, arrhythmias, conduction anomalies, pericardial effusion and sudden death.
The aim of the study was to describe the cardiac involvement in children with FAOD from Wielkopolska Region of Poland.
Material and Methods: Cardiac evaluation was done in 12 patients with FAOD (VLCAD-2, LCHAD-5, MCAD-2, SCAD-1, primary carnitine deficiency-2) compared to 30 healthy controls by ECG, Holter-ECG, standard TTE, TDI and real time 4D.
Results: Standard TTE revealed abnormalities in 8 patients: hypertrophic cardiomyopathy in 5, mild TI in 1, pericardial effusion in 2. Arrhythmia was observed in 2 cases. Additionaly in 2 patients congenital heart defects were diagnosed. Compared to controls, indexes of LV systolic and diastolic function of FAOD patients differed significantly, with reduced EF, FS as well as MV and TV E/A ratio, also MAPSE and TAPSE of LV and RV were significantly reduced. FAOD patients showed a reduction in PW-TDI measurements of lateral mitral annulus. In 2 patients reverse of cardiomyopathy have been observed.
Conclusions: Cardiac involved is common in children with FAOD. Asymptomatic children with FAOD have an increased prevalence of subclinical LV dysfunction

P-228

FATTY ACID OXIDATION DISORDERS IN ADULTS: A MULTICENTRE UK REVIEW OF 34 PATIENTS

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Background: Most fatty acid oxidation disorders (FAOD) present in childhood with cardiac or liver involvement, but exercise intolerance, myalgia, muscle weakness, and attacks of rhabdomyolysis may characterise presentation in adulthood.
Objectives: To report the clinical, biochemical and genetic findings in adults with FAOD (either diagnosed initially in adulthood (n=15), or surviving childhood (n=19)).
Methods: Clinical, biochemical and genetic data for 34 unrelated adult patients, aged 16 to 60 years, followed at four UK metabolic centres, were reviewed. Patients with MCAD deficiency were excluded.
Results: Patients with CPT2 (17/34), VLCAD (7/34), LCHAD (3/34), PCD (3/34), MAD (2/34) and CPT1 deficiencies (2/34) were reviewed. Attacks of rhabdomyolysis were the most frequent manifestation of CPT2 and VLCAD deficiencies. Although muscle symptoms were rare in other defects, fixed muscle weakness and multisystem involvement were seen. In this cohort, acylcarnitine profile was frequently though not always indicative of the FAOD, with cultured skin fibroblast assays and/or identification of a pathogenic mutation confirming the diagnosis.
Conclusions: FAOD presenting in adulthood may result in delayed diagnosis. Diagnosis can be achieved without resorting to invasive muscle biopsy. Recognition is crucial to allow appropriate management. Long-term outcome of childhood survivors is generally good, but many remain symptomatic.

P-229

OCTANOIC ACID ACTS AS A TOXIN IN BRAIN OF YOUNG RATS

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Background: Medium-chain acyl-CoA dehydrogenase deficiency (MCADD) is the most frequent fatty acid oxidation disorder. Patients present tissue accumulation of the medium-chain fatty acids octanoic (OA) and decanoic acids. Clinically, progressive encephalopathy, drowsiness and lethargy that may develop into coma and death are found.
Objective: Since the pathophysiology of the brain injury in MCADD is uncertain, in the present work we investigated the effect of intracerebroventricular OA administration on oxidative stress parameters in cerebral cortex, striatum, hippocampus and cerebral spinal fluid (CSF) of rats.
Material and Methods: Animals received a single intracerebroventricular OA injection (1.66 μmol). Control animals received artificial cerebral spinal fluid in the same volume. Animals were killed 1 h after OA administration. Thiobarbituric acid-reactive species (TBA-RS) levels, carbonyl content and superoxide dismutase (SOD) and catalase activities were evaluated.
Results: It was observed that OA increased TBA-RS levels and carbonyl content in all tissues tested. In addition, catalase activity was increased only in cerebral cortex and hippocampus. On the other hand, OA administration did not alter SOD activity.
Conclusion: Taken together, these data suggest that OA induces oxidative stress in brain. Our results may help to explain, at least in part, the characteristic brain dysfunction observed in MCADD patients.

A-016

FATTY ACID OXIDATION IN KUWAIT: A CASE SERIES AND REVIEW

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Background: fatty acid oxidation disorders (FAOD) are rare autosomal recessive inherited metabolic conditions.This paper will highlight the clues that can be obtained from history, clinical examination, and simple bedside tests characteristic for FAOD.
Method: records of 15 patients admitted between 2000 to 2003 and diagnosed as FAOD were reviewed.
Results: The final diagnosis was very long chain acyl-CoA dehydrogenase deficiency in ten patients and long chain 3-hydroxyacyl-CoA dehydrogenase deficiency in five. Eighty percent had a positive family history of either a previous sudden unexplained infant death or a similar diagnosis in a first-degree relative. History of consanguinity was positive in eleven patients. Diagnosis of FAOD can be difficult because patients tend to develop symptoms only during prolonged fasting and intercurrent acute illnesses. In our series seven patients presented with acute illness and four patients presented with respiratory distress, four had heart failure due to cardiomyopathy and another two presented with convulsions due to severe metabolic acidosis. Physical findings are usually non-specific, eight had hepatomegaly and four had marked hypotonia.
Conclusion: Our group of 15 cases accumulated within 3 years is inordinately large and suggest that Kuwait provides a promising venue in which to study the biochemical and molecular genetics of FAOD.

A-017

FATTY ACID OXIDATION DEFECT, DETECTED USING SELECTIVE SCREENING

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Background: Selective screening for Inborn Error of Metabolism was conducted among patients of pediatric clinics. Children were observed intensive care units with acute intoxication, hypoglycemia, resistant to therapy epilepsy, coma, and liver damage

Methods: Clinical observation and selective metabolic screening was performed in the Kharkiv Specialised Medical Genetic Centre: blood amino acids (HPLC, Waters), urine organic acids (GS/MS, Agilent), biochemical blood indexes, ammonia, lactate levels were measured. TMS investigation from Guthrie cards was performed in other countries.

Results: An 8 month old boy was observed by the geneticist in the intensive care unit. Clinical presentation included severe gastroenteritis, diarrhea, functional liver and kidney disorder, severe lactic acidosis, respiratory violations (artificial ventilation), fatty hepatosis seen on ultrasound observation, hypertrophic cardiopathy, hypoglycemia during infectious diseases. We suspected an inborn error of metabolism in the child. TMS analyses from the DBS sample were performed. The levels of long chain hydroxylated acylcarnitines and tetradecadienoylcarnitine (C14:2) were increased. The organic acid profile showed increased levels of suberic, 3-OH-suberic and sebacic acids. The levels of lactic acid and liver enzymes in blood were increased. Long-chain 3-hydroxyacyl-CoA dehydrogenase/Mitochondrial trifunctional protein deficiency was dignosed.

O-040

ATP SYNTHASE DEFICIENCY: DIAGNOSTIC STRATEGIES FOR NOT SUCH AN UNCOMMON CAUSE OF OXPHOS DYSFUNCTION

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Background: Until recently, ATP synthase deficiency was considered as a relatively rare cause of OXPHOS dysfunction, mainly caused by pathogenic mtDNA alterations. The last two years an emerging group of patients with complex V defects of nuclear origin, mostly TMEM70 mutations, have been reported. However, technical difficulties for evaluating complex Vactivity and the heterogeneous clinical picture of patients seriously hamper correct identification.

Methods: A diagnostic strategy combining spectrophotometrical analysis, blue native polyacrylamide gel electrophoresis (BN-PAGE) followed by in-gel activity staining, western blotting and diverse microscopic techniques was developed.

Results: We report on a selection of complex V deficient patients harbouring pathogenic mutations in either mtDNA or nuDNA. Patients with mtDNA alterations usually have normal complex V activities measured by spectrophotometric analysis and show complex V subcomplexes in the BN-PAGE gel. Complex V deficiency due to nuDNA mutations results in a significant decrease in complex V activity using spectrophotometric techniques. The amount of detectable holocomplex V with BN-PAGE or western blotting is very low. Microscopic analysis in these patients shows aberrant mitochondrial morphology and severely decreased complex V immunoreactivities.

Conclusion: Deficiencies of complex V either from mtDNA or nuDNA origin can efficiently be identified by combining several diagnostic techniques.

O-041

RESTORATION OF IMPAIRED NITRIC OXIDE PRODUCTION IN MELAS SYNDROME WITH CITRULLINE AND ARGININE SUPPLEMENTATION

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Background: MELAS syndrome is one of the most common mitochondrial disorders. Mitochondrial proliferation may result in endothelial dysfunction and decreased nitric oxide (NO) availability leading to stroke-like episodes. This study aimed to assess NO production in adults with MELAS and the effect of oral supplementation with the NO precursors arginine and citrulline.

Methods: Using stable isotope infusion techniques, we measured NO synthesis, amongst other variables, in healthy adults and subjects with MELAS before and after arginine or citrulline supplementation.

Results: Adults with MELAS had lower NO synthesis rate, plasma arginine and citrulline concentrations, citrulline flux, and de novo arginine synthesis. In subjects with MELAS, arginine and, to a greater extent, citrulline supplementation increased the de novo arginine synthesis rate, the plasma concentrations and flux of arginine and citrulline, and NO production.

Conclusions: The lower NO production in subjects with MELAS is primarily due to decreased de novo arginine synthesis secondary to decreased citrulline availability. De novo arginine synthesis increased markedly with citrulline supplementation, explaining the superior efficacy of citrulline in increasing NO production. The improvement in NO production with arginine or citrulline supplementation supports their use in MELAS syndrome and suggests that citrulline may have a better therapeutic efficacy.

O-042

RESVERATROL FOR THE CORRECTION OF MITOCHONDRIAL DISORDERS

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There are to date very few treatments for mitochondrial Respiratory Chain (RC) disorders and the management of patients remains largely supportive. In many cases, the RC defect is not total and some residual enzyme activity level can be measured. Thus, a therapeutic strategy aimed at stimulating residual capacities could correct partial RC deficiencies. In this context, one possible approach is to activate key factors capable to up-regulate the RC, using specific pharmacological agents. In line with this, we investigated the potential of resveratrol (RSV), a natural polyphenol compound, known to activate PGC-1alpha (Peroxisome proliferator-activated receptor Gamma Coactivator-1 alpha), to stimulate the RC. The effects of RSV were tested in fibroblasts of controls and of five Complex I (CI) deficient patients harboring mutations in 3 nuclear genes encoding CI subunits (NDUFV1, NDUFV2 and NDUFS3). Spectrophotometric experiments indicated that exposure to RSV (75 microM, 72 h) increased the CI residual enzyme activity in controls (+30%) and in 4 out of 5 CI-deficient cells (+20 to +78%). Western-blot analysis showed that RSV significantly increased (+45 to +100%) the expression levels of mutated proteins in 4 out of 5 deficient cell lines. Finally, preliminary experiments suggest that these RSV effects might involve a mitochondrial biogenesis.

O-043

THE SEARCH FOR BIOMARKERS FOR RESPIRATORY CHAIN DEFICIENCIES: A METABOLOMICS APPROACH

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Background and objectives: We recently reported on 24 statistical significant urinary organic acids from children with RCDs, disclosed through a metabolomics investigation (Reinecke, et al, Metabolomics, 2011—doi: 10.1007/s11306-011-0309-0). The objectives here are: (1) to describe the outcome of a metabolomics investigation of urinary amino acids and acyl-carnitines from RCD patients and (2) to propose a putative biosignature for RCDs.
Material and Methods: The urinary samples came largely from a previous patient group (Smuts, et al, J. Inherit. Metab. Dis., 2010—doi:10.1007/s10545-009-9031-8). The methods were: GC-MS analysis for the amino acids and tandem-MS for the acyl-carnitines. Statistical analysis included standard univariate analyses as well as principal component (PCA), partial least-square discriminant (PLS-DA) and a new method of concurrent class (CONCA) analyses.
Results and Conclusions: From the metabolomics analysis a putative biosignature could be formulated which comprised of 16 metabolites: lactic acid, two Krebs-cycle intermediates, four organic acids from fatty acid catabolism, seven amino acids and two acyl-carnitines. This biosignature should still be validated with more RCD cases, but is already implementable as a screening instrument to establish the need for biopsying patients for confirmative enzyme analyses on RCDs as well as for monitoring of treatment.

O-044

KEARNS-SAYRE SYNDROME CAUSED BY MUTATIONS IN THE NUCLEAR RRM2B GENE

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Background: Mutations in the RRM2B gene encoding the ribonucleotide reductase (RNR) p53R2 subunit were initially reported to cause childhood-onset mitochondrial DNA (mtDNA) depletion syndrome.
Objectives: To determine the frequency of RRM2B mutations in adults with multiple mtDNA deletions, and to report the association of RRM2B mutations with Kearns-Sayre syndrome (KSS).
Patients and Methods: RRM2B gene sequence analysis was performed in 50 adult patients with multiple mtDNA deletions in skeletal muscle, in whom mutations in known mtDNA maintenance genes (POLG and C10orf2) had been excluded. Functional studies of RNR protein included Blue-Native gel electrophoresis (BN-PAGE) and Western blot analysis.
Results: A patient with KSS had two novel missense mutations in RRM2B. BN-PAGE demonstrated reduced heterotetrameric R1/p53R2 RNR levels, despite normal p53R2 levels on Western blot, suggesting failed assembly of functional RNR as a potential disease mechanism. Another patient, who presented with late-onset progressive external ophthalmoplegia and fatigue, had a heterozygous 3 bp deletion in RRM2B.
Conclusions: 4% of our cohort had RRM2B mutations. KSS has not previously been linked to nuclear gene defects; we now show that disease pathogenesis may be caused by defective RNR assembly. RRM2B mutations should be considered in the differential diagnosis of multiple mtDNA deletions presenting in adults.

O-045

FAT SUPPLEMENTATION SLOWS DOWN DISEASE PROGRESSION IN COMPLEX I DEFICIENT-HARLEQUIN MICE

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Background: Complex I (CI) deficiency is the most frequent cause of oxidative phosphorylation (OXPHOS) defect. X-linked AIF-deficient Harlequin (Hq) mouse is a faithful model for OXPHOS defect, with tissues-specific CI defect and phenotype closely resembling that of CI deficient-patients. No therapeutic solutions are available except "neutraceuticals" among which high-fat diet (HFD) has been proposed but never evaluated.
Objectives: To evaluate the impact of HFD on disease progression in CI-defective mice.
Methods: Hemizygous (Hq/Y) males were obtained by mating Hq/X females with control males and the diets given from weaning. Control diet was 8 KCal% in fat and isocaloric HFD 30 Kcal%. From one to six months, animals were monthly evaluated using an extensive battery of phenotype characterization: rotarod (indirect cerebellar ataxia evaluation), grip-test (muscular strength) and hindlimb clasping (HL, neurodegeneration marker).
Results: HFD-treated Hq mice exhibited a significantly less severe loss of rotarod scores from one to six months (45 points of loss on average, n=21) when compared to Hq mice fed the control diet (100 points of loss, n=20). There was also a tendency to better HL-scores in HFD-fed mice.
Conclusion: Isocaloric HFD slows down clinical neurodegeneration in CI-defective mice. This strongly favors its use in CI-defective patients.

O-046

PHENYLBUTYRATE THERAPY FOR PYRUVATE DEHYDROGENASE DEFICIENCY

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Deficiency of pyruvate dehydrogenase complex (PDHC) is the most common disorder leading to lactic acidemia. Phosphorylation of specific serine residues of the E1-alpha; subunit of the PDHC by pyruvate dehydrogenase kinase (PDK) inactivates the enzyme, whereas dephosphorylation restores PDHC activity. We recently found that phenylbutyrate prevents phosphorylation of the E1-alpha subunit of the branched-chain ketoacid dehydrogenase complex (BCKDC) and reduces plasma concentrations of neurotoxic branched chain amino acids in patients with maple syrup urine disease (MSUD), due to the deficiency of BCKDC. We hypothesized that, similarly to BCKDC, phenylbutyrate enhances PDHC enzymatic activity by increasing the portion of unphosphorylated enzyme. To test this hypothesis, we treated wild-type human fibroblasts at different concentrations of phenylbutyrate and found that it reduces the levels of phosphorylated E1-alpha; as compared to untreated cells. To investigate the effect of phenylbutyrate in vivo, we administered phenylbutyrate to C57B6 wild-type mice and we detected a significant increase in Pdhc enzyme activity and a reduction of phosphorylated E1-alpha; subunit in brains and muscles as compared to saline treated mice. Being a drug already approved for human use, phenylbutyrate has great potential for increasing the residual enzymatic activity of PDHC and to improve the clinical phenotype of PDHC deficiency.

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THE PHOSPHORESCENCE OXYGEN ANALYZER AS A SCREENING TOOL FOR DISORDERS WITH IMPAIRED LYMPHOCYTE BIOENERGETICS

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This study aimed to show feasibility of using the phosphorescence oxygen analyzer to screen for disorders with impaired cellular bioenergetics. [O2] was determined as function of time from the phosphorescence decay of Pd (II) meso-tetra-(4-sulfonatophenyl)-tetrabenzoporphyrin. In sealed vials, oxygen consumption by peripheral blood mononuclear cells was linear with time, confirming its zero-order kinetics. The rate of respiration (mean ±SD, in μM O2 per min per 107 cells, set as the negative of the slope of [O2] vs. time) for adults was 2.1±0.8 (n=18), for children 2.0±0.9 (n=20), and for fetuses 0.8±0.4 (n=18), $p < 0.0001$. For an 8-year-old patient with reduced muscle NADH dehydrogenase and pyruvate dehydrogenase activities, the rate was 0.7±0.2 (n=3) μM O2 per min per 107 cells. For a 3-month-old patient with hepatocerebral mitochondrial DNA depletion syndrome (confirmed mutation in the MPV17 gene), the rate was 0.6 μM O2 per min per 107 cells. For two siblings with reduced cytochrome oxidase on muscle neurochemistry, the rates were 5.5 and 3.0 μM O2 per min per 107 cells. This novel approach allows non-invasive (preliminary) assessment of cellular bioenergetics.

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FLUORESCENT VISUALIZATION OF THE MITOCHONDRIAL MEMBRANE POTENTIAL GRADIENT IDENTIFIES OXIDATIVE PHOSPHORYLATION DEFECTS IN FIBROBLASTS

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Background: The process of oxidative phosphorylation (OXPHOS) establishes a mitochondrial membrane potential gradient (DY) which is utilized by ATP synthase to generate cellular energy.

Methods: We developed a microscopic method to evaluate DY in cultured fibroblasts using the fluorescent compound JC-1. We tested our method on controls and OXPHOS deficient cell lines.

Results: DY was significantly lower in fibroblasts from patients with isolated and combined OXPHOS complex I to IV defects, while DY values were maintained in patients with ATP synthase defects. DY reduction correlated with severity of the defect and with the percentages of heteroplasmic mtDNA mutations. Cell lines displayed differential vulnerability to rotenone. Also, this complex I inhibitor induced perinuclear mitochondrial relocalization accompanied by cytoskeletal changes. In our set-up, the complex IV inhibitor KCN had not such an effect but caused cell death when used in high concentrations. The method was found especially efficient to visualize the heterogeneity of the defect in cells from patients with heteroplasmic mtDNA mutations.

Conclusions: We found that JC-1 fluorescent microscopy allows to detect and differentiate OXPHOS defects and thus may be employed to study fibroblasts from suspected patients.

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ONE-STEP COMPREHENSIVE MOLECULAR ANALYSES OF MITOCHONDRIAL DISORDERS BY NEXT GENERATION SEQUENCING IN A CLINICAL SETTING

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Background: Mitochondrial disorders are a group of complex diseases that can be caused by mutations in nuclear or mitochondrial genomes. Current molecular diagnosis requires multiple different and complementary methods, including sequencing, qPCR, Southern blot or array CGH, for the detection and quantification of mutations. These procedures are labor intensive, time consuming, and costly.

Methods: We have developed and validated a "deep" coverage Next Generation Sequencing (NGS) technique in clinical settings. This approach allows simultaneous analysis of a set of nuclear genes targeted to mitochondria and the whole mitochondrial genome for point mutations and deletions. We instituted proper qualitative and quantitative controls to be analyzed along with each sample for quality assurance.

Results: We demonstrated an average coverage of >500X for targeted nuclear genes and >5000X for each of the 16,569 bases of the mitochondrial genome. Nucleotide changes are correctly called with quantitative information. The limit of detection of a heteroplasmic change is calculated to be about 1.5%. Small and large insertion/deletions were correctly detected with clear breakpoints and percentage of heteroplasmy.

Conclusion: Our "deep" sequencing approach provides a one-step comprehensive molecular analysis for patients with suspicion of mitochondrial diseases in a timely, accurate, and cost-effective manner suitable for clinical application.

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IS ELEVATED CITRULLINE A MARKER OF CITRIC ACID CYCLE DEFECTS, SUCH AS LIPOAMIDE DEHYDROGENASE (E3) DEFICIENCY ?

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Introduction: During the year 2010, we've diagnosed 2 infants with Lipoamide dehydrogenase (E3) deficiency.

Both presented a metabolic crisis, drowsiness & apathy.

Both showed an elevated level of citrulline in serum or whole blood.

The ACOMG recommends considering the next disorders, as for further investigation of elevated Citrulline levels in the NBS test: Citrullinemia Types I&II, Argininosuccinic Aciduria & Pyruvate Carboxylase Deficiency.

Since introduction of Citrulline level measurement in blood, as part of the NBS tests in Israel, 5 samples were found abnormal (above 40 mmol/Ltr), of which 1 was found to be linked to E3 deficiency (thanks to Dr. Almashanu for the data).

Data is being gathered from the files of thirty E3 deficienct patients. The citrulline level of each patient is then weighed in relation to time of diagnosis & metabolic crisis, if had been present.

Possible Conclusions: We highly suspect that elevated Citrulline in the NBS tests or serum analysis test for amino acids may mark a defect in the Citric Acid Cycle, such as E3 deficiency.

A reasonable mechanism may resemble that for elevated citrulline in Pyruvate Carboxylase Deficiency: a decline in Aspartate production, as a result of a blockage in the transformation of Pyruvate to Oxaloacetate.

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LPS (LIPOPOLYSACCHARIDES) AND PREMATURITY: EFFECT ON THE ACTIVITY OF RESPIRATORY CHAIN ENZYMES IN HUMAN UMBILICAL VENOUS ENDOTHELIAL CELLS

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Objective: Fetal infection is associated with considerable perinatal morbidity. As the umbilical vein regulates fetal blood flow from the placenta dysfunction of human umbilical venous endothelial cells (HUVEC) may compromise fetal blood supply. We incubated HUVEC with one of the key inflammatory mediators, LPS (Lipopolysaccharides), and measured the activity of respiratory chain enzymes.

Material and Methods: Based on gestational age, HUVEC were divided into 1 mature and 2 premature groups from otherwise uncomplicated pregnancies (4 patients each) and incubated for one hour, six or twelve hours with LPS (100 ng/ml), respectively, incubation without LPS served as control. We analyzed the activity of respiratory chain enzymes and citratesynthase as a mitochondrial marker spectrophotometrically.

Results: No significant differences in respiratory chain activities between the LPS-incubated cells and internal controls could be observed. However, we could demonstrate significant differences depending on gestational age: respiratory chain complexes C I+III ($p<0.05$) and C IV ($p<0.01$) showed higher activity at lower gestational age. No such differences were observed for C II+III, C V and citratesynthase.

Conclusion: LPS on its own had little influence on the activities of mitochondrial respiratory chain enzymes. However, complex I+III and C IV activities are increased at lower gestational age.

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PLASMA CREATINE IS ELEVATED IN MITOCHONDRIAL DISORDERS: A NEW BIOMARKER FOR THE DIAGNOSIS

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The most recent technological developments allows novel tools for biomarkers identification in mitochondrial respiratory chain deficiencies (RCD) through metabolome analysis. Using this approach, it has recently been shown in RCD that an extracellular creatine excess is in relation to intracellular P-creatine depletion. Furthermore, authors found that plasma creatine, appears to be the most specific and sensitive metabolite in RCD patients, exceeding lactate and alanine in magnitude of elevation and statistical significance (Shaham et al, PNAS 2010).

In order to confirm this single report, we tested plasma creatine as potential biomarker of mitochondrial dysfunction in samples obtained from 30 patients aged between 0.2-19 yrs. with pathogenic mutation (10 patients) or abnormally low RC enzyme activity in muscle (20 patients).

Data showed that plasma creatine concentrations was significantly higher in mitochondrial patients (mean±SEM=81.1±3.2 vs. 57.7±2.0; $p<0001$) when compared with age-matched controls. In 8/30 plasma creatine clearly exceeded the upper control limit (97th percentile), and 4 of them had normal levels of plasma lactate and /or alanine on the same blood sampling.

Our study confirms that plasma creatine is elevated in RCD and can be used in combination with other biomarkers for the diagnosis of mitochondrial diseases.

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MTDNA DEPLETION INVESTIGATION OF MYOCARDIUM SAMPLES IN CHILDREN

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mtDNA depletion syndrome (MDS) includes a group of autosomal disorders, generally recessive, characterized by a severe reduction in cellular mtDNA content in affected tissues. MDS is tissue-specific, affecting mostly tissues with higher energy demanding. As the cardiac muscle is one of the most energy requiring tissues, we aimed to establish control values for mtDNA copy number in myocardium in patients with cardiac involvement.

We have analyzed mtDNA copy number in 14 samples of myocardium from paediatric patients (age: 4 days – 11 years old) with possible mitochondrial OXPHOS disease (multiple OXPHOS deficit in 13 cases) and/or heart involvement. Total DNA was extracted by standard methods. The common mtDNA mutations were excluded and mtDNA copy number was determined by real-time PCR, using SYBR green and specific primers for target genes. The intensity of fluorescent signals was analyzed by 7500 Software v2.0.4 and relative mtDNA content was calculated by deltaCt.

We performed a normal distribution and estimate 21% of cases (median 594, min-max 40–625, compared to 1293, min-max 1039–6474 in all other samples) with mtDNA depletion.

The present work is an important contribution for gathering reference values in myocardium samples, given the scarcity of data in literature, namely in children.

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DETECTION OF MITOCHONDRIOPATHY IN PATIENTS WITH RESPIRATORY CHAIN ENZYME ACTIVITIES IN MUSCLE BIOPSY WITHIN NORMAL RANGE

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In 322 samples out of 921 muscle biopsies measured in Klinikum Schwabing between 2005 and 2009 a defect of respiration chain was found (35.0%). Isolated complex I defect was detected most frequently (118=36.6%), followed by combined complex I and IV defect (66= 20.5%).

In 139 out of the 701 genetically investigated samples a positive result was found. In 35 cases (25.2%) mtDNA deletions and in 29 cases (20.9%) a depletion of mtDNA was detected. Interestingly within the 20 deletion positive cases also measured for respiratory chain activity in 13 cases no defect was detected (65 %). In contrast, within the 19 depletion positive samples measured for respiratory chain in only 3 cases no defect was found (16 %).

In 253 samples genetic testing was performed initially without investigation of the respiratory chain activity due to clinical symptoms or histological findings. In 72 (28.5%) of these cases a diagnosis could be established.

Our results show the high value of the muscle biopsy in the elucidation of mitochondrial disease. However a normal respiratory chain activity in skeletal muscle can not rule out mitochondrial disease. From our experience testing for mtDNA deletions or depletion should be the initial step.

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IMPAIRED MITOCHONDRIAL FATTY ACID METABOLISM IN TAFAZZIN-DEFICIENT MICE: IMPLICATIONS FOR HUMAN BARTH SYNDROME

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Background: Barth syndrome is an X-linked genetic disorder presenting with cardiac and skeletal myopathies and caused by inborn errors in the tafazzin gene, resulting in defects in cardiolipin (CL) metabolism. CL is a unique mitochondrial phospholipid that is essential for assembly of electron-transport chain (ETC) and multi-enzyme complexes.
The objective of this study is to elucidate the involvement of CL in the interaction of fatty acid oxidation (FAO) enzymes with mitochondrial ETC complexes.
Methods: We investigated the effects of CL-deficiency on mitochondrial trifunctional protein (mTFP) interaction with ETC complex I in cardiac muscle of tafazzin knockdown mice.
Results: Cardiolipin deficiency resulted in cardiac dysfunction, mitochondrial abnormalities, excessive autophagy and impaired energetics in tafazzin-knockdown mice. We found that cardiolipin-deficiency destabilizes the interaction of mTFP with ETC complex I. Analysis of extracellular fluxes revealed impaired utilization of palmitate as an energy substrate for mitochondrial FAO in tafazzin-deficient cardiomyocytes.
Conclusion: Cardiolipin is essential for the normal physical association of TFP with ETC Complex I in cardiac mitochondria. Dissociation of TFP from ETC Complex I adversely affects the efficiency of fatty acid oxidation and aerobic energy production in heart. Impaired FAO may be one of the many pathogenic factors in Barth syndrome patients.

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EARLY DETECTION OF CARDIAC CHANGES IN TWO PATIENTS WITH BARTH SYNDROME BY FETAL ECHOCARDIOGRAM

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Background: Barth syndrome (BTHS)(OMIM302060)or 3-Methylglutaconic aciduria type II, is a rare x-linked recessive disorder. It characterized by dilated cardiomyopathy, skeletal myopathy, cyclic neutropenia and elevated 3-methylglutaconic and 3 methylglutric acide in the urine. Variable clinical phenotypes being reported.
Methods: Total of 3 patients, presenting with the constellation of findings suggestive of BTHS. Two had early evaluation by fetal echocardiogram (Echo).The diagnosis in all cases confirmed at the biochemical (urine gas chromatography-mass spectrometry (GC/MS)) and molecular level (TAZ gene sequencing).Periodic complete blood count, lactic acid, and Echo were performed.
Results: Two families with 3 affected children; two patients were picked up early by abnormal fetal echocardiogram. The third presented with recurrent infection in infancy and failure to thrive. Subsequently all were found to have cardiomyopathy, cyclic neutropenia and the characteristic urine organic acid changes. Molecular analysis revealed that affected patients and their mothers were having hemizygous and heterozygous changes respectively in Taz gene.
Conclusions: This is the first report of Saudi patients with Barth syndrome and their genotype.
Early detection of cardiomyopathy by fetal echocardiogram and high index of suspicion would prevent unnecessary investigations and aid to better management in patients with BTHS.

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A NEW UPLC-MS/MS CARDIOLIPIN ASSAY FOR THE DIAGNOSIS OF BARTH SYNDROME

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Background: Diagnosis of Barth syndrome (BTHS) is difficult as clinical and biochemical features are variable. Measurement of increased monolysocardiolipin (MLCL)/cardiolipin (CL) ratio and decreased CL have been described as reliable diagnostic tests using analysis by normal phase HPLC with chloroform mobile phase; however chloroform is incompatible with UPLC.
Objective: To develop a diagnostic test for BTHS using a new UPLC cardiolipin assay, avoiding chloroform mobile phase.
Methods: Internal standard (CL (14:0)4) was added to leukocyte preparations and phospholipids extracted. Analysis was performed on a Waters Acquity UPLC Premier XE system with a BEH C8 column using a gradient of water and methanol containing 0.1% ammonium hydroxide. MRM analysis used the following m/z transitions: 723.8>279.4, CL (18:2)4; 582.5>281.3, MLCL (18:1)2(16:0); 619.7>227.3 (internal standard).
Subjects: Samples from 19 BTHS patients and 61 controls were analysed.
Results: 17/19 BTHS patients had low CL (<14.5 nmol/mg protein, controls >114 nmol/mg protein). All BTHS patients had increased MLCL (18:1)2(16:0)/CL ratio (>0.07, controls <0.005).
Conclusion: A new reversed-phase UPLC method for CL and MLCL has been developed which is suitable for routine use. Calculation of the MLCL/CL ratio distinguishes patients with BTHS from controls with 100 % sensitivity and specificity, making this a reliable diagnostic test.

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CASE REPORT: SPONGIFORM LEUKOENCEPHALOPATHY CAUSED BY NDUFV1 MUTATIONS

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We report a 12 year old boy, who presented at 3 months of age with intractable seizures, optic atrophy, hypertonia, hyperreflexia in the lower extremities with clonus, and areflexia in the upper extremities. Family history was normal. Progressive cystic changes of the white matter noted on sequential MRIs was classified as a neurodegenerative spongiform leukoencephalopathy of unknown etiology. MRS demonstrated a large lactate peak and low NAA. Over the next 5 years, development was static, limited to smiling responsively. Muscle biopsy was done despite the MRI changes not considered classical of mitochondrial disease. Complex I deficiency was demonstrated and sequencing for known complex 1 genes available at that time including NDUFS 3, 4, 6, 7 failed to delineate the molecular etiology. A healthy brother was born unaffected suggesting recessive rather than maternal inheritance. In 2010, molecular analysis of NDUFV1 was pursued and the patient was found to have two heterozygous mutations: one previously reported in homozygous state in two siblings with similar presentations and MRI changes. The second mutation is a novel splice site mutation. This case report illustrates the importance of considering Complex 1 disease and in particular NDUFV1 molecular analysis in children with spongiform leukoencephalopathy.

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CASE REPORT: VARIABLE HISTOCHEMICAL FINDINGS IN TWO SIBLINGS WITH SENGERS SYNDROME

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We report on two siblings, born to consanguineous Pakistani parents, with Sengers syndrome. The proband presented at 15 months of age following a cardio-respiratory arrest and was found to have hypertrophic cardiomyopathy with severely impaired cardiac function requiring ECMO support. The history revealed bilateral cataract surgery at 3 months and gross motor delay noted at 12 months. After assessment for cardiac transplantation eligibility, cardio-respiratory support was withdrawn. The mother was 32 weeks pregnant at the time and delivered a newborn male, noted to have bilateral cataracts at 1 week of age, lactic acidosis and hypertrophic cardiomyopathy at 8 weeks of age. The histochemical studies in the proband showed scattered COX negative fibers (previously unreported in Sengers syndrome) and pleomorphic mitochondria with abnormal structure. Respiratory chain complex activities were atypically all severely reduced. Histochemical studies in the sibling showed normal COX staining, but an increased number of morphologically abnormal mitochondria. The sibling had reduced respiratory chain complex activities reminiscent of, but less severe than, the proband. The sibling has reached 15 months of age and has had few symptoms, although his cardiomyopathy is progressive. This case report illustrates intra-familial variability of histochemical findings for two siblings with the same clinical phenotype.

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TWO NOVEL COMPOUND HETEROZYGOUS MUTATIONS IN THE TWINKLE HELICASE (C10ORF2) GENE CAUSING EARLY ONSET MITOCHONDRIAL ENCEPHALOMYOPATHY

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Background: Twinkle (C10orf2) gene is involved in mitochondrial-DNA maintenance because of its mtDNA helicase function. Mutations in the gene result in autosomal dominant adult onset progressive external ophthalmoplegia and autosomal recessive early onset hepatoencephalopathy phenotypes. Additionally autosomal recessive mutations in the gene cause infantile onset spinocerebellar ataxia manifesting with ataxia after first year of life following a normal development.

Case presentation and results: This 4 year-old girl, born to non-consanguine Chinese parents, presented with global developmental delay within the first 6 months of life. She was evaluated for sensorial-neural hearing loss, ataxia, ophthalmoplegia and muscle weakness at age 3 years. In her physical examination, her growth parameters were at 50th percentile. There was no organomegaly. Her muscle tone was decreased. Deep tendon reflexes were not elicitable. She had marked ataxia and marked restriction in all gaze directions of each eye. Peripheral nerve conduction study showed generalized sensory-motor neuropathy. Her cranial MRI and MR-spectroscopy were normal. Her liver function tests were normal. She had novel compound heterozygous (c.1279 G>A; p.E427K and c.1838A>G; p.D613G) missense mutations in the TWINKLE Helicase (C10orf2) gene.

Conclusion: This is a patient with early onset mitochondrial encephalomyopathy caused by mitochondrial depletion in the TWINKLE Helicase (C10orf2) gene.

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BIOCHEMICAL AND MOLECULAR ANALYSIS IN MITOCHONDRIAL COMPLEX I DEFICIENT CHILDREN

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Background: Mitochondrial complex I assembly is an intricate process which involves incorporating 45 subunit proteins, 1 flavin mononucleotide and 8 iron–sulphur clusters into the final ~980 kDa holoenzyme. Mutations in any of the 45 subunits or in an unknown number of assembly factors and chaperones may cause complex I deficiency.

Objectives: To facilitate genetic screening in complex I deficiency by implementing biochemical and genetic approaches.

Patients and Methods: 30 complex I deficient paediatric patients with heterogeneous clinical phenotypes were studied. Complex I deficiency and assembly state were analysed using spectrophotometric assay and Blue Native gel electrophoresis. Candidate gene analysis and homozygosity mapping were used to search for the underlying gene defect.

Results: Abnormal complex I subassemblies accumulated in 3 cases: defects in NDUFS4 and a known assembly factor were causative in two cases, whereas the third defect is still unknown. Homozygosity mapping and bioinformatics analysis helped to identify a mutation in the novel complex I chaperone FOXRED1 in a fourth case.

Conclusion: By using a combined biochemical and genetic approach we have found the underlying molecular defect in 13/30 [43%] of this complex I deficient patient cohort; further studies are ongoing to identify the genetic defects in the remaining patients.

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PALMITATE PROTECTS COMPLEX I-DEFICIENT FIBROBLASTS FROM DEATH

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Background: Complex I (CI) deficiency is the most frequent cause of oxidative phosphorylation (OXPHOS) defect. No therapeutic solutions are available except "neutraceuticals" among which high-fat diet (HFD) has been proposed but never evaluated.
Objectives: To evaluate the effect of the long-chain fatty acid palmitate on human CI-deficient fibroblasts viability.
Methods: Fibroblasts were obtained from three CI-defective patients genetically identified: two with NDUFS1 mutations and one with NDUFS4 mutations. They were cultured either in glucose- and glutamine-enriched DMEM, or in glucose-free, glutamine-enriched DMEM or in glucose-free, glutamine-, palmitate-enriched DMEM. Cell viability was evaluated daily using an automated cell counter. Succinate oxidation was assessed by polarography. Complex II activity was measured by spectrophotometry.
Results: In one of the NDUFS-1 mutated cell line, death occurred within 80 hours after glucose withdrawal with full protection afforded by palmitate. In the two other CI-defective cell lines, death occurred within 170 hours after glucose withdrawal with full palmitate protection. The full protection from death provided by palmitate was associated with a slight but consistent increase (20–30%)in respiratory chain complex II activity.
Conclusion: These data show that palmitate protects CI-deficient fibroblasts from death and provide a strong rationale for performing HFD trials in CI-deficiency.

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"DOUBLE TROUBLE OR DIGENIC DISORDER IN COMPLEX I DEFICIENCY

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Complex I (CI) deficiency is a defect of OXPHOS caused by mutations in the mitochondrial or nuclear genomes. To date disease-causing mutations have been reported in all mitochondrial-encoded subunits and 21 nuclear genes. In about 50% of the patients no mutations are found, suggesting that undiscovered factors are an important cause of disease.
In this study we report a consanguineous family from Southern Portugal with three affected children where CI deficiency could not be clarified.
The affected children presented similar clinical findings with early onset of the disease having two of the patients a fatal outcome. A reduced activity of CI was detected in two of the patients, which led us to investigate, at the molecular level, some CI associated genes. However, no mutations were detected. Interestingly, all patients presented 3-methylglutaconic acid in the urinary organic acids and it is known that POLG gene is involved in the etiology of these syndromes. In only one of the patients, that also showed mtDNA depletion, the p.G848S/p.Q1236H mutations were found in POLG. It remains unsolved if this family due to the high consanguinity could have two different disorders or if a yet unknown gene, leading to complex I deficiency, could be involved.

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A NOVEL MUTATION IN BCS1L IN A PATIENT WITH AN ISOLATED MITOCHONDRIAL COMPLEX III DEFICIENCY

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Background: Isolated complex III deficiencies are caused by mutations in the mitochondrial CytB gene, in the BCS1L gene coding for a CIII assembly factor and in the UQCRQ gene that codes for the ubiquinone binding protein of complex III.
Objective: Description of clinical features, mitochondrial function and molecular genetic analysis in a patient with an isolated complex III deficiency.
Patient: A 17 year old boy, born to consanguineous parents who presented with hypoglycemia, glycosuria, deafness, growth retardation, Fanconi Syndrome and severe lactic acidosis in the neonatal period.
Methods: Activities and assembly of OXPHOS complexes were investigated spectrophotometrically and by BN-PAGE. mt-DNA was screened for deletions. Cytochrome b (CytB) and the BCS1L gene were sequenced.
Results: Isolated complex III deficiency was detected in the patient's skeletal muscle. Using BN-PAGE blotting a complex III of lower molecular weight was detected. Staining the 2D reveals a missing subunit. No mutation was detected in the mitochondrial CytB gene. Sequence analysis of BCS1L revealed a novel homozygous point mutation p.M48V.
Conclusion: The patients decreased complex III activity is most likely caused by incomplete assembly of complex III due to the homozygous p. M48V mutation in the BCS1L gene.

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FATAL HEART FAILURE ASSOCIATED WITH COQ10 AND MULTIPLE OXPHOS DEFICIENCY IN A CHILD WITH PROPIONIC ACIDEMIA

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The role of a secondary respiratory chain deficiency as an additional mechanism to intoxication, leading to development of long-term energy-dependent complications, has been recently suggested in patients with propionic acidemia (PA). We show for the first time a coenzyme Q10 (CoQ10) functional defect accompanied by a multiple organ oxidative phosphorylation (OXPHOS) deficiency in a child who succumbed to acute heart failure in the absence of metabolic stress. Quinone-dependent activities in the liver (complex I+III, complex II+III) were reduced, suggesting a decrease in electron transfer related to the quinone pool. The restoration of complex II+III activity after addition of exogenous ubiquinone to the assay system suggests CoQ10 deficiency. Nevertheless, we disposed of insufficient material to perform direct measurement of CoQ10 content in the patient's liver. Death occurred before biochemical diagnosis of OXPHOS deficiency could be made. However, this case highlights the usefulness of rapidly identifying CoQ10 defects secondary to PA since this OXPHOS disorder has a good treatment response which could improve heart complications or prevent their appearance. Nevertheless, further studies will be necessary to determine whether CoQ10 treatment can be useful in PA complications linked to CoQ10 deficiency.

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ESTABLISHMENT OF A NEURONAL CELL MODEL OF COENZYME Q10 DEFICIENCY: IMPLICATIONS FOR THE PATHOGENESIS OF DEFECTS IN COENZYME Q10 BIOSYNTHESIS

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Background: Coenzyme Q10 (CoQ10) deficiency is a rare but often treatable condition. In view of the encephalopathic presentation of this condition the aim of this project was to establish a neuronal cell model of CoQ10 deficiency in cultured neuroblastoma, SHSY-5Y cells using para-aminobenzoic acid (PABA) treatment.
Methods: Treatment of SHSY-5Y cells with 1 mM PABA induced a maximal 54% (46% residual CoQ10; $p<0.01$) decrease in celluar CoQ10 status compared to control levels.
Results: A progressive decrease in complex I, II/III and IV activities was noted with a maximal inhibition observed at 46% residual CoQ10 of 50–59%. ATP production also decreased by 67.5% in comparison to control. Residual CoQ10 levels of 77% and 46% resulted in an approximately 4x increase in mitochondrial oxidative stress. A 25% decrease in mitochondrial membrane potential was observed at 77% residual CoQ10. Conversely a 40% increase was observed at 46% residual CoQ10 compared to the controls suggesting a possible reversal of ATP synthase (complex V).
Conclusions: This cellular model has provided insight into the effect of CoQ10 deficiency on neuronal ETC function and oxidative stress and will enable the efficacy of potential treatments to restore mitochondrial function. This project is funded by Ataxia UK (www.ataxia.org.uk).

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INFANTILE MYOCLONIC EPILEPSY, AND NEPHROTIC SYNDROME: PRIMARY COENZYME Q10 DEFICIENCY WITH MUTATION IN PARA-HYDROXYBENZOATE-POLYPRENYL TRANSFERASE (COQ2).

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Objective: coenzyme Q10 (CoQ10) deficiency due to mutation in the COQ2 with a new clinical presentation.
Case report: At the age of 3 weeks, patient developed myoclonic seizures. Both plasma and CSF lactate were increased. Brain MRI was normal and muscle biopsy performed at 2 months of age did not disclose morphological or OXPHOS abnormality. Despite supplementation with riboflavin, biotin, pyridoxine and CoQ10 and several anti-epileptic drugs he continued to have partial and myoclonic seizures, evolving into epilepsia partialis continua. At 5 months he developed a nephrotic syndrome and CoQ10 was increased to 30 mg/kg /day but he expired.
Results: Renal biopsy showed focal segmental glomerulosclerosis and on ultrastructural examination enlarged podocytes with hyaline cytoplasmic vacuoles. Respiratory chain activities of renal cortex demonstrated decreased activities of complexes II+III. A homozygous mutation c.326 G>A (p. Ser109Asn) in exon 2 of CoQ2 gene was found. Both parents were heterozygous carriers. CoQ10 concentration in fibroblasts was 16nmolCoQ/gr prot (mean 67).
Conclusion: In contrast to previous reports of COQ2 nephropathy, in this case, kidney was not the only target. Therefore, this report extends in COQ2 mutation causing primary CoQ10 deficiency, the spectrum of the phenotype of severe early myoclonic epilepsy, subsequently nephrotic syndrome.

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COENZYME Q10 RESPONSIVE ATAXIA: 2-YEAR-TREATMENT FOLLOW-UP

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Background: Coenzyme Q10 (CoQ) deficiency syndromes are a heterogeneous groups of diseases that may show a good clinical outcome after CoQ supplementation.
Objectives: We assessed the clinical outcome after CoQ therapy in 14 patients presenting ataxia classified into two groups according to CoQ values in muscle (deficient or not).
Material and Methods: We performed an open-label prospective study: patients were evaluated clinically (ICARS scale, MRI and video-tape registration) at baseline and every 6 months during a period of 2 years after CoQ10 treatment (30 mg/Kg/day).
Results: Patients with CoQ10 deficiency showed a statistically significant reduction of ICARS scores (Wilcoxon test: $p=0.018$) after 2 years of CoQ10 treatment as compared to baseline conditions. In patients without CoQ10 deficiency, no statistically significant differences were observed in total ICARS scores after therapy, although one patient from this group showed a remarkable clinical amelioration.
Conclusions: Biochemical diagnosis of CoQ deficiency was a useful tool for the selection of patients who are good candidates for treatment, since all of them responded to therapy. However, the remarkable clinical response in one case without CoQ deficiency highlights the importance of treatment trials for identification of patients with CoQ-responsive ataxia.

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MULTIPLE MITOCHONDRIAL ELECTRON TRANSPORT CHAIN ENZYME DEFICIENCIES ASSOCIATED WITH A DECREASE IN SKELETAL MUSCLE COENZYME Q10 STATUS

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Coenzyme Q10 (CoQ10) functions as an electron carrier in the mitochondrial electron transport chain (ETC) as well as serving as a potent lipid soluble antioxidant. Diagnosis of a CoQ10 deficiency normally follows a finding of decreased activity of the linked ETC complex II+III and/or I-III which utilise endogenous CoQ10 and normal activities of complex I, II and complex III. Diagnosis is confirmed by HPLC analysis of muscle CoQ10. However, since all ETC complexes are susceptible to free radical induced oxidative damage a deficit in CoQ10 status may in theory result in multiple ETC deficiencies. In order to investigate this hypothesis we assessed the muscle CoQ10 status of 12 patients with combined ETC I (0.075±0.006; ref interval: 0.104-0268), II-III (0.023±0.004 ; ref interval: 0.040–0.204) and IV (0.006±0.001; ref interval: 0.014–0.034) deficiencies of as yet unknown genetic cause. ETC activities expressed as a ratio to citrate synthase activity. On assessment, 8 of the 12 patients showed evidence of decreased muscle CoQ10 status (82.0±16.6 pmol/mg; ref interval: 140–580 pmol/mg).
In conclusion, evidence of decreased muscle CoQ10 status has been detected in a subset of patients with multiple ETC deficiencies. These results may have therapeutic implications.

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EFFECT OF TREATMENT BY L-CARNITINE AND COENZYME Q10 ON EXERCISE TEST IN A WOMAN WITH MYOPATHY RELATED TO RESPIRATORY CHAIN DEFICIENCY

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We report the case of a 32 years woman in whom an exercise test as been useful for the diagnosis of mitochondrial related myopathy and for the evaluation of the treatment efficiency. This patient presented abnormal fatigability who was later shown to be related with a multiple respiratory chain deficiency on muscle biopsy. An homoplasmic mutation (m.8322 T>C) in the tRNALysine gene has been found in this patient. Initial incremental exercise test demonstrated a pathological increase in peak serum lactate (9251 μmol.L-1), consistent with a predominant glycolytic anaerobic activity. Following the diagnosis confirmation (muscle biopsy, genetic) a treatment by L-Carnitine and Co-Q10 was initiated. A second exercise test was performed 4 months later (same protocol). It showed an increase in aerobic capacity, resulting in a greater maximal work rate exercise (96% vs 85% of predicted), higher oxygen uptake at peak (96% vs 89%) and at anaerobic threshold (71% vs 51%). In contrast, no change in peak serum lactate level was assessed. Larger studies are needed to confirm whether such treatment could lead to significant improvement of exercise performance.

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DIVERSITY OF MTDNA MUTATIONS AMONG RUSSIAN CHILDREN WITH LEIGH SYNDROME

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Mitochondrial diseases are a type of neurodegenerative disorders which are associated with mitochondrial respiratory chain deficiency in muscles and in a wide variety of tissues. They are caused by mutations in nuclear or mitochondrial genome, what leads to extremely heterogeneous clinical manifestation of these diseases. A dramatic example of such disorder is Leigh syndrome which mostly has onset at first years of life and frequently induced by mutations in SURF1 gene or T8993C/G substitution in mitochondrial DNA (mtDNA). However there's a wide range of patients with rare mutations in mtDNA. The etiology of a mutation and a proportion of mutant mtDNA in a cell define the severity of a disease. Thus, point mutations in mtDNA can lead to a clinical polymorphism, thereby complicating the finding of a causative mutation. We performed mtDNA sequence analysis of a group of patients (c. 50) with Leigh/Leigh-like phenotype. A total 8 different substitutions were identified. Among these, 3 unknown mutations (C3945A, G8839C, T14441C) were found. Also 5 other mutations were identified: G3697A, T8362G, G8363A, T13094C, G13513A. On a base of our results we've developed a specific primers panel for MLPA analysis which allows us to hasten a search of mutations in mtDNA.

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ANESTHETIC CONSIDERATIONS IN MITOCHONDRIAL ENCEPHALOMYOPATHY LACTIC ACIDOSIS AND STROKE LIKE EPISODES SYNDROME (MELAS). A CASE SERIES

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MELAS could progresses to multiorgan pathology and early death. Impaired integration of pyruvate into Krebs cycle predisposes to lacticademia and increased perioperative risk. Controversy exists regarding increased susceptibility to certain anesthetics and malignant hyperthermia. Avoidance of lactated intravenous fluids (LR) was advocated because of impaired lactate metabolism. We describe outcomes of MELAS patients who underwent surgery at Mayo Clinic, USA. Perioperative data of confirmed MELAS patients were retrospectively characterized. 9 patients underwent anesthesia for 20 surgeries: general anesthesia with volatile anesthetics in 14; propofol—3; NMDA—14; succinylcholine—4. Lack of need for excessive amounts NMDA was not suggestive for resistance. Recovery from NDMR blockade appeared uneventful. Most patients were receiving levetiracetam/topiramate which do not alter response to NMDA. One had severe myopathy, succinylcholine was not used. MH triggering agents were routinely used without evidence of MH. Intraoperative LR in 13 cases did not result in acid-base decompensation. MELAS patients did not show aberrant response to muscle relaxants and to anesthesia management in this study. Response to muscle relaxants may depend on interplay between medications/antiepileptics and myopathy. NMDA should be dosed with aid of neuromuscular monitoring. Alterations of acid-base balance not encountered despite administration of LR.

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BIOCHEMICAL PARAMETERS TO ASSESS CHOROID PLEXUS DYSFUNCTION IN KEARNS-SAYRE SYNDROME

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Background: Kearns-Sayre syndrome (KSS) is a mitochondrial disorder presenting as main biochemical findings high CSF protein and low 5-methyltetrahydrofolate (5-MTHF) values, which reflect impaired transport across choroid plexus. Other compounds may also be affected. Our aim was to assess different biochemical parameters that may detect choroid plexus dysfunction in KSS patients.
Methods: We studied 7 patients genetically diagnosed with KSS. The measured parameters in CSF were total proteins, 5-MTHF, homovanillic acid (HVA) and Selenium (Se) concentrations.
Results: Together with high CSF Se values, increased CSF HVA and total protein concentrations and decreased CSF 5-MTHF values were observed in all cases. This pattern was only detected in the 7 KSS patients of 1,850 CSF samples analyzed in our laboratory during the last 6 years.
Conclusions: The application of these biochemical analyses may allow early identification of new cases with undiagnosed KSS. These impaired metabolites seem very specific and may represent a good biochemical model for evaluating choroid plexus dysfunction. The accumulated Se in CSF might have cause toxicity effects or increased dopamine turnover. This last feature would be reflected by increased CSF HVA (marker of dopamine turnover). The association between Se and HVA and its possible clinical implications deserve further investigation.

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AN EVER EXPANDING MOLECULAR AND CLINICAL SPECTRUM OF POLG REVEALED BY THE STUDY OF A LARGE PATIENT COHORT

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Background: Mutations in the POLG gene have emerged as one of the most common causes of autosomal inherited mitochondrial diseases in children and adults, with a broad clinical spectrum.
Objective: To report the molecular results from 2700 individuals suspected of having POLG related disorders.
Method: The coding regions of POLG gene were sequenced by Sanger method.
Results: Mutations in 136 unrelated families (5%) have been identified. Among them, 92 had two recessive pathogenic mutations and 3 harbored a dominant mutation. A total of 25 novel mutations were discovered, including a de novo p.Y951N dominant mutation in a patient with distal neuropathy. The 92 patients with two mutations presented with an extremely broad spectrum of diseases. Almost all patients have some degree of neuropathy, including developmental delay, dementia/encephalopathy, regression, peripheral neuropathy, seizures, ataxia, or headache/migraines. Seizures, hepatopathy, and lactic acidemia are predominantly in younger patients. By comparison, adult patients have a higher percentage of myopathy, sensory ataxia, and CPEO/ptosis.
Conclusion: POLG mutations account for a broad clinical spectrum of mitochondrial disorders. Sequence analysis of the POLG gene should be considered as a part of routine screening test for the diagnosis of mitochondrial disorders, in the absence of mitochondrial DNA abnormalities.

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TISSUE COPPER LEVELS IN CHILDREN CARRYING MUTATIONS IN SCO1, SCO2 AND SURF1

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Human cytochrome c oxidase (COX) contains two copper centers CuA and CuB that are essential for enzyme catalytic activity. Aim of our study was to analyze copper content and COX activity and amount in muscle, liver, heart and brain tissue obtained at autopsy from 17 children with mutations in SURF1 (7x), SCO2 (9x) and SCO1 (1x) genes. Copper content was analyzed by atomic absorption spectrometry. COX activity and amount were analyzed by spectrophotometric and immunoelectroforetic methods. The copper content was more than 4-fold reduced in SCO2 and SURF1 liver in comparison with age related controls and more than 3-fold in SURF1 skeletal muscle. The severe copper deficiency of SCO1 muscle (5-fold) was accompanied by 6-fold decrease of COX activity. The SCO2 heart showed 3-fold reduction of copper content and 10-fold reduction of COX activity. The SCO2 and SURF1 frontal cortex specimens displayed either normal or borderline copper levels although all of the SCO2 and SURF1 patients suffered from severe CNS involvement. While the copper deficient phenotype of SCO1/SCO2 tissues appears consistent with the function of both gene products in regulation of cellular copper homeostasis, the severe copper deficiency of SURF1 deficient tissues remains elusive. Supported by MSM0021620806, IGA-MZ-NS 10581/3, GAUK28410

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MITOCHONDRIAL PHOSPHATE CARRIER DEFICIENCY PRESENTING AS (CARDIO-)MYOPATHY IN A FAMILY WITH THREE AFFECTED CHILDREN

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In a family three children presented with severe neonatal lactic acidosis, hypertrophic cardiomyopathy and generalized muscular hypotonia. One child died in infancy, two survived a clinically severe neonatal period. At an age of 9 and 17 years, respectively, they present with exercise intolerance, proximal muscle weakness, non progressive hypertrophic cardiomyopathy and normal mental development. In a muscle biopsy normal activity of respiratory chain enzymes were found, however the amount of the mitochondrial phosphate carrier was decreased. This protein is expressed in two tissue-specific isoforms generated by mutually exclusive alternative splicing of the SLC25A3 gene transcript. We identified a homozygous mutation c.158-9A>G located in the 5'-intron next to exon 3A specific for heart and skeletal muscle. This creates a novel splice site resulting in a more than 95% decrease of the wild type allele.

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REVERSIBLE MITOCHONDRIAL LIVER DISEASE WITH TRMU GENE MUTATIONS

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Although early onset mitochondrial liver disease is often rapidly fatal, there is spontaneous reversal in a small number of cases and these have a good long term prognosis. Identification of causative genetic defects in these patients has an important bearing on management decisions. A female neonate presented on day 6 with poor feeding, weight loss, jaundice and lactic acidosis. Serum transaminases were elevated and there was a coagulopathy. Liver histology showed distorted architecture and necrosis. Cytochrome oxidase activity in liver and muscle was profoundly reduced. However, by 3 months, she was progressing well and liver function, blood lactate and muscle cytochrome oxidase activity had all normalised. She is now developing appropriately. Two pathogenic mutations, c.835 G>A (V279M) and IVS11-3, C>G, were identified in the TRMU gene. This gene encodes an enzyme involved in mitochondrial tRNA modification and was initially recognised as a modifier of deafness due to the mtDNA A1555G mutation. Recently a small number of infants have been reported with mutations in this gene and reversible liver disease. It is therefore important to screen the TRMU gene in patients with early onset liver disease and lactic acidosis to identify those who will be expected to have a benign course.

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PYRUVATE DEHYDROGENASE E3 BINDING PROTEIN DEFICIENCY ASSOCIATED WITH PROLONGED SURVIVAL, DYSTONIA AND BEHAVIOURAL DISTURBANCES

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Deficiency of the pyruvate dehydrogenase E3 binding protein (E3BP) was identified in two sisters, the offspring of consanguineous Portuguese parents. The sisters, currently aged 17 and 27 years, both have episodic weakness and painful dystonic posturing of the torso and limbs associated with intercurrent illnesses. In addition, the older sib is prone to self-mutilation and aggressive outbursts, whilst the younger girl has an anxiety disorder. Dichloroacetate and a ketogenic diet appeared to be of some benefit, but triheptanoin oil was not tolerated. Both sisters have non-progressive moderate to severe learning disability and modest elevations of blood and CSF lactate. Fibroblast pyruvate dehydrogenase activity was significantly reduced in both girls and there was a homozygous base substitution in the splice acceptor site of intron 7 of the PDHX gene, resulting in skipping of exon 8 and generation of a premature stop codon. Patients with complete E3BP deficiency have some residual enzyme activity, which may allow prolonged survival, although not usually to the extent seen in these sisters. A dystonic movement disorder developing during childhood is increasingly being recognised as a major manifestation of less acute presentations of pyruvate dehydrogenase deficiency, particularly those involving the E2 and E3BP subunits of the complex.

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BIOCHEMICAL CHANGES IN PATIENTS WITH PYRUVATE DEHYDROGENASE DEFICIENCY

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Pyruvate dehydrogenase complex (PDC) deficiency is one of the most common neurodegenerative disorders associated with abnormal mitochondrial metabolism. The key feature is gray matter degeneration with foci of necrosis and capillary proliferation in the brainstem. Lactic acidosis with normal/low Lactate/Pyruvate is the main biochemical marker. The most common cause is mutations in the X-linked E1 alpha gene (PDH1 gene).
Two girls with mutation c.904 C>T in the 10th exon of PDH1 gene have been diagnosed in Estonia. Before enzymatic/mutation analysis, organic and amino acids analysis in serum and urine were performed to determine lactate, pyruvate and alanine. For both analysis in serum protein was precipitated using 10% sulfosalicylic acid (0.25 ml for 1 ml of serum).
Lactate and pyruvate in serum were elevated: 3400/372 μmol/l and 4060/1152 μmol/l (normal 3300/160 μmol/l). L/P was relatively low (< 10). Alanine in serum was elevated only in pt. I—829 μmol/l (N<495). In urine lactate, pyruvate and alanine were elevated in pt. II—4209 (N<151), 5146 (N<130) and 358 (N<254) mmol/mol creatinine.
Conclusion: In both cases lactate and pyruvate were elevated in serum (L> 3000 μmol/l, P>300 μmol/l) with low L/P<10, which is specific to PDH deficiency.

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THE RELATIONSHIP BETWEEN THE PYRUVATE DEHYDROGENASE COMPLEX AND THE MITOCHONDRIAL RESPIRATORY CHAIN

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Background: Defects of both the pyruvate dehydrogenase complex and the mitochondrial respiratory chain are well recognised. It has been observed that patients with disease affecting the mitochondrial respiratory chain may have secondary defects of the pyruvate dehydrogenase complex, and vice versa. The interaction between these two systems is not fully understood.
Aims: To identify how common it is to find deficiencies in both assays, to determine if it is possible to establish which is the likely primary defect and to comment on possible pathological mechanisms.
Methods: The pyruvate dehydrogenase complex and mitochondrial respiratory chain assay results of patients from the north of England from January 1994 to December 2009 were matched.
Results: The final data set was from January 1999 to December 2009. In total two hundred and thirty-nine pyruvate dehydrogenase complex assays were performed. Eighty-five were abnormal of which seventeen also had an abnormal mitochondrial respiratory complex. The majority had a complex I deficiency and, in all groups, the majority presented with a Leigh like illness.
Conclusions: Advances in laboratory techniques means that more patients now receive a genetic diagnosis; this allows determination of the primary pathology and aids counseling.

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THE DIFFICULTY IN THE DIAGNOSIS OF PYRUVATE DEHYDROGENASE COMPLEX DEFICIENCY IN 6 HETEROZYGOUS FEMALES WITH NOVEL PDHA1 MUTATIONS

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We report on 6 female patients with pyruvate dehydrogenase complex (PDHC) deficiency and a heterogeneous clinical and biochemical presentation. In all patients functional investigations of the mitochondrial energy metabolism were performed. The oxidation rates of pyruvate substrates were clearly decreased in 3/6 and mildly decreased in another 2/6. One patient had normal activities of the substrate oxidation rates. The activity of PDHC, however, was reduced only in 2 of the patients. Remarkably western blot analysis showed a reduced amount of E1α in relation to the other PDHC subunit in all patients. Analysis of PDHA1 gene expression showed an expression of the mutated allele in the range of 45-75% in the muscle biopsies of our patients. Sequence analysis of the X chromosomal PDHA1 gene revealed 4 novel missense mutations at conserved positions, 1 novel insertion-deletion and 1 large deletion, which also affected several neighbouring genes. All mutations were heterozygous. Heterozygous X-chromosomal diseases are a diagnostic challenge since the severity of the diseases depends on X-inactivation, which can be heterogeneous in different tissues. Concerning biochemical investigations 83% of the patients were detected by functional investigations of intact mitochondria while PDHC enzyme activity was decreased only in 33%.

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DEFECTS IN THE OXIDATION OF PYRUVATE DUE TO NOVEL DEFICIENCIES IN COFACTOR METABOLISM

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Investigation of the mitochondrial energy metabolism from intact mitochondria by radiochemical substrate oxidation reveals defects of the pyruvate oxidation (PO) which is seen in an abnormal ratio of the oxidation of pyruvate+malate versus acetylcarnitine+malate. In approximately 3/4 of these patients defects of either one of the subunits of pyruvate dehydrogenase or of the regulatory phosphatases could be identified. Evaluation of the cofactor status in the remaining patients revealed an at least 4-fold decreased amount of thiamine pyrophosphate in the muscle of patients from 3 unrelated families. The clinical course of the 5 patients was characterised by metabolic crises after the first year of life with mild lactic acidosis. MRI showed a Leigh-like picture and lactate elevation in spectroscopy. Two of the patients died in infancy. Investigation of the known disease causing genes of the thiamine metabolism did not reveal pathogenic mutations. In another patient with reduced PO a severe deficiency of the lipoic acid prosthetic group was found. This patient presented with neonatal seizures. Brain sonography showed the development of a severe generalised multicystic encephalopathy. Echocardiography revealed an enlargement of the ventricular septum. He died at the age of 4 years.

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EARLY CEREBRAL MRI AND NEUROPATHOLOGICAL CHANGES IN PYRUVATE CARBOXYLASE DEFICIENCY PATIENTS HOMOZYGOUS FOR THE C.1828 G>A MUTATION

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Pyruvate carboxylase (PC) deficiency is an autosomal recessive disease with three subtypes. Patients homozygous for the c.1828 G>A mutation in the PC gene belong to Type A, which typically has infantile-onset of developmental delay, hypotonia, and moderate lactic acidemia. We report the early brain MRI abnormalities of three patients homozygous for the c.1828 G>A mutation at 3 days, 4 days and 2 months of life respectively. The patients had bilateral cystic abnormalities in differing locations of the brain. One patient also developed diffuse brain edema. Neuropathology is available from two additional patients who died at 11 and 32 months. One patient had mild ventriculomegaly, and focal cavitation of the right caudate and right cerebellar white matter. The other patient had scattered multifocal demyelination and gliosis of the cerebral white matter. Although our patients have identical genotype and phenotype, the areas of neurologic injury in PC deficiency are diverse and varied. Our patients experience repeated episodes of severe metabolic decompensation, leading to profound developmental delay, independent of time of treatment implementation, frequency of metabolic decompensation and presence or absence of seizures. Despite the later clinical disease onset of the c.1828 G>A mutation, neurological injury can be observed on neuroimaging in the neonatal period.

P-267

3-METHYLGLUTACONIC ACIDURIA—LESSONS FROM NEARLY 50 GENES AND MORE THAN 900 PATIENTS

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Elevated urinary excretion of 3-methylglutaconic acid (3-MGA) is considered rare in patients suspected of a metabolic disorder. In 3-MGA-uria I, 3-MGA stems from leucine degradation. In all other types mitochondrial dysfunction is thought to be the common denominator. We investigated the clinical, metabolic and genetic data of 386 patients with 3-MGA-uria. 3-MGA-uria was often found in correlation with disorders not reported earlier in association with 3-MGA-uria (organic acidurias, urea cycle disorders, haematological/neuromuscular disorders). Mitochondrial dysfunction was indeed the common denominator for most of the patients. But, also a disturbed cholesterol biosynthesis can lead to 3-MGA-uria (Smith-Lemli-Opitz syndrome, glycogen storage disorder). Furthermore, we investigated 597 patients with mutations in 48 mitochondria-associated genes, thus having genetically proven mitochondrial disorders. 8.3% of these patients presented 3-MGA-uria. We show, that it was frequently seen in Complex V related disorders, in patients with mitochondrial DNA depletion or Pearson syndrome. Deficiencies of complex I and mitochondrial translational defects were less frequently associated with 3-MGA-uria, which was also never reported in association with deficiencies of the complexes II–IV. With these data we will improve the diagnostic approach to the patient with 3-MGA-uria in general and type IV in particular. The paper further reclassifies the 3-MGA-urias.

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CLINICAL PHENOTYPE OF TWO SPANISH PATIENTS WITH 3-METHYLGLUTACONIC ACIDURIA TYPE IV AND TMEM 70 MUTATIONS

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Background: Nuclear genetic defects in TMEM70 gene have been described in a subgroup of patients with 3-methylglutaconic aciduria type IV showing ATP synthase deficiency. Clinical presentation includes: cardiomyopathy, nervous system involvement and lactic acidosis. We report the clinical findings of two Spanish patients with TMEM70 mutations.

Case reports: Patient 1 is the first son of healthy consanguineous parents diagnosed of intrauterine growth retardation and hypertrophic cardiomyopathy in neonatal period. He showed lactic acidemia with increased L/P ratio and 3-methylglutaconic aciduria, normal mitochondrial respiratory chain activities and normal brain MRI. He has developed: special facial phenotype, mild growth and psychomotor retardation and multiple decompensations. He is homozygous for c.317-2A>G mutation.

Patient 2 is the second daughter of healthy consanguineous parents born premature after intrauterine growth retardation. She presented acute deterioration with lactic acidosis, increased L/P ratio and 3-methylglutaconic aciduria. Mitochondrial respiratory chain activities showed deficiency of complex II+III. Brain MRI showed corpus callosum dysgenesis and cerebellar atrophy. She has developed: special facial phenotype, mild growth and psychomotor retardation, hypertrophic cardiomyopathy and multiple decompensations. She is compound heterozygous for the common c.317-2A>G and a new c.211-450_317-568del mutations.

Conclusion: Patients with 3-methylglutaconic aciduria and cardiomyopathy should be screened for TMEM70 mutations.

P-269

HEPATOCEREBRAL FORM OF MDS. DIAGNOSTIC DIFFICULTIES

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Today new diagnostic approaches are being developing for such "young" forms of mito diseases as mitochondrial hepatoencephalomyopathies. The algorithm for AS includes test for 4 frequent mutations (W748S, G268A, A467T, G848S) and afterwards whole POLG gene sequencing. For patients with clinical features different from AS, but still with strongly suggestion of hepatoencephalomyopathy we performed test for frequent POLG mutations and then sequencing of DGUOK and MPV17 genes. We revealed molecular defect in 9 AS patients and 5 MDS patients. The main pitfalls were: genetic heterogeneity—although AS has one gene, patients with other forms of hepatoencephalomyopathy may have mutation either in DGUOK, MPV17, POLG genes. Clinical data couldn't help us to foresee what gene is mutated; tissue specific accumulation of mtDNA depletion—we revealed only 2 patients with significant depletion of mtDNA (with DGUOK and MPV17 mutations consequently); search for 2nd allele—In our group of patients we didn't detect the second mutant allele in 4 cases (3 patients with hepatoencephalopathy and 1 patient with AS). Large POLG gene rearrangements are known being analyzed with CGH-method. 4) clinical polymorphism—differential diagnosis could be provide among several other forms if IMDs.

P-270

TISSUE-SPECIFICITY OF MITOCHONDRIAL RESPIRATORY CHAIN DISORDERS IN EACH CLINICAL DIAGNOSES

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Background: Many enzyme defects show tissue-specificity in mitochondrial respiratory deficiency (MRCD), which sometimes make difficult for diagnosis. .

Subject/Method: 158 patients were diagnosed to have MRCD out of 429 candidate patients. We classified clinically the following groups, Leigh's disease (LD), mitochondrial cytopathy (MC), neurodegenerative diseases (ND), hepatic disease(HD), cardiomyopathy (CM) and infantile mitochondrial disorder (IMD). We investigated the tissue specific pattern of enzyme defect to contribute correct diagnosis for MRCD.

Results: Clinical diagnosis of lethal infantile mitochondrial disease was the greatest in number. The number of clinical LD, MC, ND, HD, CM and non-lethal IMD was 29, 23, 9, 21, 11 and 10. In LD, 14/18, 12/15, 3/3 and 0/0 were diagnosed in fibroblasts, muscle, liver and heart, respectively. In MC, 4/11, 11/16, 4/5 and 2/4, in ND, 3/3, 5/5, 1/1 and 0/0, in HD, 1/7, 0/0, 20/20 and 1/1, in CM, 2/6, 3/5, 4/9 and 10/10, in IMD, 14/25, 17/22, 22/24 and 8/13 were diagnosed in each specimen.

Discussion: Almost HD and CM can be diagnosed only with affected organs. In other MRCD, enzyme defects can be detected in wide variety of organs and tissues, which support from many specimens can lead to greater confidence in the diagnosis achieved.

P-271

129 PATIENTS WITH NEONATAL ONSET OF MITOCHONDRIAL DISORDER: A RETROSPECTIVE STUDY

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Mitochondrial disorders (MD) may manifest in neonates, but the early diagnosis is difficult. The aims of the study were to analyse clinical and laboratory characteristics of MD with neonatal onset and identify possible association between clinical findings and specific mitochondrial diseases. Retrospective clinical and laboratory data were evaluated in 461 patients (331 families) with confirmed MD from two metabolic centers Prague and Salzburg. The neonatal onset of MD was reported in 129 patients (28%). Prematurity, intrauterine growth retardation, hypotonia necessitating ventilatory support were present in one third, cardiomyopathy in 40%, Leigh syndrome in 15% and profound lactic acidosis in half of neonates. 19 patients died in neonatal period. PDH complex deficiency was identified in 6, complex I in 15, complex III in one, complex IV in 23, complex V in 31, combined deficiency of several complexes in 53 patients. Molecular diagnosis was confirmed in 49 cases including newborns with Alpers, Barth, MELAS, MILS and Pearson syndromes, PDHc deficiency, and patients with SCO1, SCO2, TMEM70, MTATP6, ATP5E, NDUFS4, SUCLG1, and MTND1 mutations. New diagnostic algorithm was proposed. Supported by: IGA MZ NS 10561-3/2009, IGA MZ NS 9782-4/2008, IGA MZ NT 11186-5/2010

A-018

THE STUDY OF ASSOCIATION OF C677T MTHFR AND A66G MTRR POLYMORPHISMS WITH MITOCHONDRIAL DYSFUNCTION

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Objective: to study association of folate cycle polymorphisms (FCP) with mitochondrial dysfunction.
Materials and Methods: 54 patients with a primary mitochondriopathy (PM) (Kearns-Sayre, MELAS, MNGIE, MERRF, organic acidurias) and 22 patients with secondary mitochondriopathy (SM). In both groups study of polymorphisms of MTHFR C677T and A66G MTRR by method of allele-specific PCR.
Results: In 47 patients (87%) with PM were identified, compounds of allele frequencies MTHFR/MTRR as follows: N/N—7(13%); hmzg/hmzg—1 (1,9%); htrzg/htrzg—9(16.7%); htrzg/hmzg—17(31.5%); hmzg/htrzg 2 (3.7%); N/hmzg—6(11.1%); N/htrzg—6(11.1%); hmzg/N—1(1,9%); htrzg/N—5(9.3 %). In 19 (86.4%) patients with SM identified FCP, distribution of allele frequencies was as follows: N/N—3(13.6%); hmzg/hmzg—0(0%) ; htrzg/htrzg—2(9.1%); htrzg/hmzg—10(45.5%); hmzg/htrzg—0(0%); N/hmzg—3(13.6%); N/htrzg—3(13.6%) ; hmzg/N—0(0%); htrzg/N—1 (4.5%).
Conclusions: 66 (86.8%) patients with mitochondrial dysfunction had FCP and more specific to them was a polymorphism A66G MTRR—in 59 patients (77.6%) than the C677T MTHFR—in 48 patients (63.2%). This should be considered when developing an individual tactical correction energy deficit and conduct of prevention of complications associated with the presence of these polymorphisms.

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MULTIPLE RESPIRATORY CHAIN DEFECTS CAUSED BY 3-HYDROXY-ISOBUTYRYL COA HYDROLASE DEFICIENCY

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Background: Mitochondrial disorders affect ~1 in 5000 births, and frequently present with combined deficiency of multiple respiratory chain (RC) enzymes. 3-hydroxy-isobutyryl-CoA hydrolase (HIBCH) deficiency is a rare disorder of valine catabolism.
Objective: To report the association of multiple RC deficiencies with HIBCH deficiency.
Patients and Methods: Two brothers, born to healthy distantly-related parents, presented with neonatal-onset neurodegenerative disease including seizures, dystonia and developmental regression. Relentless progression led to early death in both siblings. Investigation for mitochondrial disease revealed multiple RC defects (affecting complexes I, II+III and IV) in muscle from the older brother but normal activities in his sibling. The observation of persistently elevated hydroxy-butyrylcarnitine levels in the younger sibling led to investigation of HIBCH activity.
Results: HIBCH activity was markedly reduced in cultured skin fibroblasts from both brothers. Subsequently a homozygous missense mutation affecting a highly conserved amino acid residue in the HIBCH gene was identified in both brothers. This mutation segregated with disease in the family.
Conclusion: Multiple RC deficiencies are usually thought to arise from impaired maintenance and/or expression of the mitochondrial genome. We now show that HIBCH deficiency may lead to multiple RC defects.

P-273

NEUROCHEMICAL EVIDENCE THAT PRISTANIC ACID DISRUPTS MITOCHONDRIAL HOMEOSTASIS IN BRAIN OF YOUNG RATS

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Background: Pristanic acid (Prist) concentrations are increased in peroxisomal disorders characterized by neurologic dysfunction and brain abnormalities, whose pathogenesis is poorly known.
Objectives: Prist effects on important parameters of energy homeostasis were investigated in rat brain mitochondria.
Material and methods: Purified mitochondrial preparations were obtained from brain of 30-day-old rats. Various parameters of energy homeostasis were then determined in the presence of 20–100 μM Prist.
Results: Prist markedly increased state 4 respiration and diminished the RCR using both glutamate plus malate or succinate as substrates. In addition, Prist decreased state 3 respiring mitochondria, the ADP/O ratio, the mitochondrial membrane potential and NAD(P)H levels. Prist also induced mitochondrial swelling, probably through oxidative attack to the permeability transition pore (PTP) since cyclosporine A (PTP inhibitor) and N-acetylcysteine were able to prevent this effect.
Conclusion/Discussion: The data indicate that Prist acts as an uncoupler of oxidative phosphorylation and as a metabolic inhibitor, besides causing mitochondrial swelling. It is presumed that impairment of mitochondrial homeostasis may contribute to the neurological abnormalities presented by patients affected by peroxisomal diseases in which brain Prist concentrations are increased.
Financial support: Research grants from FIPE/HCPA, CNPq, PROPESq/UFRGS, PRONEX/ FAPERGS, FINEP IBN-Net AND INCT-EN.

P-274

INHIBITION OF NA+,K+−ATPASE ACTIVITY AND MITOCHONDRIAL RESPIRATION BY PRISTANIC ACID IN DEVELOPING RAT BRAIN

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Background: Peroxisomal abnormalities are biochemically characterized by tissue accumulation of branched chain fatty acids, including pristanic acid (Prist). Affected patients usually present brain abnormalities, however the pathomechanisms of cerebral injury in these diseases are still unknown.
Objectives: We investigated the in vitro effects of Prist on Na+,K±ATPase and respiratory chain complex activities.
Material and methods: Brain of young rats was dissected and the biochemical test performed after tissue preparation.
Results: Prist markedly decreased the activities of Na+,K±ATPase (80 %) and of complexes I (65%), II (40%) and II-III (95%), without affecting complex IV activity.
Conclusion/discussion: Considering the importance of Na+,K±ATPase for the maintenance of the membrane potential necessary to normal neuronal excitability and cellular cell volume control and of oxidative phosphorylation for brain energy production, the present data indicate that Prist compromises neurotransmission and brain bioenergetics. It is presumed that these pathomechanisms may be involved in the brain damage occurring in disorders in which Prist accumulates.
Financial Support: Research grants from CNPq, PRONEX, FINEP rede Instituto Brasileiro de Neurociência (IBN-Net) and INCT-EM.

P-275

EVIDENCE THAT PRISTANIC ACID INDUCES LIPID AND PROTEIN OXIDATIVE DAMAGE AND REDUCES NON-ENZYMATIC ANTIOXIDANT DEFENSES IN RAT BRAIN

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Background: Pristanic acid (Prist) is a branched-chain fatty acid that accumulates in a variety of peroxisomal disorders. Although these disorders are characterized by neurological symptoms, the mechanisms involved in the pathophysiology are poorly known.
Objectives: We studied the in vitro effects of Prist on important parameters of oxidative stress in cerebral cortex from young rats.
Methods: Thiobarbituric acid-reactive substances (TBA-RS), carbonyl formation, sulfhydryl content, reduced glutathione (GSH) levels and nitric oxide production were measured in cerebral cortex from 30-day-old rats.
Results: Prist increased TBA-RS levels, reflecting an increase of lipid peroxidation. This effect was totally prevented by the free radical scavenger melatonin, suggesting the involvement of reactive species. Prist also provoked protein oxidative damage, as determined by increased carbonyl formation and sulfhydryl oxidation. Otherwise, it did not alter nitric oxide production. Furthermore, the concentration of GSH was significantly decreased by Prist and this decrease was prevented by melatonin and alfa-tocopherol.
Conclusions: It is therefore presumed that Prist elicits oxidative stress in the brain and that this pathomechanism may possibly be involved in the brain damage found in patients affected by peroxisomal disorders where Prist accumulates.
Financial support: CNPq, FAPERGS, PRONEX and the FINEP research grant Rede IBN-Net and INCT-EN.

P-276

X-LINKED ADRENOLEUKODYSTROPHY: IS THERE A GOOD CORRELATION BETWEEN NEUROLOGIC AND NEUROPSYCHOLOGIC IMPAIRMENT AND LOES MRI SCORE?

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Background: X-linked adrenoleukodystophy (X-ALD)(MIM 300100) results in inflammatory demyelination of the brain and spinal cord. The severity of MRI abnormality assessed with Loes score has a high predictive value.
Objective: In X-ALD localization of the initial cerebral lesion can be more predictive about the severity of neuropsychologic impairment when similar MRI scores are encountered.
Material/Methods: 28 patients with childhood cerebral X-ALD followed between 1991–2010 were studied. MR images, Loes scores; neurologic examination, X-ALD neurologic severity scale; Denver II test from 0–6 years, Wechsler Intelligence Scale for Children-Revised (WISC-R) from 6–16 years were evaluated.
Results: 28 patients were grouped according to the pattern of cerebral involvement. Groups with parietooccipital (15), frontal (4), diffuse (2), cerebellar (1), corpus callosum (3) and no involvement (3) were evaluated. Mean Loes scores were 17(3–21), 15.75(11–17), 21.5(21–22), 15, 5.6(2–12) and 0; neurologic severity scales 2.3, 3, 3.5, 3, 1.8 and 0; neurologic examination scores 8, 14, 16.5, 12, 4.3 and 0.3; WISC-R performans IQ: 65, 41, 32, 58, 89 and normal respectively. Frontal, diffuse and cerebellar involvements correlated with higher neurologic severity and lower IQ compared to parietooccipital lesions when Loes scores are similar.
Conclusion: MRI score has high predictive value with frontal/diffuse involvement indicating severity.

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PRELIMINARY FINDINGS OF A SINGLE-CENTER, OPEN-LABEL CLINICAL TRIAL OF TREATMENT OF X-LINKED ADRENOLEUKODYSTROPHY (XL-ALD) WITH LOVASTATIN AND COENZYME Q10

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Background: Biochemical evidence of the potential effectiveness of treatment of XL-ALD with lovastatin was reported by Pai et al in 2000.
Objective: To evaluate the tolerability and effectiveness of treatment of pre-symptomatic X-ALD with lovastatin/coenzyme Q10 for the prevention of acute cerebral disease.
Methods: Open-label, treatment of pre-symptomatic XL-ALD males <18 yrs old, with lovastatin, 1 mg/kg (max 40 mg) and coenzyme Q10 50–60 mg per day.
Results: Seven pre-symptomatic boys currently aged 20.3±4.0 yrs were treated with 10–40 mg lovastatin and 50–60 mg coenzyme Q10 daily from age 8.5±3.5 yrs (3.3–12.6). One developed early MRI evidence of progression at age 5 yrs and underwent hematopoietic stem cell transplantation (HSCT). A second boy developed early signs of adrenomyeloneuropathy (AMN) at age 20 yrs. Of 16 boys who were never treated with lovastatin/coenzyme Q10, 3 are deceased (mean age 9.7±1.4 yrs), 3 are asymptomatic (mean age 12 yrs), 1 has adrenal insufficiency, and 9 have severe neurological impairment (mean age 14±6.3 yrs). No effect was demonstrated on plasma very long-chain fatty acids.
Conclusion: Pre-symptomatic treatment of XL-ALD may prevent the development of acute cerebral degeneration; however, it does not appear to prevent the development of AMN. Further clinical trials are indicated.

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ADRENOLEUKODYSTROPHY X LINKED: EXPLORING THE IMPLICATION OF COPY NUMBER VARIANTS IN PHENOTYPE

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Structural variations of human genome emerge as novel major contributors to genetic diversity and disease susceptibility. Copy number variations, CNVs, are known to be involved in Mendelian disorders. Adrenoleukodystrophy X linked (XALD) is a rare disorder with two main phenotypes, a childhood form with cerebral involvement (CCALD) and a mild, adrenomieloneuropathy (AMN),adult chronic, that affects axonal tracts of the spinal cord. Both forms can be triggered by the same genetic mutation, indicating a participation of a modifier gene and or environmental factors. Therefore, to gain insight into what might be the potential source contributing to the differential phenotypes evolution we studied the CNV of genes involved in fatty acid metabolism, the inflammatory response and cell reparation.

By using Illumina SNP data, Sentrix HumanHap 1 M-SNPs, we analyzed 8 XALD patients either with CCALD and AMN pure phenotypes. For gene candidate study (34) we used the semi-quantitative screening multiplex ligament- dependent probe amplification (MLPA) applied to 41 AMN, 46 CCALD and 26 Controls from Spain and Brazil. We found variable levels of AGPTA2, ABCA2, SLC27A2, SLC25A22, PCDH17, PCDH20, PNPLA2 . The data obtained may contribute to elucidation of clinical heterogeneity in XALD patients and to design specific therapeutic approaches.

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DISTINCT INTERACTION PATTERN OF A PEX19 SPLICE VARIANT THAT IS SENSITIZED TO NMD POINTS TO COMPLEX REGULATION MECHANISMS OF PEX19 FUNCTION

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Mutations in PEX19 cause peroxisomal biogenesis disorders. PEX19 encodes a cytosolic protein that interacts with peroxisomal membrane proteins (PMPs) and functions as a cycling PMP-receptor protein. Besides full-length PEX19, the splice variant PEX19DeltaE2 accounts for a significant level of total PEX19 mRNA. Even though production of a protein was suggested by in vitro translation, PEX19DeltaE2 was shown not to complement PEX19 deficient cells.

In the present work, we quantified PEX19DeltaE2 by real-time PCR showing significant variations among tissues. Furthermore, we found an increase in PEX19DeltaE2 mRNA upon inhibition of translation suggesting a sensitization to NMD without complete degradation. Using BRET, we showed that PEX19DeltaE2 has the capacity to bind PMPs. Nevertheless, PEX19DeltaE2 shows a distinct interaction pattern to the full-length protein lacking the interaction with PEX14, PEX13, and PEX10. This finding may explain that PEX19DeltaE2 is not able to restore the import of peroxisomal matrix proteins in PEX19 deficient cells. In conclusion, PEX19DeltaE2 mRNA varies among tissues, its translation is regulated by NMD, and the protein partially retains PEX19 function. These results indicate that this PEX19 splice variant is linked to a novel regulatory mechanism.

P-280

PROLONGED SURVIVAL IN A FEMALE WITH PEROXISOMAL BIOGENESIS DISORDERS (PBD) : CASE REPORT

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We present an 18 year old female with variant Zellweger syndrome. She presented at age 5 months with hypotonia, and failure to thrive.

She was diagnosed at age 8 months on biochemical findings. Molecular analysis at 13 yrs showed compound heterozygosity for c.2528 G>A (p. Gly843Asp) and IVS2+1 G>A mutations. The child participated in a number of clinical treatment trials including Cholic acid and Docosahexanoic acid supplementation.

She eats a low phytanic acid diet and takes vitamin K. She had a cochlear implant placed at 15 years resulting in marked improvement in her behavior. Currently she is ambulatory with support. She is functionally blind and is dependent for feeding, toiletry, dressing and all aspects of daily life. She has asymptomatic nephrocalcinosis and liver fibrosis. She has biochemically adequate adrenal function but with hypotension requiring glucocorticoids during illness. She has significant osteopenia. There is periventricular leukodystrophy. Her course has been slowly progressive and although generally healthy she is very fragile during illness. Currently the family is transitioning her to adult sheltered care.

There are few reports of clinical outcome in long term survivors with PBD. Most have either one or 2 copies of the Gly843Asp mutation as in this child.

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A NEW CASE OF SCPX DEFICIENCY IN AN ADULT FEMALE PRESENTING WITH ATAXIA, LEUKOENCEPHALOPATHY AND LOWER LIMB SENSORY NEUROPATHY

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Background: In peroxisomal disorders, accumulation of branched-chain fatty acids or bile acids intermediates can lead to severe neurological disease. SCPx is the last enzyme of the peroxisomal beta-oxidation system and until now, only one patient with SCPx deficiency has been described.

Clinical description: The female patient presented at the age of 58 years with weakness of limbs, symmetrical sensory neuropathy, ataxia and moderate neuropsychological dysfunctions. Brain MRI demonstrated leukoencephalopathy. Metabolic investigations revealed increased levels of pristanic acid, whereas phytanic acid and very long-chain fatty acids were normal. The bile acid intermediates DHCA and THCA were undetectable, but there was excretion of abnormal bile alcohol glucuronides in urine. Fibroblast studies and SCP2 gene mutation analysis confirmed the diagnosis of SCPx deficiency. There was no clinical improvement on a phytanic acid restricted diet during six months.

Discussion and conclusion: Like the first SCPx-deficient patient, this patient presented with neurologic symptoms but the onset was during adulthood. Most likely, SCPx deficiency is underdiagnosed, even if a peroxisomal disorder is considered, because the biochemical abnormalities are limited, and only analyzed in specialized laboratories. The excretion of bile alcohol glucuronides was similar in both SCPx-deficient patients and can be considered typical for this disorder.

O-047

DOMINANT NEGATIVE INHERITANCE IN A FAMILY AFFECTED WITH GLYCOGEN STORAGE DISEASE TYPE IX (GSD IX) CAUSED BY MUTATION IN THE PHKB GENE OF PHOSPHORYLASE KINASE (PHK)

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GSD IX is historically considered to be a recessively inherited disorder of glycogenolysis caused by mutation in the PHKA2 (X-linked) or PHKB or PHKG2 (autosomal) genes, coding the alpha, beta and gamma subunits of hepatic PhK respectively. PhK is a decahexameric enzyme composed of four of each of the subunits: alpha, beta, gamma, delta.

A 19 month old boy with recurrent ketotic hypoglycemia had deficient erythrocyte PhK—0.75micromol/ml/gmHb (10–90) and normal glycogen phosphroylase and debrancher. He was a simple heterozygote for the novel missense mutation of PHKB (p.[=]+[Tyr167Cys]) with no mutation in PHKA2 or PHKG2. Both PHKB allelles generated stable mRNA transcripts. The mother and the half-brother (different father) experienced symptomatic hypoglycemia and PhK deficiency (3.8micromol/ml/gmHb, and undetectable respectively). Both were simple heterozygotes for the PHKB (p.[=]+[Tyr167Cys]) mutation, and both generated two stable mRNA transcripts.

In this case the stable mutated betasubunit disrupts enzyme activity. Each PhK enzyme complex contains four beta subunits. If any one of these four is mutated, the resultant enzyme complex has no activity and a total PHK activity is 1/16 of normal. The Tyr167Cys mutation causes autosomal dominant negative inheritance of GSD IX, not previously reported in GSDs, with significant genetic counselling implication.

O-048

NEXT GENERATION SEQUENCING (NGS) FOR GLYCOGEN STORAGE DISEASES (GSDS) THE FIRST UKGTN APPROVED NGS DIAGNOSTIC STRATEGY

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The diagnosis of GSDs has traditionally relied on clinical features combined with biochemical profiling and enzymatic analysis. Diagnosis may then be confirmed by gene sequencing.

Disadvantages of this approach include overlapping clinical and biochemical features; cost and time of sequential investigations; the need in some subtypes for tissue biopsy; the scarcity of laboratories offering enzyme analysis and the inherent enzyme instability. Unsupported by sequencing this can easily lead to misdiagnosis with inaccurate prognostic and recurrence risks provided and incorrectly targeted clinical surveillance.

We present a new screening process of in-solution DNA capture and NGS to simultaneously screen 18 genes known to cause GSD. 120 bp RNA probes used to create a GSD library covering over 1 Mb Validation studies detected all point mutations, sequence variations and copy number variants. The results from over 30 suspected patients, confirm this technique improves diagnostic accuracy compared to stand alone biochemistry. Improving the original GSD library has allowed multiplexing of samples to further reduce costs.

We propose using this technique early in the diagnostic pathway offers a more cost effective, efficient and accurate service and extends accurate diagnosis to patients from around the world who do not have access to the current diagnostic techniques.

O-049

GENERATION OF A NOVEL MOUSE MODEL THAT RECAPITULATES EARLY AND ADULT ONSET GLYCOGENOSIS TYPE 4

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Glycogen Storage Disease type IV (GSD IV) is caused by deficiency of the glycogen branching enzyme (GBE). The diagnostic feature of the disease is the accumulation of a poorly branched form of glycogen known as polyglucosan. The disease is clinically heterogeneous, with variable tissue involvement and age of disease onset. Complete loss of enzyme activity is lethal in utero or in infancy, affecting primarily muscle and liver. However, enzyme activity as low as 5 to 20% leads to juvenile or adult onset of the disease that affects the central and peripheral nervous system and muscles. By using homologous recombination, two mouse models of GSD IV that reflect this spectrum of enzyme activity and disease were generated. By completely eliminating GBE activity, a phenotype similar to early onset GSD IV with neonatal lethality was created. In contrast, adult animals with some residual GBE activity accumulate significant polyglucosan in virtually all tissues, including the central and peripheral nervous system, and exhibit progressive neuromuscular dysfunction. Unlike in muscle, polyglucosan in liver is a degradable source of glucose and is readily depleted by fasting, emphasizing that there are structural and regulatory differences in glycogen metabolism between these tissues.

P-282

BIOCHEMICAL FINDINGS, CLINICAL FEATURES AND GENOTYPES IN GLUT1-DEFICIENCY SYNDROME

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Background: Glucose transporter 1—deficiency syndrome (GLUT1-DS) is a cause of the reduced glucose entry into the brain. Our aim was to compare biochemical findings, clinical features and genotypes in patients detected in Slovakia.

Methods: Serum (S) glucose, cerebrospinal fluid (CSF) glucose (reference range, RR 2.5–3.9 mmol/l) and lactate (RR 0.70–2.10 mmol/l) were investigated and ratio CSF:S glucose (RR>0.65) was calculated in patients with various neurological and psychiatric symptoms. Genomic DNA for mutation analysis was obtained from blood samples.

Results: Now, patients are at the age 7(M), 7(F), 8(F), 9(M) years, their initial biochemical findings were: CSF glucose 1.1, 1.7, 1.8, 2.2 mmol/l; CSF:S glucose ratio 0.19, 0.37, 0.40, 0.48; CSF lactate 0.82, 0.93, 1.30, 1.20 mmol/l and the first clinical symptoms appeared at 2.5, 8, 9 and 48 months respectively. Patient (Pt)1 presented with infantile epilepsy, developmental delay, Pt 2 with apathy, ataxy, Pt 3 with seizures, ataxy, Pt 4 with concentration disorders. Pathogenic mutations c.798_799insC, c.505_507del, c.1119delG were identified in Pt 1, 2, 3 respectively, analysis in SLC2A1 gene is being performed in Pt 4.

Conclusions: Despite small series we suppose CSF:S glucose ratio and CSF glucose could correlate with severity of clinical course in GLUT1-DS.

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HYPERINSULINEMIC HYPOGLYCEMIA,REPORT OF 22 CASES

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Background: Hyperinsulinemic hypoglycemia is the most important cause of hypoglycemia in the neonatal period or early infancy. It is a heterogeneous disorder with two different types of histopathological lesions; focal(islet-cell hyperplasia),always sporadic, and diffuse,a heterogenous disorder, recessively inherited, or rarely dominantly HI, including glutamate dehydrogenase gene involvement, when hyperammonemia is associated. These are clinically indistinguishable.
Objective: The aim was to review clinical presentations, laboratory findings and outcome of 22 patients with hyperinsulinemic hypoglycemia.
Methods: A Retrospective study of all patients diagnosed to have (PHH) between (19902010).
Results: Total 22 patients;Male 11(50%);age at onset, 13(61%)in the first week,16(76%)in first 3 months and 22(100%)in first 6 months; related parents, 70%; birth weight >4 kg,54%. The most common presentation was seizures,95% followed by cyanosis,57%; insulin level,(10–25 micro IU/ml,mean 17.5). Plasma ammonia level was 2–3 times increased in 5/11 cases. All cases after emergency treatment were treated with Diazoxide, 13/22 were diazoxide sensitive and 9 underwent surgical intervention (pancreatectomy). Pancreatic biopsy was performed in 9 cases; Island hyperplasia,7 cases; B cells hyperplasia,2. 20 year FU; well in 18.
Conclusion: 75% became symptomatic in the first week and 100% up to 6 months. Early detection and treatment are very important to prevent severe brain damage.

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CONGENITAL HYPERINSULINISM: RETROSPECTIVE GENOTYPE/PHENOTYPE EVALUATION OF 5 CASES

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It has been reported that rapid genetic analysis of ABCC8 and KCNJ11 genes could improve the clinical management of congenital hyperinsulinism (CHI).
We evaluated retrospectively 5 patients (pts) with diazoxide-unresponsive CHI treated at our Centre in the last 30 years with respect to genetic analysis. Genetic analysis was performed after CHI management, at Royal Devon & Exeter NHS, UK and in 2 pts at Baylor College, Texas.
Between 1981–2001, 2/5 pts (pts 1,2) underwent total pancreasectomy (TP) after multiple partial resections. Pt 3 underwent TP after medical treatment awaiting spontaneous remission. These pts showed diffuse CHI at histological examination. Pts 4 and 5 did not show focal CHI at venous pancreatic sampling (pt 2, 1995) or 18 F-Dopa PET/CT (pt 5, 2008). Case 4 showed early CHI remission, pt 5 is stable on low dose octreotide.
Pts 1–3 are homozygous for nonsense mutations (E51X case 1; A187V cases 2–3, sisters) on KCNJ11 gene.
Pt 4 is heterozygous for nonsense mutation Q444H and pt 5 compound heterozygous (E529K and H125Q), both on the ABCC8 gene.
In our pts rapid genetic analysis could be helpful to indicate the correct management (near-total pancresectomy or medical treatment), in combination with 18 F-DOPA PET/CT.

P-285

INSULIN DOWNREGULATES ALAS1 EXPRESSION IN HUMAN HEPATOCYTES

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Specific treatment of the acute porphyria attack is based on oral or intravenous carbohydrate loading. The rationale for this treatment has been unveiled in experimental animals; fasting of mice induces aminolevulinic acid synthase-1 (ALAS1) through actions of the peroxisome proliferator-activated receptor-gamma coactivator-1alpha (PGC-1alpha). The aim of this study was to investigate the direct effect of glucose and insulin on mRNA levels of ALAS-1 in human hepatocytes.
The rationale for carbohydrate loading in treatment of the acute porphyria attack was confirmed in primary human hepatocytes including a direct downregulatory effect of insulin on ALAS1 and PGC-1alpha. There was a linear correlation between ALAS1 and PGC-1alpha mRNA levels confirming a coregulation of ALAS1 and PGC-1alpha. The fact that increased expression of PGC-1alpha by dexamethasone at normophysiological levels of glucose (5 mM) and insulin (0.3 nM) did not increase ALAS1 points to importance of the mechanism of insulin stimulated export of the ALAS1 transcriptional regulator, forkhead box protein O1, from the nucleus. On the other hand, the potentiation of the phorphyrogenic action of phenobarbital by dexamethasone–induced expression of PGC-1alpha suggests that the role of PGC-1alpha in ALAS1 regulation extends to induction via the nuclear receptors pregnane X receptor and/or the constitutive androstane receptor.

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CONGENITAL HYPERINSULINEMIC HYPOGLYCEMIA (HH) AS A RESULT OF GLUCOKINASE MUTATION CASE REPORT

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Glucokinase (GCK) is the enzyme controlling insulin release. Mutations in GCK gene can result in various phenotypes with autosomal dominant inheritance. Both persistent hyperinsulinemic hypoglycemia of infancy and hyperglycemia (MODY-2, PNDM) are observed, depending on the type of DNA changes. Activating mutations cause oversecretion of insulin despite hypoglycemia, with good response to pharmacological treatment. The prevalence of GCK-HH is 1.2% out of all HH cases.
We present a girl, born at time to unrelated young parents. Father's history revealed serious HH in childhood, treated initially with diazoxide but finally pancreatectomy had to be performed. He developed insulin-dependent diabetes in age of 26 years. Some family members are probably also affected but not diagnosed Hyperinsulinemic hypoglycemia was diagnosed in our patient from first days of her life but was milder that in her father. DNA analysis revealed activating mutation in GCK gene, with protein effect: Val455Leu in both patients. Diazoxide therapy was introduced with good clinical response. **Conclusions**: 1.The identification of a GCK mutation provides information on the prognosis of the disease and helps therapeutic decision. 2.It implies also the diagnostic procedures for hypoglycemia directed to other family members.
3.In each case of neonatal hyperinsulinism, genetic counseling should be recommended.

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INBORN ERRORS OF METABOLISM PRESENTING WITH HYPOGLYCEMIA

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Glucose is a metabolic fuel in the body especially for the brain. Glucose homeostasis is based on storage of carbohydrates, proteins and fats. Interconversion is regulated by several hormones and enzymes. Hypoglycemia in IEM is mainly due to inadequate stores, or immature enzymes, abnormalities in insulin production, disorders of carbohydrate, protein and fat metabolism.

Material: A total of 40 neonates, Infants and children presenting with hypoglycemia of <50 mg/dl were investigated for inborn errors of metabolism.

Results: Two of the neonates were premature at 28 weeks gestation, Beta ketothiolase deficiency in two neonates, CPT1 in one infant, 2 of MCAD deficiency, 2 of Glycogen storage disease (GSD 1 one case, GSD3 one case). 17.5% of children with hypoglycemia had an inborn error.

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MOLECULAR GENETIC ANALYSIS OF HEPATIC GLYCOGEN SYNTHASE, GLYCOGENIN-2 AND GLYCOGENIN-1 IN PATIENTS WITH UNCLASSIFIED KETOTIC HYPOGLYCEMIA (KETHG)

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Background: The classification of hypoglycemia into ketHG and non-ketHG is important for the diagnostic approach. Among ketHG are disorders of glycogen formation, clinically recognizable by supranormal postprandial rise of plasma glucose and lactate concentration. In addition to hepatic glycogen synthase (encoded by GYS2), other proteins play a role in glycogen formation. In liver, glycogenin-2 (GYG2), glycogenin-1 (GYG1), shown to interact with glycogenin-2, and a glycogen synthase kinase are important. Defects of GYS2, GYG2 and GYG1 might result in ketHG, although GYG1 mutations have recently been associated with myopathic symptoms (Moslemi et al, NEJM 2010).

Methods/Results: Nineteen consecutive patients with ketHG and typical laboratory findings were investigated (the majority without liver biopsy and/or enzymatic studies). When GYS2, GYG1 und GYG2 were systematically sequenced, only one patient with GYS2 deficiency was diagnosed. Defects of glycogenins were not detected.

Conclusion: Glycogen synthase deficiency is a rare cause of ketHG even if the presentation is typical. In contrast to muscle glycogenin deficiency, recently reported for the first time, a deficiency of the most important hepatic glycogenin, GYG2, has not yet been diagnosed.

We continue to offer GYG2 sequencing in suspicious patients, particularly those with low hepatic glycogen content and normal liver glycogen synthase activity.

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PPARγ AGONIST PIOGLITAZONE IN THE MANAGEMENT OF SEVERE DYSLIPIDEMIA IN A PATIENT WITH GLYCOGENOSIS TYPE 1A

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Dyslipidemia, with increased triglycerides (TG) and cholesterol (total [TC], VLDL- more than LDL-C, but decreased HDL-C), is a typical biochemical finding in glycogen storage disease type 1 (GSD1a). Intriguingly, this dyslipidemia is not associated with increased cardiovascular morbidity and mortality.

We describe a 15-year-old female patient with a GSD1a chronically difficult to manage, whose blood lipids increased massively at puberty, with TG up to 29 mmol/L and TC up to 13 mmol/L. For the last 18 months, she has been repeatedly hospitalized for episodes of acute abdominal pain with nausea and vomiting; but no biological signs of pancreatitis, the much feared complication of massive hypertriglyceridemia, nor of any other underlying cause were found. Various therapeutic trials, including optimizing continuous glucose uptake, supplementation with medium chain triglycerides, or omega fatty acids, improved the clinical picture and the lipid profile only transitorily.

PPARγ agonists have been suggested to act positively on lipid metabolism in this context. We have used pioglitazone (Actos.) and studied its impact on both glucose and lipid homeostasis in our patient: TG were reduced by 40%, TC by 25%, compared to baseline. Changes were also observed in adiponectin and insulin levels (and HOMA), whereas glycemia remained stable.

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BONE METABOLISM AND HYPOVITAMINOSIS D IN PATIENTS AFFECTED BY GLYCOGEN STORAGE DISEASE TYPE I (GSD I)

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Osteopenia and osteoporosis are known complications of GSD I; a recent study (Banaguria et al, 2009) showed a vitamin D deficiency in a group of GSD I patients. Aim of our study was to correlate bone mineral density (BMD) [DEXA (Dual Energy X-ray Absorptiometry) scans] with metabolic balance and bone status, [including 25-hydroxy-vitaminD (25 (OH)D) levels] in 13 GSD I patients (Ia/Ib=6/7, M/F=8/5, range 8–30 years).

9 out of 13 (69,2%) patients (Ia/Ib=4/5) presented reduced BMD values (4 osteopenia [Ia/Ib=3/1] and 5 osteoporosis [Ia/Ib=1/4]); 6/9 presented low metabolic control. 5/9 patients (Ia/Ib=2/3) showed low levels of 25(OH)D (< 30 ng/ml), despite of supplementation with oral vitamin D and calcium, but we also reported the same findings in 4/4 patients with normal BMD. Statistical analysis didn't show a significant correlation between BMD, metabolic control, bone markers and 25(OH)D values ($p>0,05$).

Considering the essential role of vitamin D in bone homeostasis and the prevalence of hypovitaminosis D in our GSD I patients, we suggest to routinary evaluate this parameter in all patients.

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LYMPHOPENIA AND IMPAIRED LYMPHOCYTES PROLIFERATION CAUSE INCREASED RISK FOR AUTOIMMUNE DISORDERS IN PATIENTS AFFECTED BY GLYCOGEN STORAGE DISEASE TYPE 1B

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Background: Glycogen storage disease type 1b (GSD1b) is caused by mutations in the glucose 6-phosphate translocase (G6PT) gene. GSD1b patients present autoimmune disorders including inflammatory bowel disease (IBD), thyroid disease and Myastenia Gravis. Although these manifestations impact significantly on patients' quality of life, their pathophysiology has remained obscure.
Patients and methods: Seven GSD1b and 14 age and sex-matched controls were enrolled. The presence of autoimmune disorders, serum levels of different autoantibodies, classes and subclasses of circulating lymphocytes, T cells activity in vitro, activity and number of T-cells-regulating system (TRegs) were investigated.
Results: The CD3, CD4, CD8, NK, CD4CD28, CD3CD45RA, CD3CD45RO, CD4CD45RO lymphocytes counts were lower in patients than in controls. A weak proliferation activity was observed in both homologous and heterologous sera. The patients also showed reduced TReg cells counts in the periphery. The presence of autoimmune thyroid disease correlated with CD8DR cells prevalence. The diagnosis of IBD inversely correlated with NK and CD4CD8 cells prevalence. The serum levels of acetylcholine receptor antibodies inversely correlated with CD4CD8, CD4CD45RA cells prevalence.
Conclusion: The obtained data suggest that the association of lymphopenia, impaired T cell proliferation and reduced TReg cells is responsible for the development of autoimmune disorders in GSD1b patients.

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A CASE OF GLYCOGENOSIS TYPE IB ASSOCIATED WITH MGUS

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Glycogen storage disease Ib (GSD Ib) is characterized, in addition to the signs and symptoms of GSD I, by recurrent infections and inflammatory bowel disease (IBD), associated with neutropenia/neutrophil dysfunction. MGUS (Monoclonal Gammopathy of Unknown Significance) is a biochemical condition without clinical expression often described in patient with chronic diseases, with higher risk of transformation in myeloma. There are no cases of MGUS in GSD Ib patients described in literature.
We describe the case of a 31 years old boy with diagnosis of GSD Ib at 3 months of age and of IBD at the age of 19, when anti-inflammatory therapy was started. Since 2007 the protein electrophoresis showed a monoclonal not determinable IgAλ component. Since 2010 a policlonality, attributed to his chronic inflammatory condition, was evidenced. The plasma and urine determination of κ and λ light chains showed higher values than in the normal range, with a normal κ/λ rate and a monoclonal component lower than 1,5 g/dl. The patient doesn't show either kidney insufficient function or peripheral neuropathy or bone lesions.
This report confirms that GSD Ib is a clinical condition that still needs further investigations in order to better understand its evolution in adult patients.

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USING THE GAITRITE WALKWAY SYSTEM TO ASSESS FUNCTIONAL CHANGES IN CHILDREN WITH GLYCOGEN STORAGE DISORDER III (GSD)

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Background: GSD III has both hepatic and muscle manifestations of varying degrees. Muscle disease affecting function is not
always clinically apparent early in childhood and may go undetected. One aspect of the musculoskeletal
system that lends itself to monitoring is gait. Importantly, objective assessment of gait has the potential to quantify clinical change.
Method: Four children with a diagnosis of GSD III were assessed using the GaitRite system.
Specific parameters of gait—velocity, cadence, step length and base of support were measured. Changes for each individual were analysed and compared with age-matched controls.
Results: All patients had slower and less efficient gait patterns than their peers. The most notable differences were in velocity and asymmetry in step length and base of support indicating an unstable and inefficient gait. These differences
were not apparent on routine clinical examination. Creatinine kinase levels were elevated in all patients.
Conclusion: The GaitRite identified abnormalities in gait parameters in GSD III, highlighting alteration in musculoskeletal function prior to overt clinical signs. This reliable instrument can monitor the muscle disease in this rare disorder. It could have considerable potential in clinical management and as an outcome tool in evaluating potential therapies.

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GROWTH IN GLYCOGEN STORAGE DISEASES DIET OR DISEASE?

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Introduction: Dietary treatment of GSD is variable according to type and severity. There are few reports examining growth in GSD.
Aim: A cross-sectional audit reviewing growth in children with GSD.
Methods/Subjects: 18 children (10 boys) were studied. GSD Ia n=5; GSDI b, n=4; GSD III, n=5; GSD VI, n=1; GSD IX, n=3). Median weight/height z scores were calculated.
Results: In GSD Ia (median age 8.3y, 4 Asian, 1 Caucasian), on 1.5 g/kg/dose uncooked cornstarch (UCCS), normal diet and overnight tube feeds (CNTF), median height/weight z score was −1.63/-0.24. In GSD Ib (median age 7.2y; 4 Asian) on 0.7 g/kg/dose UCCS (n=2), milk-free diets (n=2) and CNTF, median height/weight z score was −2.04/0.68. In GSD III (median age 9.1y; 1 Arabic, 4 Caucasian) on 1.9 g/kg/dose UCCS, normal diets and CNTF (n=3) median height/weight z score was −0.5/-0.25. In GSD VI (age 6.9y; Asian) on 1.2 g/kg/dose UCCS only, height/weight z score was −3.31/-2.26. In GSD IX (median age 8.7y, 3 Caucasian) one dose of UCCS pre-bed only, median height/weight z score was −2.09/-0.52.
Conclusion: Suboptimal growth occurred in all GSD irrespective of dietary treatment. The impact on growth of metabolic control, infections, impaired exercise tolerance need investigation by collaborative trials.

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THE MALE REPRODUCTIVE SYSTEM IN CLASSIC GALACTOSEMIA: CRYPTORCHIDISM AND LOW SEMEN VOLUME

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Background: Previous studies examining reproductive parameters in men with galactosemia have inconsistently demonstrated abnormalities. We hypothesized that men with galactosemia would demonstrate evidence of reproductive dysfunction.
Methods: Pubertal history, physical examination, hormone levels and semen analyses were assessed in 26 galactosemic men and compared to those in 46 controls.
Results: The prevalence of cryptorchidism was higher in men with galactosemia than in the general population [9.1% vs. 1.0% (95%CI: 0.75-1.26; p=0.007)]. Testosterone (461±125 vs. 532±133 ng%; p=0.04), inhibin B (144±66 vs. 183±52 pg/mL; p=0.002) and semen concentration (46±36 vs. 112±75 x10^6 spermatozoa/mL; p=0.01) were lower and SHBG was higher (40.7±21.5 vs 26.7±14.6; p=0.002) in galactosemic men compared to controls. Semen volume was lower than normal in 7 out of 12 patients.
Conclusions: Despite the limited sample size, this is the second time that a higher than expected prevalence of cryptorchidism is reported in men with classic galactosemia. The subtle decrease in testosterone and inhibin B levels and sperm count may mark mild defects in Sertoli and Leydig cell function. Follow-up studies are needed to determine the clinical consequences of these abnormalities. The low semen volumes may be an indicator of pathophysiological abnormalities in this disease and deserve further study.

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OVARIAN INSUFFICIENCY IN FEMALE CLASSIC GALACTOSEMIA PATIENTS: TIMING OF THE LESION

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Background: Most female classic galactosemia patients suffer from ovarian insufficiency from an early age on. We characterized the phenotype of female galactosemia patients at different ages by MRI and/or abdominal or transvaginal ultrasound
Patients and methods: 23 patients were included. MRI data of 14 patients were compared to different control patients (prepubertal, age-matched and postmenopausal) who underwent MRI for other reasons. Data on 17 ultrasounds were obtained retrospectively.
Results: When comparing the ovarian volumes on MRI, the ovaries of the galactosemic girls were significantly smaller than those of the age matched controls (p=0.001) and the prepubertal ovaries (p=0.008), but did not differ significantly from postmenopausal ovarian volumes (p=0.161). Also, evidence for follicle activity was regularly seen in both the prepubertal and age matched control groups, but only rarely in the galactosemia patients and postmenopausal controls. On ultrasound, the ovaries were detected only occasionally.
Conclusions: Imaging results point to early onset of ovarian abnormalities in female galactosemia patients. Little evidence for maturation of follicles was seen. Combined with the significantly smaller volumes of the ovaries we found no support for a maturation arrest alone as the main pathophysiological mechanism. This study supports the mechanism of increased follicle loss in these patients.

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THE SPECTRUM OF MOVEMENT DISORDERS IN ADULT PATIENTS WITH CLASSICAL GALACTOSEMIA

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Objectives: To define the prevalence and characteristics of motor complications in adult patients with galactosemia.
Background: Early restriction of galactose prevents the neonatal complications of galactosemia, but long term problems, such as premature ovarian failure and neuropsychiatric disorders, often involving motor function, continue to develop. Their exact prevalence and phenotype are not well defined.
Methods: All patients with classical galactosemia attending the Charles Dent Adult Metabolic Unit (London) from September 2010 to April 2011 were examined by a neurologist and their medical records reviewed.
Results: 42 patients were examined. 26 patients (62%) had motor complications. This group also had a higher prevalence of other neurologic problems (65% vs 12%). 13 patients had symptoms related to their movement disorders and 8 reported progressive worsening. The remaining 13 had convincing signs on examination. The most common motor manifestation was tremor (N=21; 81%), followed by dystonia (N=14; 54%) and cerebellar signs (N=7; 27%). Several patients responded to standard treatments for tremor and dystonia.
Conclusions: A high proportion of patients have central nervous system damage despite adhering to a galactose-restricted diet. Many develop symptomatic motor complications, mainly tremor, dystonia and cerebellar ataxia, which may progress, leading to disability and suggesting ongoing neurodegeneration.

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LANGUAGE PRODUCTION IN CLASSIC GALACTOSEMIA REVISITED: A COGNITIVE NEUROSCIENCE APPROACH

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Classic galactosemia is an inborn error of galactose metabolism. Language production problems are among the most burdensome complications. This study hypothesized that galactosemia patients have language difficulties at the level of syntax (i.e. where grammatical information is retrieved and a sentence is constructed). Electroencephalography (EEG) was recorded in a group of galactosemia patients (n=22,mean age=14.9) and healthy controls (n=21,mean age=14.3), during task performance. The participants were instructed to either passively watch an animated visual scene or to utter an overt response describing this scene, requiring either minimal (separate words) or maximal syntax (sentence). Event-related-potentials (ERPs) were extracted from the continuous EEG, reflecting the brain's preparatory response to this task. Results indicate that the galactosemia ERPs start to diverge as early as 120 ms after scene presentation (P1). Also, the patient data deviates in the P2 and P6 ERP time windows (i.e. around 200 ms and 400–600 ms post-stimulus, respectively). Behaviourally, the patients need more time to prepare the utterance and make more mistakes. We suggest that galactosemia patients have not (only) late articulatory problems, but also impairments of early conceptualisation (P1), lexical access of the words (P2) and syntactic processing (P6), resulting in delays and more troublesome language production.

P-299

A STUDY OF CLASSICAL GALACTOSAEMIA IN CYPRUS: EPIDEMIOLOGICAL, BIOCHEMICAL AND MOLECULAR DATA

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Classical galactosaemia is an autosomal recessive inborn error of carbohydrate metabolism caused by mutations in the galactose-1-phosphate uridyl tranferase gene (GALT). The objective of the present study was to investigate galactosaemia in Cyprus at the epidemiological, biochemical and molecular level. We identified two mutations in Cypriot patients with classical galactosaemia: A novel large deletion of 8489 bp encompassing all exons of the GALT gene, and the p.Lys285Asn mutation. Microsattelite analysis revealed the presence of a common haplotype suggesting a founder effect for this deletion in Cyprus.

The frequency of galactosaemia carriers in the general population was estimated at 1/105 by means of GALT activity measurement in red blood cells. Molecular analysis of all carriers identified biochemically revealed three additional mutations, on one allele each: the p.Pro185Ser and the c.[820+13A>G], previously found in Portuguese patients, as well as a new transition, the c.[378–12 G>A], not encountered in the general population. Thus, five mutations account for all galactosaemia alleles studied: a novel whole gene deletion (55% of alleles), p.Lys285Asn (30%), p.Pro185Ser (5%), c. [820+13A>G] (5%) and c.[378–12 G>A] (5%). Furthermore, the allele frequencies of the p.Asn314Asp, the Los Angeles variant and the Duarte 2 variant were determined at about 8%, 5.5% and 2.5% respectively.

P-300

PRODUCTION AND FUNCTIONAL CHARACTERIZATION OF RECOMBINANT HUMAN GALT MUTANT FORMS: MOLECULAR BASIS OF DISEASE-CAUSING MUTATIONS IDENTIFIED IN PORTUGUESE GALACTOSEMIC PATIENTS

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Herein we report comparative functional studies on wild-type and mutant forms of recombinant human galactose-1-phosphate uridylyltransferase (GALT), seeking a molecular basis for the phenotypes assigned to classical galactosemia patients bearing the respective mutations.

GALT plays a key role in galactose metabolism. Mutations in the GALT gene resulting in GALT deficiency set the basis for classical galactosemia, an autosomal recessive disorder. To date, more than 200 mutations have been described, the majority being missense. Novel mutations were identified in the Portuguese galactosemic population (e.g. G175D and P185S), which will be studied in parallel with other known mutations (R148Q, Q188R and S135L). To characterize the wild-type and mutant forms, GALT wild-type cDNA was cloned into pET24b(+), bearing an N-terminal 6xHis-tag, the mutations generated by site directed mutagenesis, and expressed in E. coli BL21(DE3) Rosetta cells, in LB medium supplemented with iron and zinc. After purification by affinity-chromatography, comparative functional studies were employed to evaluate the cofactor loading, oligomeric profiles, conformational stability and enzymatic activity of wild-type vs. mutant GALT.

The results provided biochemical information confirming that the selected mutations are disease-causing, and contributed for the understanding of how the mutational spectrum in galactosemic patients modulates GALT functional properties.

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P-301

GALACTOKINASE DEFICIENCY EXPERIENCES OVER TEN YEARS IN A GERMAN SCREENING LABORATORY

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Background: Galactokinase deficiency (GALK) is a rare autosomal-recessive disease with an estimated incidence 1:150,000 to 1:1,000,000. Cataract formation due to accumulation of galactitol may already occur in early childhood. This can be prevented, reversed or ameliorated by early diagnosis and therapy with a galactose restricted diet.

Methods: From 2000 to 2010 the Screening-Laboratory Hannover analyzed 2 million dried blood spot samples from neonates. Enzymatic activities of galactose-1-phosphate uridyltransferase (GALT) and galactose plus galactose-1-phosphate were quantified. Indirectly screening for GALK was accompanied; in cases with elevated galactose plus galactose-1-phosphate and normal GALT activity, GALK activity was measured in erythrocytes to confirm GALK deficiency.

Results: In 9/11 cases with elevated galactose levels and normal GALT activity the diagnosis of GALK was confirmed. In two cases screening results were false positive. 3/9 children developed cataracts. Two children were treated with lentectomy. Under a galactose-restricted diet existing cataracts were stable or reversed; concentrations of galactose normalized. Galactokinase activities and cataract formation did not correlate.

Conclusion: For galactokinase deficiency a therapy with galactose restricted diet is available and useful to ameliorate ophthalmological complications. Measurement of elevated galactose levels as well as normal GALT activity in newborn screening may be helpful to detect galactokinase deficiency early

P-302

A FOLLOW UP OF PATIENTS PREVIOUSLY DESCRIBES AS RELATIVE MILDER PHENOTYPE OF TRANSALDOLASE DEFICIENCY (TALDO)

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Background: Transaldolase (TALDO) deficiency is a rare inborn error of pentose phosphate pathway. So far 14 patients from 8 families were described. In the majority of these cases clinical course of the disease was severe with neonatal presentation and early death. In 2009 we described two transaldolase deficient brothers (6 and 4 years of age) with relatively mild phenotype.

Patients: Three years of follow up revealed in the older brother (currently 8 years old) advanced liver (nodular) cirrhosis, hyperbilirubinemia, persisted moderate anaemia and thrombocytopenia, clotting disturbances, splenomegaly, low body mass. Since the age of 7 the boy developed tubulopathy, proteinuria and progressing renal damage, mild pulmonary fibrosis and cardiomegaly. His intellectual development and social functioning are adequate to the calendar age. He has persisted tendency to generalized edema, and ascites requiring diuretics administration. His younger brother (6 years old) presents less advanced liver cirrhosis, as well as anaemia, thrombocytopenia, clotting disturbances, tubulopathy, proteinuria and osteopenia.

Conclusions: TALDO deficiency is mostly pronounced in liver but taking into account the severe renal, pulmonary and cardiac involvement, TALDO deficiency should be considered as severe multisystemic disease.

P-303

HEREDITARY FRUCTOSE INTOLERANCE: NEW MUTATIONS IN THE ALDOB GENE

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Background: Mutations in the ALDOB gene impair the activity of Aldolase-B, causing Hereditary Fructose Intolerance (HFI). HFI is an inherited recessive disease of carbohydrate metabolism resulting in hypoglycemia, liver and kidney failure, coma and death. Diagnosis is possible by identifying mutant ALDOB alleles in suspected patients.

Objectives: Molecular characterization of ALDOB gene in patients with suspected HFI.

Methods: PCR, long-PCR, direct sequencing of exons/intron-exon boundaries and Multiplex Ligation-dependent Probe Amplification (MLPA) of ALDOB gene in a cohort of 46 suspected HFI patients.

Results: Genetic lesions were identified in 18 patients. 15 patients carried, at homozygous or compound-heterozygous level, the known mutant alleles p.Ala150Pro, p.Ala175Asp, p.Asn335Lys, c.360-363del4, p.Tyr204X, g.10196A>G(IVS6-2A>G). Three remarkable cases were found. Patient1 carried a new exon1-deleted allele, identified by MLPA, compound-heterozygous with p.Ala149Pro allele. Patient2 carried a new single nucleotide insertion in exon8 causing frame-shift, compound-heterozygous with p.Ala174Asp allele. Patient3 carried, in homozygosis, a largely deleted allele, from exons 2 to 6. Deletion was confirmed by multiplex-PCR assay. Breakpoint detection was carried out by long-PCR and direct sequencing, and heterozygous patient's parents were identified.

Conclusion: Two new mutant ALDOB alleles, carrying one deletion and one small insertion respectively, were identified in patients with HFI.

P-304

IDENTIFICATION OF NOVEL MUTATIONS IN PROMOTER AND CODING REGIONS IN ALDOB GENE CAUSING HEREDITARY FRUCTOSE INTOLERANCE

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Hereditary fructose intolerance (HFI) is an autosomal recessive disease caused by mutations in the ALDOB gene encoding aldolase B enzyme. If undiagnosed, the persistent ingestion of fructose can lead to severe liver and kidney damage and death. Molecular genetic analysis are essential and useful diagnostic tool for HFI because of less invasive methods. Recently, promoter mutations in ALDOB gene were identified in patients with HFI beside over 50 different type of known mutations. In this study, mutation screening of 2–9 exons and promoter region (untranslated exon 1) in ALDOB gene was carried out in 13 Turkish patients with HFI by using direct sequence analysis. Six different type of mutations in ALDOB gene (one splice site, three missense and two deletion) were detected. In this new cohort with HFI, ALDOB gene harbored for three novel mutations including IVS2-3deltagG, c.71delTTGCins11GAATCTCTGGG, IVS1+1 G>A and three known mutation, p.A175D and p.C135R in coding region and g. -132 G>A mutation in promoter region of the ALDOB gene.

P-305

INFANTILE HYPOLACTASIA: A CASE WITH CHALLENGING DIAGNOSIS

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Background/Objectives: Lactose intolerance is the inability to metabolize lactose, because of the lack of enzyme lactase in the digestive system. There are known three major types of lactose intolerance. We report a patient with adult type of hypolactasia who was picked up by selective screening for galactosemia.

Case report: 2-month-old boy with recurrent complaints of restlessness and meterorism. Urinary Benedict's test was positive and urinary mono- and disaccharide analysis revealed the elevation of galactose. Repeated analysis showed even larger excretion of galactose and also galactitol. Lactose-free diet showed positive impact. All galactosemia subtypes were ruled out by enzymatic analysis, but hypolactasia was confirmed on molecular level. He is homozygous for c.-13910 C>T polymorphism in MCMG gene, which regulates lactase gene transcription.

Methods: Urinary galactose/galactitol content was evaluated by HPLC with refractive index/ultraviolet-visible spectrophotometrical detectors.

Results: concentration of glucose in urine was 0.08 mmol/l (ref<0.8), galactose 334 mmol/mol cr (ref<377); 1-month later urinary glucose 1 mmol/l (ref<0.8), galactose 782 mmol/mol cr, galactitol 597 mmol/mol cr (ref<30); sugar derivatives in organic acid GC/MS analysis. Lactose contents in urine were respectively 44.3 mmol/mol cr and 20.3 mmol/mol cr.

Conclusion: Urinary galactose analysis might be a valuable tool for picking up infantile hypolactasia cases.

P-306

URINE GLUCOSE TETRASACCHARIDE ANALYSIS BY HIGH PRESSURE LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION (HPLC-ECD) FOR THE IDENTIFICATION AND MONITORING OF PATIENTS WITH POMPE DISEASE

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Patients with Pompe disease (Glycogen storage disease (GSD) type II) have been shown to excrete increased amounts of glucose tetrasaccharide (Glc4) in urine, thought to be derived from the action of serum amylase on glycogen accumulated as a result of lysosomal acid alpha glucosidase deficiency.

We have developed a simple, rapid and reproducible method for the analysis of Glc4 by HPLC-ECD, by adaptation of an established method for urine and faecal sugars analysis.

Using this method, we were able to demonstrate elevated urinary Glc4 concentrations in all Pompe patients studied (n=11), with all control patients having concentrations below the limit of detection (5 μmol/mmol creatinine) (n= 21); and importantly, we were able to show a distinction in Glc4 excretion between Pompe patients and an individual harbouring a pseudo-Pompe mutation. A clear decrease in Glc4 excretion was observed following commencement of enzyme replacement therapy (ERT) in 3 Pompe patients studied.

Our results therefore support the use of urinary GLc4 measurement by HPLC-ECD as an adjunct to enzymatic analysis in the diagnosis of Pompe disease, and as a biomarker for monitoring ERT in these patients. Additionally, raised urinary Glc4 was observed in patients with GSD type Ia and III, suggesting possible applications in other GSDs.

O-050

WHOLE-EXOME-SEQUENCING IN CONGENITAL DISORDERS OF GLYCOSYLATION

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Identification of disease genes in patients with a Congenital Disorder of Glycosylation type I commonly follows a series of biochemical assays, several of those with limited availability. After extensive biochemical and genetic research, still 10% of CDG-I families in our cohort remain unsolved.

Next-generation-sequencing techniques offer new alternatives to identify disease genes, thus far mostly applied to several families with identical clinical features. We hypothesized that the functional knowledge of a glycosylation abnormality in CDG-I patients would greatly facilitate gene identification in single cases. Whole-exome sequencing was applied on 6 single cases with CDG-I. After removal of known public and in-house polymorphisms, ~600 private variants remained. A number of ~60 possible CDG-I gene candidates was selected, based on their location in ER glycosylation, dolichol synthesis or cytoplasmic monosaccharide conversions. In 4 patients, the genetic cause of disease was identified: one as DPAGT1-CDG (a single case reported so far) and 2 novel gene defects. Mutation analysis and biochemical assays confirmed the pathogenicity of the identified variants. In the 2 remaining patients, no variants were found in the 60 candidate genes.

This approach shows the huge potential to apply whole-exome-sequencing in metabolic disease, where functional knowledge of the disease is available.

O-051

ABNORMAL DYSTROGLYCAN O-MANNOSYLATION IS THE CAUSE OF ISOLATED DILATED CARDIOMYOPATHY DK1-CDG

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In a group of patients with dilated cardiomyopathy, a diagnosis of Congenital Disorders of Glycosylation (CDG) type I was found. After homozygosity mapping in the consanguineous families, pathogenic mutations were identified in DK1 in all individuals, while enzyme activity was deficient in fibroblasts. DK1 encodes the dolichol kinase responsible for formation of dolichol-phosphate. In comparison with the severe multisystem presentation in CDG, the clinical presentation in our cohort was dominated by dilated cardiomyopathy, a rare feature in CDG. Expression analysis of DOLK in different tissues showed highest expression in fetal and adult brain, thereby not explaining the tissue specific phenotype. The availability of patient heart biopsy material allowed us to study other dolichol-phosphate dependent glycosylation pathways in affected tissue. N-glycosylation was affected as shown by western blotting of CD63. In addition, reduced O-mannosylation of alpha-dystroglycan was found with functional loss of its laminin-binding capacity. Loss of functional dystroglycan is known in a subgroup of the congenital muscular dystrophies, commonly presenting with dilated cardiomyopathy.

Conclusions: Dolichol kinase deficiency results in decreased availability of dolichol-P-mannose thereby leading to abnormal O-mannosylation of alpha-dystroglycan. We thus describe a combined deficiency of protein N-glycosylation and O-mannosylation, presenting as late-onset dilated cardiomyopathy.

O-052

DOLICHOL SYNTHESIS DISORDERS: A RECENT GROWING FAMILY OF CDG I

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Background: CDG I are inborn errors of metabolism affecting the early steps of N-glycan synthesis. Recently, it has been shown that CDG I can harbor a defect in the synthesis of dolichol.

Patients and Methods: Among the 24 CDG Ix patients of the French cohort, we sequenced three known genes implicated in dolichol synthesis: DK1, SRD5A3 and DHDDS.

Results: We typed 5 patients. One presented a dolichol kinase deficiency (DK1-CDG) with new mutations: c.1564-1565delCA (p.Gln522fsX25) and c. 1023 C>G (p.His341Gln). Three other patients presented a deficiency of the steroid 5 alpha-reductase type 3 (SRD5A3) with the following new mutations at the homozygous state c.894-908delCTA-CAAAAGCAAATT (p.Tyr299-Phe303delFYKSK) (simultaneously detected by Cantagrel et al), c.620 T>G (p.Met207Arg) and c.224-225insA (p.Tyr75X). The last patient presented a cis-prenyltransferase deficiency with new mutations in DHDDS gene c.192 G>A (p.Trp64X) and c.441-24A>G (p.Cys148GlufsX11).

Conclusion: Regarding our results, testing steps of the dolichol synthesis allowed the identification of the disorder for 20% of our CDG Ix patients. According to the published patients, dolichol synthesis disorders present with a severe clinical phenotype with extra neurological manifestations including liver, cardiac, renal, eyes or skin involvement associated, and death in early infancy as for our DK1 and DHDDS deficient cases.

P-307

SRD5A3-CDG: A PATIENT WITH A NOVEL MUTATION

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Congenital disorders of glycosylation (CDG) are genetic diseases with an extremely broad spectrum of clinical presentations due to defective glycosylation of glycoproteins and glycolipids. Some 45 CDG types have been reported since the first clinical description in 1980. Protein glycosylation disorders are defects in protein N- and/or O-glycosylation. Dolichol phosphate is the carrier of the N-glycans during their assermbly first at the outside and subsequently at the inside of the endoplasmic reticulum (ER) membrane, and hence is a key molecule in protein glycosylation. Recently, defects have been identified in the last three steps of the dolichol phosphate biosynthesis: dolicholkinase deficiency (DK1-CDG), steroid 5alpha-reductase type 3 deficiency (SRD5A3-CDG), and dehydrodolichyl diphosphate synthase deficiency (DHDDS-CDG). We report on a patient with SRD5A3-CDG carrying a novel (homozygous) mutation. The diagnostic features of this novel inborn error of glycosylation are psychomotor retardation, nystagmus, visual impairment due to variable eye malformations, cerebellar abnormalities/ataxia, and often ichthyosiform skin lesions.

P-308

THREE SIBLINGS WITH EXT1- CDG

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EXT1/EXT2-CDG (HME, hereditary multiple osteochondroma) are common defects of O-glycosylation. HME is a rare autosomal dominant disorder characterized by the occurence of multiple benign cartilage capped tumors, that are typically located at juxta-epiphyseal regions of long bones. The diagnostic criteria are at least two osteochondromas of the juxta-epiphyseal region of long bones within the majority of cases with a positive family history and/or mutation in one of the EXT genes. Herein we report three siblings with an age range of 9–14 years who respectively manifested the features of hereditary multiple exostoses. HME affected siblings were referred to outpatient clinic with a lower lumbar spine BMD (z scores of −1.66, -1,9 and −3.18 respectively) and painless progressively increasing bony swellings located at the juxta-epiphyseal regions of long bones as well as other sites. A mutation in EXT1, c.1659 delC (p. Tyr553X), which cosegregated with the disease phenotype, was detected in three siblings. The EXT-1 gene may have a possible additional role in bone metabolism and we should follow these patients not only for the malignant change of exostoses but also for the other complications such as reduction in skeletal growth, restricted joint motion, premature osteoarthrosis and compression of peripheral nerves.

P-309

CLINICAL AND BIOCHEMICAL CHARACTERIZATION OF THE SECOND CDG-IJ(DPAGT1-CDG)PATIENT

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Background: DPAGT1-CDG (CDG-Ij) is caused by a deficiency of the first GlcNAc transferase in the ER N-glycosylation pathway. Only one patient has been reported with microcephaly, dysmorphia, exotropia, hypotonia and seizures.

Case report: The boy born as the second dizygotic twin by Caesarean section with BW 1410 g and asphyxia. Respiratory distress with frequent apnoeas occurred since birth. Physical examination showed a hypotrophic child with bilateral cataract, cryptorchism, dysmorphia, extremities hypertonia and joint contractures. During the first 3 months: jaundice treated by phototherapy, persistent anaemia—frequent transfusions, feeding difficulties—parenteral and naso-gastric feeding.

Then refractory tonic seizures appeared, apnoeas and respiratory insufficiency. Cataract surgery (pars plana lensectomy) at 6 months showed calcification of lenses nuclei. Chest X-ray revealed broncho-pulmonary dysplasia. Biochemical tests showed low serum total protein, mildly elevated aminotransferases and elongated APTT. Metabolic work-up revealed abnormal, type 1 profile of transferrin isoforms suggesting CDG. Activities of PMM, PMI and LLO profile in fibroblasts were normal and patient was classified as unsolved CDG-Ix. He died at age of 2.5 years due to respiratory insufficiency. Genetic defect has been recently identified by whole-exome-sequencing.

Conclusions: Congenital cataract is an uncommon manifestation of CDG and a novel finding is calcified nuclear cataract.

P-310

DHDDS-CDG: A NEW TYPE OF DOLICHOL SYNTHESIS DISORDER

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Background: CDG I are inborn metabolism errors affecting the early steps of N-glycan synthesis, classified in three subgroups: oligosaccharide moiety synthesis, dolichol synthesis, and oligosaccharide transfer on the nascent protein.
Case report: We describe here a patient presented with a new CDG I subtype, DHDDS-CDG (cis-prenyltransferase (CPT) deficiency) (first step of the dolichol synthesis). The patient had a severe clinical phenotype with extra neurological manifestations including liver, cardiac, renal, eyes or skin involvement associated, and died in early infancy.
Results: Mutations in DHDDS gene from paternal and maternal origin were identified: respectively a nonsense mutation c.192 G>A (p.Trp64X) and c.441-24A>G confirmed by RNA study to be a splicing mutation by creating a cryptic donor splice site (with score of 0.99 rather normal exon 6 donor site is 0.65) leading to loss of exon 6 and 63 bases of intron 5 insertion, and a premature stop: c.440_543del102ins63 (p.Cys148GlufsX11). The CPT activity measured in the fibroblasts of the patient was lower compared to control cells, and Q-PCR of the CPT mRNA confirmed a very low level of transcription.
Conclusion: This patient's clinical phenotype, coherent with the other CDGs contrasts with the retinis pigmentosa phenotype of the first described patient.

P-311

CONGENITAL DISORDER OF GLYCOSYLATION RFT1-CDG AT TEENAGER SIBLINGS WITH PROFOUND MENTAL RETARDATION AND HEARING IMPAIRMENT

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RFT1 is an enzyme involved in glycosylation pathway. Its defect causes one of the CDG syndromes. So far there were described six patients all up to the age of six. We present clinical, biochemical and molecular findings of two siblings with RFT1-CDG. First is 18 years old boy with severe mental retardation, profound bilateral hearing loss, epilepsy, obesity, craniofacial dysmorphy, hypothyreosis and mild coagulopathy. His sister is 16 and shows similar symptoms; just the hearing problems are milder. Both patients showed abnormal a type 1 transferrin isofocusing pattern. LLO analysis showed accumulation of DolPP-GlcNAc2Man5 at the cytosolic side of the ER membrane. Molecular analysis revealed two novel heterozygous missense mutations (c.1222A>G/c.1325 G>A) in the 12th exon of RFT1 gene. Our patients show milder phenotype and longer survival compare to the previously published patients. Supported by: IGA MZ NT-12166

P-312

ABNORMAL THYROID FUNCTION IN PATIENTS WITH CONGENITAL DISORDERS OF GLYCOSYLATION

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Several glycoproteins are essential in the normal production and regulation of thyroid hormones, including TSH, TBG, thyreoglobuline and the thyroid receptor. We prospectively examined glycosylation, the endocrine and clinical aspects of thyroid function, energy expenditure, and evaluated growth and development in 32 patients with congenital disorders of glycosylation.
Thyroid function was abnormal in 19 patients with consistently elevated TSH in ten children, mostly in PMM2-CDG. In four patients, TSH elevations resolved without intervention. Enzymatic desialylation of TBG and TSH had no effect on the laboratory results in these patients and controls. Low free thyroxine was noted in three patients, corresponding to decreased energy expenditure and weight-increase. Low TBG was noted in half of the patients. Thyroid function tests in CDG patients are often abnormal. Most patients appeared to be clinically euthyroid even in the presence of abnormal TBG and TSH values. Still, in certain cases of significantly elevated TSH or low FT4, manifesting in clinical hypothyreoidism, thyroxine supplementation was necessary. TBG deficiency did not appear to have any clinical consequences.
Hypoglycosylation of thyroid-proteins may affect laboratory values but may not always be of clinical importance for patients. Measuring energy expenditure in patients helps to decide on the appropriate therapeutic approach.

P-313

IDENTIFICATION OF C1INHIBITOR AS A POTENTIAL BIOMARKER FOR N- AND O- LINKED CONGENITAL DISORDERS OF GLYCOSYLATION (CDG)

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Isoelectric focusing of transferrin and apolipoprotein CIII are used to diagnose N-linked and / or core-1- and 2 O-linked glycosylation defects. Plasma 2D-PAGE studies revealed altered charge and mass forms of C1-inhibitor in CDG I and II patients. C1-Inhibitor is a protease inhibitor that plays a crucial role in various physiological pathways. It is heavily glycosylated: 49% of its molecular weight (MW) is composed of six N- and seven core-1 O-glycans. Investigations of C1-inhibitor as a potential marker for both N- & O-linked glycosylation defects was performed on patients with CDG type I and II, including congenital muscular dystrophies, by Western blotting. CDG I cases revealed reduced intensity of the native 100 kDa MW band and a lower MW band. In the CDG II group, samples from patients with FKRP and POMGnT1 mutations showed a normal profile (as expected as these are defects in O-mannosylation). However, reduced MW bands were observed in the plasma from a patient with a LARGE mutation (which have defects in both O-mannose and O-GalNAc glycosylation) and also from a muscular dystrophy patient of undefined genetic cause. This demonstrates C1-inhibitor could be a combined potential marker for various N- and O-glycosylation disorders.

P-314

MASS SPECTROMETRY OF SERUM N-GLYCANS AND EVALUATION OF THE ADULT PHENOTYPE IN CMP-SIALIC ACID TRANSPORTER DEFECT

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SLC35A1-CDG has been described in a single patient with spontaneous massive bleedings in the posterior chamber of the right eye, cutaneous and pulmonary hemorrhages, thrombocytopenia, neutropenia and recurrent infections. This new type of congenital disorder of glycosylation affects the transport of CMP-sialic acid into the Golgi apparatus. Due to the lethal outcome in the initial patient the genetic background could not be fully confirmed.

We evaluated a patient of consanguineous origin, presenting with multiple dysmorphic features, psychomotor retardation, thrombocytopenia, persistent proteinuria and aortic insufficiency.

Isoelectric focusing of transferrin showed a type II pattern, while the apolipoprotein C-III isofocusing pattern was normal. Mass spectrometry of serum N- glycans demonstrated severely abnormal sialylation. Mutation analysis in SLC35A1 confirmed a homozygous missense mutation. Functional analysis of the mutations in a CMP-sialic acid transporter assay in yeast showed a 50% reduction in transport activity. Expression studies showed significant gene expression in the central nervous system, and the kidneys, comparable with mental retardation and chronic proteinuria in our patient.

We report on the second SLC35A1-CDG, and first adult, patient, presenting with intellectual disability, thrombocytopenia, renal and cardiac involvement due to a CMP-sialic acid transporter defect. The bleeding disorder remained asymptomatic until adulthood.

P-315

ERNDIM QC SCHEME FOR THE CONGENITAL DISORDERS OF GLYCOSYLATION

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In the framework of the European program Euroglycanet, a quality control scheme was set up for the screening of Congenital Disorders of Glycosylation by analysis of transferrin glycosylation. Reproducibility problems after 3 rounds among Euroglycanet members necessitated an elaborate stability experiment. Serum samples were stored at different temperatures and lyophilisation conditions, after which the stability was tested using transferrin isofocusing during a 1-year follow up. Samples lyophilized in the presence of cryoprotectant were stable at shipment conditions for at least 2 months.

Since 2009, the scheme is supervised by ERNDIM and open to other laboratories world-wide. Currently, 51 centres participate. In view of the very limited amount of patient material, the scheme operates with 20 microliter serum per sample. Different techniques are used for analysis of transferrin glycosylation, including isofocusing, capillary electrophoresis, HPLC and mass spectrometry.

The samples originate from patients with a known CDG subtype or a secondary cause of abnormal transferrin glycosylation. The results are interpreted against a clinical information, provided with the samples. Scoring is based on profile interpretation and suggestions for follow-up diagnostics, and showed clear improved over the years. The results of this novel CDG QC scheme in ERNDIM's regular QC program will be presented.

P-316

PRENATAL PRESENTATION AND DIAGNOSTIC EVALUATION OF SUSPECTED SMITH-LEMLI-OPITZ SYNDROME

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Background: Smith-Lemli-Opitz syndrome (SLOS) is a multiple malformation syndrome due to a deficiency of 7-dehydrocholesterol reductase resulting in an accumulation of 7- and 8-dehydrocholesterol in serum and amniotic fluid (AF). In affected pregnancies free estriol is often reduced and in prenatal ultrasound intrauterine growth retardation (IUGR) and malformations of CNS, heart, kidney, genitals and limbs are often detected. Objectives: Evaluating the specificity of features leading to a suspicion of fetal SLOS.

Material and Methods: From 2002 to 2011 we determined sterol concentrations in AF samples from 69 pregnancies suspicious for SLOS by gas chromatography mass spectrometry.

Results: Sterol analysis in AF was done in 24 cases because of the family history (affected sibling: n=14, other affected family member: n=5, lethal malformations in a sibling: n=5). In 39 cases prenatal abnormalities were determined (reduced estriol: n=10, multiple fetal malformations: n=15, IUGR: n=11). In 6 cases there was no clinical information. SLOS was diagnosed in 6 fetuses (8.7 %). 3 of those had an affected sibling, 3 had multiple malformations, one additionally a reduced estriol concentration.

Conclusion/Discussion: The highest rate of confirmed SLOS was in at risk families, whereas fetal malformations were in most cases not caused by SLOS.

P-317

PHENOTYPICAL PROPERTIES AND RESPONSE TO CHOLESTEROL THERAPY OF SMITH-LEMLI-OPITZ SYNDROME CASES

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Background: Smith-Lemli-Opitz Syndrome is an autosomal recessive disorder characterized by cholesterol synthesis defect. Yet there is no current proven therapy, studies so far indicate cholesterol therapy and HMG-CoA reductase inhibitors like Simvastatin improve growth, behavior and general wellness.

Methods: Our study included four patients between 31 and 71 months of ages.

Results and Conclusions: Male to female ration is 1/3 and consanguinity is present in 25 percent of cases. All patients presented around 2–3 months of ages with failure to gain weight and received a diagnosis before age 1. A characteristic facial appearance, microcephaly, submucosal cleft palate, cutaneous syndactyly, mental retardation, behavioral and feeding problems, postnatal growth retardation were present in all cases; and prenatal growth retardation was seen in half of cases. Cholesterol therapy of 100 mg/kg/day was started. During follow up increase in cholesterol level was observed while 7-dehydrocholesterol level was not decreasing. Patient's parents didn't consent with Simvastatin therapy due to unknown efficiency and possible side effects. With therapy; growth parameters, head circumference increased in accordance with growth chart rates. Except for one case, none of the patients reached to the 3 rd percentile. With regard to behavior, improved skills in every area was detected albeit little.

P-318

GENOME WIDE EXPRESSION PROFILING IN THE SMITH-LEMLI-OPITZ SYNDROME (SLOS)

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Background: SLOS is caused by inactivating mutations of the DHCR7 gene leading to cholesterol deficiency. It is characterized by a broad phenotypic spectrum typically including congenital malformations and mental retardation. SLOS pathophysiology remains poorly understood and there is no effective treatment.
Objectives: to characterize the molecular changes caused by DHCR7 deficiency. Specifically, to obtain baseline gene expression profiles in affected and non-affected cells.
Methods: Genome wide expression profiling (RNA-Seq) was performed using skin fibroblasts isolated from a patient with severe SLOS and neural stem cells (NSC) isolated from Dhcr7−/− mice (E18).
Results: The SLOS fibroblast gene expression profile showed down regulation of genes involved in mitochondrial function, calcium homeostasis, cytoskeleton, lysosomal function, signaling (caveolin1, Na+/K+ ATPase), folate metabolism and defense against oxidative stress. Genes related to apoptosis, ubiquitination and extracellular matrix synthesis were upregulated. Such expression profile was not observed in Dhcr7−/− NSCs. In these cells, stem-cell marker nestin and cell-replication genes were downregulated whereas genes implicated in synaptic transmission, signaling, and cell differentiation were upregulated, suggesting abnormal NSC-to-neuron differentiation in vivo.
Conclusion: Cell biology is profoundly altered in SLOS. Future studies with cell lines from other patients and from NSC-derived neurons are needed to confirm and expand these findings.

P-319

PLASMA 25, 27 AND 24S-HYDROXYCHOLESTEROL MEASUREMENT BY ULTRA-PERFORMANCE LIQUIDE CHROMATOGRAPHY ELECTROSPRAY TANDEM MASS SPECTROMETRY

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Background: An increase of plasma 25- and 27-hydroxycholesterols (25-OHC and 27-OHC) have been reported in hereditary spastic paraplegia type 5 (SPG5). In addition, 24-S-hydroxycholesterol (24-OHC) may be a biomarker of disease progression in patients with Huntington disease (HD) and Niemann Pick C1 (NPC1). Therefore, we have developed a highly sensitive and specific method to measure simultaneously these 3 plasma oxysterols.
Method: Plasma oxysterols 24, 25 and 27-OHC were analysed by a LC-MSMS method with isotopic dilution after sterols derivatization into picolinyl esters (Honda et al; 2008). Derivatized sterols were analysed by an UPLC-TQD system, consisting of a triple quadrupole mass spectrometer equipped with an ESI probe and UPLC- system. Oxysterols were separated on a C-18 RP-BEH column and detected by using multiple reaction monitoring.
Results: This method provides high precision and accuracy (intra-assay reproducibility 7% CV, inter-assay reproducibility 10%, accuracy 93–106%). We identified a 15 fold increase of 25-OHC and a 7 fold increase of 27-OHC ratios to cholesterol in 8 SPG5 patients compared to 10 controls.
Conclusion: The present UPLC-MS method provides a reliable and reproducible tool to quantify oxysterols in order to identify new SPG5 patients and to monitor disease progression in HD and NPC1 patients.

P-320

THE EFFECT OF COMBINED THERAPY OF BILE ACIDS AND STATINES IN THE TREATMENT OF CEREBROTENDINOUS XANTHOMATOSIS (CTX).

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Background: CTX is an autosomal recessive defect in bile acid biosynthesis with accumulation of cholestanol in most tissues and body fluids. The enzyme Sterol 27-hydroxilase is deficient and mutation analysis is possible of the CYP27A1gene The clinical picture of juvenile cataract, xanthomas and a progressive neurologic disorder is very suggestive.
Patients: Two brothers were diagnosed at the age of 12 and 14 years with cataract, a cerebellar ataxia, a peripheral neuropathy, absence of xanthomas, and a chronic diarrhea from early childhood. They had a low IQ (range 60–78), speech disturbances and normal neuroimaging. Molecular studies established the diagnosis of CTX.
Treatment objectives and results: They first received only chenodeoxycholic acid which resulted in an improvement of well being, strength, growth and speech and a spectacular disappearance of the steatorrhea. When a HMG-coA reductase inhibitor Atorvastatin was added to the treatment, the bile alcohols excretion in urine normalized which could not be achieved with Simvastatin. Follow-up under the combined therapy showed an improvement of several cognitive functions although the IQ remained in the same range. The peripheral neuropathy disappeared under treatment.
Conclusion: Chenodeoxycholic acid and a HMG- coA reductase inhibitor, especially Atorvastatin, should be combined in the treatment of CTX patients.

P-321

CHYLOMICRON RETENTION DISEASE: A NOVEL POLYMORPHISM AND A MANAGEMENT CHALLENGE

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We present a case of a consanguineous child diagnosed on newborn screening with medium chain acyl-CoA dehydrogenase deficiency (MCADD) who went on to develop significant failure to thrive. His weight, height and head circumference were all below the 0.4th centile. Investigations revealed a low vitamin E but only marginally low apo-B, not consistent with either abetalipoproteinaemia or hypobetalipoproteinaemia. Attempts at nasogastric, lactose free and hydrolysed feeds failed to achieve weight gain. An endoscopy and small bowel biopsy demonstrated lipid laden enterocytes. Further investigation demonstrated he fulfilled the criteria for chylomicron retention disease (CRD). Mutation analysis showed him to be homozygous for the SAR1b gene (c.409 G>A) with a polymorphism in exon 7. This mutation has previously been seen in eight French-Canadian patients, but the polymorphism seen is novel. Management of CRD usually requires a medium chain triglyceride (MCT) based diet however this was impossible due to his MCADD. He has instead been treated with a non-MCT based, high carbohydrate diet and high dose vitamin E. He has made excellent progress in terms of growth. This case is of interest as it provides additional insight into intracellular trafficking and describes the unique challenges in its management due to co-morbidity with MCADD.

P-322

BIOCHEMICAL CONTROL OF TRIGLYCERIDES AND ASSOCIATED MORBIDITY IN TYPE 1 HYPERLIPOPROTEINAEMIA

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Background: Primary disorders of exogenous lipoprotein metabolism can cause recurrent morbidity including pancreatitis. Currently, restriction of long chain fat intake is the only established management.

Methods: A retrospective review of the clinical course and biochemistry of all children who presented with type 1 hyperlipoproteinaemia over a 15 year period.

Results: Of the 14 patients, 8 were diagnosed before age 6 months (4 presented incidentally, 1 on family screening and 3 with rectal bleeding). Of those diagnosed after age 6 months, 2 presented with pancreatitis, 2 on family screening and 2 were fortuitous. No patient whose serial triglyceride levels remained <10 mmol/l (n=6) was symptomatic, but 7 of the 8 patients whose levels were recurrently >10 mmol/l had episodic abdominal pain and 5 had pancreatitis. There was no significant difference (p=0.524) in mean triglyceride levels between those with abdominal pain (17.93+/−2.339) and those with pancreatitis (15.59 +/−2.766). No adverse effects on growth were seen.

Conclusions: In over half of all patients adherence to a strict low long chain fat diet was not achievable and was associated with increased morbidity, especially pancreatitis.

P-323

TWO NOVEL MUTATIONS IN TURKISH PATIENTS WITH SJVGREN LARSSON SYNDROME

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Background: Sjogren Larsson syndrome (SLS) that is a rare inherited disorder caused by mutations in the ALDH3A2 gene for fatty aldehyde dehydrogenase (FALDH) presents with mental retardation of variable severity, spastic or quadriplegia, and generalised ichthyosis at birth. FALDH catalyzes long-chain aldehydes derived from lipid metabolism. More than 80 mutations in ALDH3A2 have been reported worldwide. Here we report two novel mutations in Turkish patients with SLS.

Case Report: Patient 1: 7 years old male patient born of consanguineous marriage had generalised ichthyosis at birth. Evaluation because of mental retardation, spastic quadriplegia and ichthyosis at the age of 6 years demonstrated profoundly low level of FALDH [0.02 nmol/(min.mg)] in skin fibroblasts. Molecular analysis confirmed the diagnosis of SLS and revealed a novel mutation (homozygous c.274_281del8ins1) in the ALDH3A2 gene.

Patient 2: 10 years old male patient born of non-consanguineous marriage had generalised ichthyosis at birth. SLS was suspected with the findings of severe mental retardation, spastic quadriplegia and generalised ihthyosis at 9 years of age. Subsequent molecular analysis revealed homozygous c.153+5_385+361del3011ins19 mutation (full-length exon 2 deletion) in the ALDH3A2 gene.

Conclusions: We predicts that described novel mutations in our patients with typical clinical features of SLS cause premature termination codon.

A-019

FIRST CASE REPORT OF CEREBROTENDINOUS XANTHOMATOSIS IN IRANIAN FAMILY WITH 3 AFFECTED (STEROL 27-HYDROXYLASE DEFICIENCY)

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Background: Cerebrotendinous xanthomatosis is a rare genetic disorder of cholesterol and bile acid metabolism that results in systemic and neurologic abnormalities. It was first described by Van Bogaert and has been characterized clinically, biochemically, and genetically (mutations in the gene CYP27A1). The disease begins in infancy with chronic diarrhea. Cataracts become evident in childhood or adolescence, and xanthomata develop in the second and third decades of life. Significant neurologic impairment includes seizures, dementia, and extra pyramidal dysfunction and begins in the third decade of life and progresses until death. The presentation and course widely varies, and treatment can dramatically alter, especially with early initiation.

Case Report: We report an Iranian family with three affected child who are suffering from Cerebrotendinous Xanthomatosis. Cardinal features were: Motor dysfunction, ataxia, spastic paresis, Xanthomas of the Achilles tendon, Cataracts double. MRI of the brain show diffuse cerebral atrophy and increased signal intensity in the cerebellar white matter on T2-weighted scans. In this report, we present 3 patients with Cerebrotendinous Xanthomatosis, who confirmed by Molecular Analysis. The patients are suffering from disease resulting from a homozygous splice- mutation in intron 2 of the CYP27A1 gene.

Key word; Cerebrotendinous Xanthomatosis, Motor dysfunction, CYP27A1 gene.

P-324

FIRST FULLY VALIDATED (GLP) ASSAY TO MEASURE KIDNEY SPECIFIC ISOFORMS OF GL-3 IN URINE IN FABRY DISEASE

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Background: Fabry disease (FD) is characterized by deficiency of alpha-galactosidase A leading to accumulation of globotriaosylceramide (GL-3). Urine levels of GL-3 are elevated in males and females.
Objectives: To develop a fully validated GLP assay to measure isoforms of GL-3 (C22:0 and C24:0) that are representative of GL-3 found in the kidney. This assay is designed to accurately quantify GL3 in urine from females with high residual enzyme activity resulting in lower levels of GL-3.
Methods: Well characterized synthetic reference standards were used to validate for C22:0 and C24:0 isoforms of GL-3. The assay is validated as per the 2001 US FDA Bioanalytical Method Validation guidance.
Results: The validated assay is able to accurately, specifically and reproducibly quantify C22:0 and C24:0 isoforms. Lower limit of quantitation for this assay is 1 ng/mL for both isoforms. Inter-assay precision and accuracy for six validation runs is as follows: C22:0 Precision (4.5 to 15.1%) Accuracy (−0.92 to −4.9%), C24:0 Precision (5.6 to 7.1 %) Accuracy (−3.5 to −11.6%). Normal GL-3 range was determined in 38 healthy males and females using the validated assay.
Conclusion: We have successfully validated an assay that can discriminate between healthy individuals and males and females with suspected FD.
Conflict of Interest declared.

P-325

RATE OF DECLINE OF GFR IN PATIENTS WITH FABRY DISEASE (FD): PROSPECTIVE NATURAL HISTORY DATA FROM THE CANADIAN FABRY DISEASE INITIATIVE (CFDI)

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Background: Retrospective studies report discordant rates of change of GFR (ΔeGFR) (−2.9–12.2 ml/min/year) in untreated males with FD. Most studies of ERT rely on historical controls, so prospective data not affected by ascertainment biases on ΔeGFR are needed.
Objectives: Describe prospectively ΔeGFR in FD subjects
Methods: CFDI subjects not meeting criteria for ERT (Cohort1c) and those who met treatment criteria and were started on ERT through the CFDI (Cohort1b) were included. Data on ΔeGFR were analyzed by gender and stratified by baseline proteinuria (using a threshold of 0.3 g/day).
Results: 97 subjects (84 F, 13 M; median followup 35 months) did not meet criteria for ERT (Cohort 1c). 44 subjects (29 F, 15 M; median followup 38.5 months) were randomized to ERT through the CFDI (Cohort 1b). ΔeGFR rates were: Cohort 1b: M—3.96 ml/min/1.73 m2/year F +1.56 ml/min/1.73 m2/year Cohort 1c: M- 2.16 ml/min/1.73 m2/year F 0.0 ml/min/1.73 m2/year. As expected, ΔeGFR tended to be higher in subjects above the proteinuric threshold then those below but these trends were not statistically significant due to limited numbers.
Conclusions: ΔeGFR is lower than previously reported. ERT does not restore ΔeGFR to values seen in subjects with no clinically apparent renal disease.
Conflict of Interest declared.

P-326

HIGH RESOLUTION MELTING ANALYSIS- A SIMPLE AND RAPID METHOD TO DETECT FEMALES WITH FABRY DISEASE

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Female carriers of Fabry disease have a high tendency of developing vital organ damage causing severe morbidity and mortality. According to our newborn screening study, we found most female carriers were not detected by enzyme assay.
In this study, we developed a streamlined method for HRM analysis to screen the mutations of GLA gene using a single PCR programme and a single melting profile.
A total of 299,007 (156,179 males) newborns were screened for Fabry disease at our newborn screening centers. From this screening, we identified 121 (106 males) newborns carrying Fabry mutations. A total of 20 different mutations were identified in these patients. Both male and female patients were enrolled in the HRM analysis study. Primer sets were designed to cover the 7 exons and the Chinese common intronic mutation, IVS4+919 G>A of GLA gene. PCR and HRM analyses were performed using a Roche LightCycler. 480.
Both the heterozygous and hemizyous patients of these 20 mutations could be easily identified by HRM analysis. We also successfully used this method to test the dry bloodspots of the newborns with Fabry mutations without the need of determining DNA concentration before PCR amplification.

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DELAYED MENARCHE AND EARLY MENOPAUSE IN FEMALES WITH FABRY DISEASE

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Background: Females with Fabry disease (FD), an X-linked lysosomal storage disorder, exhibit variable degrees of symptoms (eg, asymptomatic to equally severe as males but generally with delayed [~10 years] onset). Effects of FD on female life events (menarche, menopause) are not documented.
Methods: Fabry Outcome Survey (FOS; Shire HGT-sponsored) post-enrolment data were evaluated for relationships between mean age of menarche, and menopause, age-adjusted Mainz Severity Score Index (aa-MSSI) score and other clinical parameters in females with FD.
Results: Of 159 girls (baseline age [mean±SD years] 11.6±4.5), 58 experienced menarche after enrolment (age of menarche 12.8±1.7 [n=46]; aa-MSSI 5.5±6.4 [n=58]). In more severe females treated with agalsidase alfa (agalα) (aa-MSSI 7.5±6.7 [n=21]), age of menarche (13.7±1.6 yrs [n=17]) was delayed versus less severe, untreated females (aa-MSSI 4.4±5.9 [n=37]; age of menarche 12.3±1.4 yrs, [n=29]) (p=0.004). Of 891 women (baseline age 44.0±14.7), 45 underwent menopause after enrolment. In more severe, agalα-treated patients (aa-MSSI 12.4±9.2 [n=30]) versus less severe, untreated patients (aa-MSSI 2.7±9.3 [n=15]), age of menopause occurred earlier (44.8±6.5 [n=27] vs 49.4±5.6 [n=13]) (p=0.036).
Conclusion: In this study, females with significant FD manifestations experienced delayed age of menarche or earlier menopause.
Conflict of Interest declared.

P-328

MULTIDIMENSIONAL ANALYSIS OF CLINICAL SYMPTOMS IN PATIENTS WITH FABRYS DISEASE

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Background: Fabry's disease is an X-linked inherited lysosomal storage disorder due to the deficient activity of alpha-galactosidase A. The interrelationships between clinical symptoms in individual Fabry patients have not yet been fully established.

Objectives: To determine association between clinical symptoms in a cohort of Fabry patients.

Methods: Retrospective collected data from 108 Fabry patients analyzed using multiple correspondence analysis and hierachical ascendant classification.

Results: The cohort comprised 41 males (aged 28.9±11.6 years) and 67 females (aged 40.4±15.5 years). A first cluster grouped digestive disorders and exercise intolerance, which were found in 30% and 32% of the patients, respectively. A second cluster aggregated asthenia (50%), dyshidrosis (47%), acroparesthesia (67%), angiokeratoma (44%) and cornea verticillata (54%), while a third cluster was characterized by kidney (30%), cardiac (39%) and brain abnormalities (25%), and hearing loss (44%). Acroparesthesia, angiokeratoma and dyshidrosis were more frequent in male than in female patients but not other clinical signs or organ complications. Asthenia and hearing loss were found to be independent significant variables in explaining cardiomyopathy, kidney disease and cerebrovascular complications.

Conclusions: Among the various interrelated clinical symptoms occurring in Fabry patients, asthenia and particularly hearing disorders appear as important symptoms predicting disease severity.

Conflict of Interest declared.

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DEMOGRAPHIC CHARACTERIZATION OF BRAZILIAN PATIENTS ENROLLED IN THE FABRY REGISTRY

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Background and Methods: We analyzed Fabry Registry data from patients enrolled in Brazil, to characterize the demographic and baseline clinical characteristics of this patient population. As of October 2010, 126 Brazilian patients were enrolled in the Fabry Registry (61 males, 65 females).

Results: The mean age at symptom onset was 13±11.5 years in males (±SD, n=55) and 14±9.5 years in females (±SD, n=49). The mean age at Fabry diagnosis was 32±14.1 years in males (±SD, n=56) and 31±15.8 years in females (±SD, n=58). The mean time from the onset of symptoms until diagnosis was 20±13.6 years in males (±SD, n=48) and 19±14.8 years in females (±SD, n=44). Neurologic pain was the presenting symptom most frequently reported by both genders (80% of males and 62% of females). Renal signs or symptoms were reported by 12% of male and 2% of female patients in Brazil.

Conclusion: Fabry disease is treatable, and the long delay from symptom onset until diagnosis in Brazilian patients is very concerning, as irreversible damage may occur during this time.The prevalence of neurological pain as a presenting symptom among Brazilian Fabry Registry patients is consistent with previous reports from the overall Fabry Registry population.

Conflict of Interest declared.

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THE PATHOLOGICAL FINDINGS ON PATIENT WITH E66Q MUTATION IN ALPHA-GALACTOSIDASE GENE IS E66Q MUTATION A FUNCTIONAL POLYMORPHISM?

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Background: Fabry disease is an X-linked lysosomal disorder resulting from mutations in alpha-Galactosidase A (GalA) gene. Recent reports described that E66Q mutation in GalA gene is not a disease-causing mutation based on enzymatic studies. We carried out enzymatic and pathological studies on a patient with E66Q mutation in GalA gene.

Case: A 34 years old male patients with end-stage renal failure and cardiomegaly who referred to our hospital for mutation analysis of GalA gene. He was diagnosed as gout in 15 years old and hemodialysis was started for gouty nephropathy from 31 years old. We carried out enzymatic and genetic analysis for GalA and cardiac biopsy.

Result: He had E66Q mutation in GalA gene. GalA activity in leukocytes was 46.2% of average of normal controls and serum globotriaosylceramide (GL3) level was not elevated. The pathological study on cardiac biopsy sample showed no characteristic findings of Fabry disease. The immunostaining for GL3 of cardiac biopsy sample showed no positive cells.

Conclusion: Although E66Q mutation reduced enzyme activity, the characteristic pathological findings of Fabry disease and the abnormal accumulation of GL3 were not detected. E66Q mutation of GalA gene was thought to be a functional polymorphism based on enzymatic and pathological studies.

Conflict of Interest declared.

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PREIMPLANTATION GENETIC DIAGNOSIS OF FABRY DISEASE (GLA GENE)

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Background: Since 2007, we have performed 122 IVF cycles with scheduled preimplantation genetic diagnosis (PGD) for 40 different monogenic diseases all types of indications. Main outcome measure hearth beat was reached in 43%.

We present PGD of Fabry disease (FD). Female in her thirties is FD carrier with positive family history. Her previous pregnancy was aborted. The risk 50% for offspring resulted in her informed decision for PGD in conjunction with genetic counseling.

Our aim was to design specific-family test based on linkage analyses with GLA adjacent polymorphic markers avoiding the need to detect the mutation itself.

Methods: Molecular analysis identified the pathogenic familial mutation c.1025 G>A (p.Arg342Gln) located in exon 7 of the GLA gene (GLA; EC 3.2.1.22), locus Xq22. In the IVF cycle we used genetic haplotyping technique by specific FD multiplex PCR on products of multiple displacement amplification (MDA) from one blastomere biopsied from the cleavage-stage embryo.

Results: There was at least one unaffected embryo available for transfer on day 3, resulting in ongoing pregnancy after first attempt.

Conclusion: The karyotype was denoted 46,XX from amniocentesis. Direct DNA analysis of the GLA gene did not confirmed causal mutation. The pregnancy is followed till delivery.

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SCREENING FOR FABRY DISEASE IN JAPAN

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Fabry disease is an X-linked disorder of alpha-galactocidase A which causes the accumulation of glycolipids in lysosomes. The incidence of the classical type of the disease is approximately 1 in 40,000 males. Recent studies have revealed the late-onset type of the disease to have a higher frequency than previously known. To determine the disease incidence in Japan, we screened newborns to measure alpha-galactosidase A activity in dried blood spots from Japanese neonates. Enzyme-deficient infants were retested, and infants who were double-screening positive were diagnostically confirmed by enzymatic activity and mutation analyses. Thirty eight neonates had a deficiency in alpha-galactosidase A activities and specific mutations, including 5 neonates with classical mutations identified previously. Based on our newborn screening in Japan, the incidence of alpha-galactosidase A deficiency was 1 in 5,600 male. Based on enzymatic activities, the incidence was 1 in 6,000 male. These results suggest that the late-onset phenotype of Fabry disease is underdiagnosed among both males and females in Japan. The recognition of the existence of these patients suggests the need for both early diagnosis and therapeutic intervention. However, ethical issues need to be taken into consideration in terms of when and whom the screening should be performed.

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HENOCH-SCHVNLEIN PURPURA: AN UNUSUAL PRESENTATION OF FABRY DISEASE

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Introduction: Fabry disease(FD) is a lysosomal disorder caused by the deficiency of a-galactosidase A, which leads to storage of globotriaosylceramide(Gb3) and endothelial disease with involvement of kidney, heart and the nervous system. Presentation in female patients can be very late in life and usually is suspected from family history. We report a female patient(20yo) with an unusually presentation of FD.

Case report: Patient was admitted to the ER with purpuric rash in buttocks and lower extremities. After seven days she started with joint and abdominal pain. Lab exams showed proteinuria and hematuria and the diagnosis of Henoch-Schonlein Purpura was made; metilprednisolone 1 mg/kg IVand anti-hypertensive were started. Skin biopsy was performed and showed neutrophilic vasculitis with storage of IgM and granular C3. A kidney biopsy showed vacuolated podocites, suggesting FD. The assay of α-galA showed normal enzyme activity in leukocytes. Molecular analysis has shown one heterozygous mutation in exon 5(c.644A>Gp.N215S).

Discussion:The patient has no other signs or symptoms of FD, and has no family history of FD. The clue to the diagnosis was a kidney biopsy, performed as she did not improve of the proteinuria with the standard treatment. Although unusual, Fabry disease may be considered in the evaluation of Henoch-Schönlein purpura.

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MEASURING PATIENT EXPERIENCES IN FABRY DISEASE: VALIDATION OF THE FABRY OUTCOME SURVEY (FOS) PAEDIATRIC HEALTH AND PAIN QUESTIONNAIRE

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Introduction: Subjective symptoms in paediatric Fabry Disease (FD) can only be assessed by patients using a valid instrument. To date, no such instrument exists.

Methods: A 28-item measure of symptoms, Fabry Outcome Survey (FOS) Paediatric Health and Pain Questionnaire was developed. FOS is a registry for all patients with Fabry disease who are treatment naïve or receiving enzyme replacement therapy (ERT) with agalsidase alfa. A battery of psychometric analyses was performed to assess the measurement properties of this new instrument.

Results: 87 children (age 4–18 years) completed the questionnaire. 23 items in three subscales emerged: Pain associated with heat or exertion; pain associated with cold; abdominal pain and fatigue. Internal consistency reliability for all three subscales was good ($\alpha \geq 0.84$) and was high for all age groups (4–7, 8–12, 13–18 years). Test-retest reliability was high (intraclass correlation coefficient$\geq$0.74). Construct validity showed that each subscale measured unique patient symptom experiences.

Conclusions: Psychometric analyses indicate that the measurement properties of the three subscales are valid and reliable for measuring patient-reported symptoms of FD. The questionnaire could be a useful tool for clinicians to understand the progression of disease and monitor treatment effects.

Conflict of Interest declared.

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CARDIAC MANIFESTATIONS ARE UNDER-RECOGNIZED IN FEMALES WITH FABRY DISEASE

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Background: Fabry disease, X-linked deficiency of the lysosomal enzyme alpha-galactosidase A, results in progressive accumulation of globotriaosylceramide, and affects eyes, skin, kidneys, CNS and heart. Cardiac involvement is most frequent among males after age 40, due to changes of myocardium, conduction system and valves. Females are suggested to have an attenuated disease. We explored the contribution of cardiac manifestations in presentation and disease progression in a cohort of female Fabry patients.

Subjects and methods: Records of 25 female patients (9–62 yr) including ECG, echocardiogram and Holter, were reviewed. LVMI and LVWT were used to assess myocardial involvement.

Results: The mean age-of-onset was 25, and most common finding was bradycardia.

20 % presented with primary cardiac involvement, and more than 50 % had low resting heart rate. 47% (9/19) had EKG abnormalities: LVH (5/9), T wave (4/9) and conduction abnormalities, but dysrhythmias were rare. Abnormal LVMI, though common, did not correlate with age. ERT was administered only in 24% , mostly due to pain.

Conclusions: In females, cardiac manifestations are the silent symptom of Fabry disease. Cardiac morbidity is rarely a reason for consideration of ERT despite its progressive nature. Treatment guidelines should be re-assessed for female Fabry patients.

Conflict of Interest declared.

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ARE GLOBOTRIAOSYLCERAMIDE CONCENTRATIONS USEFUL AS SURROGATE MARKERS TO EVALUATE THERAPY FOR FABRY DISEASE?

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Background: Globotriaosylceramide (Gb3) was assessed as a surrogate marker to predict change from baseline after 12 months of agalsidase alfa (agalα) treatment (CFB-M12) in estimated glomerular filtration rate (eGFR) and left ventricular mass index (LVMI) using pooled data from three 24-week, randomized, placebo-controlled trials (RCTs; TKT003/TKT005/TKT010) and their open-label extension studies (EXT; TKT006/TKT007/TKT013/TKT015) of patients with Fabry disease (FD).

Methods: Males (≥18-year-old) with confirmed FD received agalα (0.2 mg/kg every other week) for 12 months. A backward elimination approach evaluated potential predictors (baseline and CFB-M12 Gb3 [urine; plasma], urine protein; baseline eGFR; age at first dose; baseline LVMI). Subgroups included patients randomized to placebo during RCTs (pbo–>agalα) or to agalα (agalα–>agalα), or with stage 2/3 chronic kidney disease (CKD2/3).

Results: In the analysis population (n=73), eGFR (baseline) and plasma Gb3 (baseline; CFB-M12) significantly predicted CFB-M12 eGFR (all $P < 0.05$). No predictors of CFB-M12 LVMI were significant (n=39). In the pbo–>agalα subgroup (n=36), only plasma Gb3 (baseline; CFB-M12) significantly predicted CFB-M12 eGFR ($P<0.05$). No significant predictors were found in other subgroups.

Conclusion: Urinary and plasma Gb3 concentrations were not useful biomarkers for FD progression in agalα-treated patients.

Conflict of Interest declared.

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PHARMACOKINETICS AND PHARMACODYNAMICS OF MIGALASTAT, A PHARMACOLOGICAL CHAPERONE OF α GALACTOSIDASE A, IN HEALTHY VOLUNTEERS

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Background: Migalastat hydrochloride (AT1001) is being evaluated as an oral agent for treatment of Fabry Disease in patients with responsive α-Gal A mutant forms.
Objective: Characterize migalastat pharmacokinetics and pharmacodynamics in healthy volunteers.
Methods: Healthy volunteers from five Phase 1 studies received single doses of 25–2000 mg (FAB-CL-101, FAB-CL-104); single 100 mg doses fasting and after a high fat meal (FAB-CL-103, AT1001-010); and 50 and 150 mg twice daily for 7 days (FAB-CL-102). Plasma and urine AT1001 concentrations and PBMC α Gal A activity were measured using validated assays.
Results: 139 subjects (104 males, 35 females), 26–34 years old. Bioavailability was ≥50%; food reduced bioavailability by 40%. Plasma levels increased proportionally up to 1250 mg; no further increase was observed at 2000 mg. Steady-state was achieved within 5 days; accumulation ratios were 1.2 to 1.8. Unchanged drug was eliminated by the kidney with a half-life of 4 hours. Dose-related increases in WBC α-Gal A activity were observed after 7 days of dosing. AT1001 was well tolerated, with dizziness, headache and skin irritation the most commonly reported adverse effects.
Conclusion: Migalastat was rapidly absorbed and exhibited linear pharmacokinetics. Dose-related increases in α-Gal A activity in PBMCs were observed.
Conflict of Interest declared.

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A PHARMACOGENETIC APPROACH TO IDENTIFY MUTANT FORMS OF α-GALACTOSIDASE A THAT RESPOND TO A PHARMACOLOGICAL CHAPERONE FOR FABRY DISEASE

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Background: Fabry disease is caused by mutations in the gene (GLA) that encodes alpha-galactosidase A (alpha-Gal A). The iminosugar AT1001 (GR181413A, migalastat hydrochloride, 1-deoxygalactonojirimycin) is an orally-available pharmacological chaperone that selectively binds and stabilizes alpha-Gal A, increasing total cellular levels and activity for some mutant forms. AT1001 is currently under clinical development for the treatment of Fabry disease.
Methods: A validated, transient transfection-based assay was developed in HEK-293 cells to test 460+ Fabry disease-causing mutant forms (predominantly missense) for response to AT1001. Responses were measured by enzyme assay and western blot. Transfection efficiencies across independent assays and mutations were assessed by quantitative real-time PCR.
Results: Increases in both alpha-Gal A protein level and enzyme activity were seen for ~60% of the mutant forms. Importantly, the HEK-293 cell responses of 19 alpha-Gal A mutant forms to a clinically-achievable concentration of AT1001 (10 μM) were generally consistent with observed increases in alpha-Gal A activity in peripheral blood mononuclear cells from male Fabry patients administered AT1001 during Phase 2 clinical studies.
Conclusions: These results suggest that the alpha-Gal A increase in cultured cells may be used to identify Fabry patients that could benefit from AT1001 therapy.
Conflict of Interest declared.

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NEW URINE GL-3 ASSAY TO SCREEN FABRY PATIENTS FOR FACETS, A CLINICAL STUDY WITH MIGALASTAT AT1001

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Background: Fabry disease (FD) is an X-linked lysosomal storage disorder characterized by deficiency of alpha-galactosidase A with accumulation of globotriaosylceramide (GL-3) in tissues and body fluids, leading to progressive, multi-organ disease.
Objective: To demonstrate the utility of a validated GLP assay to quantify two isoforms of urinary GL-3 (uGL-3).
Methods: Two kidney-related isoforms were measured in total 24 h urine in 120 untreated Fabry subjects from the FACETS clinical study and 38 healthy controls. C22:0 and C24:0 isoforms were quantified after urine homogenization and sonication; uGL-3 concentration is expressed relative to creatinine (ng/mg Cr).
Preliminary Results: Males with Fabry disease have elevated levels of uGL-3 (n=38; mean+SD=888+865; median=846; range 9.8–3504) when compared to FD females and controls. Levels of uGL-3 in females ranged from 3.8 to1079(n=82; mean=183+192; median=143), overlapping with the range for the affected male population. In females, the mean level of uGL-3 was higher than that of the control group (control group mean= 17.1+8.3; median 14.5; range 7.3–50.3).
Conclusion: This new analytically validated uGL-3 assay was used as a study screening tool to assess baseline uGL-3 levels in males and females with Fabry disease. It is anticipated that this assay may be useful to monitor treatment effects in FD subjects.
Conflict of Interest declared.

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SUSPICION INDEX TO AID DIAGNOSIS OF NIEMANN-PICK TYPE C DISEASE (NP-C), AN AUTOSOMAL RECESSIVE NEUROVISCERAL DISORDER

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Background: NP-C diagnosis can be delayed for years due to heterogeneous presentation. A Suspicion Index was developed, ranking specific symptoms within and across domains, and with family history, providing a predictive score to help identify suspected patients with NP-C.
Objectives: To construct a Suspicion Index and validate its sensitivity and specificity using patient data.
Methods: A retrospective chart review in seven centres in Europe and Australia (N=216). Three patient types were selected: classical or variant filipin staining NP-C–positive cases (n=71); suspected NP-C cases that were filipin staining negative (n=65); or non-cases (without NP-C suspicion) with ≥1 characteristic symptom of NP C (n=80). Signs and symptoms of NP-C were categorized into visceral, neurological or psychiatric domains, and scores assigned according to their relative prediction for NP-C.
Results: The Suspicion Index has good discriminatory performance (area under the ROC curve >0.75) with cut-points for grading suspicion of NP-C. Neonatal jaundice/cholestasis, splenomegaly, vertical supranuculear gaze palsy, cataplexy, and cognitive decline/dementia were strong predictors of NP-C, as well as symptoms occurring in multiple domains in individual patients, and siblings/parents with NP-C.
Conclusion: The Suspicion Index is a valid tool that can help identify patients who may warrant further investigation for NP-C.
Conflict of Interest declared.

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NIEMANN-PICK TYPE C IN BRAZIL: NATURAL HISTORY AND CLINICAL COURSE IN 42 PATIENTS

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Background: Niemann-Pick type C disease (NPC) is a rentless neurodenerative disorder characterized by a defect in cholesterol trafficking; it is a complex disorder with wide range of clinical phenotypes. Here we describe clinical characteristics and natural history of 42 Brazilian NPC patients.
Methods: Retrospective study and review of laboratory/neuroimaging data were carried out in NPC patients diagnosed in the last 6 years.
Results: Twenty-six patients were confirmed to have NPC by filipin staining (14 patients required molecular analysis). Regarding clinical form, 7 were perinatal , 16 infantile, 11 juvenile and 8 adults. Prolonged neonatal jaundice was a common feature and five of them were diagnosed with "neonatal hepatitis". Hepatosplenomegaly was present in all perinatal/infantile patients, but absent in 6 adults. Ocular abnormalities were seen in 38 patients (mostly, vertical supranuclear gaze paralysis). Leukoencephalopathy and progressive cerebral/cerebellar atrophy were the main MRI features. Other systemic manifestations included dystonia, cataplexia, immunodeficiency, dysphagia. At the time of the study, 13 patients were deceased.
Conclusions: Onset of neurological disease in NPC patients is extremely variable, even in the same sibship. Better understanging of the natural history of the disease is crucial for evaluation of potential therapeutic approaches in such devastating disorder.

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CROSS-SECTIONAL STUDY WITH FOCUS ON EPILEPTIC MANIFESTATIONS IN PATIENTS WITH NIEMANN-PICK C (NPC)

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Background: NPC is a rare lysosomal lipid storage disorder with a broad clinical spectrum ranges from death in neonatal period to adult- onset chronic neurodegenerative disease. Characteristic neurological symptoms are vertical supranuclear gaze palsy, ataxia, dysarthria, progressive dementia, dysphagia, dystonia and seizures.

Patients and results: We investigated 23 patients with NPC in terms of epileptic seizures. 13 patients developed seizures. 10 patients were assigned to the classical juvenile form and 3 patients to the infantile form. Patients with the classical NPC developed epileptic seizures with 10 years on average and patients with the infantile form with 3 years. The seizures include: tonic- clonic seizures, abscences, atonic seizures, focal and complex-focal seizures with or without secondarily generalization. 5/13 had exclusivly focal seizures, 3/13 generalized seizures and 5/13 focal and generalized seizures in combination.

The EEG of ten patients was pathological, 5 patients had normal EEGs.

Except one- because of infrequence of seizures- all patients were treated with anticonvulsive drugs.

4/12 patients become free of seizures, 8/12 still have seizures in reduced frequency.

Conclusion: A broad spectrum of epileptic seizures was observed in NPC patients. Onset of seizures is usually seen several years after neurological manifestation. Anticonvulsants may show partial efficacy.

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CORRELATION BETWEEN SPHINGOMYELINASE, BETA-GLUCOSIDASE, BETA-GALACTOSIDASE AND CHITOTRIOSIDASE ACTIVITY AMONG NIEMANN-PICK TYPE C PATIENTS AND NORMAL SUBJECTS

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Niemann-Pick type C (NPC) is a rare autosomal-recessive disorder caused by accumulation of unesterified cholesterol and glycosphingolipids in the lysosomes. Clinically is characterized by progressive neurological deterioration and hepato-splenomegaly. The aim of this study was to analyze the activity of sphingomyelinase, beta-glucosidase, beta-galactosidase and chitotriosidase in blood samples from patients with NPC and correlate this activity with that of normal individuals. Our results showed that NPC patients have a decreased sphingomyelinase activity (63%) than normal subjects (NPC=1.52 ±0.52 nmol/h/mgprot; normal=2.39±1.14 nmol/h/mgprot) and this difference was significant ($p<0.04$). NPC patients have 11.40±2.82 nmol/h/mgprot of beta-glucosidase activity and 1882±2461 nmol/h/mL of chitotriosidase activity compared to 14.8±4.49 nmol/h/mgprot for beta-glucosidase and 58.5±34.6 nmol/h/mL of chitotriosidase from normal individuals. The differences between the two groups are significant for both enzymes ($p< 0.02$ and $p<0.0002$ for beta-glucosidase and chitotriosidase, respectively). Beta-galactosidase did not differ between the two groups (NPC=152.6± 52.8 nmol/h/mgprot; normal=125.0±38.6 nmol/h/mgprot). These results allow us to conclude that cholesterol accumulation may be interfering with the metabolism of other sphingolipids such as glucosylceramide and sphingomyelin, affecting the activity of their degrading enzymes.

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NPC BRAZIL NETWORK: A COMPREENSIVE PROGRAM FOR THE DETECTION OF NIEMMAN-PICK C DISEASE IN BRAZIL

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Niemann-Pick disease type C (NPC) is an autosomal recessive condition caused by defects on cholestherol trafikking, which leads to a progressive and usually severe visceral and/or neurologic syndrome. The signs and symptoms overlap with other conditions, and diagnosis is difficult as usually requires a staining test performed on cultured fibroblasts and/or comprehensive molecular studies of the two NPC genes (NPC1 and NPC2). The possibility of treating affected patients with substrate reduction therapy (miglustat) makes more important the correct and timely identification of affected patients. With this aim we set up a comprehensive diagnostic program for NPC in Brazil, which includes: 1) providing information on diagnostic procedures, including a collection and transportation kit (for skin biopsy and blood collection); 2) assay of plasma chitotriosidase; 3) culture of skin fibroblasts and Filipin staining; 4)DNA isolation and molecular analysis of NPC1 and NPC2 gene, depending on results of the Filipin testing. This protocol was performed in 140 patients with suspected NPC, referred from physicians from all Brazilian regions. Diagnosis of NPC was confirmed in 15 cases, being abnormal but not conclusive in further 12 patients. These 27 cases were referred to molecular analysis of both genes to complete investigation (molecular analyses in progress).

Conflict of Interest declared.

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NIEMANN-PICK TYPE C: PHENOTYPE HETEROGENEITY IN A FAMILY WITH DIFFERENT GENOTYPES AND A NOVEL MUTATION

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Niemann-Pick C (NPC) disease is an autosomal recessive condition related to cholesterol trafficking defects. We describe a male patient and her aunt with NPC, with different genotypes and phenotype heterogeneity. Case 1: male,born from non-consanguineous parents,presented with normal development until the age of 2 years when started generalized seizures. The brain MRI showed hyperintense periventricular signal. At 3 years of age showed hepatosplenomegaly,umbilical hernia and spastic quadriplegia. Plasma chitotriosidase was increased (1749 nmoles/mL)and Filipin test in fibroblasts was strongly positive. Molecular analysis showed different mutations on the NPC1 gene(G1140V and L1157P). The patient died at 5 years due to respiratory complications. His aunt(father's sister),daughter of non-consanguineous parents,began at the age of 4 years with seizures, jerky movements who progressed with cognitive impairment, motor disorders,hallucinations and aggressiveness. At the age of 26 years she presents ataxia, dysmetria, choreoathetosis and absence of convergence and vertical saccades. The Filippin test in fibroblasts was positive. Molecular analysis of the NPC1 gene showed only one mutation (L1157P),indicating that the patient would be a carrier or would present a complex genotype. Although genetic heterogeneity could explain the different phenotypes observed in this family,further molecular studies(in progress)should help to clarify this interesting situation.

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PEDIATRIC NIEMANN PICK C DISEASE: CLINICAL AND MRI OUTCOME USING TENSOR DIFFUSION IMAGING (DTI) IN A SERIES OF 11 PEDIATRIC PATIENTS, 6 OF THEM TREATED WITH MIGLUSTAT

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A series of 10 NPC patients [6 boys and 4 girls, mean age (M) 7.3 years (2–13.5y)] were followed in our centre between 2007 and 2011. They fell into 4 subgroups: 5 patients presented only with visceral symptoms (mean age of onset: 1 month) followed by neurological symptoms in the early infantile period in 3 of them (M: 12 m). Four patients (4–12 y) presented with neurovisceral symptoms and 2 patients presented with pure neuropsychological symptoms (3–7y). Six patients were treated by miglustat for a mean period of 14 months (6–22 m). All patients underwent clinical assessments including neuropsychological tests, Pineda disability scale and MRI with diffusion tensor imaging (DTI) and spectroscopy (MRS). The mean dose of miglustat was 443 mg (457 mg/m2). In treated patients neurological deterioration was observed in 4 and stabilization in 2. MRI showed retarded myelination but normal MRS. Fractional anisotropy (FA) was low in most patients compared to controls and seemed to correlate to age and disease severity. FA increased in some treated patients. Miglustat was well tolerated except in one patient who interrupted treatment. Response to treatment was variable with a trend for stabilization in patients treated at an early stage of the disease.

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M. NIEMANN-PICK TYPE C: RESTORATION OF LIPID RAFTS AND OTHER BIOCHEMICAL ANOMALIES BY N-BUTYL-DEOXINOJIRIMYCIN

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Morbus Niemann –Pick type C (NPC) is a neurovisceral lysosomal storage disorder caused by mutations of either NPC1 or NPC2 gene. It results in abnormal cellular cholesterol trafficking and secondary accumulation of glycosphingolipids. The precise mechanism leading to clinical symptoms is unknown. One potential mechanism may be altered composition of cellular membrane lipids with subsequent impairment of lipid raft function and protein trafficking. Miglustat (N-butyl-deoxynojirimycin, NB-DNJ), an N-alkylated imino-sugar, inhibits the ceramide-specific glucosyltransferase has been advocated in the treatment of NPC.

Methods and results: We examined the effects of NB-DNJ on membrane trafficking in fibroblasts of NPC patients and controls. We found substantial reduction in glycosphingolipid levels in the patient's fibroblasts after treatment with NB-DNJ. Furthermore, NB-DNJ resulted in a partial restoration of cholesterol-and sphingolipid- enriched membrane microdomains (lipid rafts) as assessed by increased levels of flotillin 2 (a protein marker of lipid rafts) in the floating fractions of sucrose density gradients in Triton X-100 lysates of NB DNJ-treated NPC fibroblasts.

Finally, the enzymatic activities of mitochondrial inner membrane complexes II and IV were partially restored by NB-DNJ.

Conclusion: Our study demonstrates that NB-DNJ as a potential therapeutic agent for NPC patients reverses membrane lipid abnormalities in NPC fibroblasts.

Conflict of Interest declared.

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PERSISTENT EFFECT OF MIGLUSTAT ON CHILDREN WITH NIEMANN-PICK C DISEASE

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Niemann-Pick C disease (NP-C) is characterized by the accumulation of unesterified cholesterol and glycolipids in the lysosomes of cells in the nervous system and visceral organs. Miglustat, an iminosugar reversibly inhibits glucosylceramide synthase, has been approved for the treatment of Niemann-Pick C disease.

We treated five children with NP-C disease. The median age for the first neurological symptom onset was 7.5 years (range 5–9 years). They were treated by miglustat since the median age of 12.3 years (range 9–14).

Before treatment, all cases had mental impairment, 4 cases had motor impairment, and 4 cases had difficulties in swallowing. After treatment, improvement in swallowing was the most prominent and immediate finding. Although the condition of swallowing quality deteriorated in some patients after 1 year of treatment, probably due to drug interruption, both penetration-aspiration scales and dysphagia severity scores did remain stable in 3 of 5 patients after 3.7–5.7 years of therapy. Other domains which improvement has been found include motor function, cognitive function, and borderline horizontal eye pursuit movement.

This study suggests that miglustat can provide therapeutic benefits in neurological symptoms and stabilize the disease in children affected by NP-C.

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NIEMANN PICK TYPE C IN ADULT PATIENTS: RESULTS EVALUATION OF MIGLUSTAT THERAPY

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Introduction: Niemann-Pick type C (NPC) is an autossomal recessive disorder of cholesterol intracellular trafficking. The age of presentation extend into adulthood and the late forms have dominant neurological and psychiatric affectations. Substract deprivation therapy with miglustat could changed the course of the disease

Case Reports: We present two brothers with the diagnosis of NPC at 21 and 26 years old. The first patient presented at 21 years old. He had an excellent school performance until 19 years old, when he begun a slow neurological deterioration associated with dysartic speech, saccadic eye movements, limb hypotonia, dismetry and an ataxic broad based gait. The biochemical study of cholesterol intracellular trafficking in fibroblasts confirmed NPC.The mollecular study identified a mutation in NPC1 gene. His oldest brother was being followed in Psychiatric consultation for psycosis and the same NPC1 gene mutation was confirmed. Both started therapy with miglustat. Four years later, the neurological picture is stabilized with a significative improvement in brain metabolic pattern in positron emission tomography study (PET).

Discussion: NPC late presentations present a diagnostic chalange for the physician. Substract deprivation therapy with miglustat determined a favorable evolution. PET scan give good information to monitor neurologic progression and treatment response.

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EPIDEMIOLOGICAL FINDINGS OF SAMPLES REFERRED TO THE LABORATORY OF A BRAZILIAN REFERENCE CENTER FOR INBORN ERRORS OF METABOLISM (LEIM) FOR DIAGNOSIS OF POMPE DISEASE (PD) USING DRIED BLOOD SPOTS ON FILTER PAPER (DBFP)

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Background: The LEIM is a reference center for the diagnosis of PD in DBFP, according to the technique described by Chamoles et al (2004) which results are expressed both in a Neutral Glicosidase/Acid Glicosidase ratio (N/A) and by the percentage of inhibition of Neutral Glicosidase (% INH) using acarbose. Values of N/A>40 and %INH>87 establish the diagnosis of PD.

Methods: Retrospective analysis of information provided on the requisition form for each sample referred to LEIM from 2006 to 2010.

Results: We have received 735 samples of possible PD patient. Forty seven (6.4%) samples were in the affected range, 16 were from patients below 1y of age, 12 between 1 and 18y and 19 above 18y. The N/A (mean±SD) and the % INH (mean±SD) of the positive samples below 1y of age, between 1 to 18y and above 18y were respectively: 75.3±18.6 and 91.6±3.8, 65.7± 19.5 and 91.2±2.8, 57.3±12.1 and 89.9±2.8.

Conclusion: This technique in DBFP is accurate, time-saving and suitable for establishing the diagnosis of PD, and the samples' handling and shipment are much easier than the traditional technique, these later facts very important logistics matters in a large continental country like Brazil.

Financial support: FAPESP, CNPq, CAPES, AFIP and IGEIM
Conflict of Interest declared.

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INVOLVEMENT OF AKT SIGNALING PATHWAY IN AUTOPHAGY INDUCTION IN FIBROBLASTS FROM INFANTILE-ONSET POMPE DISEASE PATIENTS

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Backgroud: Pompe disease is an autosomal recessive lysosomal storage disorder caused by a deficiency of acid α-glucosidase. Accumulation of autophagosomes is a key factor for poor response to Enzyme replacement therapy in Pompe disease. We and others previously found that autophagy is also activated in patient fibroblasts. However, induction mechanism of autophagy in patient fibroblasts is not fully elucidated. In this study, we analyzed the glucose metabolism-related signaling pathway in patient fibroblasts to clarify their involvement in regulation of autophagy.

Methods: Skin fibroblasts derived from infantile-onset patients with Pompe disease and normal infant were cultivated in the presence or absence of insulin, and were lysed with 2% SDS. These samples were analyzed by Western blotting using the antibodies against akt, and LC3.

Results: The levels of phosphorylated akt were about 30-fold lower in patient fibroblasts than in normal fibroblasts, whereas the levels of LC3-II were about 7-fold higher in patient fibroblasts than in normal fibroblasts. When patient fibroblasts were treated with insulin, increased levels of phosphorylated akt and decreased levels of LC3-II were observed in them.

Conclusion: These results suggest that activation of autophagy in fibroblasts from infantile-onset patients with Pompe disease is caused by downregulation of akt signaling.

Conflict of Interest declared.

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CHARACTERIZATION OF UBIQUITIN-PROTEIN CONJUGATES AS A BLOOD BIOMARKER FOR DETECTION OF AUTOPHAGIC BUILDUP IN POMPE DISEASE MOUSE

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Pompe disease is an autosomal recessive myopathic disorder arising from the deficiency of acid a-glucosidase (GAA). Autophagic buildup is a key factor for poor response to Enzyme replacement therapy in Pompe disease. This finding highlights the need for blood biomarkers which reflect autophagic dysfunction in skeletal muscles. In this study, we characterized the ubiquitin-protein conjugates in GAA-KO mouse plasma and skeletal muscles to investigate the possibility of them as a biomarker for autophagic buiidup. Skeletal muscle and plasma of GAA-KO and wildtype mice were lysed with 2% SDS and analyzed by using the antibodies against ubiquitin, K63-linked polyubiquitin, and LC3. Smears of ubiquitin–protein conjugates were more abundant in skeletal muscles of GAA-KO mice than those of wildtype mice. Additionally, the levels of specific ubiquitin-protein conjugates (approximately 58 kDa) were markedly increased in skeletal muscles of GAA-KO mice. Increased levels of the 58 kDa proteins were also observed in plasma from GAA-KO mice, and were prominently detected by anti-K63-linked polyubiquitin antibody. By immunohistological analysis, both LC3 and K63-linked ubiquitin-protein conjugates was colocalized in the myofibers from GAA-KO mice. These results suggest that the 58 kDa ubiquitin-protein conjugates in plasma may reflect the autophagic buildup in skeletal muscles from GAA-KO mice.
Conflict of Interest declared.

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ALTERED GLYCAN STRUCTURES IN PATIENTS WITH POMPE DISEASE

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Background: N-linked oligosaccharides of glycoproteins represent important molecules that show significant alterations in a great number of conditions.
Objectives: Given the fact that Pompe disease is characterized by altered glycogen metabolism, we hypothesized that N-glycan fingerprinting might represent a useful biomarker for the diagnosis and follow-up of Pompe patients.
Patients and Methods: N-glycans in the serum of 2 patients with Pompe disease and an equal number of controls were isolated and digested with sialidase. The N-glycan profiles were analyzed by DNA sequencer-assisted fluorophore-assisted carbohydrate electrophoresis. Peaks corresponding to glycans were quantified and normalized to the total signal intensity.
Results: We have found that the levels of profiles for peaks 1, 2, 4, 6, 7 and 9 displayed an increase, while those for peaks 3, 5 and 8 exhibited a decrease as compared to healthy controls. Peak 8, corresponding to a trigalactosylated, tri-antennary N-glycan structure (NA3), exhibited the most prominent difference, showing a 1.7-fold decrease. Measurements in consecutive blood samples before the initiation and during enzyme replacement therapy, showed no significant alterations in the concentrations of sugars.
Conclusions: These data suggest that the changes in the serum N-glycans, especially NA3, could provide a useful biomarker for the diagnosis of Pompe patients.

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RECURRENT BRONCHIOLITIS REVEALING AN INFANTILE FORM OF POMPE DISEASE

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Background: Pompe disease (PD) is an autosomal recessive muscle disorder of glycogen metabolism caused by a deficiency of lysosomal alpha-glucosidase A (GAA) that results in the accumulation of glycogen primarily in muscle tissue leading to a severe hypertrophic cardiomyopathy associated with myopathy leading to death in early life.
Objetive: We report a case of 8 months old baby affected by PD misdiagnosed as recurrent bronchiolitis episodes.
Case report: Born of consanguineous parents (first cousin) without specific family history.
He was admitted to hospital at 3, 4 and 5 months old affected by bronchiolitis; the last episode was associated to marked hypotonia. An echocardiography was performed and documented hypertrophic non obstructive cardiomyopathy and lab examinations showed a 3 fold increased CK levels. He was transferred to the regional centre of pediatric cardiology. Muscle biopsy suggested glicogen storage disease and blood spot revealed undetectable GAA activity. At 6 months of age he started enzyme replacement treatment in association of cardiac therapies and after 2 months the cardiac situation doesn't present any evolution.
Conclusion: Respiratory disorder and progressive hypotonia could hide hypertrophic cardiomyopathy due to infantile PD form. The prognosis is strictly related to the early start of enzyme replacement treatment.

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GENERATION OF INDUCED PLURIPOTENT STEM (IPS) CELLS DERIVED FROM A MURINE MODEL OF POMPE DISEASE AND DIFFERENTIATION OF POMPE-IPS CELLS INTO SKELETAL MUSCLE CELLS

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Background: Pompe disease (GSD-II) accumulates glycogen due to a deficiency of acid-α-glucosidase(GAA).Induced pluripotent stem (iPS) cell technology facilitates the study of the pathogenesis of Pompe disease and might eventually enable the development of autologous cell transplantation therapy.
Objectives: We generated iPS cells from somatic cells by introducing reprogramming factors (Oct3/4, Sox2, Klf4 and c-Myc). Pompe-iPS cells were differentiated into skeletal muscle(SM) cells in Matrigel.-coated plates.
Results: Our study first demonstrates the ability to generate iPS cells from a mouse model of Pompe disease. Initially, mouse fibroblasts were harvested from GAA knockout mice, and three reprogramming factors (Oct3/4, Sox2 and Klf4) were transfected into the isolated donor cells using a retroviral vector. These iPS cells also showed decreased levels of GAA enzymatic activity and strong staining with PAS and ACP. Spindle-shaped SM cells were successfully generated from Pompe-iPS cells and showed spontaneous contraction and positive staining with the MHC antibody. EM picture of SM cells showed typical morphological features. Furthermore, Pompe SM cells accumulated massive glycogen in lysosomes.
Conclusions: This study indicates that the iPS and skeletal muscle cells generated in this study could also be a useful disease model for studies investigating the pathogenesis and treatment of SM in Pompe disease.

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HISTOLOGICAL ASSESSMENT OF SKELETAL MUSCLE IN PATIENTS RECEIVING ENZYME REPLACEMENT THERAPY FOR INFANTILE ONSET POMPE DISEASE

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The heterogeneous response of skeletal muscle to enzyme replacement therapy (ERT) causes variable disability, from hypotonia to respiratory failure, in patients with infantile onset Pompe disease (IOPD). Histomorphometrical analysis reflects the extent of glycogen clearance and muscle damage, and may have a prognostic value.

Two patients with IOPD, who started ERT at 2 months of age, had quadriceps biopsy during gastrostomy for feeding problem. Patient 1 responded well to ERT until late infancy, with motor regression and dependency on non-invasive ventilation since 14 months of age. Muscle biopsy at 15 months showed muscle fibres with abundant glycogen and minimal residual myofibril. He succumbed at 22 months of age. Patient 2 was well in the first 3 years. She then developed recurrent aspiration pneumonia and had gastrostomy at 43 months of age. She had satisfactory cardiorespiratory status and could walk independently. Light microscopy revealed variable glycogen content in muscle fibres and patchy involvement. More extensive glycogen overload was observed in the ultrastructural study. She eventually required walking-aid and non-invasive ventilation by 4 years of age.

Our findings support the correlation between histology and clinical state, but caution the risk of sampling errors. Further studies are needed to evaluate its prognostic value.

Conflict of Interest declared.

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POMPE DISEASE IN THE NEWBORN: CLINICAL DIAGNOSIS, ENZYME ACTIVITY ASSESSMENT AND TIMELY INTRODUCTION OF ENZYME REPLACEMENT THERAPY (ERT) ON THE VIII DAY OF LIFE

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Background: Pompe disease (glycogenosis type II) is a lysosomal storage disorder due to deficiency of glucosidase enzyme activity which leads to accumulation of glycogen in the cardiac and skeletal muscle. In the newborn predominant clinical presentation is hypertrophic cardiomyopathy, hypotonus and macroglossia.

Case report: Our patient presented on the II day of life on a routine clinical examination with splitting of the second heart sound without any other clinical signs or abnormal fetal heart ultrasound. Electrocardiogram (ECG) showed typical Pompe disease related abnormalities and the heart ultrasound severe cardiac hypertrophy. Reduced enzyme activity (0,027, range 2,31-27,38 mcmol/L/h) on the blood spot was assessed within 48 hours by Tandem MS, further investigations (enzyme activity in leucocytes and fibroblasts, mutation analysis) were subsequently performed. In the meantime ERT was started on the VIII day of life.

Conclusions: In the newborn unspecific cardiac findings may be Pompe disease related. Earliest detection of the reduced glucosidase activity by Tandem MS is a reliable method which allows timely introduction of the only therapeutic approach, the enzyme replacement therapy and normalisation of cardiomyopathy. Mutation analysis in our patient revealed a condition of compound heterozygousity of two novel splicing mutations: c.955+1 G>A and c.1438-2A>G.

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ENZYME REPLACEMENT THERAPY DURING PREGNANCY AND LACTATION IN POMPE DISEASE

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In 2006 Enzyme Replacement Therapy (ERT) with alglucosidase alfa was registered as a treatment for Pompe disease. We present a primiparous 40-year-old woman with adult-onset Pompe disease who continued receiving alglucosidase alfa in a dose of 20 mg/kg every other week during pregnancy and lactation.

The patient had moderate limb-girdle weakness and used nocturnal ventilation. The mother's clinical condition remained stable until the 25th gestational week. Thereafter she experienced more mobility problems and increased respiratory effort. Fetal growth was normal as monitored by regular ultrasound investigations. At a gestational age of 38 weeks and 5 days, a healthy baby-boy was born. There were no maternal complications and the child developed normally. After delivery pharmacokinetic studies were performed in breast milk and plasma. In breast milk, alpha-glucosidase activity levels peaked 2 hours after the end of the infusion, which was 2 hours later than in plasma. The alpha-glucosidase activity in breast milk disappeared over a period of 24 hours after the infusion. This case report indicates that ERT with alglucosidase alfa can be administered safely during pregnancy. We recommend refraining from breastfeeding on the day of the infusion, since Alglucosidase alfa transfers in small amounts into breast milk.

Conflict of Interest declared.

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IMMUNE RESPONSE TO ENZYME REPLACEMENT THERAPY (ERT) IN 4 PATIENTS WITH INFANTILE POMPE DISEASE

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Recent studies show that immune response to rhGAA is associated with infusion related reactions and limited efficacy of treatment. Our study focus on the immune response to ERT in 4 patients with classical infantile phenotype followed up 1–6 years with ERT. All 4 patients had infusion-rate dependend IREs, temporally for 6–9 months in 3 patients, ongoing in 1 patient. No Ig-E antibody formation. Patient 1–3 had low titer IgG antibody formation in the first year of ERT and immuntolerizied there after. Patient 4 had high titer antibody formation without trend for immuntolerization. Patient 1 had partial response to ERT. Patients 2 and 3 had favourable outcome, as they are able to walk. Patient 4 showed initially some improvements followed by rapid detoriation after onset of IREs. Patient 1 is tested CRIM positve. In patients 2–4 CRIM status is not tested, but the mutations are highly suggestive for CRIM negative. In patients 2 and 3 alternative infusion protocols and preventive premedication were used.

Immune response to rhGAA was associated with infusion related events. Poor outcome was observed in a patient with high antibody titer. Patients with immuntolerization had favourable outcome. For future immunmodulation strategies are mandatory to prevent high antibody formation.

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CHITOTRIOSIDASE IN PATIENTS WITH TYPE I GAUCHER DISEASE IN MINAS GERAIS, BRAZIL

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Background: Chitotriosidase (ChT) activity is 100 to 1000-fold higher than normal in Gaucher disease (GD) patients and falls with enzyme replacement therapy. It is not a useful marker in 6% of the population that is homozygous to a 24 bp duplication in exon 10 of CHIT1. The Hospital das Clínicas da Universidade Federal de Minas Gerais is a reference center in Minas Gerais state—Brazil for the treatment of GD type I. Only recently this center has included ChT in the follow-up.
Objective: To establish the ChT activity and genotype for CHIT1 in patients with GD type I in treatment (n=26) in the state of Minas Gerais, Brazil.
Methods: ChT activities (Hollak et al, 1994) and genotyping of CHIT1 duplication (Hise et al., 2003) in 26 GD patients.
Results: Among 26 patients with GD, 18 were homozygous wild-type (HH=69%), 6 heterozygous (Hh=23%) and 2 homozygotes (hh=8%) for CHIT1 duplication. The ChT activity in patients HH (115–32,577, mean= 6,798 nmol/h/ml), Hh (200–4,417, mean=2,106 nmol/h/ml) was, respectively, around 200 and 62-fold greater than in controls (3–68, mean= 34 nmol/h/ml). The hh produced 1–1,4 nmol/h/ml.
Conclusions: Our results are consistent with previous studies. The introduction of ChT marker certainly improves the clinical management of GD patients.
Financial Support: Fapemig

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CHITOTRIOSIDASE AS SCREENING PARAMETER FOR DIAGNOSIS OF LSDS

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Background: In the last years lysosomal labs use Chitotriosidase as diagnostic screening parameter for LSDs. We evaluated the median and interquartile range (IQR) of chitotriosidase in 8 lysosomal storage disorders.
Methods: We investigated 101 patients with confirmed diagnosis of Gaucher disease, Sphingomyelinase deficiency, Niemann-Pick disease type C, Fabry disease, GM1-Gangliosidosis, A-Mannosidosis and CESD. In 5% of the patients we found lack of chitotriosidase activity due to a common chito1-gen mutation.
Results: It is a retrospective cross-sectional study in children and adults. Median chitotriosidase activity in the control group was 56 nmol/ml/h (IQR 42 nmol/ml/h) reflecting normal values. In Gaucher disease (GD: N=26) 18563 (IQR 13020), in Sphingomyelinase deficiency (SMD: N=9) 1111 (IQR 2009), in Niemann-Pick disease type C (NPC: N=35) 827 (IQR 1204), in GM1-Gangliosidosis (N=6) 776 (IQR 1533), in Fabry disease (N=10) 130 (IQR 151), in A-Mannosidosis (N=13) 171 (IQR 121), in one patient with Farber disease 3279 and in CESD 249 nmol/ml/h.
Conclusion: Excluding those patients with lack of chitotriosidase activity 100 percent sensitivity was observed for elevated chitotriosidase in GD, SMD, GM1-Gangliosidosis, Farber disease and CESD. Limited specificity was seen in NPC, Fabry disease and A-Mannosidosis. Extreme high activity>5000 nmol/ml/h was exclusively noted in GD.

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CHITOTRIOSIDASE IN GAUCHER TYPE 1- A STUDY FROM INDIA

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Background: Monitoring efficacy of ERT in Gaucher type 1 by assaying plasma chitotriosiase activity is complicated by prevalence of its benign deficiency in all populations. Alternately HDL cholesterol measurement is advocated and observed to be increased on ERT.Study Design: We evaluated 102 suspected Gaucher type 1 patients and 165 healthy controls to assess chitotriosidase activity amongst Indians. ERT was initiated in 4 patients and both HDL cholesterol and chitotriosidase were assayed in baseline and follow up samples.
Results: Gaucher type 1 was diagnosed in 32 patients based on the β glucocerebrosidase activity. Benign chitotriosidase deficiency was obtained in 21% of Gaucher type 1 and 16% of healthy controls. Chitotriosidase activity in remaining patients was significantly elevated and ranged from 5504–28,340 nmoles/hr/ml in Gaucher type 1 as compared to 8–87 nmoles/hr/ml in healthy controls. Three out of the four patients of ERT showed a significant decrease in chitoriosidase (17–24%) and a parallel increase in HDL cholesterol (32–42%) as compared to the pre treatment values.
Conclusion: Chitotriosidase deficiency is consistent in India. Chitotriosidase reduces remarkably on ERT. Larger studies are required to assess utility of HDL cholesterol as a marker for monitoring the therapy.

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β-GLUCOCEREBROSIDASE GENE MUTATIONS IN TWO COHORTS OF GREEK PATIENTS WITH SPORADIC PARKINSONS DISEASE

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β-Glucocerebrosidase gene (GBA) mutations have been identified as a risk factor for the development of Parkinson's disease (PD) in different ethnic groups. In Northern Greece significant association was only found in early onset PD patients (EOPD).

Eight different GBA mutations were investigated in two cohorts of ethnic Greek patients with sporadic Parkinson's disease from Thessaly (n=100, cohort A) and Athens (n=105, cohort B). Age-gender-ethnicity matched healthy individuals from the same areas were included as controls (n=206). Statistically significant overrepresentation of GBA mutation carriers in the PD patients compared to controls was observed (p=0.006; OR=3.24, 95% CI=1.35–7.81). The association was reinforced in EOPD patients (p=0.000; OR=11.37, 97% CI 3.73–34.6). PD patients harboring GBA mutations had an earlier onset of symptoms than non-carriers (p=0.034, p=0.004). Differences regarding the type of mutations and/or their relative frequencies were observed between cohorts.

In conclusion, GBA mutations were identified with increased frequency in both geographical cohorts of sporadic PD patients compared to controls, the difference being statistically significant only in cohort A. An impressive association with EOPD was found, one third of the EOPD patients harboring a GBA mutation. Genetic and/or environmental factors may account for the observed differences in the two geographical cohorts studied.

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THE SACCADIC AND NEUROLOGICAL DEFICITS IN TYPE 3 GAUCHER DISEASE

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Objective: To characterize the saccadic eye movement abnormalities in patients with chronic neuronopathic Gaucher disease (GD3) in relationship to neurological and neurophysiological abnormalities.

Methods: N=15 patients with GD3, age 8–28 years, prospective follow up (up to 4 years), saccadic eye movements (search coil method), neuropsychological and neurophysiological testing.

Results: Patients with GD3 had a significantly higher regression slope of duration vs amplitude and peak-amplitude vs amplitude compared to healthy controls for horizontal/vertical saccades. Saccadic latency was significantly increased for horizontal saccades. Downward saccades were more affected than upward saccades. Regression slopes of horizontal vs vertical saccades for duration vs amplitude and peak-amplitude vs amplitude were significantly correlated (r=0.31, p<0.005) as was horizontal vs vertical saccade latency (r=0.68, p<0.005). These abnormalities became more pronounced over time in some patients. There was a significant correlation between verbal IQ (p=0.03), performance IQ (p=0.04), full-scale IQ (p=0.02) and the downward saccade slope and between vertical saccade slope and the performance on the Purdue Pegboard when patient uses both hands (p=0.01). There was poor correlation between BAER, SSEP and saccadic eye movement parameters.

Conclusions: Saccadic function and oculo-manual dexterity abnormalities are likely useful for studying neurological function in patients with GD3.

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GAUCHER DISEASE DIAGNOSIS ON DRIED BLOOD SPOTS: ESTABLISHMENT OF SPECIFIC ACTIVITY

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Diagnosis for Gaucher Disease(GD) is based on beta-glucosidase(GBA) assay on leukocytes and chitotriosidase(CT) on plasma. Lately dried blood spots(DBS) are been used as screening, but not as final diagnosis because it's not established a measurement of specific activity.

The aim of this study is to establish the best conditions of DBS elution to measure the specific activity of GBA and CT using total protein assay.

DBS from 15 healthy volunteers and 8 GD patients were used. One punch of each sample was eluted using 40μL of sodium-phosphate-buffer(pH7) and tested for four incubation periods(10, 20, 30 and 60minutes) and two temperatures(30°C and 37°C). A microplate 4-MU fluorimetric assay for CT and GBA and a protein assay using 10μL of each elution was performed after.

It was possible to measure activity of GBA and CT on DBS using a previous elution with pH7 buffer. The enzymes had higher activity with the 37°C elution than 30°C, so was protein(p<0.05). Because the determination of specific activity takes in consideration the protein amount, there was no statistically significant difference between temperatures and elution time. It was able to differentiate the healthy volunteers from the GD patients with a p=0.002 for CT and p=0.0002 for GBA.

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REFERENCE VALUES AND CORRELATION BETWEEN STANDARD TECHNIQUES AND DRIED BLOOD SPOTS FOR GAUCHER DISEASE DIAGNOSIS

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Gaucher Disease(GD) is caused by β-glucosidase(GBA) deficiency and chitotriosidase(CT) is increased in these patients. The dosage of GBA (leukocytes) and CT(plasma) are considered as standard for diagnosis. This measure can also be held in dried blood spots(DBS) just as screening.
This study aims to establish reference values of GBA and CT activity in leukocytes, plasma and DBS and its correlation.
We used heparinized-blood of 18 healthy-volunteers(HV), 18 GD-patients and 4 carriers. The techniques used 4-Metilumbeferil substrates. GBA were incubated for 60 min(leukocytes) or 5 h(DBS) and CT for 15 min(plasma) or 30 min(DBS), at 37°C and stopped with pH10.3 buffer. Reactions were read at 365 and 450 nm spectrofluorimeter.
The reference values for GBA in leukocytes raged from 8.6 to 18.4(HV), 0.4 to 9.0(carriers) and 0.0 to 4.6 nmol/h/mg protein(GD); while in DBS were 2.7 to 7.1(HV), 2.6 to 6.2(carriers) and 0.2 to 2.6 nmol/h/mL(GD). CT in plasma ranged from 0 to 246.9(HV), 165.1 to 338.7(carriers) and 0 to 49.58 nmol/h/mL(GD) and in DBS from 4.6 to 44.5(HV), 4.5 to 21.4 (carriers) and 0.0 to 2249.5 nmol/h/mL(GD). For both enzymes, the correlation was significant with $p<0.0001$, $r=0.78$(GBA) and $r=0.89$(CT). The results showed that DBS enzyme assay are reliable and can be useful as screening methods for high-risk populations.

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ORGANIZATION OF MEDICAL CARE TO PATIENTS WITH TYPE I GAUCHER DISEASE IN A UNIVERSITY HOSPITAL IN BRAZIL

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Background: In Brazil there are 610 patients with Gaucher disease type I (GDI). Minas Gerais state (MG), with 21 million inhabitants, has 93 patients—38 at the main university hospital.
Objective: Own organization of medical care of GDI patients in MG, starting by the Hospital das Clinicas-Universidade Federal de Minas Gerais (HC-UFMG).
Material and Methods: Patients: 30 adults and 8 children monitored according to the GDI protocol of the Brazilian Health System. Collected data: clinical information, blood count, chitotriosidase, DNA analysis for CHIT1 and SF36 instrument. Database: developed with PHP-Myskl system.
Results: The database stocked clinical and laboratory data of patients allowing the doctors to visualize graphically the progress of each patient. Imiglucerase had been performed in 36 patients and miglustat in 2 patients. During the recent shortage of imiglucerase, 32 patients discontinued their treatment and 4 had dosage reduced in half.
Conclusions: Our goal of monitoring of all patients with GD type I of MG state turned possible with the development of an own database. Its expanded use will allow a better management of individual patients. It may also reflect in the costs of treatment and facilitate the evaluation of new drugs.
Financial Support: Fapemig

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FREQUENCY OF GBA GENE MUTATION N370S, CAUSING GAUCHER DISEASE, IN LATVIA

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Background: Gaucher disease is an autosomal recessive disorder of lysosomal degradation of glucosylceramide. It results in a progressive accumulation of glucosylceramide in the lysosomes, leading to hepatosplenomegaly, anemia, thrombocytopenia and various skeletal complications. The most common mutation is the substitution A to G at nucleotide position 1226, which produces aAsn-Ser change at amino acid 370. There is variable frequency of this mutation: in Ashkenazic Jews carrier frequency 1: 15; 1 in 100 people in the United States.
Objectives: Detect carrier frequency of mutation N370S in Latvian population
Material and Methods: We studied 150 volunteers, aged 19–25 years. DNA was extracted from whole blood and purified by standard phenol/chloroform extraction protocol. The presence of mutation N370S in gene GBA was analyzed using PCR with subsequent restriction enzyme XhoI digestion and detected in polyacrylamide gel.
Results: One patient out of 150 was heterozygous for mutation N370S. Estimated Gaucher disease frequency when patients are homozygous for mutation N370S is 1 : 90 000
Conclusions: 1. Carrier frequency of mutation N370S in Latvian population is low.
2. Gaucher disease in Latvian population may be caused by other mutations
3. To obtain more precise results about mutation frequency control population should be enlarged.

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SPECTRUM OF MUTATIONS IN THE GLUCOCEREBROSIDASE GENE OF A COHORT OF BRAZILIAN PATIENTS WITH GAUCHER DISEASE

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Gaucher disease (GD), an autosomal recessive lysosomal storage disorder, characterized by glucocerebrosidase (GC) deficiency due to mutations in the glucocerebrosidase (GBA) gene. To date, more than 250 mutations have been identified. The aim of present study was to identify sequence alterations in the GBA coding region in GD patients from Brazilian. We have evaluated a group of 125 unrelated patients from a total of over 800 individuals tested that were shown to have GC deficiency through enzyme assay. DNA samples from these patients were analyzed through long-range PCR, followed by specific screening for common mutations and direct sequencing. We have identified common and rare mutations in the mutant alleles. Besides p.N370S and p. L444P, p.G377S mutation was also frequently found in this cohort. Among rare mutations, alterations include insertions, deletions, complex alleles as well as single base changes. In addition, we have also described novel sequence variations that are likely to be disease causing mutations. As these sequences alterations are located in conserved residues of the protein or at a splicing site, these changes are likely to be responsible for changes in the structure and/or function of GC. Full screening of GBA gene is relevant to identified additional rare mutations.

P-370

A 2-YEAR, PROSPECTIVE, OPEN-LABEL, NON-INFERIORITY STUDY OF MIGLUSTAT AS MAINTENANCE THERAPY IN ADULTS WITH TYPE 1 GAUCHER DISEASE (GD-1) STABILISED ON ENZYME REPLACEMENT THERAPY (ERT)

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Forty-two adults with GD-1 stable after ≥3 years of ERT were enrolled. Endpoints were: change from baseline in liver volume (primary) and in spleen volume, haemoglobin concentration and platelet count (secondary). Mean (SD) patients' age was 45.1 (12.7) years and previous ERT duration was 9.5 (4.0) years. Median exposure to miglustat (100 mg t.i.d.) was 658 (range 3–765) days. Thirteen patients discontinued treatment as a result of adverse events (principally gastrointestinal), six for protocol-mandated safety criteria for suspected disease progression, one for non-compliance and one withdrew consent. The primary endpoint was met, as the upper 95% confidence limit of mean percent change in liver volume from baseline to End of Treatment was below the non-inferiority margin of 10% [−1.1%; 95%CI −6.0, 3.9%; mean (SD) baseline volume 1775 (484) cm3, mean absolute change −47.5 cm3 (95%CI −151, 56)]. Baseline values [mean (SD)] for spleen volume, haemoglobin concentration and platelet count were 510 (372) cm3, 14.8 (1.6) g/dL, and 198.9 (95.7) W10(9)/L, respectively. Mean (95%CI) absolute changes in these three parameters, respectively, were 102 (24,180) cm3, 0.95 (−1.38,-0.53) g/dL, and 44.1 (57.6, 30.7) W10(9)/L. Miglustat is a useful option for maintenance treatment in patients with GD-1 previously stabilised by enzyme therapy.

Conflict of Interest declared.

P-371

BONE MINERALIZATION IN A PEDIATRIC COHORT OF GAUCHER PATIENTS UNDER ENZYME REPLACEMENT THERAPY

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Background: Skeletal involvement is a frequent complication of Gaucher disease (GD), and represents an important cause of morbidity and disability.

Objectives: To evaluate retrospectively the long-term efficacy of enzyme replacement therapy (ERT) in correcting bone mineralization in a paediatric cohort of patients with GD type 1.

Patients and methods: 18 patients (age 9.2±4.7), receiving ERT (20–60 U/kg) biweekly, up to a maximum of 17 years, were included. Bone symptoms, growth, imaging and lumbar bone mineral density (BMD) were assessed.

Results: At baseline BMD was pathological (Z-Score range: -3.80 to −2.21) in 7/9 patients who started ERT after pubertal spurt, 5 of them showed growth delay. After 2 years of ERT, BMD significantly improved in 13/18 patients: mean values from −1.3 to −1.0 (p=0.02). A significant difference was found between baseline and last reading data (p=0.01). At the end of the study BMD normalized in all but 2 splenectomized siblings; growth delay normalized in all patients.

Conclusions: ERT started in paediatric age correct osteopenia, maintain normal BMD and growth in the vast majority of patients (16/18). The improvement of BMD is mainly achieved during the first 2 years of treatment. Early splenectomy may hamper a normal bone mineralization.

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COMBINED ENZYME-REPLACEMENT AND SUBSTRATE-REDUCTION THERAPIES IN GAUCHER DISEASE TYPE II/III

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The patient from non-consanguineous Albanian parents presented at four months with isolated splenomegaly. Oculomotor apraxia and hepatomegaly progressively appared during the second half of the first year. Hematological values showed intermittent bicytopenia (hemoglobin 103–113 g/L [reference >100], neutrophils 1.25–1.80 G/L [>1.50] and thrombocytes 71–116 G/L [>150]). Chitotriosidase was 27.5 mU/ml (reference <1.03). Decreased beta-glucosidase activity in lymphocytes confirmed the diagnosis of Morbus Gaucher (residual activity 10%). The double homozygous mutations c.882 T>G and c.1342 G>C detected in the GBA gene of our patient (AMC Amsterdam) have been described earlier in Gaucher type II patients.

At one year, tip-toe-posture and slight extension reaction of the upper limbs were the only additional neurologic abnormalities in an otherwise well developing girl, suggesting an intermediary rather than type II Gaucher, and prompted us to introduce combined enzyme-replacing (ERT) and substrate-reduction (SRT) therapies.

Since treatment initiation (follow-up 9 months), both ERT and SRT are well tolerated, hepatosplenomegaly and blood cell counts have normalized almost completely. Moreover, psychomotor development is near to normal and neurological signs stable. Notwithstanding the necessarily cautious interpretation of the short follow-up, these findings suggest that early initiation of combined ER- and SR- therapies might improve outcome in Gaucher disease.

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ELIGLUSTAT, AN INVESTIGATIONAL ORAL THERAPY FOR GAUCHER DISEASE TYPE 1 (GD1): PHASE 2 RESULTS AFTER 3 YEARS

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Background: In GD1, deficient acid β-glucosidase activity causes glucosylceramide accumulation. Eliglustat, a potent and specific glucosylceramide synthase inhibitor, is under development as an oral substrate reduction therapy for GD1.

Objective: Report long-term efficacy and safety results.

Methods: Efficacy outcomes included changes in hemoglobin and platelet levels, spleen and liver volumes, and bone mineral density (BMD). Achievement of therapeutic goals was measured.

Results: Of 26 enrolled patients, 19 completed 3 years. After 3 years, mean hemoglobin level and platelet count increased by 2.6 g/dL and 91%, respectively; mean spleen and liver volumes decreased by 61% and 29%, respectively (all $P<.0001$). The majority of patients met long-term therapeutic goals for these parameters; all met ≥3 long-term therapeutic goals. Mean lumbar spine BMD increased by 0.6 Z-Score; femur dark marrow was reduced or stable. There were no bone crises; new lytic lesions or bone infarcts; or worsening of pre-existing lytic lesions or bone infarcts. Eliglustat was well-tolerated. Most adverse events (AEs) were mild and unrelated to treatment. Eight drug-related AEs, all mild, occurred in 6 patients.

Conclusions: Eliglustat has shown promising efficacy and safety, with clinically meaningful improvements in hematologic, visceral, and bone parameters. Three Phase 3 trials are enrolling patients.

Conflict of Interest declared.

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GAUCHER DISEASE IN UKRAINIAN PATIENTS: CLINICAL AND BIOCHEMICAL INDICATORS OF ERT EFFICIENCY

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Introduction: Gaucher disease (GD) is an inherited glycolipid storage disorder resulting from the deficiency of glucocerebrosidase. The GD accounts for 71% of all lysosomal disorders diagnosed in Ukraine.

Materials and methods: The 17 GD patients (7 children, 10 adults) have been treated with ERT and monitored since 2004. The diagnoses of all patients were established by clinical, laboratory and morphological ("Gaucher cells" in bone marrow) data. The mean activity of glucocerebrosidase deficiency in leukocytes was 2,7±1,4 nmol/h/mg protein (reference range—7,2±2,1 nmol/h/mg protein). All patients were screened for gene mutations in the GBA gene.

Results: The regularity of ERT with the recombinant glucocerebrosidase has reduced manifestations of hepatosplenomegaly and pancytopenia, bone pain and bone crises in the most GD patients, and led to significant improvements. The chitotriosidase activity has decreased from 4552–29614 nmol/h/ml pl (before the ERT) to 870–4897 nmol/h/ml pl (after the ERT).

Conclusions: The efficacy of treatment depends on the regularity, the dose and the severity of the disease at the beginning of therapy.

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BIOMARKER RESPONSES AFTER 3 YEARS OF TREATMENT WITH ELIGLUSTAT, AN INVESTIGATIONAL ORAL THERAPY FOR GAUCHER DISEASE TYPE 1 (GD1)

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Background: In GD1, certain disease-related biomarkers (GL-1, GM3) have been identified that reflect perturbation of the glycosphingolipid metabolic pathway caused by acid beta-glucosidase deficiency, whereas others (chitotriosidase, CCL18, ACE, TRAP) represent secondary effects on tissues. Various biomarkers in GD1 are responsive to imiglucerase enzyme replacement therapy and are used clinically to monitor patients. Eliglustat, a novel, small molecule inhibitor of glucosylceramide synthase (GCS), is under development as an oral substrate reduction therapy for GD1.
Objectives: Evaluate biomarker responses to eliglustat in GD1 patients.
Methods: A Phase 2 study enrolled 26 adult patients with GD1 not on treatment; 19 completed 3 years of treatment with eliglustat. Plasma levels of GL-1, GM3, chitotriosidase, CCL18, ACE, and TRAP were measured periodically throughout the study.
Results: GD1 biomarkers were elevated in most patients pre-treatment. Statistically significant decreases ($P<.0001$) following eliglustat treatment were seen in median plasma GL-1 and GM3 (80% and 64%, respectively), which normalized; and median chitotriosidase, CCL-18, and ACE (80%, 73%, and 62%, respectively), which all remained above normal. Two-year median TRAP decreased by 51% ($P<.0020$), but remained above normal.
Conclusions: Eliglustat treatment led to reductions in GD1-related biomarkers that reflect both primary and secondary effects of the underlying enzyme deficiency.
Conflict of Interest declared.

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A PATIENT WITH ADULT ONSET GM2-GANGLIOSIDOSIS: 24 YEARS OF FOLLOW-UP

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Sandhoff disease is a lipid-storage disorder caused by a defect in ganglioside metabolism due to mutations in the HEXB gene that disrupt the function of the N-acetyl-beta-D-glucosaminidase isoforms Hex A and Hex B. A late-onset form of Sandhoff disease is rare and its symptoms are heterogeneous, including cerebellar syndrome, motor neuron disease and autonomic dysfunction.
We report on a 25 year-old man who suffered from mild proximal lower limb muscle weakness (4 MRC) and intense fasciculations. EMG demonstrated signs of denervation in lower limbs, whereas brain MRI was normal. Biochemical studies documented a 13% residual Hex activity in leukocytes; HEXB genetic analysis revealed a compound: delta5' deletion and C1214T (Pro405Leu) missense mutation.
Twenty years later appeared bilateral Babinski sign and neurophysiological study showed peripheral sensory neuropathy. Twenty-four years from the onset, he was independent in deambulation, his weakness in lower limbs is slightly worsened (3 MRC) and mild weakness appeared in both arms. He didn't develop neither cerebellar or autonomic signs and brain MRI remained normal.
To our knowledge, this is the longest follow-up in a patient with Sandhoff disease. It underlines how a clinical history of pure motor neuron disorder, in GM2-gangliosidosis, may have a relative benign course.

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LATE INFANTILE GM1 GANGLIOSIDOSIS PRESENTING AS A PRIMARY LEUCODYSTROPHY

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Background: Late infantile GM1 Gangliosidosis presents after the 1st year with neuroregression, absence of dysmorphism, and typical MRI changes affecting the thalami and basal ganglia. We present a case where diagnosis was delayed because of MRI findings of primary leucodystrophy.
Case Report: The patient presented at 14 months with delayed walking & hypotonia without dysmorphism. After 2, she lost the ability to cruise, crawl and sit. Brain MRI showed typical features of leucodystrophy without abnormalities of the thalamus, basal ganglia and distal long tracts. Investigations for MLD, peroxisomal disorders and other metabolic causes were negative. Between 2.5–4 years she had progressive intellectual decline and onset of seizures, dystonia and spasticity. Further MRI scans at 4 and 5.5 years showed progressive loss of white matter with cerebral atrophy; the basal ganglia and thalami remained relatively preserved. At 8 years, she was in a near-vegetative state with spasticity, dystonia and complex epilepsy with mild dysmorphism; spinal Xray showed anterior beaking of vertebrae. Persistent oligosaccharidiuria and low leucocyte beta-galactosidase led to confirmation of diagnosis by demonstration of 2 mutations, c75+2_3insT/c.601 C>T in the GLB1 gene.
Conclusion: GM1 Gangliosidosis should be considered in the differential diagnosis of primary leucodystrophy presenting in late infancy

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THE FIRST CASE OF INFANTILE TAY-SACHS DISEASE CONFIRMED BY GENETIC ANALYSIS IN KOREA

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Tay-Sachs disease (TSD) is a metabolic disease, inherited in an autosomal recessive way, that is caused by a defect of hexosaminidase A. The gene responsible for the disease, HEXA gene, is located on chromosome 15q23-q24. Though TSD is rare in the general population, the frequency is reported to be 100 times higher in the Ashkenazi Jewish and eastern Quebec French Canadian. The disease is characterized by progressive weakness, loss of motor skills, decreased attentiveness, and increased startle response with progressive evidence of neurological degeneration. Hexosaminidase A activity study was performed in a boy who had shown delayed development since 6 months of age, regression since 9 months and exaggerated startle response. He had no unusual history nor medical event at birth. His hexosaminidase A activity was decreased and progressive brain atrophy was noted in serial brain MRI. HEXA gene study was performed and direct sequencing of the amplified genomic DNA revealed 2 kinds of mutations being in compound heterozygosity (c.913_915delTTC and IVS5-1 G>T). Family members received HEXA gene study and all were diagnosed as carriers. We would like to report the very first case of the classic infantile form of TSD that was confirmed by HEXA gene study in Korea.

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A NOVEL MUTATION IN A TURKISH PATIENT WITH THE INFANTILE FORM OF TAY-SACHS DISEASE

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Background: Hexosaminidase A deficiency results in a group of inherited neurodegenerative disorders characterized by impaired lysosomal catabolism of ganglioside GM2. Tay Sachs disease corresponds to deficiency of the alpha-subunit of beta-hexosaminidase. The infantile form of Tay-Sachs disease presents with progressive weakness, loss of motor skills, decreased attentiveness, and increased startle response beginning between three and six months of age with progressive evidence of neurodegeneration. More than 100 mutations have been described in HEXA gene which encodes the alpha subunit of beta-hexosaminidase. We here report a novel mutation in HEXA in a patient with infantile Tay-Sachs disease.

Case Report: 34 month-old male patient, appeared to be completely normal at birth, presented with progressive weakness, severe psychomotor retardation, myoclonic jerks, an exaggerated startle reaction, and retinal cherry red spots. Leukocyte beta-hexosaminidase A activity in patient was profoundly low (1.72 nmol/h/mg protein of patient). The diagnosis of hexosaminidase A deficiency confirmed by a novel mutation (homozygote c.A1306G;I436V) in HEXA gene.

Conclusions: The great majority of specific mutations described in the alpha subunit of the HEXA gene are associated with the acute infantile form. We here report a novel mutation associated with infantile form of Tay-Sachs disease.

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PRENATAL SCREENING OF SIALIC ACID STORAGE DISEASE, SIALIDOSIS AND GALACTOSIALIDOSIS AND CONFIRMATION OF SASD IN CULTURED FIBROBLASTS BY LC-MS/MS

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Background: Inborn errors of sialic acid metabolism include three lysosomal storage disorders: sialic acid storage disease (SASD), sialidosis and galactosialidosis. The phenotypical spectrum of these three diseases ranges from severe forms, which may present as hydrops fetalis, to relatively mild forms. Screening can be performed by determination of free (FSA) and conjugated (CSA) sialic acid in urine or amniotic fluid supernatant (AFS). Diagnosis of SASD can be performed by quantification of FSA in cultured fibroblasts.

Objective: To develop simple quantitative procedures to determine FSA as well as CSA in AFS, and FSA in cultured fibroblasts, using isotope dilution LC-MS/MS.

Results: In 6 SASD AFS samples FSA was 2-9-fold elevated compared to normal controls. CSA levels in AFS of 3 sialidosis and 4 galactosialidosis cases were all higher than the reference samples. The method was also validated for determination of FSA in fibroblast homogenates. In SASD fibroblasts FSA was clearly elevated compared to normal controls.

Conclusion: We report simple quantitative procedures to determine FSA and CSA in AFS as well as FSA in fibroblasts, improving both prenatal diagnostic efficacy for sialic acid disorders and confirmatory testing in cultured fibroblasts following initial screening in urine or AFS.

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CROSS SECTIONAL-STUDY OF PATIENTS WITH ALPHA-MANNOSIDOSE: CLINICAL PRESENTATION UND COURSE OF DISEASE

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Introduction: α-Mannosidosis is a rare lysosomal storage disorder caused by deficiency of α-mannosidase. Characteristic symptoms of a-Mannosidosis are hearing loss in early childhood, skeletal abnormalities, immunodeficiency and mental retardation.

Patients and Results: We investigated 31 patients, aged 3–44 years, with a-Mannosidosis.

Common mutations found in 28 non-related patients were c.2248 C>T (9/56) and c.1830+1 G>C (6/56) in homozygous or heterozygous form.

Mean age of onset was 1.5 years. Diagnosis was confirmed on average with 6.4 years. Most common first symptom was hearing loss, followed by skeletal abnormalities and development delay. Patients suffer from recurrent infections of upper airways in childhood and purulent infections of teeth and skin.

30 patients developed ataxia. 10 patients need a wheelchair. 6 patients showed psychiatric symptoms. Inguinal or umbilical hernia, mental retardation, amblyopia, gingivahyperplasia, mild hepatomegaly or splenomegaly can also be found in patients with a-Mannosidosis.

Conclusion: Combination of hearing loss, recurrent infections, skeletal abnormalities and development delay are characteristic manifestations of a-Mannosidosis.

A phase II- trial with enzyme replacement therapy for a-Mannosidosis is currently in process. Experience with other enzyme replacement therapies revealed that early diagnosis is important for efficacy of this therapy.

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SIGNIFICANT ACCUMULATION OF CHOLESTEROL ESTER IN FETAL TISSUES IN WOLMAN DISEASE IMPLICATION OF ENZYME REPLACEMENT THERAPY

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Background: Wolman disease is an autosomal recessive disorder of breaking down cholesteryl esters and triglycerides, resulting in accumulation of fat in the several organs. Major clinical manifestations are hepatomegaly and liver dysfunction. Enzyme replacement therapy is now under development. Its effectiveness is controversial because it is unclear whether significant accumulation and organ dysfunction initiate in fetal period or not. We had opportunity to analyze fetal liver and kidney of Wolman disease.
Case report: A 36 year-old woman had two babies diagnosed as Wolman disease based on clinical manifestations. Gene test showed that the proband is a homozygote of 890 insert G of acid lipase gene. Prenatal genetic diagnosis was performed and the fetus was diagnosed as affected and aborted at 16 gestational weeks. An autopsy was done.
Results: Liver and kidney tissue were obtained from the fetus. Cholesterol, Cholesteryl esters and triglycerides were separated by thin-layer chromatography. Apparent cholesterol ester accumulation was detected in liver and kidney tissue. In the liver, accumulation of triglycerides and cholesterol was also observed, indicating the accumulation had already started in fetal period.
Conclusion: These data suggest enzyme replacement therapy for Wolman disease may not be effective when it starts after birth.

P-383

TARTRATE-RESISTANT ACID PHOSPHATASE DEFICIENCY IN A PATIENT WITH BONE DYSPLASIA, LEUKOENCEPHALOPATHY AND AUTOIMMUNITY

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Background: Tartrate-resistant acid phosphatase (TRAP), a lysosomal enzyme expressed in osteoclasts and macrophages, is a marker for growth and bone turnover, macrophage activation, and hairy cell leukemia. Recently, biallelic mutations in APC5, the gene encoding TRAP, have been found in patients with spondyloenchondrodysplasia (SPENCD), cerebral calcifications, spasticity and autoimmunity1. Abolished TRAP activity in serum and loss of expressed protein in patients' cells was demonstrated. TRAP deficiency causes hyperphosphorylation of osteopontin, a bone matrix protein, which appears to be the key pathogenetic factor for autoimmune disease in these patients. Here we report clinical characteristics of one of these patients.
Case Report: The 15-years-old female patient was born to healthy consanguineous Turkish parents. First clinical findings evolved during the second year of age when chronic thrombocytopenia, progressive gait disturbance, spasticity, leukoencephalopathy, SPENCD, growth retardation, proteinuria, and episodes of severe hemolytic anemia developed. Her karyotype was found to be 46, XXX. Recently, homozygosity for an APC5 mutation was detected1; both parents were heterozygous for this mutation.
Conclusion: SPENCD with leukoencephalopathy and autoimmunologic symptoms such as lupus erythematosus, hemolytic anemia and thrombocytopenia is highly suggestive of TRAP deficiency and requires further investigations with enzymatic and molecular genetic analyses.

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MUCOLIPIDOSIS TYPE II (I-CELL DISEASE) MASQUERADING AS CONGENITAL INFECTIONS IN A NEWBORN

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Background: Mucolipidosis-type-II (ML-II) is rare progressive neurodegenerative lysosomal storage disease (LSD). The characteristic clinical features become evident within the first year of life.
Case presentation and results: This 6-month-old girl was born at term by Caesarean-section. She had blueberry muffin rash with multiple non-blanching purplish lesions and 2 cm palpable liver. Laboratory investigations revealed neutropenia, marked thrombocytopenia, unconjugated and conjugated hyperbilirubinemia and moderately elevated lactate dehydrogenase at age 4 days. Skeletal radiographs showed diffuse periostitis of all long bones suggesting congenital infections. However all serological investigations were negative. There were large, very prominent cytoplasmic vacuoles within the lymphocytes on peripheral smear, which led us to investigate for LSD at age 5 days. Serum total hexosaminidase, hexosaminidase A and alpha-N-acetylglucosaminidase enzyme activities were markedly elevated suggesting ML-II at age 10 days. She had known homozygous disease causing mutation in the GNPTAB gene (c.3503_3504delTC) confirming the diagnosis of ML-II.
Conclusion: This is the first patient with ML-II presented with blueberry muffin rash. Peripheral blood film examination is a simple, cheap and readily available screening test to diagnose patients with LSD in the neonatal period. As bone marrow transplantation is widely used treatment option for the most LSD, early diagnosis is crucial.

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LONG-TERM SURVIVAL AND RESTORATION OF GLCNAC-1-PHOSPHOTRANSFERASE ACTIVITY IN PERIPHERAL LYMPHOCYTES OF A PATIENT WITH I-CELL DISEASE WHO RECEIVED ALLOGENIC BONE MARROW TRANSPLANTATION

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Background: Therapeutic effect of allogenic bone marrow transplantation (BMT) on I-cell disease has not been determined.

Objective: To evaluate effects of BMT in a patient with I-cell disease.

Case report: This girl, diagnosed with I-cell disease at age 8 months, underwent BMT at 14 months of age. She had achieved psychomotor development, though delayed, until 11 years of age, but her psychomotor function began to deteriorate after that time and she died at the age 21 years. Her affected elder sister achieved no psychomotor development at all and died at the age 2 years and 11 months. They both carried p. Q104X/p.R1189X mutations of the GNPTAB gene.

Results: Activity of GlcNAc-1-phosphotransferase, alfa-mannosidase and beta-hexosaminidase in peripheral lymphocytes increased to a maximum value of 2.28-, 4.8- and 2.8-folds, respectively, after BMT. Chimerism analysis revealed that 94.8% of genome in the peripheral blood was of donor origin, as assed at 15 years of age.

Conclusion/Discussion: BMT apparently restored enzymatic activity in the observed periods. Achievement of psychomotor development until the age 11 years may be attributable to possible therapeutic effect of BMT, as compared with the outcome of the affected sister. The reason of the deterioration remains unclear, as rejection was excluded.

Conflict of Interest declared.

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TANDEM MS SCREENING OF BOUND SIALIC ACID IN URINE SAMPLES OF PATIENTS WITH MUCOLIPIDOSIS II AND III

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Mucolipidosis (ML) II and III are inherited lysosomal storage disorders caused by a deficiency of the enzyme N-acetylglucosaminyl-1-phosphotransferase (GlcNac-1-phosphotransferase), whose activity is required for the post translational processing of many hydrolases to form the mannose 6-phosphate (M6P) recognition site that permits lysosomal targeting. As a result of this enzyme deficit, lysosomal enzymes are secreted by cells and abnormal amounts of carbohydrates and fatty materials (lipids) accumulate in lysosomes, resulting in damaged tissues causing symptoms that range from joint problems and mild learning disabilities to severe retardation and skeletal deformities. Biochemical analysis of ML II/III patients show increased hydrolase (eg. beta-D-hexosaminidase, beta-D-glucuronidase, beta-D-galactosidase, and alpha-L-fucosidase) activities in blood plasma and elevated excretion of oligosaccharides in urine. Quantitative analysis using LC-MS-MS of free and bound sialic acid concentrations in urine samples of ML II/III patients reveal excessive excretion of bound sialic acid in 80% of investigated patients. In contrast, mildly elevated excretion of glycosaminoglycans (GAGs) detected by spectrophotometry after dimethylmethyleneblue staining and abnormal oligosaccharides detected by thin layer chromatography were each present in 60% of our patients. Excessive urinary excretion of bound sialic acid in a metabolic screen indicates, apart from sialidosis (ML I), also ML II/III as a putative diagnosis.

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I-CELL DISEASE AND MULTIPLE SULFATASE DEFICIENCY ARE PART OF THE DIFFERENTIAL DIAGNOSIS IN PATIENTS WITH MULTIPLE ABNORMAL LYSOSOMAL ENZYME ACTIVITY LEVELS

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I-Cell disease is caused by defects in the targeting of lysosomal enzymes to lysosomes due to deficiency in the enzyme UDP-N-acetylglucosamine:N-acetylglucosaminyl-1-phosphotransferase. Multiple sulfatase deficiency (MSD) is caused by mutations in the sulfatase-modifying factor 1 (SUMF1) which encodes formylglycine-generating enzyme (FGE).

Patient 1 is a 7-year old female with extremetly elevated serum total hexosaminidase and α-N-Acetylglucosaminidase activity. Patient 2 is a 5-year old girl with deteriorating clinical symptoms whose Arylsulfatase-B activity was only slightly below the normal range which triggered us to measure serum hydrolases. The activity of several serum hydrolases (Hexosaminidase, α-galactosidase, and α-N-acetylglucosaminidase) was high. A third patient had low enzyme activity for α-N-Acetylglucosamindase,α-L-Iduronidase, α-glucoronidase and Arylsulfatase B. Total serum hexosaminidase and α-Galactosidase activities were high which was consistent with a diagnosis of I-Cell disease. Patient 4 had reduced Arylsulfatase A and B, and α-NAcetylgalactosamine enzyme activities, findings suggestive of MSD.

Conclusion: These cases demonstrate the difficulty of diagnosing as well as differentiating between I-Cell disease and MSD because of the overlap in abnormal enzyme activity levels in both conditions. It is very important to include I-Cell disease and MSD in the differential diagnosis of any abnormal lysosomal enzyme activity, especially if two or more enzymes are abnormal.

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INCIDENCE OF I-CELL DISEASE (MUCOLIPIDOSIS TYPE II) IN THE IRISH POPULATION

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Cases of I-cell disease diagnosed on the island of Ireland over 13 years (1/1/199-31/12/2010) were identified in collaboration with the clinical diagnostics laboratory. A database documenting details of diagnosis and clinical course where available was compiled and correlated with published birth rates including that of Irish Travellers (available only for the Republic). Twenty infants from 14 families were diagnosed with I-cell disease during the study period. 18 were born to Irish Traveller parents, one to non-Traveller Irish parents and one to parents from Southern Europe. Mutation analysis was available for 7 cases, of whom 6 (all Travellers) were homozygous for the c3503_3504delTC mutation. Median age of death in patients of the Traveller community was 232 days (range 3–936).

Incidence estimated using population data for the Republic (ROI) and Northern Ireland was 1.56 per 100,000 live births. The incidence amongst Travellers (based on ROI cases and population data) was 114 per 100,000 live births, suggesting a carrier frequency of the common mutation of 1 in 15. The carrier rate amongst Irish non-Travellers remains rare at 1 in 512. This high incidence and carrier rate found in the Irish Traveller population is relevant for genetic counselling of this consanguineous community.

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MUCOLIPIDOSIS TYPE II AND III: ECHOCARDIOGRAPHIC STUDY OF BRAZILIAN PATIENTS

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Mucolipidosis (ML) types II and III are lysosomal diseases due to GlcNAc-phosphotransferase deficiency. Multisystem complications are present, including cardiopulmonary ones.

Objective: To characterize echocardiographic abnormalities in Brazilian ML II/III patients.

Methods: Cross-sectional study based on echocardiograms and medical records of ML II/III patients seen in a Brazilian Reference Center for LSD. Results: 9 patients were included; 5/9 ML II (aged 0.8 to 5 years) and 4/9 ML III (11–42 years). **Clinical exam**: systolic murmurs (n=6/8), cyanosis and dyspnea (n=1/8). Echocardiography: some alteration (n=9/9), systolic function preserved (n=9/9), patent foramen ovale (n=1/8, 10 months, MLII), mitral thickening (n=5/9, of whom one MLIII), mild mitral regurgitation (n=8/9), aortic valve thickening (n=3/9, and 2/3 MLIII). Two MLII patients presented LVH (aged 4 and 5 years). PH was present in 1/9 patient (MLIII, good evolution after treatment with Sildenafil and continuous positive airway pressure during sleep).

Conclusion: Our study is unprecedented in Brazilian patients and confirmed that ML II/III patients may present HP, aortic and mitral thickening, and mitral insufficiency. Differently from the literature, LVH occurred in one child in our sample. Following these patients is important to detect early abnormalities, especially PH, which can be adequately treated.

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MUCOLIPIDOSES TYPE II AND III: MOLECULAR ANALYSIS OF THE GNPTAB AND GNPTG GENES IN 15 BRAZILIAN PATIENTS

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Mucolipidoses α/β (ML II or III) and γ (ML III) are lysosomal diseases (LSD) due to deficiency in GlcNAc-phosphotransferase, which is encoded by GNPTAB and GNPTG genes.

Objective: To identify mutations in the GNPTAB and GNPTG genes in Brazilian patients with MLII/III.

Methodology: We analyze both genes in gDNA of MLII/III patients diagnosed in a Brazilian Reference Center for diagnosis of LSD. Exons and flanking regions are being amplified by PCR and sequenced in automated sequencer ABI3100..

Results: Fifteen patients were included: 7/15 have ML II (4 female; mean age±SD=2.2±1.9 years) and 8/15, MLIII (1 female; mean age±SD=20.9 ±12.3); 2/15 were siblings. Both pathogenic mutations in GNPTAB were identified in 8/14 unrelated patients and only one in 4/14. The most frequent pathogenic mutation was c.3503_3504delTC (exon 19); two novel pathogenic mutations are being described: c.2269_2273delGAAAC; c.2808A>G. To date, 9/11 exons of GNPTG were sequenced in 14 patients, but only 3 different mutations were found in 4/28 alleles.

Discussion/Conclusions: MLII/III α/β appears to be the most common type of ML in Brazil. Our findings suggests that, in our country, screening for GNPTAB gene mutations should be initiated in exon 19.

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EVALUATION OF ENDOTHELIAL FUNCTION BY ENDOTHELIAL PULSE AMPLITUDE TESTING IN PATIENTS WITH MUCOPOLYSACCHARIDOSIS

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Background: Progressive cardiovascular changes have been known as one of the major causes of death in patients with mucopolysaccharidosis (MPS). All types of MPS have been reported to present with cardiovascular manifestations. Evaluation of endothelial function (EF) by flow-mediated dilation (FMD) of the brachial artery has been studied and endothelial dysfunction (ED) characterized by decreased percent mediated dilatation was demonstrated in individuals with risk factors for atherosclerosis. Close relation of EF in coronary and brachial artery has been demonstrated. Studies to evaluate peripheral vascular EF with finger arterial pulse wave amplitude (PWA) with a finger plethysmograph (PAT) have been performed and showed that PAT hyperemia and FMD were significantly correlated.
Objectives: To evaluate coronary artery ED in MPS patients for early detection of coronary artery lesions.
Material and Methods: Finger arterial PWA with a PAT device (Endo-PAT2000) was used to evaluate coronary EF. Total of 17 patients were studied: MPS-I (11), II (2), III (2), VI (1), and Mucolipidosis (1).
Results: Ten out of 11 patients with MPS-I, and one MPS-II, one MPS-III, and one MPS-VI patients showed abnormal results.
Conclusion: a high prevalence rate of ED was demonstrated in patients with MPS by the finger PAT device.
Conflict of Interest declared.

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EFFECTS OF ENZYME REPLACEMENT THERAPY (ERT) ON JOINT MOBILITY IN 27 PATIENTS(PTS) WITH MUCOPOLYSACCHARIDOSIS (MPS) I, II AND VI

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Twenty-seven MPSpts (6MPS I, 16 MPS II, 5 MPS VI) on ERT were evaluated regarding urinary glycosaminoglycans(ur.GAGs) and the passive articular range of motion.
Pts' mean age: MPS I 30.5 years(y)(range 16.7–44.0); MPS II 17y (11.3–29.9), MPS VI 7.4y (4.5–9.4). ERT mean duration: MPS I 7.5y (6.3–8.7); MPS II 4.8y (3.2–7.1); MPS VI 3.7y (2.4–5.2).
ERT resulted in a mean reduction in ur.GAGs of 89% for MPS I (78.9–96.7); 76.1% for MPS II (30.4–97.2); 65.4% for MPS VI (37.5–83.4).
All the pts increased shoulder abduction: mean gain of 27.9degrees in MPS I; 20.8 in MPS II; 8.5 in MPS VI.
In MPS I there was a hip mobility improvement (mean 11.2degrees) and a moderate improvement of wrists (6.7degrees) and elbows (4.9degrees).
In MPS II there was a stabilization for each joint (gain of 2-3degrees), except for the foot (−1.6). In MPS VI, there was a gain of 2 degrees in foot mobility, there was a 1-3degree loss for the other joints.
ERT is effective in decreasing ur.GAGs and in improving shoulder abduction. The best improvement was in MPS I pts (the ones treated longer), whereas there was a joint mobility stabilization in MPS II and MPS VIpts

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MUCOPOLYSACCHARIDOSES IN THE STATE OF MINAS GERAIS, BRAZIL

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Introduction: In Brazil there are around 540 patients with mucopolysaccharidoses (MPS). The state of Minas Gerais (MG), with 21 million inhabitants, has 58 MPS patients.
Objetive: To establish the prevalence of MPS, their differential diagnosis and treatment in MG state, Brazil.
Material and Methods: Review suspected cases of MPS in the clinic for inborn metabolic disorders of Hospital das Clínicas da Faculdade de Medicina da Universidade Federal de Minas Gerais (HC-UFMG) from 1999 to 2010.
Results and Conclusions: From 1999 to 2010, 47 patients were diagnosed: MPSI-17, MPSII-10, MPSIII-9, MPSIV-2, and MPSVI-9. MPSII is the most prevalent type in Brazil with 30% of the cases (Rede MPS Brasil, 2010), but in MG the most common is MPSI with almost 35% of the MPS. Most patients have classic phenotypes, but rare symptoms as heart aneurysm of the tip of the left ventricle and liver cirrhosis were observed in MPSII. As a differential diagnosis, GM1 gangliosidosis, mucolipidosis II, Geleophysic dysplasia, Dyggve-Melchior-Clausen syndrome, congenital hypothyroidism and Costello syndrome patients were sent to HC-UFMG. Enzyme replacement therapy has been indicated to most patients with MPSI, II and VI. One MPSI patient received successful stem cell transplantation 15 months ago at age of 1 year.

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PSYCHOMETRIC PROPERTIES OF A NEW MEASURE OF QUALITY OF LIFE FOR PATIENTS WITH MUCOPOLYSACCHARIDOSIS (MPS) THE BRAZILIAN MPS-QOL: PRELIMINARY RESULTS OF THE PILOT STAGE

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Background: There are few studies on the impact of enzyme replacement therapy (ERT) on the QOL of MPS patients. There are no specific measures to assess their QOL.
Objectives: To evaluate psychometric properties (PP) of MPS-QOL.
Methods: Sample was collected during a meeting of MPS patients and consisted of 27 patients (8 children, 11 adolescents, and 8 adults). They completed 2 measures for QOL including the MPS-QOL. There are three MPS-QOL versions: for adolescents (MPS-QOL-AD), for children (MPS-QOL-CH), and for adults (MPS-QOL-ADU). PP were analyzed by percentage of missing value, frequencies, inter-item correlation, and exploratory factor analysis.
Results: Patients had different types of MPS: MPS I=4, MPS II=7, MPS IIIB=3, MPS IVA=7, MPS VI=6. Measures were applied by proxy in nine patients due to cognitive impairment. Twelve patients were on ERT. Items presented significant percentage of missing values in "Finances" for MPS-QOL-CH, "Affective Life" and "Sexual Behavior" for MPS-QOL-AD and "Accessibility" and "Work Capacity" for MPS-QOL-ADU. MPS-QOL-AD and MPS-QOL-CH presented a 5-factor solution (89.8% of variance explained).
Conclusions: Patients of different ages value different aspects of QOL, which keeps the need for specific versions. MPS-QOL shows good PP.

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CHLORAMPHENICOL ENHANCES IDUA ACTIVITY ON FIBROBLASTS FROM MUCOPOLYSACCHARIDOSIS I PATIENTS

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Background: Mucopolysaccharidosis I (MPS I) is caused by a deficiency of α-L-iduronidase (IDUA), mainly due to nonsense mutations. Stop codon read through (SCRT) is an alternative to increase enzyme activity by the suppression of a premature stop codon.
Objective: To treat MPS I fibroblasts with geneticin and chloramphenicol, which were described to induce misreading.
Methods: Fibroblasts from three MPS I patients (p.W402X/p.W402X, p. Q70X/c.1739 G>T, p.R89W/p.W402X) were treated with geneticin (200ug/mL), chloramphenicol (200ug/mL) or had no treatment (n=4/ group). IDUA activity was measured by fluorimetric assay and mRNA expression by quantitative PCR. cDNA sequencing of compound heterozygous was performed. Statistical analysis was done using Friedman with pairwise compairisons (differences: $p<0.05$).
Results: Geneticin was not able to enhance IDUA activity or expression and chloramphenicol enhanced 100-fold IDUA activity on compound heterozygous fibroblasts. cDNA sequencing showed that only the missense allele was being amplified.
Conclusion: Nonsense alleles are probably being target to nonsense-mediated mRNA decay (NMD). Thus, positive response to SCRT is dependent on the patient's genotype and on the efficiency of NMD. Chloramphenicol apparently acts through a mechanism other than SCRT. This drug may be used ancillary to enzyme replacement therapy, as it is able to cross the blood brain barrier.

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ALFA-L -IDURONIDASE GENE MUTATIONAL ANALYSIS OF 10 EGYPTIAN PATIENTS

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Introduction: The various disorders of mucopolysaccharidosis (MPS) share many clinical features. MPS-I is an autosomal recessive disorder caused by a deficiency of the lysosomal enzyme α-L-iduronidase.
Aim of work: Diagnosis of MPS-I among suspected cases referred to Biochemical Genetics Department. Detection of the mutation in the α-iduronidase gene among some of the diagnosed Egyptian patients.
Subjects and Methods: 10 patients were included; each one was subjected to quantitative determination of urinary GAGs, two dimensional electrophoretic separation, assay of α-L–iduronidase enzyme activity and mutational analysis.
Results: Mutational analysis proved that 1 patient was homozygous for the mutation E299, 1 patient homozygous for the mutation C 593 ins 4 del 8 and another one for P533R. MPS-I mutations were not detected in the 4 other patients. 3 novel mutations (c. 854delC in exon 6, T141S in exon 4, and IVS2+6c>t) and G51D in exon 1 were detected. In addition; 9 sequence variants including 5 previously unreported polymorphisms (N73H, N297N, R363S, IVS10 (3025) g>t, and IVS11 (3318) c>a) were identified.
Conclusion: Sequencing of the whole gene of α-L-iduronidase is needed to diagnosed patients to detect the commonest mutations among our population and their correlation with the response to enzyme replacement therapy when available.

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ENZYME REPLACEMENT THERAPY DURING PREGNANCY IN A MUCOPOLYSSACHARIDOSE I SCHEIE PATIENT

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We present a primiparous 37-year-old woman with Mucopolysaccharidose type I, Scheie disease (MPSI-S) who continued to receive enzyme replacement therapy (ERT) with Aldurazyme in a dose of 100 I/U every week during pregnancy.
Fetal development was normal and amniocentesis showed a normal karyoptype and enzyme activity for alpha-L-Iduronidase (IDUA).
The patient underwent regular cardiac evaluation by echocardiography, which showed moderate mitral and aortic valve regurgitation with normal LV function. At the end of pregnancy she developed signs of pre-eclampsia with hypertension and albuminuria, which was treated with intravenous magnesium sulfate. After a gestational period of 37 weeks and 5 days a Caesarean section was performed and a healthy boy was born. After delivery the patient was admitted to the ICU because of congestive heart failure. Echocardiography revealed significant aggravation of the preexisting mitral and aortic valve regurgitation. After rigorous treatment, the patient recovered without sequelae and was discharged eight days after delivery.
This case report indicates that ERT with Aldurazyme can be administered safely during pregnancy, but demonstrates that pregnant MPS I patients should monitored closely especially during the last months of pregnancy because of hemodynamic changes that may occur.

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LONG-TERM EFFICACY OF HEMATOPOIETIC STEM CELL TRANSPLANTATION IN THE PATIENTS WITH MUCOPOLYSACCHARIDOSIS TYPE II: A NATIONWIDE SURVEY IN JAPAN

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Objectives: To evaluate the efficacies of hematopoietic cell transplantation (HSCT) in mucopolysaccharidosis II (MPS II).

Methods: A nationwide survey was conducted in Japan to collect clinical data of the patients with MPS II who received HSCT. The ability of daily life (ADL), joint mobility, height, IQ, MRI lesions, regurgitations of heart valves, and urinary glycosaminoglycans (GAG) were analyzed at the baselines and at the most recent visit in each patient.

Results: The records of 26 patients collected from 8 distinct hospitals. The follow-up period was from 5 years 5 months to 16 years 3 months (mean, 9 years 6 months). ADL was maintained almost around baseline levels, joint mobility was improved, growth of height was better than untreated patient cohort. Cribriform changes and ventricular dilatation in brain MRI were improved in 9 and 4 patients, respectively. No improvement was shown in brain atrophy. Valvular regurgitations were diminished in 20 valves out of 66 valves, while worsening was observed frequently in the patients transplanted at older ages. The values of urinary GAG were remarkably lower in HSCT-treated patients than age-matched untreated patients.

Conclusion: HSCT is effective and worthwhile in MPS II patients with both of attenuated and severe forms.

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ENZYME REPLACEMENT THERAPY IN A 3-MONTH-OLD BOY WITH PRESYMPTOMATIC MPS II: 3-YEAR FOLLOW-UP

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Background. Mucopolysaccharidosis type II is an X-linked, progressive lysosomal storage disease caused by a deficiency of the lysosomal enzyme iduronate-2-sulfatase. In recent years, clinical trials and overall experience with enzyme replacement therapy (ERT) have been shown to clinically benefit patients with considerable preexisting disease, but no data exists on the effect of beginning ERT before the onset of significant clinical signs of disease.

Material and Methods. We present the 3-year follow-up of a boy with MPS II who had idursulfase therapy initiated at the age of 3 months and compare his clinical course to his healthy twin brother.

Results. After 3 years of treatment, the patient has not developed any clinical manifestations of MPS II. The most impressive findings were the preservation of normal ranges of movement for most joints, cardiac valves and facial appearance for our patient. The only difference when compared with his healthy twin brother was lower IQ and mild deformity of one vertebrae.

Conclusion. We suggest that early treatment of MPS II may significantly delay or prevent the onset of the major clinical signs, substantially modifying the natural history of the disease.

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GENISTEIN IN THE TREATMENT OF A CASE OF MPS IIIC

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Introduction: Mucopolyssacharidosis type III (MPS III), known as Sanfilippo syndrome results from the deficiency of several enzymes needed to break down the glycosaminoglycan (GAG) heparan sulphate, which is particularly important in the central nervous system. MPS IIIC results from deficiency of acetylCoA:alpha-D-glucosaminide acetyltransferase. No specific treatment is available and is therefore limited to the management of symptoms. Genistein an isoflavone that inhibits glycosaminoglycans synthesis has been tried in some patients with MPS IIIA.

Case report: We report a seven year old girl presenting with dysmorphia, mild developmental delay mainly of speech, hyperactivity, hepatomegaly high liver enzymes and mild bone abnormalities. Biochemical and molecular analysis showed increased urinary heparin sulphate, no residual activity in PBTL and two causal splicing mutations confirming the diagnosis of type C Sanfilippo disease.

Having started genistein supplementation (10 mg/kg/day, in two daily doses) a significant decrease of GAGs and heparan sulphate in the urine were noticed three months later. This decrease was not sustained at a 6 month control evaluation and did not seem to have any clear clinical benefit.

Conclusions: Although genistein may decrease the excretion of GAGS in the urine in patients with MPSIII, this effect may be transient and have no effect on symptoms.

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VALIDATION OF AN LC/MS/MS ASSAY FOR DETECTING RELEVANT DISACCHARIDES FROM KERATAN SULFATE AS A BIOMARKER FOR MORQUIO A SYNDROME

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Mucopolysaccharidosis IVA (MPS IVA, Morquio A syndrome) is an inherited lysosomal storage disease caused by deficiency of N-acetylgalactosamine-6-sulfatase (GALNS), an enzyme required for step-wise degradation of keratan sulfate (KS). KS is an important biomarker for MPS IVA as it appears in the circulation and urine, and KS levels can be used to differentiate between MPS IVA patients and unaffected individuals. We have developed a selective, sensitive, accurate and precise liquid chromatography-tandem mass spectrometry (LC/MS/MS) assay for the KS-derived disaccharides Galb1-4GlcNAc(6S) and Gal(6S)b1-4GlcNAc (6S) in human urine and plasma using keratanase II digestion. This method expands on the existing LC/MS/MS method by allowing quantitation in both urine and plasma and using improved internal standards (heavy isotope-labeled Galβ1-4GlcNAc(6S) and Gal(6S)b1-4GlcNAc(6S)). The lower limit of quantitation was 0.026 μg/mL (plasma) and 0.104 μg/mL (urine), with a quantitation range of 0.026–5 μg/mL (plasma) and 0.104–20 μg/mL (urine). Clinical sample analysis in 168 MPS IVA patients and 225 healthy controls demonstrates the clinical utility of this method in identifying reference ranges for KS in urine and plasma and discriminating between individuals with MPS IVA and unaffected individuals. It also has the potential for longitudinal disease progression assessment.
Conflict of Interest declared.

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REPORT OF TWO COMMON MUTATIONS AND FIVE NOVEL MUTATIONS OF GALNS GENE IN KOREAN PATIENTS WITH MUCOPOLYSACCHARIDOSIS TYPE IVA

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Background: Mucopolysaccharidosis IVA (MPS IVA; OMIM #253000) is caused by the deficiency of N-acetylgalactosamine-6-sulfate sulfatase (GALNS). The GALNS gene encodes GALNS, a lysosomal enzyme involved in the catabolism of keratan and chondroitin sulfate. Herein, we examined biochemical and genetic aberrations from six Korean patients presenting with classic MPS IVA.
Methods: GALNS activity was measured in peripheral blood leukocytes or skin fibroblasts. PCR-direct sequencing was performed to investigate the molecular defects of GALNS gene. We also investigated the mutational status for the family members of the patients as well as 50 healthy subjects.
Results: The mean age of six female patients was 8.0±5.2 years (range: 2–17 years). Severe reduction of GALNS enzyme was found in all patients. A total of 12 mutant alleles were identified, belonging to 7 different mutations. Five novel mutations were c.218A>G (p.Y73C), c.451 C>A (p.P151T), c.725 C>G (p.S242C), c.752 G>A (p.R251Q) and c.1000 C>T (p.Q334X). The others were c.1156 C>T (p.R386C) and c.1243-1 G>A mutations. Two mutations, c.451 C>A and c.1000 C>T, consist of 58.3% of all mutations.
Conclusions: We firstly identified the Korean patients with MPS IVA by clinical, biochemical and genetic analyses. Sequencing analysis of GALNS gene revealed five novel mutations, of which two was common.

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CHANGE IN FUNCTIONAL ABILITIES IN A PATIENT RECEIVING INVESTIGATIONAL ENZYME REPLACEMENT THERAPY (ERT) FOR MUCOPOLYSACCHARIDOSIS IVA (MPS IVA) IN A PHASE 1/2 MULTICENTER, OPEN-LABEL, DOSE-ESCALATION STUDY TO EVALUATE THE SAFETY, TOLERABILITY AND EFFICACY OF BMN 110

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Introduction: MPS IVA is characterized by deficient activity of Nacetyl-galactosamine6sulfatase (GALNS), causing excessive lysosomal storage of keratan sulfate (KS). Patients experience impaired endurance and respiratory function.
Case Description: This 16 y/o male patient with genotype homozygous c.452>T (p.Pro151Leu) started ERT with weekly infusions of GALNS (BMN 110) on 10Jun2009. Medical history included aortic regurgitation, genu valgum, limited mobility and overall weakness. Baseline physical exam showed short stature, cardiac murmur, shortness of breath, and joint laxity. Before ERT, he could not sign the consent form, was completely non-ambulatory, and required full assistance for self-care and activities of daily living.
During treatment, he became stronger, gaining ability to rise from wheelchair and use toilet without assistance. After 79 weeks, mobility, breathing, and strength improved. He could crawl on his knees, ascend 30 steps, and he was able to sign the consent form for the extension study. Screening urine KS of 24.5 ug/mg creatinine had decreased to 10.8 by Week 72.
Conclusions: ERT is a potential new treatment option for MPS IVA, which is expected to reduce KS storage, leading to improvements in respiratory function and endurance. This patient's markedly improved strength and endurance during trial participation improved his daily function.
Conflict of Interest declared.

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A PRACTICAL FLUORIMETRIC METHOD FOR THE SCREENING OF MPS IVA IN DRIED BLOOD SPOTS

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Background:Mucopolysaccharidosis IV A(MPS IVA) is a rare autosomal recessive disease caused by deficiency of the lysosomal enzyme N-acetylgalactosamine-6-sulfatase(GALNS),resulting in storage of keratan-sulfate in many tissues and organs.This accumulation causes a severe skeletal dysplasia with short stature,and affects the eye, heart and other organs. Clinical trials with ERT for this disease are in progress. We describe an innovative fluorometric method for the assay of GALNS in dried blood spots(DBS).

Materials and Methods: We used DBS as the enzyme source and compared it with leukocytes,having studied 25 MPS IVA patients and 54 healthy controls.We optimized the assay conditions in DBS,including incubation time and stability on different storage temperatures and along time.Results in DBS were compared to the ones obtained in leucocytes using the standard technique.

Results and conclusions: The described fluorescent methodology was validated in our laboratory and the assay was found sensitive and specific, allowing reliable detection of MPS IVA patients.The use of DBS simplifies the collection and transportation,and is especially useful for testing patients from more remote areas of large countries,and when samples need to cross country borders.We believe this assay could be easily incorporated by reference laboratories and play a role in the screening for MPS IVA, contributing to the earlier detection of affected patients.

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MUCOPOLYSACCHARIDOSIS VI (MAROTEAUX-LAMY SYNDROME): DEVELOPMENT OF CLINICAL AND LABORATORY GUIDELINES FOR DIAGNOSIS

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Mucopolysaccharidosis (MPS) VI is a lysosomal storage disease in which deficient N-acetylgalactosamine 4-sulfatase (arylsulfatase B, or ASB) impairs degradation of dermatan sulfate. Accurate diagnosis is critical to ensure that patients receive appropriate enzyme replacement therapy. Review of laboratory tests for mucopolysaccharidoses and their use in diagnosis of MPS VI was the topic of a recent expert panel meeting, inclusive of clinicians and laboratory directors.

Clinicians completed a survey on MPS VI diagnosis practices. There was consensus on the requirement to evaluate ASB enzyme activity to obtain a final diagnosis. While the reported use of additional tests was variable, such as urinary glycosaminoglycans (uGAG) analysis (qualitative and quantitative), molecular testing, and multiple lysosomal enzymes testing, it was recommended that these laboratory tests be included as part of diagnosis of MPS VI. All participants emphasized the importance of direct communication between the diagnostic laboratory and clinician.

In addition to high clinical suspicion, diagnosis of MPS VI requires quality results from tests run at laboratories with expertise in MPS testing and result interpretation. The use of multiple specimens and tests, such as qualitative and quantitative uGAG, multiple lysosomal enzyme activities, and molecular testing, when possible, was the unanimous recommendation of the panel.

Conflict of Interest declared.

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HIGH CORRELATION OF ARSB ACTIVITY IN DBS ACROSS BRAZILIAN LABORATORIES SUPPORTS AN IMPORTANT ROLE FOR THIS MEASUREMENT ON THE DETECTION OF MPS VI

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Mucopolysaccharidosis VI (MPS VI) is a lysosomal storage disorder (LSD) caused by arylsulfatase B (ARSB) deficiency. Patients can be identified through ARSB activity measurement in different specimens. Because of their many advantages, dried blood spots (DBS) on filter paper have been considered a useful tool for LSD detection. Our aim was to validate DBS activity for MPS VI detection by comparing samples measurement in two different Brazilian laboratories. DBS samples from three countries were sent to Hospital de Clínicas de Porto Alegre (Laboratory 1) and Universidade Federal de São Paulo (Laboratory 2) which developed their reference values for normal and MPS VI affected individuals. A total of 26 DBS samples with clinical suspicion of MPS VI were evaluated. Positive values ranged between 0.8–3.5 nmol/h/mL (normal: 5.3 to 22) for Laboratory 1 and 1.19–2.75 nmol/h/mL (normal: 5.3 to 14) for Laboratory 2. Although the differences in the absolute values obtained in each laboratory, both were able to identify normal individuals and MPS VI patients in all cases, with results showing a high correlation (r=0.92). These results confirm DBS as a valuable diagnostic tool which enables the discrimination among normal individuals and MPS VI patients.
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Conflict of Interest declared.

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AN UNUSUAL PRESENTATION OF MUCOPOLYSACCARIDOSIS VI WITH A NOVEL MUTATION

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Mucopolysaccharidosis(MPS) VI is caused by deficient arylsulfatase B. Albinism is characterized by the absence of pigment in the skin, hair, eyes due to production of melanin.

Case: The patient was the first of consanguineous parents. She had sensorineural deafness, generalized albinism., coarse face at the age of eighteen months old. Dysostosis multiplex by skletal X-Ray, high excretion of mucopolysaccarides in the urine, reduced arylsulfatase B enzyme activity in leucocytes (73,75 nmol/hour/mgprotein (209±96)) confirmed the diagnosis of MPS VI. The other sulfatase enzyme levels were normal. ARSB gene sequencing revealed homozygous c.908 G>A change in exon 5. This variant, predicted to result in an amino acid change from glycine to glutamic acid (p.G303E), has neither been shown to be associated with MPS VI nor has it been listed in the Ensembl SNP database.

Conclusion: MPS type VI is caused by mutations in the ARSB gene, located in chromosome 5 (5q13-5q14). Over 130 ARSB mutations have been reported, causing absent or reduced arylsulfatase B activity. On the other hand the gene accounting for oculocutaneous albinism type 4 is locatad in chromosome 5 (5p13.2).

We speculated that, possible chromosomal rearrengements should be further investigated in this case because of this extremely rare co-existence.

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NEWBORN SCREENING PILOT PROGRAM FOR MPS VI IN A HIGH-INCIDENCE AREA OF NORTHEAST BRAZIL

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Mucopolysaccharidosis VI(MPS VI,or Maroteaux-Lamy syndrome)is caused by the deficiency of the lysosomal enzyme N-acetylgalactosamine 4-sulfatase (ARSB).This deficiency causes the storage of dermatan sulphate in tissues, leading to a progressive and severe bone dysplasia and to problems in many organs and systems.MPS VI is a very rare condition,which was a relatively high incidence(13 cases identified so far)in the county of Monte Santo (50,000 inhabitants),in Bahia state,Northeast region of Brazil, where a common mutation(H178L)was found in all cases.As MPS VI could be treated with ERT and as there are indications that a better outcome may be expected in early treated cases,a newborn screening program for MPS VI was set up on this specific area.To the program already in place for PKU,hypothyroidism and hemoglobin disorders,a screening for MPS VI was added by ARSB activity assay and by the detection of the common mutation, both performed on DBS. The standardization of the techniques of enzyme assay,DNA extraction and mutation detection were already completed and the test on newborn samples has started on January 1st,2011.The possibility of detecting carriers will help to calculate the expected frequency of this disease in the area, and will also be a tool to target genetic counseling to the more susceptible families.

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MUTATION ANALYSIS IN ARSB GENE IN TURKISH PATIENTS WITH MPS TYPE VI: HIGH PREVALENCE OF L321P MUTATION

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Mucopolysaccharidosis type VI or Maroteaux-Lamy syndrome is an autosomal recessive lysosomal storage disorder resulting from impaired function of the enzyme N-acetylgalactosamine-4-sulfatase (arylsulfatase B, ARSB). In this study, the mutation spectrum of ARSB gene in 19 Turkish patients from 15 different families diagnosed with MPS type VI was determined by using direct sequence analysis. In total, 6 different types of disease-causing mutations (c.1036delG, p.L321P, p.R160X, p.R191X, p. C192R and p.E390K) and 4 polymorphisms (p.V358M, p.V376M, IVS1-26 T>C, IVS5–28A>C) were identified in the patients with MPS type VI. One of the six mutations (p.E390K) is novel in Turkish patients. As an interesting result, the most frequent mutation in our screened cohort was p. L321P (c.961 T>C) with 53% frequency. Our findings are shown inconsistency with the knowledge of the literature, since p.L321P (c.961 T>C) is not one of the common mutation in Caucasians.The mutational analyses on ARSB gene will identify structure/function relationships of the enzyme, more accurate interpretation of genotype/ phenotype correlations and for choosing appropriate treatments.

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HEART FAILURE: AN EARLY PRESENTATION FORM OF MPS VI

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Background: Cardiac abnormalities are frequent in patients with MPS VI and are an important cause of morbidity and mortality.

Case Report: A four months old girl was admitted due to failure to thrive, bronchilolitis and heart murmur. The diagnosis of congenital mitral anomaly with severe regurgitation and severe dilatation of left atrium and ventricle was defined. Symptomatic treatment was started with high doses of diuretics and ACE-inibithor, but patient maintained severe heart failure, several respiratory infections and poor weight gain. At the age of 18 months, heart surgery was proposed to valve repair but was refused by parents. Meanwhile it became apparent a phenotype suggestive of MPS VI; diagnosis was established by enzymatic assay and ERT-Galsulfase was started. Now she accomplished 18 months of treatment and clinically is greatly improved, but still maintains aggressive therapeutics of congestive heart failure.

Conclusions: This case presents with an unusual early severe valvular involvement with few systemic signs of MPS VI misleading the etiologic diagnosis initially. We emphasize the value of identification of a systemic disease in the context of apparently isolate mitral anomaly in an infant to timely begin specific treatment. Adequate management of these patients is still a challenge to multidisciplinary teams.

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GENOTYPE-PHENOTYPE CORRELATION IN MUCOPOLYSACCHARIDOSIS TYPE VI (MAROTEAUX-LAMY SYNDROME)

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Background: Mucopolysaccharidosis type VI (MPS VI; Maroteaux-Lamy syndrome) is a rare autosomal recessive disorder caused by a deficiency of N-acetylgalactosamine-4-sulfatase (ARSB). Over 130 ARSB gene mutations have been identified thus far. Genotype-phenotype correlation for most MPS VI patients has been difficult due to a large number of mutations, which are often private or novel.

Objective: We aimed to analyze the genotype-phenotype correlation in 21 unrelated MPS VI families from Central and Eastern Europe.

Material and Methods: We studied 21 families with MPS VI patients with fourteen different disease-causing mutations in the ARSB gene (p.A33V, p.W57C, p.Q88X, p.T92K, p.Q97X, c.375_376insT, p.R152W, p.R160Q, p.R160X, p.G167R, p.Y210C, c.750_754delinsCCTGAAGTCAAG, p.Y266S, p.G302R).

Results: Mutations p.R152W and p.Y210C were associated with relatively attenuated MPS VI phenotype. Homozygosity for p.R152W mutation yielded very mild phenotypes in patients, while heterozygous patients presented with intermediate phenotype, even despite the fact that the other mutation detected in them was severe.

Mutation p.Y210C was also associated with relatively attenuated phenotype. Nonsense mutations (p.Q88X, p.Q97X, p.R160X) as well as a double-mutated allele [p.A33V; p.Y266S] were associated with severe MPS VI phenotype.

Conclusion: Our study has provided evidence to support genotype-phenotype correlation, namely the milder phenotype may be associated with the p.R152W mutation.

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OUTCOMES OF ENZYME REPLACEMENT THERAPY FOR MUCOPOLYSACCHARIDOSIS TYPE VI:GAZI UNIVERSITY EXPERIENCE

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Mucopolysaccharidosis type VI (Maroteaux-Lamy syndrome) is an autosomal recessive disorder caused by a deficiency in the lysosomal enzyme N-acetylgalactosamine 4 sulfatase (arylsulfatase B, or ASB). We reviewed 10 patients with MPS VI (five males and five females; age range 1–15.5 years) treated with weekly intravenous infusions of rhASB (1.0 mg/kg) for at least 6 months. Gestational and perinatal data were normal for all patients. During the course of the disease, all patients except one whom we diagnosed at 4 months because of his sibling with the diagnosis of MPS VI developed coarsened facial features, short stature, heart valve disease, eye problems, musculoskeletal problems, hepatosplenomegaly and neurological abnormalities. We assesed the biochemical and clinical response every 3 months. All patients received rhASB enzyme replacement therapy (ERT) and showed improvement or stabilisation in clinical manifestations after onset of therapy. The most frequently noted benefits were improvement of endurance, reduced hepatosplenomegaly and decrease in urinary glycosaminoglycan excretion. ERT was well tolerated by all patients. This treatment is thus beneficial and appears to be safe for treatment of MPS VI in Turkish patients. These results indicate that the earlier ERT is started, the greater the response.

P-413

SIBLING COMPARISON STUDY OF 7 YEARS OF ENZYME REPLACEMENT THERAPY FOR MUCOPOLYSACCHARIDOSIS TYPE VI STARTING AT 8 WEEKS AND 3.5 YEARS OF AGE

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Two siblings with mucopolysaccharidosis type VI (Maroteaux-Lamy syndrome) have had seven years treatment with enzyme replacement therapy (ERT) using galsulfase (Naglazyme) weekly at a dose of1mg/kg starting from 8 weeks and 3.5 years of age respectively. Treatment has been well tolerated by both siblings with no infusion associated reactions recorded. In the younger sibling ERT has preserved joint movement, cardiac valves, liver and spleen size, height (10th centile) and facial morphology. He has mild scoliosis (23 degrees), stable mild corneal clouding but has recently developed evidence of carpal tunnel syndrome and has significant skeletal disease with a waddling gait from avascular necrosis of the hips. The older sibling initially had a marked improvement in joint and scoliosis mobility which have been maintained and has softening of her facial features and stabilised cardiac valve pathology and corneal clouding. Her height is 6 cms below the 1st centile after correcting for scoliosis which has progressed. Rodding of her spine, which was considered necessary prior to initiating ERT, has been deferred until completion of growth. She has required surgery for severe pes cavus. This paper adds further evidence that early initiation of ERT will slow or prevent the natural pathological progression of MPS VI.

P-414

THE FIRST REPORTED TWIN CASES WITH MUCOPOLYSACCHARIDOSES VI ON ENZYME REPLACEMENT TERAPY

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Mucopolysaccharidoses VI is an inherited lysosomal storage disease, associated with deficiency of arylsulphatase B. The accumulation of dermatan sulphate leads the symptoms of coarse face, short stature, dysostosis multiplex, contractures, cardiac valve disease, reduced pulmonary function, hepatosplenomegaly, hearing loss, sleep apnoea, corneal clouding, hernias, and generally normal intelligence. Diagnosis based on clinical findings, deficient enzyme activity and demonstration of normal activities of other sulphatase enzymes. With enzyme replacement therapy the prognosis is getting better.

Case report: This 33 month-old-monozygotic-twin sisters are from a first degree cousin's marriage. They admitted with complaints of frequent respiratory infections, and limited mobility. Their older sister who had a similar facial appearance died from respiratory insufficiency at the age of 13. They have coarse face, short stature, corneal clouding, pectus carinatum, mitral valve insufficiency, claw hands, dysostosis multiplex, and normal intelligence. Enzyme analysis revealed undetectable levels of arylsulphatase B, with other sulphatases within normal limits. They have been on enzyme replacement therapy for one year.

Discussion: With enzyme replacement therapy their effort capacity, and mobility have improved and the frequency and the severity of respiratory infections have decreased. To the best of our knowledge these patients are the first reported twins with mucopolysaccharidoses.

P-415

ENZYME REPLACEMENT THERAPY IN 10 DUTCH MUCOPOLYSACCHARIDOSIS TYPE VI PATIENTS

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Background: The common treatment for MPS VI (Maroteaux-Lamy syndrome) is enzyme replacement therapy (ERT) with galsulfase.

Objectives: We studied the effects of galsulfase on several outcome measures in an open-label study.

Results: Ten patients were included. The median follow-up was 2.4 years (range 1.1-3.8 years).

Galsulfase had a significant positive effect on the flexion of the right shoulder ($p<0.001$), liver size ($p<0.001$) and spleen size ($p=0.017$). Flexion of the left shoulder showed a positive trend ($p=0.07$). Two patients showed a significant decrease of left ventricular mass index (LVMI) Z-score. The one patient who showed a decreased forced vital capacity (FVC) at baseline, showed better FVC results during ERT ($p=0.023$). For the total group a mean decline of 84% in urinary glycosaminoglycan values was observed. ERT did not have an effect on cardiac valve regurgitation and hearing function.

Conclusion: Galsulfase had a positive effect on several outcome measures in our Dutch patient group with MPS VI.

P-416

EXCRETION OF CHONDROITIN SULPHATE AND HEPARIN SULPHATE IN PATIENT WITH MUCOPOLYSACHARIDOSIS TYPE VII (SLY SYNDROME) CASE REPORT

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Mucopolysaccharidosis type VII (MPS VII) is an autosomal recessive disorder caused by the deficiency of beta-glucuronidase. A small number of patients have been reported since the first case described by Sly in 1973. Patients with MPS VII described in the literature excreted chondroitin sulphate and dermatan sulphate predominantly.

We report mildly mentally retarded (IQ- 52) 19 year old girl with MPS VII. She was referred for the genetic counseling because of suspected lysosomal storage disorder. Macrocephaly, coarsened facial features, short stature 146 cm (<3‰), dysostosis multiplex, sternal protrusion, short trunk, oversized arms and legs, mild restriction of joints, corneal clouding were noticed at examination. Liver and spleen were normal. Analysis of urinary glycosaminoglycans (GAGs) showed increased excretion of GAGs −10,57 mg/mmol creat (ref 1.6±0.8). One- and two-dimensional electrophoresis performed in different laboratories showed increased excretion of chondroitin sulphate and heparan sulphate. Diagnosis of MPS VII was confirmed by the reduced activity of beta glucuronidase in serum—0,04 nmol/mg protein/h (ref. 79±39).

P-417

IMPACT OF LYSOSOMAL STORAGE DISORDERS ON DAILY LIVING OF PATIENTS AND THEIR FAMILIES AND PERCEPTIONS ON ENZYME REPLACEMENT THERAPY

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Background: Lysosomal storage disorders (LSDs) affect patient quality of life (QoL) but little is known about the disease impact on family life. We assessed the impact of LSDs on daily living of patients and their families and evaluated parent perceptions on the disease and enzyme replacement therapy (ERT).

Patients and Methods: Adaptive behaviour, mental development, and cognitive function were assessed in 12 patients with LSDs (MPS I, MPS VI, Pompe, and Gaucher) over a 4-year period. Fifteen parents were evaluated for emotional well-being, satisfaction with life, perception on the severity and prognosis of the disease, disease impact on family QoL, and expectations on and satisfaction with ERT.

Results: Adaptive behaviour, mental development and/or cognitive function were impaired in most patients. Depression and emotional disturbance was common among the mothers. Most parents considered their QoL as satisfactory. The disease impact and stress on the family was high, but appeared to decrease after onset of ERT. The high expectations of ERT before the start of treatment decreased to a more realistic level thereafter.

Conclusions: LSDs greatly affect the lives of patients and their families. Psychological counselling of parents and providing realistic information about the expectable benefits from ERT is very important.

Conflict of Interest declared.

P-418

FROM PUNCH TO RESULT IN LESS THAN A HALF DAY—SIMPLIFIED LC-MS/MS ASSAY FOR LYSOSOMAL STORAGE DISORDERS

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Background: During the past years various approaches for high throughput tandem mass spectrometry (MS/MS) screening of lysosomal storage disorders (LSDs) have been developed. However, newborn screening for LSDs is still a technological challenge. One of the major challenges of implementing MS/MS-based multiplex enzyme-assays using the currently available substrates is the complexity of sample preparation.

Methods: We present two protocols which were refined and optimized for the analysis of lysosomal enzyme activity in dried blood spots. They were developed for the screening of Pompe, Gaucher, Niemann-Pick A/B, Fabry, Krabbe and Mucopolysaccharidosis Type I disease.

Results: We developed an innovative assay introducing TurboFlow technology in a clinical diagnostic setting in the form of an online two-dimensional chromatography to eliminate the need for time-consuming off-line sample preparation including liquid-liquid and solid-phase extraction, and the use of UHPLC to separate excess substrate from all other analytes eliminating the major potential source of analytical interference or ion suppression.

Conclusion: The protocols can be used for both high-throughput analysis in newborn screening with several hundred samples per day or for fast analysis of single samples in case of emergency within a few hours from "punch to result" without the need for overnight incubation, respectively.

Conflict of Interest declared.

P-419

INFLUENCE OF PRE-ANALYTICAL FACTORS ON ALPHA-GALACTOSIDASE A, ARYLSULFATASE B ALPHA-GLUCOSIDASE ACTIVITIES MEASURED ON DRIED BLOOD SPOTS ON FILTER PAPER

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Background: Lysosomal storage disorders (LSDs) are a heterogeneous group of inherited metabolic diseases. The definitive diagnosis of these diseases includes the determination of enzyme activity in plasma, leukocytes, or cultured fibroblasts. In recent years, dried blood samples collected on filter paper (DBS) have been utilized. This technique offers a series of advantages, like the low transportation requirements which explain the increasing preference it enjoys currently.
Objectives: To analyze the effect of storage conditions on activity of α-galactosidase A, arylsulfatase B and α-glucosidase.
Material and Methods: Blood was collected and directly spotting on filter paper and stored at different temperatures (−20, 4, 25 and 37:C) and storage times (3, 10, 17 and 180 days). The influence of filter paper size was also assessed (3.0 and 1.2 mm).
Results: α-glucosidase A activity significantly decreased after the 10th day, while arylsulfatase B activity only differed significantly after the 180th day, and α-galactosidase A activity remained constant throughout this storage time. Excellent correlation coefficients were observed for the two filter paper sizes used.
Conclusions: Both paper sizes may be employed. Filter paper specimens should be transported under refrigeration as soon as possible after blood collection.

P-420

ETIOLOGY OF CARPAL TUNNEL SYNDROME IN CHILDREN AND ADOLESCENTS IN BRITISH COLUMBIA

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Background: Mucopolysaccharidosis (MPS) and mucolipidosis (ML) are the most common causes of carpal tunnel syndrome (CTS) (35–70%) in children in the absence of trauma.
Methods: All nerve conduction studies (NCS) in the Electrophysiology-Laboratory (1992–2010) were reviewed to screen individuals for MPS and ML by using CTS and/or tarsal tunnel syndrome (CTS/TTS) as a screening indicator. All individuals underwent CTS release surgery were included.
Results: 2254 individuals (age 0–18 years) underwent NCS. The reason for referral was CTS/TTS in 1.6%. 3% (68/2254) had CTS/TTS. There was history of trauma in 47% (32/68). 26% (18/68) had an underlying disease. In the latter group 38.8% (7/18) had diagnosis of MPS (3 with MPS I, 2 with MPS II, 1 with MPS IV and 1 with MPS VI). 17/68 individuals underwent carpal tunnel release surgery. 5/17 had diagnosis of MPS in the latter group. One additional patient with MPS IIIA had normal NCV at age 4.5 years who will be followed with periodic NCS.
Conclusion: CTS/TTS may have a role as a screening indicator for diagnosis of MPS and ML. Enzyme replacement therapy is available for MPS type I, II, and VI to improve patients' quality of life and disease related morbidity.
Conflict of Interest declared.

P-421

MULTIPLEX ANALYSIS OF OLIGOSACCHARIDOSES BASED ON TANDEM MASS SPECTROMETRY AND A UNIVERSAL BUFFER SYSTEM

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Background: Oligosaccharidoses are Lysosomal Storage Disorders (LSDs) caused by mutations in genes encoding exoglycosidases and lead to accumulation of N-linked oligosaccharides within lysosomes. Due to overlapping clinical symptoms, diagnosis of oligosaccharidoses is challenging, involving individual enzymatic assays or urinary oligosaccharide analysis. Here, we aim to determine a universal buffer for the development of an in vitro multiplex assay to measure exoglycosidase activities from human tissues using tandem mass spectrometry (MS/MS).
Methods: Classical 4-methylumbelliferyl (4MU) fluorometric assays were compared to determine an optimal universal buffer used to develop a MS/MS based multiplex assay involving commercial substrates targeting exoglycosidases from fibroblasts.
Results: A 30 mM citrate-phosphate buffer with a pH at 4.3 was the universal condition. Additionally, a MS/MS multiplex assay was established for beta-galactosidase and beta-hexosaminidase using substrates composed of 4MU and paranitrophenol (PNP) linked to particular monosaccharides. Enzyme activities from fibroblasts were successfully assayed in one vial by simultaneous measurement of 4MU and PNP reaction products.
Conclusions: A universal buffer allows investigation of several exoglycosidases in a single vial. This was demonstrated for beta-galactosidase and beta-hexosaminidase using distinctive starting substrates and MS/MS technology. Substrates giving mass differentiable products for other exoglycosidases will delineate a multiplex assay that can rapidly identify oligosaccharidoses.

P-422

ALLOGENIC HEMAOPOTIETIC STEM CELLTRANSPLANTATION FOR PATIENTS WITH LYSOSOMAL AND PEROXISOMAL STORAGE DISEASES: A SINGLE INSTITUTE EXPERIENCES (NIHON UNIVERSITY)

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Over the last 15 years, 19 children of lysosomal and peroxisomal storage diseases have received hematopoietic stem cell transplantations (HSCT) at Nihon University Hospital.

Patients and Methods: 12 mucopolysaccharidosis (MPS) type II, 3 MPS III, 1 MPS VI, 1 adrenoleukodystrophy and 2 Krabbe disease were included. The median age was 5.7 years (2 months to 15 years) at the time of transplantation. The donors were HLA-matched siblings in 4 cases, unrelated bone marrow in 10 cases and cord blood in 5 cases. The conditioning regimen consisted of cyclophosphamide (CY) 200 mg/kg, total body irradiation 10 Gy and antilymphocyte globulin 10 mg/kg in 13 patients, CY 200 mg/kg, Buslufan 16 mg/kg and Fludarabin 180 mg/kg in 4 cases, and others in 2 patients.

Results: Median follow-up was 4.25 years (1 month to 14 years). Eleven out of 19 patients (57.9%) achieved complete donor-type engraftment and 9 (47.4%) are still alive. Grade II-IV acute graft-versus-host disease (GVHD) was observed in 3 patients and chronic GVHD occurred in 3 patients. Joint mobility and hepatosplenomegaly were improved in all engrafted recipients with MPS. Protein level in cerebral spinal fluid decreased in Krabbe disease.

Conclusion: HSCT is effective but requires further long-term studies to measure the metabolic effects.

P-423

GLYCOSAMINOGLYCAN QUANTIFICATION BY LC/MS-MS FOR MUCOPOLYSACCHARIDOSES

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Background: MPS disorders are the result of primary defects in lysosomal enzymes. Depending on the specific gene defect, the catabolism of one or more of the MPS is blocked leading to accumulation of the corresponding substrate(s).

Objectives: To develop a LC-MS/MS method for glycosaminoglycan quantification: dermatan sulfate (DS) and heparan sulfate (HS) using urine filter paper samples.

Methods: A urine filter paper disc was eluted, evaporated under nitrogen. Methanolysis was performed using methanol-HCl•3 N with heating at 65°C for 75 min. Deuterated DS and HS internal standards were used. An Alliance 2795XE-LC coupled to a Quattro micro MS/MS (Waters) was used. A chromatographic gradient allowed good separation with an Atlantis T3 column (9 min run). Data were recorded in positive electrospray ionization.

Results: Validation of the method provided good results: linearity ($r^2 > 0.995$), accuracy (Bias<20%) and precision (CV<15%). Levels of DS and HS in controls were below LOQs. We analyzed 28 MPS patients: 9 MPS I, 2 MPS IH; 6 MPS IH/S; 1 MPS IS, 5 MPS II and 7 MPS VI. In every case, we were able to differentiate MPS patients from controls.

Conclusion: A robust methodology was devised to measure glycosaminoglycans using urine filter paper samples.

P-424

THE UKRAINIAN REGISTRY OF MUCOPOLYCACCHARIDOSES

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Introduction: The Mucopolysaccharidoses (MPS) are genetically determined disorders in which glycosaminoglicans are stored in the tissues. Pathological storage leads to effects on wide variety of systems.

Objective: To present the results of Ukrainian registry of patient affected with different MPStypes.

Methods: Retrospective review of patients diagnosed of MPS in Ukraine since 1996. The patients were confirmed by quantitative and qualitative GAG analysis with subsequent detection of lysosomal enzymes activity. DNA analysis was carried out only in MPSI and MPSIIIA.

Results: 60 patients from 61 families (37 male, 23 female).
The MPS registry includes: 6 patients with MPS I, 18—with MPS II, 22—with MPS III (12 MPS IIIA, 4 MPS IIIB, 1 MPS IIIC, 5—unidentified), 8—with MPS IV, 6—with MPS VI. Genetic testing revealed Q70X (4/10 alleles) in 5 cases with MPS1 and R74C (18/24 alleles) as the most common mutation in 12 cases with MPSIIIA. The three MPS I patients have been treated with enzyme replacement therapy (ERT) since 2004.

Conclusions: The creation of the MPS registry allows to investigate the spectrum of different MPS types in population.

P-425

MPS-BRAZIL NETWORK INTERNATIONAL ACTIVITY IN LATIN AMERICA

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Objectives: The MPS BRAZIL-NETWORK(MBN) was created in 2004 to improve the diagnosis and management of MPS diseases in Brazil. Since then, physicians from many Latin American(LA) countries have contacted the MBN get help confirm the diagnosis in patients in whom they suspect of a MPS disease.

Methods: services from other LA countries sent biological samples, usually dried blood spots, to the Medical Genetics Service of Hospital de Clínicas de Porto Alegre, Brazil, where are carried out the laboratory tests needed for the MPS diagnosis. The contact between the referring services and the MBN is possible through the website www.redempsbrasil.ufrgs.br or through the email redempsbrasil@ufrgs.br.

Results: From April/2004 to April/2011, samples from 216 patients with MPS suspicion sent from other LA countries were investigated. The diagnosis was confirmed in 176/216(81,9%) patients. The most frequent type of MPS diagnosed was MPS II, confirmed in 72/176(40,7%) of the MPS patients, followed by MPS VI(23,7%) and MPS I(15,2%). The diagnosed cases came from 12 different countries, with most cases coming from Chile(18,5%) and México(18,2%).

Conclusions: MBN is improving the diagnosis of MPS also in other LA countries, helping patients to get access to the appropriate management tools which could bring a better outcome for their lives.

P-426

MOST COMMON LYSOSOMAL STORAGE DISEASES IN OUR COUNTRY: RESULTS OF LAST FIVE YEARS

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Background: Lysosomal storage diseases (LSD) are charecterized by storage of substrates in lysosomes related with defects of lysosomal enzyme activity or defects of proteins of lysosomal membran receptor.
Material (Patients) and Methods: We are analyses enzyme activity in leukocytes samples for five years. Enzyme activities were determined by spectrofluorometric and spectrophotometric method using 4-methylumbelliferyl dervatized substrate. Enzyme activities were determined in 1793 suspicious patients.
Result: During this period 420 LSD's are diagnosed in 1793 suspicious patients. 129 of them are Gaucher, 84 are Niemann-Pick A-B, 40 are Fabry, 3 are Fucosidosis, 12 are GM I , 23 are MPS I, 31 are MPS II, 29 are MPS VI, 2 are MPS VII, 23 are Sanflippo B, 11 are Sanflippo C, 4 are Sanflippo D, 9 are Metachromatic leukodystrophy, 5 are Sandhoff disease 13 are Mucolipidosis and 2 are Tay Sachs disease.
Conclusion/Discussion: Early diagnosis is very important for treatment of LSD's. In our country the incidance of LSD's is considerably high because of consanguineous marriage are very often. Most common LSD's for our country are Gaucher, Niemann-Pick A-B, Fabry, MPS type I, MPS type II, MPS type VI and Sanflippo B and C.

P-427

HOME MECHANICAL VENTILATION IN PATIENTS WITH LYSOSOMAL STORAGE DISEASES

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Background: Many patients with lysosomal storage diseases (LSDs) have respiratory problems. They may need tracheotomy and mechanical ventilation in the advanced stages of respiratory disease.
Patients: We have treated 22 patients since 2000:mucopolysaccharidosis (MPS) type I, 2 patients; MPS II, 5 ; MPS III,1; Gaucher disease type I, 2 ; type II, 1 ; type III; 1 ; I-Cell disease, 4 ; GM1 gangliosidosis, 1 ; Tay-Sachs disease, 2 ; ceroid lipofuscinosis, 2 ; Salla disease, 1. Eight patients underwent tracheotomy. Five of these eight patients developed repeated severe lower respiratory tract infection and were treated by invasive home mechanical ventilation therapy.
Results: The frequency of hospitalization for respiratory infections decreased in the 1 year following start of home mechanical ventilation. We have encountered no severe ventilator-related issues.No patient died during this study
Conclusion: Home mechanical ventilation therapy is very helpful ; this approach helps patients to spend their lives calmly with their family at home. However, gaining regional support is paramount as many local services, such as ambulance departments, schools and regional nursing centres need to provide support to these patients and their families for home mechanical ventilation to be possible. Families often also require financial and psychological support.

A-020

A CASE OF THE CHITOTRIOSIDASE DEFICIENCY IN PATIENT WITH GAUCHER DISEASE TYPE I

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Introduction: Chitinases are enzymes that hydrolyze chitin and have been found in a wide variety of non-vertebrate species; recently a human analog of chitinases, chitotriosidase (CT) has been identified. Extreme elevations of plasma CT activity are observed in patients with Gaucher's disease (GD). The 24 bp duplication in the CT gene, resulting in an inactive protein, has been reported. The carrier prevalence is as high as 30 to 40% and the CT activity is half that in individuals with the wild-type gene. However no systematic evaluation of plasma CT activity has been carried out in GD patients taking into account the status of the allele defective for CT and dose in patients on enzyme replacement therapy (ERT).
Case report: A 63 year old patient presented mild clinical presentation of GD: splenomegaly, thrombocytopenia. "Gaucher-like cells were revealed by bone marrow biopsy. The glucocerebrosidase activity—3,7 nmol/h/mg protein (normal 5,5–9,5 nmol/h/mg protein) and chitotriosidase activity—0 nmol/h/ml pl. Genotype was N370S/N370S. The duplication 24 bp in both alleles was detected in CT gene.
Conclusion: For monitoring treatment efficacy GD in this patient should be used in other biochemical markers, such as tartrate-resistant acid phosphatase.

A-021

INTERNATIONAL DISEASE REGISTRY FOR NIEMANN-PICK DISEASE TYPE C IN CLINICAL PRACTICE: AN UPDATE

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As of 3 February 2011, 77 patients (median(range) age 20.1(0.9–46.3) years) were enrolled in this registry; 60/77(78%) were confirmed as receiving miglustat at enrolment; mean(SD) exposure 2.0(1.2) years. Fifteen of 48(31%) patients with available data had hepatosplenomegaly. History of neonatal jaundice was recorded in 3/10(30%) patients with onset of neurological manifestations at age 0–6 years, 6/17(35%) with onset at 6–15 years and 1/16(6%) with onset <15 years. The median(range) age at neurological disease onset among 70 patients was 10.1(<1–44.6) years; median(range) age at diagnosis among 74 patients was 14.9(0.1–44.7) years. Among 45 patients with available data on specific neurological manifestations 38 (84%) had at least 1 of: ataxia(71%); vertical gaze palsy (60%); cognitive impairment(60%); dysarthria (51%); dysphagia(49%). Median(range) composite disability scores at enrolment by age at neurological disease onset were: 0.29(0–0.56) in early infantile-onset patients (aged <2 years; n=5); 0.35(0–0.69) in late infantile-onset patients (2 to <6 years; n=13); 0.38(0.15–0.85) in juvenile-onset patients (6 to <15 years; n=23); 0.29 (0.06–0.67) in adolescent/adult-onset patients (>/=15 years; n=20). Low numbers of patients had normal ambulation (15/72(21%)), language (9/71(13%)) and/or manipulation (13/71(18%)) at enrolment; 26/71(37%) had normal swallowing. This registry will provide valuable information on long-term disease course and treatment outcomes in NP-C.

Conflict of Interest declared.

A-022

HOW CAN SOCIOECONOMIC STATUS AFFECT THE QUALITY OF LIFE OF MPS PATIENTS IN A DEVELOPING COUNTRY? PRELIMINARY RESULTS OF THE PILOT STUDY OF THE MPS-QOL CHILDREN VERSION

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Background: There are only few studies on quality of life (QOL) for MPS and evaluating how individual socioeconomic level correlates to QOL in MPS patients.

Objectives: To present the preliminary results on QOL and socioeconomic status of the pilot study of the MPS-QOL.

Methods: A cross-sectional design was used. The sample was composed of 8 children aged between 8 and 12 years. Patients were classified according to the Brazil Economic Classification Criterion (BECC), which ranges from 0 to 34. Patients completed the CHQ and the MPS-QOL for children, a new instrument, which generates a score from 42 (worst QOL) to 134 (best QOL). The impact of the socioeconomic level in QOL was analyzed by Pearson's correlation.

Results: Male patients were 6. Mean age was 9.5. Instruments were assessed by proxy in 4 patients due to mental retardation. Mean BECC score was 17.5± 2.976. Mean MPS-QOL score was 101±14.223. The MPS-QOL score had inverse correlation to the BECC score ($r=-0.823$; $p=0.012$).

Conclusions: Surprisingly, QOL assessed by the MPS-QOL and the socioeconomic score presented a strong inverse correlation. A possible explanation for this is the overvaluation of small victories of patients with lower socioeconomic status and a higher demand on higher class patients.

A-023

HURLER SYNDROME (MPS IH)—BONE MARROW TRANSPLANT AND ERT

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Background: Hurler syndrome is the most severe form of mucopolysaccharidosis type I. Treatment options include enzymatic replacement therapy (ERT) and bone marrow /stem cells transplantation.
Case report: Female child with dilatated cardiomiopathy diagnosed at one month of age. Coarse features and growth failure suggested MPS I, confirmed at biochemical, enzymatic and molecular level. ERT with laronidase was started at 5 months of age, improving general clinical condition; patient was proposed to transplant. Ductus arteriosus surgery was done for stabilization of pulmonary involvement. and a bacillus Calmette-Guérin lymphadenitis postponed the bone marrow transplantation, successfully performed at the age of 14 months. A cutaneous graft versus host disease was controlled with immunosuppression but at 24 months a severe lung disease brought the doubt of pulmonary graft versus host disease. Despite leukocyte analyses showed non pathological α-L-iduronidase activity after transplant, there was an urinary GAG accumulation when ERT was postponed and so the patient is going on ERT until now.
At the age of 36 months heart function and psychomotor development is close to normal, pulmonary symptoms are controlled with inhaled therapy, and she is growing well.
Discussion: questions are highlighted concerning biomarkers significance and recommendations to ERT after graft.

A-024

MUCOPOLYSACCHARIDOSIS TYPE I REPORT OF A SUCCESSFUL HEMATOPOIETIC STEM CELL TRANSPLANTATION AFTER 11 MONTHS OF ENZYME REPLACEMENT THERAPY

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Mucopolysaccharidosis type I (MPS I) is a rare autossomal recessive lysosomal disease. Here we report a case of a female infant who, at the age of 8 months, was noticed to have hepatomegaly, failure to thrive, development delay and coarse facies. She was referred to our genetic clinics for investigation. At that time, physical examination showed: weight 6425 g, height 64 cm, OFC: 43 cm, mild coarsening of facial features, enlarged tongue, gibbus, hepatomegaly and development delay. The laboratory exams showed evidence of MPS I, with a deficient activity of α-L-iduronidase (0,17 nmol/hr/mg) and two null mutations at the IDUA gene (Hurler (W402X/ W402X), a genotype compatible with Hurler type preditction. The enzyme replacement therapy (ERT) with laronidase was started when she was 16 months old, while she was under evaluation for HSCT (Hematopoietic Stem Cell Transplantation), and stabilized her clinical status and improved respiratory condition. The allogeneic HSCT was performed after she was on ERT for 11 months (the time needed to find a donor), at the age of 27 months . Five months after HSCT the patient shows a very good outcome with leucocyte enzyme activity on the normal range. We emphasize the benefits of ERT before HSCT to stabilize the respiratory symptoms.

A-025

PITFALL IN PRENATAL DIAGNOSIS OF METACHROMATIC LEUKUDYSTROPHY: IS THAT NEW FORM OF MLD?

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Two siblings of consanguinous parents were noted to have a neurologic syndrome marked by regression of psychomotor performance from the first year, marked spasticity and progressive central nervous system degeneration (clinical manifestation was like late infantile Metachromatic Leukodystrophy.) Markedly delayed nerve conduction times were evident. Brain MRI showed diffuse white matter lesions without involvement of subcortical U-fibers., activity of arylsulfatase A white blood cells and cultured skin fibroblasts were below 5%. Molecular investigation of ARSA gene showed a homozygote DNA alteration in A905G position in exon 5 which changes amino acide Lys302Arg. The parents were heterozygote for this alteration. Because of some problem with prenatal diagnosis with enzyme assay molecular diagnosis according to sequencing finding was done for this family. Prenatal Diagnosis was done for this family and fetus was heterozygot for this mutation. This child was affected according to enzyme assay after birth. Molecular investigation for multiple sulfatase deficiency and Saposin B deficiency were negative in this family. Thus, we believe these patients may represent a new form of ARSA deficiency.

O-053

EFFECTS OF TREATMENT WITH TALIGLUCERASE IN 29 GAUCHER DISEASE PATIENTS, 6 MONTH FOLLOW-UP FROM FRENCH ATU PROGRAM

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Background: Taliglucerase alfa is under evaluation by the FDA & EMA for Gaucher Disease (GD).

Methods: We report follow-up of 29 French adult patients with stabilized type 1 GD treated with taliglucerase during 6 months.

Results: 29 patients (12 females; median age 43.9). 7 were splenectomized, 2 had bleeding and 10 had a history of bone episodes (1 pathological fracture, 5 avascular necrosis & 4 bone infarcts). Previous treatment was discontinued for a few months because of a shortage of imiglucerase. Median posology was 120 U/kg/month. Biological evaluation at inclusion and at 6 months of treatment, median (min–max) was: hemoglobin G/dl: 14.1 (10.8–16.7) /13.9 (11.9–16.4); platelets 103/dl (only in 22 patients without splenectomy): 124 (50–182) /122.5 (58.0–207.0); chitotriosidase: 1519 (110–12500) /1000 (108–11050). No clinical event related to the disease evolution was notified. 6 patients experienced 21 adverse events (AE) among which 2 patients had serious drug-related AEs with treatment discontinuation: severe anaphylactic reaction and chest pain.

Conclusion: Taliglucerase seems to be an effective enzyme replacement therapy in 1GD stabilized patients. Potential side effects should be monitored in all patients and, in particular, those who have received previous exogenous ERT treatment.

Conflict of Interest declared.

O-054

ENZYME REPLACEMENT THERAPY AND PROGNOSTIC FACTORS FOR RESPONSE AN ONGOING OPEN-LABEL COHORT STUDY IN ADULTS WITH POMPE DISEASE

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Enzyme Replacement Therapy (ERT) for Pompe disease was approved for clinical use in 2006 based on the efficacy of alglucosidase alfa in classic infantile patients.

In this ongoing prospective study, we included 71 adults who were treated with 20 mg/kg alglucosidase alfa every other week. Effects of ERT were compared with natural course.

The median treatment duration was 23 months (range 5–47 months). Muscle strength increased significantly after start of ERT: 1.4% points/year for manual muscle testing and 4.0% points/year for hand held dynamometry. The calculated difference between natural course and treatment course was 3.3% points/year for manual muscle testing ($P<0.001$) and 7.9% points/year for hand held dynamometry ($P<0.001$). During ERT, FVC remained stable in upright position and declined further in supine position but less than before. For FVC in upright position, the calculated difference between natural course and treatment course was 1.75% per year ($P=0.08$). Favorable prognostic factors for treatment response were female gender for muscle strength and younger age for pulmonary function. Our study shows that ERT significantly alters the natural course in adult patients with Pompe disease. Muscle strength increased and pulmonary function in upright position stabilized, while both parameters declined before start of ERT.

Conflict of Interest declared.

O-055

TREATMENT OF HYPOPHOSPHATASIA (HPP) IN INFANTS AND YOUNG CHILDREN WITH SUBCUTANEOUS ENZYME REPLACEMENT THERAPY, ENB-0040: SKELETAL RADIOGRAPHIC OUTCOMES AT 6 AND 12 MONTHS

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Background: HPP, heritable rickets due to TNSALP gene mutations, may present in infancy with failure to thrive, fractures and respiratory compromise. ENB-0040 (bone-targeted, recombinant TNSALP) treatment is associated with respiratory function and motor development improvements (Greenberg, ASHG 2010). Primary efficacy, radiographic change in rickets is reported.
Objective: Evaluate rickets severity after 6 and 12-mos of ENB-0040 therapy.
Design/Methods: 6-mo Ph2 study (with extension phase on-going); patients received 1 iv infusion, then thrice weekly subcutaneous injections (1–3 mg/kg/dose). A Radiographic Global Impression of Change (RGI-C) was developed to assess rickets in HPP. This is a 7-point scale, where −3 represents severe worsening and +3 complete healing of rickets.
Rickets Severity Scale (RSS)(Thacher, 2000) is a 10-point scale scoring metaphyseal changes from 0(no evidence of rickets) to 10(severe rickets).
Results: 11 patients enrolled. One withdrew and another died of sepsis unrelated to ENB-0040. “Responders” had a mean RGI-C score of>+2. At 6 and 12-mos, 9/10 and 8/9 patients were responders. Mean(±SD) RSS score for 9 patients at baseline was 8.5 (± 2.4) and significantly improved by 6 [4.06(± 2.4)] and 12-mos [0.88(± 1.4)](p=0.0039).
Conclusion: Significant improvement in HPP bone disease occurred with ENB-0040 as evaluated by RGI-C and confirmed with validated RSS.
Conflict of Interest declared.

P-428

CLINICAL EXPERIENCE (PRE AND POST LICENSING) IN PREVIOUSLY ERT-NAOVE PATIENTS RECEIVING VELAGLUCERASE ALFA FOR TYPE 1 GAUCHER DISEASE

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Introduction: Gaucher disease is an inherited metabolic disorder in which a deficiency of the lysosomal enzyme glucocerebrosidase leads to pathology, primarily within the spleen and bone. Due to a global shortage, from 2009 to mid 2010, of the widely used enzyme replacement therapy (ERT), patients were allowed to be treated with VPRIV. (velaglucerase alfa, Shire Human Genetic Therapies), which was then in the late trial phase.
Discussion on patient group: The patients receiving velaglucerase alfa at this time were newly presenting patients requiring ERT. All six patients were naïve to ERT. The subjects ranged in age from 17 to 69 years at start of treatment.
Results: Average treatment duration for this group is now 11.5 months. Infusions have been well tolerated. The average dose used for this patient group is 30 units per kilogram two-weekly. The only adverse event reported, fatigue (by 1 patient), was present before ERT. Five subjects have shown improvement in platelet count within the first 10 months. Anaemia, where present (in 2 patients), has resolved. Visceral scans of these subjects and measurement of plasma chitotriosidase revealed a corresponding response.
Conclusions: Initial results of velaglucerase alfa treatment in patients, now treated beyond the clinical studies, are encouraging.

P-429

EFFECTS OF A SHORTAGE OF IMIGLUCERASE ON THREE PATIENTS WITH TYPE I GAUCHER DISEASE

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Background: Children with Gaucher disease type I (GD1) are usually treated with enzyme replacement therapy (ERT) at a dose of 30–60U/Kg/2 W. Recently, due to an acute shortage supply of imiglucerase, a reduced dose or a reduced infusion frequency was recommended.
Objective: To evaluate the effects of a reduced infusion frequency of imiglucerase over 15 months of follow-up.
Patients and Methods: Three patients were treated with ERT since a median age of 7 years (range 5–12). Only one had bone crisis and Erlenmeyer deformations. Median duration of treatment before dose reduction was 3 years (range 1–8). ERT resulted in total regression of symptoms, normalization of hematological parameters and progressive improvement of chitotriosidase in all patients. In August 2009 infusion schedule was changed from a media 45U/Kg every two weeks to every four weeks.
Results: All patients remained asymptomatic and with no major change on hematological parameters except for one patient who presented subnormal platelet count. All patients showed an upward trend in chitotriosidase values.
Comments: Although a longer follow-up is needed, is probable that even children completely stabilized can probably not be kept on lower doses even though the reduction of frequency of the infusions represent a lower social burden.

P-430

SIGNIFICANT, CONTINUOUS IMPROVEMENT IN BONE MINERAL DENSITY (BMD) AMONG TYPE 1 GAUCHER DISEASE PATIENTS TREATED WITH VELAGLUCERASE ALFA: 69-MONTH EXPERIENCE, INCLUDING DOSE REDUCTION

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Background: Bone pathology is a major concern in type 1 Gaucher disease.
Methods: We evaluated BMD in all 10 patients enrolled in the ongoing, interventional study TKT025EXT (4 men, 6 women; median age 35 years [18–62]). Through 69 months, the mean velaglucerase alfa dose was 40 U/kg; 4 patients received concomitant bisphosphonates.
Results: At baseline, at the lumbar spine (LS) and femoral neck (FN) respectively, 1 and 4 patients had osteoporosis; 8 and 5 had osteopenia; and 1 and 1 were normal (per WHO T-score-based categorization). Status change occurred only in patients not receiving bisphosphonates: osteopenic-to-normal (2 LS and 1 FN) and osteoporotic-to-osteopenic (1 FN) by Month 69. BMD improved significantly at the LS by Month 24 and at the FN by Month 33. In linear mixed models, Z-scores were significantly lower than the reference population at baseline and improved with treatment (LS and FN both $P<0.01$); subgroup analysis of patients not receiving bisphosphonates showed similar results.
Conclusions: Velaglucerase alfa was associated with clinically meaningful and statistically significant LS and FN BMD improvements as early as Month 24 (LS) and 33 (FN), despite dose reduction (60 to 30 U/kg during Year 2 of therapy) and significant baseline skeletal pathology.
Conflict of Interest declared.

P-431

MULTICENTER, OPEN-LABEL TREATMENT PROTOCOL (HGT-GCB-058) OF VELAGLUCERASE ALFA ENZYME REPLACEMENT THERAPY (ERT) IN TYPE 1 GAUCHER DISEASE (GD1): 1-YEAR ANALYSIS OF SAFETY AND TOLERABILITY

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Background: In 2009, the FDA approved HGT-GCB-058, a US treatment protocol, to offer velaglucerase alfa (then an investigational drug; FDA-approved February, 2010) to GD1 patients affected by imiglucerase shortages.
Methods: GD1 patients >2-years-old with no prior anaphylactic reaction to ERT were eligible. Treatment-naïve patients received velaglucerase alfa 60U/kg every other week (EOW); patients previously on imiglucerase (switch) received 15–60U/kg EOW (same dose as their prior imiglucerase regimen).
Results: From Sept 2009 through Jun 2010, 211 patients began velaglucerase alfa (baseline: age 6–89 years; 72 splenectomized; 6 naïve; 205 switch). Patients could discontinue the protocol when commercial therapy was available to them (median [range] treatment duration: naïve patients, 106 days [27–232]; switch patients, 182 days [1–365]). Most AEs were mild or moderate: most common were nasopharyngitis in 15 patients, headache in 15, nausea in 12. 11 patients (5%) experienced a severe AE, considered possibly drug-related in 3 (1%); 7 (3%) a serious AE, considered possibly drug-related (migraine) in 1 (0.5%). Mean hemoglobin and platelets improved in naïve patients and remained stable in switch patients.
Conclusion: A clinically heterogeneous group of 211 GD1 patients successfully transitioned to velaglucerase alfa, which was generally well tolerated, supporting this ERT as a GD1 treatment option.
Conflict of Interest declared.

P-432

ACHIEVEMENT OF THERAPEUTIC GOALS IN PATIENTS WITH TYPE 1 GAUCHER DISEASE (GD1) ON VELAGLUCERASE ALFA OR IMIGLUCERASE: PHASE III TRIAL HGT-GCB-039 AND EXTENSION

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Background: Therapeutic goals for GD1, a heterogeneous, multisystem disease, facilitate an individualized approach to long-term management.
Methods: In HGT-GCB-039, treatment-naïve GD1 patients aged>=2 years were randomized to imiglucerase or velaglucerase alfa (60U/kg every other week [EOW]; 9 months). HGT-GCB-039 completers could enroll in extension HGT-GCB-044, receiving velaglucerase alfa (60U/kg EOW; ongoing).
Results: 34 patients received study drug (17 imiglucerase, 17 velaglucerase alfa). 16 patients from each group entered HGT-GCB-044 and are included in this analysis. Hemoglobin concentration, platelet count, liver and spleen volumes were evaluated against therapeutic goals (based on Pastores et al, 2004). At HGT-GCB-039 baseline, 0/32 patients met all goals (3 goals for splenectomized, 4 goals for non-splenectomized patients). After 9 months, 13/16 patients achieved all goals in both the continuous velaglucerase alfa and imiglucerase-to-velaglucerase alfa groups. After additional 15 months' exposure (in HGT-GCB-044; 24 months total), 16/16 and 15/16 achieved all goals, respectively; 1 splenectomized patient failed to achieve the hemoglobin goal.
Conclusions: Most velaglucerase alfa-treated GD1 patients achieved all hematologic, liver and spleen therapeutic goals by Month 9, with comparable achievement in imiglucerase-treated patients. After 2 years, all patients who had received continuous velaglucerase alfa, and the majority transitioned from imiglucerase, had achieved all goals.
Conflict of Interest declared.

P-433

EFFICACY OF VELAGLUCERASE ALFA IN PATIENTS WITH TYPE 1 GAUCHER DISEASE (GD1) TRANSITIONED FROM IMIGLUCERASE: PHASE II/III TRIAL TKT034 AND EXTENSION 2-YEAR RESULTS

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Background: We report outcomes in type 1 Gaucher disease (GD1) patients transitioned from imiglucerase to velaglucerase alfa.
Methods: In trial TKT034, GD1 patients aged>=2 years, on imiglucerase (>=22 consecutive months at 15-60U/kg every other week), with a stable dose and hematologic parameters for>=6 months, switched to velaglucerase alfa (same U/kg, 60-minute intravenous infusion). TKT034 completers could enroll in the extension HGT-GCB-044.
Results: 38/40 patients treated in TKT034 continued into HGT-GCB-044 (prior imiglucerase exposure 22–192 months; ages 9–71 years; 24% <18 years; 47% male; 8% splenectomized). After 2 years, the mean changes (95% CI) in hemoglobin concentration, platelet count, and spleen and liver volumes normalized to body weight were 0 g/dL (−0.3, 0.3), 8.0% (1.1, 15.0), -8.1% (−14.1, -2.2), and −1.2% (−4.8, 2.4), respectively—all within the pre-specified clinically significant limits of stability: ±1 g/dL, ±20%, and ±15%, respectively. The majority of patients maintained all therapeutic goals met at baseline for these 4 parameters. The mean change (95% CI) in CCL18 concentration was −50.9% (−55.5, -46.3) and in chitotriosidase activity, -34.5% (−42.2, -26.9).
Conclusion: 38 GD1 patients transitioned from long-term imiglucerase to velaglucerase alfa maintained key clinical parameters through 2 years while biomarkers of glucosylceramide burden declined.
Conflict of Interest declared.

P-434

2-YEAR SAFETY AND TOLERABILITY OF VELAGLUCERASE ALFA ENZYME REPLACEMENT THERAPY (ERT) IN PATIENTS WITH TYPE 1 GAUCHER DISEASE (GD1) SWITCHING FROM IMIGLUCERASE

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Background: Adverse events (AEs) are important considerations for physicians treating type 1 Gaucher disease (GD1) with ERT.
Methods: In TKT034, 40 GD1 patients receiving stable imiglucerase for>=22 months switched to velaglucerase alfa for 12 months (protocol-specified continuous 60-minute infusion; otherwise, same regimen and U/kg as prior imiglucerase treatment). 2 patients discontinued (1 for personal reasons, 1 due to a hypersensitivity reaction during the first infusion); all 38 patients completing the study elected to continue in the extension. AEs were elicited by non-leading questions or discovered through observation, laboratory reports, or patient complaint. IRAEs were defined as beginning<=12 hours after infusion start and possibly or probably related to study drug.
Results: In the 38 patients receiving 24 months of velaglucerase alfa, median prior imiglucerase exposure was 65 months (range 22–192). The most common AEs (>20% of patients) were headache, nasopharyngitis, pharyngolaryngeal pain, cough, arthralgia, myalgia, and fatigue. Most AEs were mild or moderate and none led to study discontinuation. 6 SAEs occurred in 5 patients (none study drug-related). 9/38 patients experienced>=1 IRAE.
Conclusions: In GD1 patients transitioned from imiglucerase, velaglucerase alfa was generally well tolerated through 2 years; no drug-related SAEs occurred and most patients experienced no IRAEs.
Conflict of Interest declared.

P-435

FIRST YEAR OF CLINICAL THERAPEUTIC EXPERIENCE USING VELAGLUCERASE-ALPHA FOR THE TREATMENT OF GAUCHER DISEASE

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Background: Standard of care for Gaucher disease(GD), the deficiency of lysosomal enzyme glucocerebrosidase, is enzyme replacement therapy (ERT). ERT is highly effective in reversing the visceral and hematologic manifestations, but skeletal and pulmonary complications, and inflammatory component respond variably. We report the first-year experience with velaglucerase-alpha(VPRIV®), a gene- human glucocerebrosidase by providing specific case examples.
Methods: 15 (12 F: 3 M) GD1 patients were treated with VPRIV®, 30 to 60 IU/kg, for one year. At initiation of therapy, splenectomized patients (n=4), 2 naïve to ERT, had significant disease-load, presenting with transfusion dependency, increased pulmonary pressures, systemic inflammation and skeletal involvement. Chitotriosidase(CHITO) was followed as a GD marker, and antibodies were obtained in selected patients.
Results: Among patients with mild-moderate GD, hematological parameters and CHITO remained stable. In severely afftecteds, the clinical response was observed usually within first 3 months of therapy. Changes in CHITO ranged between normal levels to a more than 50% decrease. There were three infusion-related events, but antibody development was not observed.
Conclusions: The success in clinical response may concur with better in vitro internalization of velaglucerase-alpha, into macrophages. Nevertheless, having different enzymes will provide an opportunity to re-evaluate initiation of ERT, and management of non-responders in GD.
Conflict of Interest declared.

P-436

THE PHARMACOLOGICAL CHAPERONE AT2220 INCREASES TISSUE UPTAKE OF RECOMBINANT HUMAN ACID ALPHA-GLUCOSIDASE AND LEADS TO GREATER GLYCOGEN REDUCTION IN A MOUSE MODEL OF POMPE DISEASE

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Background: Pompe disease is caused by a deficiency in the lysosomal enzyme acid alpha-glucosidase (GAA), which results in progressive accumulation of glycogen in lysosomes of various tissues, mainly muscle. Recombinant human GAA (rhGAA) given as bi-weekly infusions, known as enzyme replacement therapy (ERT), is the primary treatment for Pompe patients. While generally effective, the rhGAA has a short circulating half-life, low tissue uptake, and can elicit immune responses that adversely affect tolerability and efficacy. AT2220 (1-deoxynojirimycin) is a small molecule pharmacological chaperone that binds endogenous GAA in cells and tissues, resulting in increased GAA lysosomal levels and activity.
Objective: To determine if AT2220 also interact with exogenous rhGAA (i.e ERT) to improve its pharmacological properties.
Results: In human plasma, AT2220 increased the stability and minimized denaturation of rhGAA at neutral pH/37°C. In rats, oral co-administration of AT2220 increased the circulating half-life of rhGAA by ~2-fold. In GAA KO mice, oral co-administration of AT2220 resulted in up to 2.5-fold greater tissue uptake and glycogen reduction compared to rhGAA alone.
Conclusions: Collectively, these data indicate that AT2220 stabilizes rhGAA, and leads to improved uptake and glycogen turnover. Based on these findings, a Phase 2a study of AT2220 with rhGAA has been initiated.
Conflict of Interest declared.

P-437

ANTI-CD3 ANTIBODY REDUCES ANTIBODY FORMATION AND PREVENTS LETHAL HYPERSENSITIVITY REACTION IN ENZYME REPLACEMENT THERAPY FOR POMPE DISEASE IN MOUSE

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Background: The aim of this study is development of immune tolerization therapy for enzyme replacement therapy (ERT) of Pompe disease.
Methods: Wild type mice and murine model of Pompe disease were used. Anti CD3 antibody was administered to mice, followed by enzyme infusion up to 20 weeks. After ERT, anti CD3 antibody was also administered to reduce the pre-existing immune reaction to enzyme. IgG and IgE antibody titer against enzyme was assayed by ELISA. CD4+cell, CD8+ cell, and CD4+CD25+ cells were analyzed by flow cytometry. We tested if immune tolerance induction by anti CD3 antibody was achieved after depletion of CD25+ cells.
Results: Anti CD3 antibody administration prior to ERT reduced IgG and IgE antibody formation against enzyme. This effect was persisted for study period (40 weeks). Lethal hypersensitivity reaction of ERT was also prevented and titer of pre-existing antibody against enzyme was reduced. Regarding mechanism, CD4+ and CD8+ cells were reduced and CD4+ CD25+ regulatory T cells were increased. The immune tolerance induction was cancelled if CD25+ cells were depleted.
Conclusion: Parenteral administration of anti CD3 antibody reduced immune reaction against enzyme, even pre-existing antibody and prevent lethal hypersensitivity. CD4+CD25+ cells play an important role in this immune tolerance induction.
Conflict of Interest declared.

P-438

LONG TERM EFFECTS OF ENZYME REPLACEMENT THERAPHY IN JUVENILE POMPE PATIENTS: A 48 MONTHS FOLLOW UP STUDY

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Background: Enzyme replacement therapy (ERT) in glycogen storage disease type 2 (GSD2) has been demonstrated to be safe and effective in short term studies.
Objectives: To evaluate effects of ERT in juvenile GSD2 patients after 48 months of treatment.
Patients and methods: 8 patients (mean age 10.9±1.9 years) treated with 20 mg/Kg of ERT biweekly up to 48 months were included. Walton Scale (WS), 6 minutes walking test (6 MWT), vital capacity, forced expiratory volume, arterial pCO2, and muscle enzymes were analyzed retrospectively. Results: After 48 months ERT was well tolerated in all subjects. Significant improvement of 6 MWT ($p<0.03$), and a stabilization or reduction of WS were observed in all patients. Parameters of respiratory function remained stable, and shortened time of required ventilatory support was observed in the 2 patients already ventilatory dependent at the beginning of the study. A significant reduction in muscle enzymes ($p<0.03$) was evidenced.
Conclusions: Although the relative small number of patients included, this study demonstrate that ERT in juvenile GSD2 is able to improve motor function and stabilize ventilatory function after 4 years of therapy. The best results were achieved when ERT was started before the onset of the clinical manifestations of the disease.

P-439

CRIM STATUS, ANTIBODY FORMATION AND NEUTRALIZING ANTIBODIES IN PATIENTS WITH CLASSIC INFANTILE POMPE DISEASE TREATED WITH ENZYME-REPLACEMENT THERAPY

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Some patients with classic infantile Pompe patients do not express any endogenous acid alpha-glucosidase (GAA) (CRIM-negative). They are prone to develop a high antibody titer against the recombinant human GAA, counteracting the effect of enzyme-replacement therapy (ERT).
We have treated 11 patients for 0.3–12.0 years (median 4.2y). All infants developed antibodies with titers ranging from 1:1,250–1:156,250. Two, possibly 3, of the 11 patients proved to be CRIM-negative. Of the two patients with the highest antibody titer, one was CRIM-positive and is still alive at the age of 12y, although ventilator dependent and tetraplegic. The other was CRIM-negative and died at the age of 4y. The patient with the lowest titer responded best to ERT and is at present 6y old. In only one of the 7 cases that we investigated did the antibodies inhibit the uptake of alglucosidase alpha in vitro. All CRIM-positive patients are still alive, with variable clinical outcome, whereas all CRIM-negative patients have died.
We conclude that antibody formation occurs frequently in classic infantile Pompe patients receiving ERT; both in CRIM-negative as well as in CRIM-positive patients. Overall, CRIM-positive patients and patients with a low antibody titer seem to have the best clinical outcome.
Conflict of Interest declared.

P-440

SUCCESSFUL ENZYME REPLACEMENT THERAPY IN A CRIM NEGATIVE CHILD AFTER 2=YEARS 0F TREATMENT

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Maltase acid deficiency (Pompe disease), leads without treatment to hypertrophic cardiomyopathy muscle weakness, and premature death. Enzyme replacement therapy (ERT: rhGGA) is poorly efficient in cross-reactive immunological material (CRIM) negative patients. In these patients, immunologic reaction can neutralize rhGGA . We described a CRIM negative (proven on fibroblasts) 30-months-old boy treated by ERT since two months of age. The disease is due to a homozygous mutation in the GAA gene (p.R870x). The cardiomyopathy rapidly improved, while the muscle weakness improved partially. Intially, he presented pulmonary infections, asthma, swallowing difficulties which led to a feeding by gastrostomy. The second year of life was marked by a real improvement in muscle weakness without anymore pulmonary infections. At 2 years old, he presented an anaphylactic reaction to the treatment while there was a slight increase of rhGGA antibodies (IgG: 1/100). Under steroid therapy he supported the next ERT infusions. Despite moderate psychomotor retardation, he clearly continue to progress on the gross motor development and on respiratory function. This is the first case of successful ERT in a CRIM negative child. Immunologic studies are pending to assess the immunological functions of this child who did not produce high levels of rhGGA antibodies.

P-441

SURVIVAL OF ADULT POMPE PATIENTS WITH AND WITHOUT ENZYME REPLACEMENT THERAPY

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Background: There is paucity of information on the impact of Enzyme Replacement Therapy (ERT) on survival of adult patients with Pompe disease. We assessed whether there is differential mortality in adult patients treated with ERT compared to untreated patients.
Methods: Data of 270 patients with a median age of 48 years (range 19–79 years) were collected in an international observational study between 2002 and 2011. Kaplan-Meier survival curves were plotted and multivariate logistic regression analyses were performed..
Results: The ERT-group included 195 patients with 15 deaths and the non-treatment group 75 patients with 26 deaths. Survival was higher for patients on ERT compared to patients never on ERT and for patients with lower compared to higher levels of disease severity. For each level of disease severity (based on wheelchair and ventilator dependency) greater survival on ERT was observed. In logistic regression analyses controlling for age, gender, and national treatment patterns, both treatment with ERT and disease severity were associated with survival.
Conclusion: Our results suggest that ERT expands the life span of adult patients even when they are severely affected. Longer follow-up is needed to further elucidate the relationship between ERT, disease severity and survival of adults with Pompe disease.
Conflict of Interest declared.

P-442

SAFETY AND TOLERABILITY OF AGALSIDASE ALFA IN PATIENTS WITH FABRY DISEASE FORMERLY TREATED WITH AGALSIDASE BETA

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Background: Safety and tolerability were evaluated in patients with Fabry disease (FD) switched from agalsidase beta (agalβ) to agalsidase alfa (agalα; switch patients) in a clinical trial setting.
Methods: An ongoing, multicenter, open-label, treatment protocol (HGT-REP-059) collected 6-month data on adverse events (AEs) and other clinical parameters for switch patients (59.2%, 71/120 of enrollees), who received intravenous agalα (0.2 mg/kg every other week).
Results: 71 patients were evaluated (aged 5–84 years; male:female, 40:31; in 69 patients, mean prior duration of agalβ was 4.6 years [range 0.3–12.3]). 66.2% (n=47) of patients experienced 159 total treatment-emergent AEs (mostly mild-or-moderate severity); 33.8% (n=24) had possibly/probably treatment-related AEs; and 25.4% (n=18) had infusion-related AEs. The most common treatment-emergent AEs were dizziness (7.0%; n=5), headache (5.6%; n=4), and vomiting (5.6%; n=4). 10 (14.1%) patients experienced serious AEs; one (transient ischemic attack [TIA] in a patient with history of TIA) was considered possibly drug-related and resolved without sequelae. No deaths or AE-related permanent drug discontinuations occurred.
Conclusions: Agalα was generally well tolerated over 6 months of treatment in 71 patients with FD who had previously received agalβ.
Conflict of Interest declared.

P-443

THE PHARMACOLOGICAL CHAPERONE AT1001 INCREASES THE CELLULAR ACTIVITY OF RECOMBINANT HUMAN ALPHA-GALACTOSIDASE A IN VITRO AND IN A MOUSE MODEL OF FABRY DISEASE

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Background: Fabry disease is an X-linked lysosomal storage disorder caused by mutations in the gene that encodes alpha-galactosidase A (alpha-Gal A). Regular infusion of recombinant human alpha-Gal A (rh-alpha-Gal A), termed enzyme replacement therapy (ERT), is the primary treatment for Fabry disease. However, rh-alpha-Gal A is unstable at neutral pH, and has a short circulating half-life and variable uptake into different tissues. The pharmacological chaperone AT1001 (migalastat hydrochloride, GR181413A) selectively binds endogenous alpha-Gal A, increasing physical stability, lysosomal trafficking, and cellular activity.
Objective: To determine if AT1001 can improve the pharmacological properties of rh-alpha-Gal A.
Results: AT1001 increased rh-alpha-Gal A physical stability and minimized denaturation in vitro. In cultured Fabry fibroblasts, AT1001 co-incubation with rh-alpha-Gal A further increased the cellular levels of the enzyme by ~3-fold. In rodents, AT1001 co-administration increased the circulating half-life of rh-alpha-Gal A. In GLA knockout mice, co-administration increased the tissue enzyme activity and resulted in greater substrate reduction (globotriaosylceramide and globotriaosylsphingosine) compared to rh-alpha-Gal A alone.
Conclusions: These data indicate that AT1001 co-administration with rh-alpha-Gal A results in more enzyme activity and greater substrate turnover in cells and animals. Thus, AT1001 co-administration with ERT warrants further clinical investigation as a treatment for Fabry disease.
Conflict of Interest declared.

P-444

SWITCH FROM FABRAZYME TO REPLAGAL IN THE TREATMENT OF PATIENTS WITH FABRY DISEASE: THE NEED FOR AN INDIVIDUALIZED APPROACH

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Introduction: An acute shortage of Fabrazyme has occurred as a result of viral contamination of Genzyme's production facility in June 2009. Due to further manufacturing problems, deliveries of Fabrazyme have been delayed several times. In April 2010, it became clear that the Fabrazyme shortage was set to continue. This resulted in updated management recommendations that were issued by the European Medicines Agency (EMA).
Methods: Discussions on therapy and switch guidelines were provided by key experts in the field. Based on these guidelines we switched four patients (3 males/1 female) from Fabrazyme, reduced dose of 0.3 mg/kg/2 weeks (registered dose 1 mg/kg/2 weeks) to Replagal 0.2 mg/kg/2 weeks. Except for the reoccurrence of neuropathic pain the switch was well tolerated and the disease status remained stable in most patients during a follow-up of at least 12 months. One patient, a male with high concentrations of neutralizing antibodies towards the enzymes, showed deterioration of his hypertrophic cardiomyopathy and increase of urinary Gb3 while treated with the reduced dose of Fabrazyme. His condition remained stable under Replagal.
Conclusions: Switching between Fabrazyme and Replagal didn't influence the clinical evolution in our patient group. Special attention however should be given to patients with neutralizing antibodies.

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HEALTH TECHNOLOGY ASSESSMENT FOR RARE DISEASES: A MARKOVIAN MODEL EVALUATING NEPHROPATHY IN FABRY DISEASE (FD) CONSIDERING ENZYME REPLACEMENT THERAPY (ERT)

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Background: Fabry Disease (FD) is a lysosomal disease, with X-linked inheritance, caused by deficiency of alphaGAL-A. This deficiency causes the accumulation of GB3 in the cells. The diasease evolves with peripheral neuropathy, cerebrovascular and cardiovascular disease and renal failure. Renal failure is the major cause of morbi-mortality in this population. Worldwide there are 2 recombinant enzymes licensed for enzyme replacement therapy (ERT) in FD: agalsidase beta and agalsidase alfa. The preliminary findings in literature about ERT in FD indicate the possibility of stabilization of renal disease.
Methods: A Markovian model with possible states of nephropaty was built. The probabilities with ERT x without ERT were compared.
Conclusions: The model was able to show a reduction in the likelihood of progression of renal dysfunction over a period of 3 years in male patients with FD with very initial involvement in the glomerular filtration rate, translated by proteinuria only, when treated with ERT.This model was build considering clinical outcomes, something new on rare diseases. The discussion on how to evaluate and on the value of 'classical modeling' in rare diseases is still an on going issue with a lot to be elucidated.Support CNPq/MS-SCTIE-DECIT n. 33/2007 and 37/2008

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INFUSION ASSOCIATED REACTION (IAR) DURING ENZYME REPLACEMENT THERAPY (ERT) WITH AGALSIDASE BETA RELATED TO UPPER-AIRWAY TRACT INFECTION: A CASE REPORT

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Background: The role of infections in IAR during ERT with the exogen protein is still not clear. We report a case of a patient who had been under ERT with Agalsidase Beta for more than four years who had an IAR when presenting an intercurrent infection.
Case report: Male, 19 years, on ERT for 4 year. During his 90th infusion, he presented high fever, tremor, skin rash, which was treated with cortico-steroids e antihistamine. On that day, even before ERT, he presented cough and rhinorrhea without fever. Acute rhinosinusitis was diagnosed, antibiotics were prescribed and the patient was discharged home. On the next infusion, he had already overcome the rhinosinusitis, was asymptomatic, didn't take any pre-medication and received the ERT without any other IAR.
Discussion: The pharmacologic interaction with immune receptors concept (P-I concept) is a recently proposed addition to drug hypersensitivity classification. Infections can incite T lymphocytes to change their link to some drugs and this process may cause adverse reactions.
Conclusion: ERT is often complicated by immune responses to the enzymes and the P-I concept must be reminded as an explanation to IAR in patients with infections, because in these cases the use of pre-medication is not necessary.
Conflict of Interest declared.

P-447

INTRATHECAL ENZYME REPLACEMENT THERAPY FOR NEUROLOGICAL IMPAIRMENT IN MUCOPOLYSACCHARIDOSIS 1

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Bachground: MPS1 is an autosomal recessive disorder caused by deficient activity of the lysosomal enzyme alpha-L iduronidase, which leads to accumulation of heparan sulfate and dermatan sulfate, resulting in progressive multisystem disease with respiratory, skeletal, and neurologic manifestations. Treatment for MPS1 consists of supportive care and enzyme—replacement therapy with Laronidase. Bone marrow and hematopoietic stem cell transplantation is the treatment of choice for patients suffering from MPS1 with no or minimal central nervous system manifestation.
Case Report: A 50 month old Iranian boy with coarse facial features, prominent forehead, corneal cloudy, sleep disturbance, hepatosplenomegaly, inguinal hernia, joint stiffness, and dysostosis multiplex congenital. He was diagnosed with MPS1 on the basis of clinical findings, an elevated urinary glycosaminoglycan level and low alpha- L- iduronidase activity in leukocytes. Mutation analysis revealed a homozygous splice mutation in the IDUA gene.
He has been started on injection of aldurozyme intravenously every week from age 26 months. To attempt to prevent neurological impairment before bone marrow transplantation, he receives intrathecal enzyme replacement of aldurozyme and samples of his CSF are obtained for testing monthly. He tolerates intrathecal ERT with no adverse events. At age 50 months he will receive a bone marrow transplantation.

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FURTHER REPORT OF INTRATHECAL ERT IN A BRAZILIAN MPS I (HURLER-SHEIE) PATIENT

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Mucopolysaccharidosis I(MPS I)is a lysosomal disease caused by α-iduronidase deficiency, leading to glycosaminoglycans(GAGs)storage in several organs.This may affect the vertebrae and meninges and cause spinal cord compression (SCC).As intravenous enzyme replacement therapy (ERT)is not expected to cross the blood–brain barrier,other approaches of drug delivery are necessary to treat SCC.We report a case of a 23 yo MPS I patient(Hurler-Scheie phenotype)with SCC who choose to stop intravenous ERT after 5 years of treatment since his perception was that symptoms were not improving.Laminectomy and occipital craniotomy were then performed, again with no improvement of his condition.Intrathecal infusions were offered on a compassionate use basis,and he agreed to perform 4 infusions with one month intervals.At the baseline and after the 4th infusion,CSF biochemistry(with GAGs measurement),clinical neurology assessments and some additional studies were performed.The patient referred improvement of his lower limbs sensitivity every first week after infusions with regression to previous status thereafter.Somatic sensitive potentials in upper and lower limbs showed improvement Intrathecal infusions showed to be safe and may be offered as an option when the neurosurgical risk is high or as an alternative to improve patient's QOL. We think that adjustments in dosis and infusion frequency may improve outcome of this approach.

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NOVEL ENZYME REPLACEMENT THERAPY (ERT) PROCEDURE FOR MUCOPOLYSACCHARIDOSIS TYPE II (MPSII) BY INTRAVENTRICULAR ADMINISTRATION (IVA) IN MURINE MPSII

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The MPSII is a lysosomal storage disorder caused by a deficiency of iduronate-2-sulfatase (IDS), characterized by the accumulation of glycosaminoglycans (GAGs). The major clinical manifestations are joint contractures and often mental retardation. The MPSII has been treated by HSCT/ ERT, but this effectiveness in CNS is limited because of poor uptake of enzyme to cross the BBB. To increase the efficacy of ERT in the brain, we tested intraventricular administration (IVA) procedure of IDS in Ids knockout mice. 20ug of IDS was administered into the ventricle of MPSII mice repeatedly 4 times every 3 weeks. The IDS enzyme activities, GAGs assay and histopathology were measured in mice tissues. The IDS activities were significantly increased in MPSII mice cerebral regions with IVA. In cerebellum, the activities were also elevated to similar levels of normal mice. The accumulation of total GAGs was decreased in the brain treated with IDS. The over expression of Lamp2 was also observed in Purkinje cell of MPSII mice. In MPSII mice tissues with IDS multiple IVAs, the high level IDS activities were maintained and GAGs storage levels were also decreased. These results demonstrate the possibility and efficacy of novel ERT procedure with IVA for MPSII treatment.
Conflict of Interest declared.

P-450

IDURSULFASE THERAPY IN A PATIENT AFFECTED BY MUCHOPOLYSACCARIDOSIS (MPS) TYPE II

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Introduction: MPS II is a X-linked lysosomal storage disorder causing a progressive multisistemic accumulation of glycosaminoglicans (GAGs). Enzimatic replacement therapy (idursulfase) has been introduced in the last years.

Case report: We report the case of a 19 years old boy who received diagnosis of MPS II at the age of 4 years by the presence of an occasional heart murmur (R443X mutation). At 14 years he began therapy with idursulfase (dose 0,5 mg/kg/die): urinary GAGs have been decreased significantly. Despite the severe aortic and mitral regurgitation, no heart failure has been detected. The severe bilateral mixed hearing loss and the restrictive respiratory impairment are both stable, while the mild form of obstructive sleep apnea syndrome has been resolved. Hepatomegaly is still present, splenomegaly has been reduced and so the pancytopenia from hypersplenism. A mild thrombocytopenia is still present. Lower limbs muscular trophysm has improved but neither joint stiffness nor bone dysmorphisms did.

Mild mental retardation is present.

Conclusion: In this patient Idursulfase therapy can be considered effective even if started in adolescence.

P-451

A SYSTEMATIC LITERATURE REVIEW: ENZYME REPLACEMENT THERAPY IN MUCOPOLYSACCHARIDOSIS TYPE II

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Enzyme replacement therapy with idursulfase (IDS) (0.5 mg/kg/every other week) is a therapeutic option for mucopolysaccharidosis (MPS) type II.

Methodology: In order to examine the efficacy and safety of ERT, a systematic literature review was conducted until February 28, 2011. The inclusion criterion was being a randomized controlled trial (RCT); in the absence of at least 5 RCTs, prospective case series with ≥5 patients that evaluated relevant endpoints, previously defined by our research team, were also included.

Results: Two RCTs comparing IDS to placebo and 2 open label trials were included. One RCT was a phase I/II trial (n=12 patients; 72 weeks), and the other a phase II/III trial (n=94; 54 weeks). Both demonstrated the following: reductions in urine glycosaminoglycans and hepatosplenomegaly, increase in the 6-minute walk test distance and pulmonary function; however, disease severity was heterogeneous between the groups at baseline. Both open-label studies revealed analogous benefits as described by the RCTs.

Conclusion: MPS II is a rare disease, and some difficulties in the conduction of RCTs can occur. IDS benefits MPSII patients; however, most studies reviewed had surrogate endpoints and clinical heterogeneity, thus limiting our conclusions. Additional studies should be conducted. A metanalysis is planned. Support: MCT/CNPq/MS-SCTIE-DECIT037/2008

P-452

UPDATE ON A MULTICENTER, MULTINATIONAL, LONGITUDINAL CLINICAL ASSESSMENT STUDY OF SUBJECTS WITH MUCOPOLYSACCHARIDOSIS IVA (MORQUIO SYNDROME)

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299 subjects were enrolled through 10/31/2010 (53 % F; 47 % M) in the Morquio Clinical Assessment Program. Mean age was 14 years (r: 1 – 65). Height in most subjects 18 and younger was below 3%ile. Average adult (>18 y) height was below 120 cm. Endurance was impaired with mean 6 Minute Walk Test (median): 220±152 m (226), and mean 3 Minute Stair Climb Test: 39±21 steps/min (35). Respiratory function was decreased with mean Forced Vital Capacity [FVC]: 1.2±0.88 L (0.9) and mean Maximum Voluntary Ventilation: 35±26.3 L/min (26). Mean plasma keratan sulfate [KS]: 1.4±1.05 ug/mL (1.3) and mean urine KS: 34±25.0 ug/mg Creatinine (31) were elevated. Urine KS decreased with age. Higher uKS values were associated with decreased height and FVC results. Adults with uKS <10 had greater height, endurance and respiratory function than those with higher levels while in younger subjects, the uKS threshold appears higher. Further analyses and continued study enrollment with patients returning for longitudinal annual assessments are ongoing. Results are consistent with prior findings from 129 subjects, supporting the choice of clinical and biomarker endpoints in the ongoing Phase 3 Study (MOR-004). Conflict of Interest declared.

P-453

OUTCOME OF ENZYME REPLACEMENT THERAPY IN PATIENTS WITH MUCOPOLYSACCHARIDOSIS TYPE VI

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Background: Mucopolysaccharidosis (MPS) Type VI is a lysosomal storage disorder characterized by a chronic, progressive course with multiorgan involvement.
Methods: In our study, clinical, biochemical and radiological findings and response to enzyme replecement therapy for at least six months were analyzed in 20 patients with MPS VI. Any changes on clinical findings such as liver and spleen sizes, cardiac and respiratory parameters, endurance tests (6-minute walk test), changes in urinary glycosaminoglycan excretions, visual and auditory changes, and joints' range of motions before and after ERT were analyzed.
Results: There was significant improvement in physical endurance with ERT ($p<0.05$). With ERT, there was an increase in growth rate but this was not statistically significant ($p>0.05$). Cardiac, respiratory, visual, auditory parameters were not been changed and there were no improvement on joint mobility ($p>0.05$). All patients and parents pointed out an increased quality of life, which did not correlate with clinical results.
Conclusions: ERT caused increased physical capacity and decreased urinary dermatan sulphate/keratan sulphate ratios. No significant changes were observed in growth parameters, cardiac, respiratory, visual, auditory findings and joint mobility. ERT was safe but an expensive method for the treatment of MPS VI with mild benefits on severely affected cases.

P-454

THE MPS VI CLINICAL SURVEILLANCE PROGRAM: CURRENT STATUS

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Mucopolysaccharidosis VI (MPS VI) results in accumulation of the glycosaminoglycan (GAG) dermatan sulfate. Storage of GAG may be relieved by Naglazyme® treatment. The Clinical Surveillance Program (CSP) is a voluntary observational program which collects data to characterize progression of MPS VI and collect long term efficacy and safety outcomes of Naglazyme treatment based on patient standard of care assessments.
Of the 151 patients enrolled in the CSP through 22 March 2011, 12 entered enrollment in the prior year and 139 have received Naglazyme treatment. To date, 6 patients have had bone marrow transplant (BMT), 2 of which were reported to not remain grafted, and 5 of the 6 patients have received subsequent Naglazyme treatment. There was an approximately 50% decrease in median urinary GAGs from baseline after Naglazyme treatment for up to 9 years.
Improved endurance measurements (12-minute walk and 3-minute stair climb tests) and increased height and weight from baseline were observed in Naglazyme treated patients. Elevated antibody levels were not correlated with changes in urinary GAGs. Occurrences of serious adverse events related to Naglazyme treatment, including infusion related events, remain low.
Naglazyme treatment appears to slow progression of MPS VI when compared to untreated patients in the CSP
Conflict of Interest declared.

P-455

CLEARANCE OF LYSOSOMAL STORAGE FROM BONE AND CARTILAGE WITH ENZYME REPLACEMENT THERAPY IN A CHILD WITH MUCOPOLYSACCHARIDOSIS TYPE VI (MPS VI)

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Background: MPS VI is a multisystemic lysosomal storage disorder characterised by the accumulation of glycosaminoglycans (GAG) in lysosomes. The disorder affects most tissues of the body including the viscera, bone, cartilage and fibrous tissue. Enzyme replacement therapy (ERT), has drastically changed the disease course in MPS VI, however much remains unknown about longterm therapy.
Case Report: We describe a female patient with rapidly progressive MPS VI who commenced ERT at 9 years of age and died at 16. Electron microscopy (EM) was performed on the tissues of a toe removed 6 months after commencing enzyme replacement therapy and on a contralateral toe at autopsy after almost 7 years of ERT.
Results: Intital EM demonstrated significant staorage of GAGs in the cells of the bone, cartilage and fibrous tissue. Autopsy microscopy showed minimal to no storage of GAGs in bone or cartilage, but persistent storage in fibrous tissue.
Conclusions: In this case there appeared to be microscopic clearance of storage from bone and cartilage, but not fibrous tissue. The differences observed may reflected differential cell turnover in bone and cartilage compared to fibrous tissue. This finding provides human evidence that ERT may influence the disease process in bone and cartilage.

P-456

EXPERIENCE IN THE MANAGEMENT OF LYSOSOMAL STORAGE DISORDERS BY ENZYME REPLACEMENT THERAPY FROM INDIA

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Lysosomal storage diseases constitute about 50 different diseases, with a specific lysosomal enzyme deficiency. They have characteristic features, and diagnosis must be established with specific biochemical assay or by DNA analysis. There is no specific cure for these disorders, however, a new era has emerged in recent years in the management with Enzyme replacement therapy (ERT).Although it is not a cure but it can modify or attenuate the disease phenotype and its progression. The treatable disorders include the non-neuronopathic Gaucher Disease, MPSI, MPSII, MPSVI, Fabry disease, and Pompe Disease by ERT.
Results: ERT for Gaucher, Pompe and MPS I, and Fabry is being given in India by Genzyme, USA as a charity. At rainbow children Hospital, Hyderabad, India, a total of six patients were under ERT, Response to therapy was excellent with Gaucher disease, and not very encouraging with Pompe disease and reasonably satisfactory with MPS I.
Conclusions: Treatable LSD is more often diagnosed in India and option for treatment is limited in view of the cost.

P-457

ENB-0040 TREATMENT CHANGES THE NATURAL HISTORY OF PERINATAL/EARLY-INFANTILE ONSET HYPOPHOSPHATASIA IN CANADIAN MENNONITES: A RETROSPECTIVE COHORT ANALYSIS

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Hypophosphatasia (HPP) is the metabolic bone disease caused by loss-of-function mutation within the gene encoding the "tissue nonspecific" isoenzyme of alkaline phosphatase (TNSALP). Perinatal/early infantile-onset HPP is usually fatal due to respiratory insufficiency. There is no approved medical therapy. We reviewed our 80-year (1927–2007) retrospective cohort of 19 Canadian patients with perinatal/early infantile-onset HPP. 14/19 were of Mennonite descent and those available for study were homozygous for the TNSALP gly317asp mutation with profound skeletal hypomineralization, severe rickets, and respiratory insufficiency. All died by 9 months-of-age, most often soon after birth, from pulmonary failure. Clinical trials of enzyme-replacement therapy for HPP using human, recombinant, bone-targeted TNSALP (ENB-0040) began in 2008. To date, 20 infants and young children (10 perinatal; 10 infantile-onset HPP) who met eligibility criteria have been enrolled and treated with thrice weekly subcutaneous injections. 1 withdrew after the first injection and 1 died of sepsis unrelated to ENB-0040. In the remaining 18 patients, including 2 perinatal homozygous for the Canadian Mennonite mutation, marked improvement in their radiological findings and respiratory function is paralleled by dramatic clinical gains with minimal side effects. Thus, comparison with our Mennonite controls indicates that ENB-0040 is transforming lethal HPP into a treatable disorder.
Conflict of Interest declared.

O-056

TOWARDS NON-VIRAL LIVER-DIRECTED GENE THERAPY FOR HEPATIC DISEASES

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The liver is a potential target for transgene delivery and expression for gene therapy of hepatic and various metabolic diseases, including amino acid metabolism or urea cycle disorders. Currently, we are developing and evaluating highly efficient non-viral gene transfer methods by targeting the murine liver as a potential alternative gene-therapeutic approach. In our study, we report the use of the minicircle (MC) technology for the gene therapy of phenylketonuria (PKU) mouse model. Our MC-DNA vectors contain a liver-specific promoter, Pah, the luciferase reporter gene plus a downstream mammalian scaffold/matrix attachment region (S/MAR) element for episomal maintenance and/or extra-chromosomal stability. Delivery was mediated by hydrodynamic tail vein (HTV) injection as a liver-targeted approach. Luciferase expression was monitored by quantitative bioluminescence in a non-invasive imaging technology in living mice (IVIS screening). We compared longitudinal luciferase expression between MC-DNA vector and conventional plasmid DNA (pDNA). Our data showed that vectors were exclusively delivered to the liver and the luciferase expression in mice injected with MC was more than 20-fold higher than mice injected with pDNA for at least 9 months. MC gene delivery for maximizing safety and sustained gene expression is a potential new approach for the hepatic treatment.

O-057

ALIPOGENE TIPARVOVEC (AAV1-LPLS447X GENE THERAPY) IS LONG-TERM SAFE AND EFFICACIOUS IN PATIENTS WITH LIPOPROTEIN LIPASE DEFICIENCY

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Background: Lipoprotein lipase deficiency (LPLD) is a rare, monogenic lipid disorder causing hyperchylomicronaemia, increased risk of acute pancreatitis (360 x) and cardiometabolic complications. Alipogene tiparvovec (Glybera®) contains a gain-of-function LPL-gene mutation in an AAV-vector. We conducted two interventional trials and one case note review study for pancreatitis (CT-AMT-011-03).
Methods: In the interventional trials involving 19 LPLD adults, alipogene tiparvovec was administered IM one-time. These patients and subjects participating in an earlier observational study were enrolled in CT-AMT-011-03. Historical data for all hospital presentations due to abdominal pain were collected. Pancreatitis definition was according to modified Atlanta Diagnostic Criteria for acute pancreatitis. Statistical analysis to model the A Cox regression gap-time-model estimating hazard of pancreatitis pre/post therapy was used.
Results: Alipogene tiparvovec was well tolerated. Adverse events were mainly injection site related, mild-moderate and transient.
A statistically significant reduction in the risk of acute pancreatitis was seen comparing the period from the first pancreatitis event to administration with the post-therapy period (median=2.9 years). The hazard ratio indicated a 63% reduction in risk of acute pancreatitis (95% CI 0.142–0.971).
Conclusion: Alipogene tiparvovec was well tolerated, safe and reduced the risk of pancreatitis during long term follow up post one-time administration.
Conflict of Interest declared.

O-058

CORRECTION OF MURINE PHENYLKETONURIA FOLLOWING LIVER-DIRECTED ADMINISTRATION OF A NOVEL RECOMBINANT ADENO-ASSOCIATED VIRUS SEROTYPE 8 (RAAV2/8) VECTOR THAT DIRECTS SITE-SPECIFIC INTEGRATION INTO RIBOSOMAL DNA

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We have previously demonstrated the successful treatment of Pahenu2 mice, a model of human PKU, following liver-directed administration of a phenylalanine hydroxylase (PAH)-expressing rAAV2/8 vector. However, the therapeutic effect of this treatment was only temporary with transgene loss and reemergence of elevated blood phenylalanine within 4–6 months after vector injection. rAAV vectors that harbor 28S ribosomal DNA (rDNA) sequences flanking the therapeutic transgene cassette have been shown in other mouse models to permanently integrate at least ten times more frequently than standard rAAV. Portal vein injection into Pahenu2 mice of a novel rAAV2/8 vector that contains a PAH expression cassette flanked by rDNA sequences yielded complete correction of blood phenylalanine within one week. The treatment effect lasted almost one year after injection. Site-specific integration of the novel vector genome into mouse 28S rDNA has been demonstrated using nested PCR, but the true integration frequency is currently being evaluated. The inclusion of rDNA sequences into rAAV2/8 vectors to direct increased frequency of site-specific integration events is a promising novel step in the development of safe, effective rAAV-mediated liver-directed gene therapy for PKU and other inborn errors of metabolism.

P-458

LENTIVIRAL-MEDIATED CORRECTION OF METHYLMALONIC ACIDURIA IN VIVO

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Introduction: Current treatments for Methylmalonic Aciduria (MMA) remain unsatisfactory and research on novel therapies remains a high priority. A lentiviral (LV) vector was developed to treat an in vivo model of MMA.
Aim: To examine the therapeutic effect of lentiviral vector-mediated transfer of methylmalonyl CoA mutase (MCM) gene into a MCM knockout mouse.
Methods: A LV-MMA vector was developed and injected intravenously into 5 MMA mice. Untreated MMA (n=6) and normal mice (n=6) were used as controls. Mice weight was measured weekly after treatment. Plasma and urine MMA were measured using mass spectrometry and post-treatment MCM enzyme activity was determined by HPLC, according to published methods.
Results: The LV-MMA treated mice achieved near-normal weight for sex. Median plasma MMA levels in the LV-MMA treated group were reduced from 909+/−365uM at baseline to 377+/−166uM at 6 months, compared to untreated MMA mice 1114+/−328.54uM. Hepatic MCM enzyme 6 months post LV therapy ranged from 1.8 to 166.1 nmol/min/ug of protein (median 130.56+/−70uM), compared to not detectable in the untreated mice (N=6).
Conclusion: These results confirm that the use of LV-MMA may be a viable approach for the treatment of this form of MMA.

P-459

EFFICACIOUS AND SAFE GENE THERAPY FOR CANAVAN DISEASE: A NOVEL APPROACH

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Background: Canavan Disease (CD), caused by aspartoacylase (ASPA) deficiency is a severe leukodystrophy with no effective treatment. Earlier gene therapy attempts using rAAV2 failed.
Objective: To develop novel rAAV-based therapeutics for efficacious and safe gene therapy of CD.
Materials Methods and Experimental Design: ASPA−/− KO mice created by us mimic the neuropathology and clinical manifestation of CD patients, as early as the 2nd week of life and die early. Novel rAAVs developed by us efficiently cross the BBB and transduce the CNS after an i.v. injection. We used miRNAs to regulate i.v. delivered rAAV towards CNS-restricted gene expression. This novel gene therapy approach was evaluated in the CD mice.
Results: Single i.v. injection of rAAVASPA postnatally corrected metabolic, psychomotor deficits and spongy degeneration of the CNS. New findings of severe retinopathogy and renal pathology that were observed in the CD mice, were corrected with Gene Therapy. This treatment prolonged the life of the mice and restored their vision.
Conclusion/Discussion: Our data demonstrate the feasibility, safety and efficacy of this novel approach for gene therapy in CD. Development of clinical trials for CD patients should be the outcome of these on-going studies.

P-460

EVALUATION OF AUTOPHAGY USING EXPRESSION OF LC3 PROTEIN IN NEONATAL GENE THERAPY OF MPS VII MICE BY LENTIVIRAL VECTOR

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Mucopolysaccharidosis type VII (MPS VII, Sly syndrome) is one of lysosomal storage diseases (LSDs) caused by deficiency of beta-glucuronidase (GUSB), resulting in progressive accumulation of glycosaminoglycans (GAGs) in various tissues including the brain. In this study, we evaluated the potential to perform gene therapy for MPS VII.
Methods: We constructed a lentiviral vector, SMPU-R-MND-HBG -IE, to transduce cells with the normal human HBG cDNA. We injected the neonatal mice of MPS VII, and sacrificed 7 weeks and 31 weeks after treatment and analyzed GUSB activity by 4-MU method, Lentiviral DNA bio-distribution by real-time PCR and evaluation of autophagy performed by Western- Blotting and Immune stain of LC3 protein in brain and liver.
Results: GUSB enzyme activities of the treated mice were elevated in all organs and decrease GAGs in all organs and a significant deceased LC3 protein in the treated mice and indicated the organs failure in MPS VII due to autophagy. We detected lentiviral DNA expression in all organs of treated mice 7 weeks and 31 weeks.
Conclusion: We have demonstrated that the neonatal gene therapy for MPS VII mice using lentiviral vector resulted in long-term and efficient GUSB expression, which corrects the biochemical defects, and decreased LC3 protein.

P-461

EXPERIMETAL GENE THERAPY TO CORRECT PTPS/BH4-COFACTOR AND BRAIN NEUROTRANSMITTER DEFICIENCY IN PTS-KI/KO MICE BY TARGETING THE LIVER

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Tetrahydrobiopterin (BH4) is an essential cofactor for phenylalanine hydroxylase (PAH), tyrosine hydroxylase (TH), and tryptophan hydroxylase (TPH). BH4 is synthesized de novo from guanosine triphosphate (GTP) by three enzymes: GTP cyclohydrolase I (GTPCH), 6-pyruvoyltetrahydropterin synthase (PTPS) and sepiapterin reductase (SR). Conventional treatment of patients with BH4 cofactor deficiency due to autosomal recessive mutations in the PTS gene, encoding PTPS, requires control of elevated blood-phenylalanine (Phe) levels by BH4 supplementation and oral replacement therapy with L-dopa and 5-OH-tryptophan, precursors of dopamine (catecholamines) and serotonin, respectively. We have recently developed a viable mouse model for PTPS deficiency, the compound heterozygous Pts-ki/ko mouse, which exhibit low PTPS activity, hyperphenylalaninemia and low brain dopamine and serotonin levels. Currently we are testing a triple-gene transfer to target liver using AAV2 pseudotype 8 vectors (rAAV2/8) in combination with luciferase co-expressed for in vivo hepatic imaging. Coordinate expression of PTPS, TH and TPH is thought to lead to hepatic synthesis of BH4, L-dopa plus 5-OH-tryptophan, respectively, and eventually to restoration of systemic phenylalanine clearance and endogenous supply of the essential precursors for monoamine neurotransmitter biosynthesis in the CNS.

P-462

HELPER-DEPENDENT ADENOVIRAL VECTORS FOR LIVER-DIRECTED GENE THERAPY OF PRIMARY HYPEROXALURIA TYPE 1

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Primary hyperoxaluria type 1 (PH1) is an inborn error of liver metabolism due to deficiency of the peroxisomal enzyme alanine:glyoxylate-aminotransferase (AGT), which catalyzes the conversion of glyoxylate to glycine. Organ transplantation as either preemptive liver transplantation or combined liver/kidney transplantation is the only therapeutic strategy available to prevent kidney failure. Gene therapy is an attractive option to provide a definitive cure for PH1. Towards this goal, we are investigating helper-dependent adenoviral (HDAd) vectors for liver-directed gene therapy of PH1. We have injected PH1 mice with an HDAd encoding the AGT under the control of a liver-specific promoter and observed a sustained reduction of oxalate urinary excretion and reduction in kidney stone formation. Recently, we have developed a minimally invasive method to improve the therapeutic index of HDAd (Brunetti-Pierri et al., 2009). This approach based on balloon occlusion catheter to achieve preferential delivery of the vector to the liver, results in higher efficiency of hepatocyte transduction using clinically relevant low vector doses. Therefore, this method may permit correction of PH1 using clinically relevant doses of HDAd and may thus pave the way to clinical application of HDAd for PH1 gene therapy.

P-463

APPLICATIONS AND DELIVERY OPTIONS FOR ANTISENSE THERAPY IN CELLULAR MODELS OF DISEASE

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The use of antisense genetic therapy for RNA mis-splicing diseases has gained increased attention as the splicing changes account for up to 15% of all mutations and with massive parallel genomic sequencing of individual patients the number of splicing mutations will be increased. Although the number of patients who can be potentially treated is low, it represents an excellent therapeutical option representing a type of personalized molecular medicine which is especially relevant for diseases for which there is to date no efficient treatment. In this work we summarize the splicing modulations explored to date especially targeted to deep intronic changes and the potential use to reprogram the splicing process using antisense therapy against intronic and exonic new or cryptic splice sites. In addition, we present our recent data in the investigation of new transporter structures that are thought to provide effective in vivo delivery. We are working on an octa-guanidine dendrimer covalently linked to specific morpholinos and also a new approach using locked nucleic acids monomers (LNA) bound to carbosilane dendrimers. We have successfully recovered the splicing process in MUT, PTPS, PCCA and PCCB disease cellular models suggesting that we are closer to applying the antisense therapy in animal models.

P-464

PTC124 INCUBATION TOWARDS RIBOSOMAL READTHROUGH OF NONSENSE MEDIATED TERMINATION CODONS IN THE SLC6A8 AND GAMT GENES

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A recently discovered chemical entity, PTC124 (Ataluren) was shown to selectively induce ribosomal readthrough of premature but not normal termination codons. In vitro experiments and subsequent treatment of mdx mice showed a promising improvement in the form of functional recovery. This led us to investigate the effect of PTC124 on nonsense mediated premature termination in two of the three cerebral creatine deficiency syndrome genes, namely the creatine transporter gene (SLC6A8) and the guanidinoacetate methyl-transferase gene (GAMT). We obtained PTC124 from Selleck Chemicals (Texas, USA) and incubated patient's cell-lines containing nonsense mutations with 17 μM of PTC124 for 46 days (SLC6A8, n=2) and 14 days (GAMT, n=3) and collected cells every 7 days. We measured intra-cellular creatine content and formation of 2H3-13 C2-creatine using a two-step derivatization procedure, followed by quantification with GC-MS. With all cell-lines we found no increase of function, indicated by low intra-cellular creatine content (SLC6A8) and no formed 2H3-13 C2-creatine (GAMT) as compared to the wild-type cell-lines. This led us to conclude that PTC124 (Selleck Chemicals) cannot induce ribosomal readthrough of the five tested nonsense mediated termination codons in the SLC6A8 and GAMT gene. However, it should be taken into account that we were unable to validate the obtained compound.

O-059

AMLODIPINE PREVENTS APOPTOTIC CELL DEATH BY CORRECTION OF ELEVATED INTRACELLULAR CALCIUM IN A PRIMARY NEURONAL MODEL OF BATTEN DISEASE

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Background: Batten disease (Juvenile Neuronal Ceroid-Lipofuscinosis, JNCL), is a recessively inherited untreatable neurodegenerative disorder caused by mutations in the CLN3 gene. The mechanism by which the abnormal or missing protein leads to neuronal cell death remains unclear. It has been shown that a downstream effect is abnormal intracellular calcium accumulation which may lead to apoptosis. Previously, we demonstrated reversal of the calcium effect in a neuroblastoma cell line using amlodipine and other calcium channel antagonists.

Objectives: The aim of this study is to investigate the neuroprotective role of amlodipine in a primary neuronal model of CLN3. We studied calcium changes and indicators of apoptosis in amlodipine-treated primary rat neurons with siRNA CLN3 knock down.

Results: We demonstrated that intracellular calcium is elevated in CLN3 siRNA-silenced primary neurons. We also demonstrate that amlodipine; an L-type calcium channel antagonist can reverse the aberrant calcium elevations at pharmacological levels. Furthermore, we demonstrated that amlodipine can protect from etoposide-induced apoptosis in the siRNA-silenced neurons.

Conclusion: This study indicates that amlodipine, in addition to its well-established role as a calcium channel antagonist, can also function as a neuroprotective drug. It suggests that currently available calcium channel antagonists may be potential therapeutic agents for Batten disease.

O-060

TRYING TO ELUCIDATE THE MECHANISM OF KIDNEY DAMAGE IN FABRY DISEASE THE USE OF GLYCOLIPID ARRAYS TO STUDY PROTEIN:LIPID INTERACTIONS IN THE KIDNEY

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Background: Fabry disease (FD) is a lysosomal storage disease resulting from a deficiency of the enzyme alpha-galactosidase A. Defects in this enzyme lead to accumulation of ceramide trihexoside (CTH) in the endothelium and with patients becoming susceptible to early onset stroke, cardiovascular and kidney disease.

Objectives: The aetiology of the kidney damage associated with FD and CTH accumulation is poorly understood. We describe the development of novel glycolipid arrays by immobilising glycolipids to a chip surface and using them to study proteins which interact with CTH in the kidney.

Methods: CTH and the ganglioside GM1 (positive control) were immobilised on RS100 arrays and incubated with adolescent rat kidney homogenates. Those proteins interacting with each glycolipid were then identified using QTOF mass spectrometry.

Results: A number of proteins were identified that bound specifically to CTH including hamartin, chloride channel protein 1 and tubulin alpha 3 chain; which have been implicated previously in kidney disease. Interestingly, several other proteins were found to bind specifically to GM1.

Conclusions: Glycosphingolipid arrays show much potential for studying protein: lipid interactions. Using this technology we have identified several proteins that could play a role in the complex pathogenesis of kidney disease in FD.

P-465

NEW MULTIPLATE AGGREGATION TEST TO SCREEN FOR GSALPHA DYSFUNCTION: SCREENING OF PATIENTS WITH UNEXPLAINED MENTAL RETARDATION FOR GSALPHA HYPERFUNCTION

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Jaeken (2003) described children with psychomotor retardation (PMR) and bleeding problems linked to Gsalpha hyperfunction. Signal transduction defects (STD) due to GNAS1 mutations can cause Gsalpha hyperfunction. Screening for Gsalpha-associated STD requires a platelet aggregation-inhibition test (PAIT) and cAMP measurement. We developed a multiplate PAIT to screen for Gsalpha defects in patients with unexplained PMR.

Material and methods: Controls were healthy volunteers (33), 20–50 years, not taking alcohol, aspirin/NSAIDs. Patients—Two Albright hereditary osteodystrophy patients (AHO) with 50% reduced Gs bioactivity. Five patients with unexplained PMR.

PAIT was performed with 3 ml of hirudin-anticoagulated blood in a multiplate aggregation(collagen)-inhibition(PGE1) assay. cAMP measurement in PGE1-stimulated platelets was performed with a cAMP enzyme immunoassay.

Results: In healthy volunteers, PGE1 gave a dose-dependent inhibition of collagen aggregation of 33±14% (30nM PGE1) and 48±17% (50nM PGE1). AHO patients showed reduced PGE1 inhibition of 17 resp. 8% (30nM PGE1) and 9 resp. 0% inhibition (50nM PGE1). Maximal cAMP in PGE1-stimulated thrombocytes was lower in AHO patient (20 pmol/ml) than controls (65 pmol/ml). 3/5 patients with PMR tested normal in PAIT; 2 showed an increased response.

Conclusion: We developed a multiplate aggregation-inhibition assay to screen patients with PMR and/or bleeding for Gsalpha dysfunction.

P-466

A NOVEL MUTATION OF THE CLAUDIN 16 GENE IN FAMILIAL HYPOMAGNESEMIA WITH HYPERCALCIURIA AND NEPHROCALCINOSIS MIMICKING RICKETS

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Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) is caused by a mutation in the gene CLDN16, which encodes paracellin 1 (claudin-16), a tight junction protein mediating paracellular transport which is expressed in the thick ascending loop of Henle and in the distal convoluted tubule, where reabsorption of magnesium occurs. Familial hypomagnesemia with hypercalciuria and nephrocalcinosis is a rare disorder first described in 1972 which can result in renal failure usually in the second decade of life. Symptoms usually begin in the first few months of life in affected individuals. The frequent symptoms include recurrent urinary infections, polydipsia, polyuria, hypomagnesemia, hypercalciuria, hyperuricemia, nephrolithiasis, failure to thrive, rickets, convulsions, muscle weakness, elevated paratyroid hormone levels. We present a 4 years old Turkish female child with a chief complaint of hypocalcemic tetany. A diagnosis of FHHNC was confirmed by genetic testing for a mutation in claudin 16 gene. Claudin 16 gene revealed homozygosity for the p.K183E(AAA>GAA) c. 547A>G indicating the diagnosis of hypomagnesemia with hypercalciuria and nephrocalcinosis. To our knowledge, this is the first case of FHHNC reported in Turkish population diagnosed at molecular level.

P-467

A NOVEL HOMOZYGOUS MISSENSE MUTATION IN THE POTASSIUM CHANNEL RELATED GENE KCTD7 IN PROGRESSIVE MYOCLONIC EPILEPSY

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Progressive myoclonic epilepsy (PME) is characterized by myoclonic seizures and progressive neurological dysfunction. The recognized causes are rare and account only for a small number of PMEs.

We report a 5 year-old boy with therapy resistant PME and severe developmental delay, first child of healthy consanguineous Turkish parents. Epilepsy started with 10 months after normal development. He shows muscular hypotonia and dystonia and dyskinesia. He cannot sit or speak; visual interaction is preserved. Extensive repeated metabolic workup was normal. Conventional cytogenetic analysis and high-resolution molecular karyotyping were normal. SNP array analysis showed an increased number of autozygosity regions. Genome-wide parametric linkage analysis under a recessive model with complete penetrance identified several candidate regions with LOD scores >1,9. Data mining on the basis of gene function and known PME causing genes indicated the potassium channel tetramerization domain containing 7 gene (KCTD7) in the 7q11.21 linkage region as a suitable candidate (Van Bogaert et al., 2007). Sequencing revealed a novel homozygous missense mutation in a highly conserved segment of exon 2 (p.R94W), not present in 100 control samples. This is the second family with PME caused by KCTD7 mutations. We speculate that KCTD7 mutations may be a recurrent cause of PME.

P-468

METABOLARRAY.: TARGETED ARRAY-CGH FOR THE DETECTION OF DELETIONS/DUPLICATIONS IN 205 GENES INVOLVED IN INHERITED METABOLIC DISEASE

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Clinical molecular testing in IMD is currently PCR-based precluding the identification of deletions which account for a variable fraction (1–25%) of mutant alleles depending on the gene involved. We have developed a high-resolution comparative genomic hybridization array (Metabolarray®) for the detection of exonic copy number changes in 205 genes involved in IMD which are currently diagnosed in the laboratory. The array consists of 62,979 oligos spread genome wide, with 40,555 hybridizing to target genes with an average spacing of about 250 bp and 26,678 covering the rest of the genome. For validation, we have retrospectively analyzed a series of IMD patients carriers of exonic deletions previously genotyped by different methods (MLPA, SNP-arrays). All the heterozygous and homozygous deletions even of a single exon were detected using the Metabolarray®. In a series of prospectively evaluated patients, we have identified a novel 2 Kb deletion in the PCCB gene encompassing exons 4 and 5. Our results show that this new molecular tool which can be easily modified to analyze additional genes may be highly efficient in screening for exon copy number changes in IMD patients with incomplete genotype.

P-469

OXIDATIVE STRESS-INDUCED DEGRADATION OF MITOCHONDRIAL LEUCYL-TRNAS IS INDEPENDENT OF PTCD1

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Background: Degradation of cytoplasmic tRNAs and of mitochondrial mRNAs are part of a cellular response to environmental stress, such as oxidative stress and may regulate protein synthesis or lead to apoptosis. PTCD1 is a novel human protein that was recently shown to decrease the levels of mitochondrial Leucyl-tRNAs.
Objectives: To investigate, whether targeted degradation of mitochondrial tRNAs is part of the cellular response to oxidative stress, and whether PTCD1 might be involved in this process.
Methods: We compared the effect of oxidative stress (induced by 500 μM tert-butyl hydroperoxide for 1 h followed by 0–3.5 h recovery) on PTCD1 knockdown , on PTCD1 over-expressing and on the respective control HepG2 cells. RNA levels were quantified by quantitative real-time PCR.
Results and Discussion: Oxidative stress treatment rapidly decreased the steady state levels of the mitochondrial Leucyl-tRNAs (tRNALeuUUR, tRNALeuCUN), but increased the mRNA steady state levels of the mitochondrial superoxide dismutase (SOD2) and of the transcription factor ATF4 in mock-transfected control cells. PTCD1 knockdown could not prevent the decrease of tRNALeuUUR and tRNALeuCUN in response to oxidative stress. These results suggest, that PTCD1 is not involved in a targeted degradation of mitochondrial Leucyl-tRNAs in response to oxidative stress.

P-470

MICRORNAS PROFILING IN HUMAN ANENCEPHALY

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Objective: To investigate the globle expression profiling of microRNAs in brain tissues of fetuses with anencephaly and normal control by using microRNA microarray, and predict the target genes of some specific miRNAs with bioinformatic method.
Methods: The profiling of miRNAs from brain tissues of 3 fetuses with anencephaly and normal brains was detected using an Affymetrix GeneChip® miRNA microarray. Then some of the significantly misexpressed miRNAs were selected to validate the microarray assay results by real-time RT-PCR analysis. Furthermore, the target genes of these miRNAs were predicted with some online databases.
Results: 1. Compared to the normal group, anencephaly group has a specific miRNA expression profile. There were 73 up-regulated miRNAs and 14 down-regulated miRNAs. 2. The microarray findings were extended using Real-time RT-PCR for 10 miRNAs. Of these miRNAs validated, 8 miRNAs were up-regulated, whereas one was down-regulated. In addition, mir-125a was found to be higher in anencephaly group than in the normal group, which contradicted the result detected by the microarray.
Conclusion: This study shows that brain tissues of anencephaly group have a significantly misexpressed miRNA expression profile compared to that of normal group. And these misexpressed miRNAs may involve in the pathogenesis of NTDs.
Conflict of Interest declared.

P-471

UNVEILING THE REGULATORY MECHANISMS OF PDHA2 GENE

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PDHA2 gene encodes the testis-specific isoform of E1α, the target subunit for most pyruvate dehydrogenase complex deficiencies, and it was postulated that its activation in somatic tissues would represent a conceptual therapy. Accordingly, we aimed to unveil the mechanisms regulating PDHA2 tissue-specific expression.
Basal transcriptional activity of different PDHA2 promoter constructs was assayed in three different somatic cell lines, and in vitro methylation experiments were also performed. A cell line (SH-SY5Y) was treated with 5'-aza-2'-deoxycytidine(DAC). PDHA2 methylation status was evaluate by MS-PCR, and mRNA levels analyzed by qRT-PCR; ChIP assays were performed and the recovered DNA analyzed by qRT-PCR.
We found that PDHA2 promoter-directed transcription occurred in cultured somatic cells, with no obvious differences between cell lines, and that in vitro methylation clearly reduced transcriptional activity of the constructs. We observed that DAC treatment induced a significant demethylation of an exonic CpG island, while the core promoter remained fully methylated. Moreover, this demethylation was concomitant with PDHA2 gene derepression. Finally, ChIP assays confirmed the involvement of DNA methylation in PDHA2 gene expression.
In conclusion, these results show that PDHA2 tissue-specific expression is strongly controlled by epigenetic modification and demonstrate the possibility of activating its expression in somatic cells.
FCT (SFRH/BD/31264/2006;POCI/SAU-MMO/57052/2004)

O-061

DISSECTING MOLECULAR BASIS OF METHYLMALONIC ACIDEMIA (MMA) BY PROTEOMIC ANALYSIS

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Hereditary Methylmalonic Acidemias, MMAs, are severe autosomal recessive inborn errors of metabolism caused by the deficiency of methylmalonyl—CoA mutase (MUT). MUT converts L—methylmalonyl—CoA into succinyl—CoA. A block at this enzymatic step results in elevated plasma levels of methylmalonic acid as well the accumulation of other propionyl—CoA—derived metabolites. The disorders are caused by mutations in the MUT apoenzyme or defective metabolism of the enzymatic cofactor, 5'-deoxyadenosylcobalamin. Two enzymatic phenotypes of apoenzyme deficiency are recognized: mut0 patients have no detectable residual enzyme activity while mut—patients have reduced enzyme activity that may be cobalamin—responsive. Mut0 patients can experience life-threatening metabolic instability.

Liver specimens from six healthy donors and six mut0 patients were employed to identify deregulated proteins by using DIGE (Differential Gel Electrophoresis. We identified 150 deregulated spots, 80 down-regulated and 70 up-regulated. The spots were excised and identified by mass spectrometry. Proteomic results revealed decreased levels of proteins involved in energetic metabolism and cellular detoxification. A metabolic profiling is underway. The protein data set will be analyzed in the frame of metabolic networks to identify the most differentially altered cellular pathways. Defining altered protein profiles could lead to the identification of new therapeutic targets for MMA.

O-062

INTERACTION OF WILDTYPE AND MUTANT GLUTARYL-COA DEHYDROGENASE WITH ELECTRON TRANSFER FLAVOPROTEIN SUBUNITS ALPHA AND BETA

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The enzyme glutaryl-Coenzyme A-Dehydrogenase (GCDH) is a homotetrameric mitochondrial matrix protein, for which so far more than 150 diseases-causing mutations have been described, which lead to amino acid substitutions in different regions of the protein.

Crosslink experiments show that distinct GCDH mutations with high residual enzymatic activity and amino acid substitutions localized on the surface of the protein affect the homotetramerization as well as the heteromeric complex formation with so far unidentified proteins. The electron-transfer flavoprotein (ETF) composed of the two subunits alpha-ETF and beta-ETF appeared as a promising candidate as interaction partners of GCDH. Pull down experiments with alpha- or beta-ETF immobilized to affinity columns and subsequent incubations with GCDH-overexpressing cell extracts revealed the first experimental evidence for a direct interaction of wildtype GCDH with alpha- as well as beta-ETF. The binding of alpha- or beta-ETF to distinct mutant GCDH proteins was reduced. The affinity of interactions between ETF and wildtype or mutant GCDH was characterized by surface plasmon resonance spectroscopy. Characterization of the interaction between alpha- and beta-ETF and different mutant GCDH proteins provided data on the site of the GCDH protein which is responsible for binding to ETF subunits.

O-063

NATURAL ARGININE MUTATIONS IN IEM: RESTORING IN VITRO FUNCTION WITH AMINOACID SUPPLEMENTATION

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Herein we describe the use of aminoacid supplementation in restoring the in vitro folding and activity of natural arginine mutant proteins related to inborn errors of metabolism (IEM).

Missense arginine mutations are the most frequent mutations in IEM-linked conformational disorders. Our group has observed that aminoacid supplementation reversed the clinical phenotypes of pyruvate dehydrogenase deficiency patients carrying mutations in arginine residues.

Following this, we selected a range of arginine mutations in different proteins related to IEM, namely: E1 alpha subunit of pyruvate dehydrogenase complex (PDH-E1α), cystathionine-beta-synthase (CBS) and galactose-1-P uridyltransferase (GALT). The respective cDNA sequences were cloned into pET-based vectors, and the selected mutants generated by site-directed mutagenesis. By expressing the target proteins in E. coli BL21 (DE3) Rosetta cells, we evaluated the effect of aminoacid supplementation in the protein levels, folding and activity of WT and mutant proteins. The results on the in vitro restoration of protein function upon supplementation are currently being probed in a small set of patient fibroblasts. Altogether these results should provide clues as whether to employ aminoacid supplementation as a general therapeutic for IEM-conformational disorder patients carrying arginine missense mutations.

O-064

CONGENITAL MYOPATHY EHLERS-DANLOS OVERLAP SYNDROME CAUSED BY THE DEFICIENCY OF AN ENZYME INVOLVED IN PROTEIN FOLDING IN THE ENDOPLASMIC RETICULUM: IDENTIFICATION AND CHARACTERIZATION OF A NOVEL DISORDER

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The extracellular matrix (ECM) is a complex meshwork of non-cellular material that surrounds cells in all tissues and organs. Correct assembly of ECM proteins is known to involve posttranslational processing in the endoplasmic reticulum (ER), but the exact mechanisms are only partially known. Recently it has been shown that autosomal-recessive forms of osteogenesis imperfecta may be due to deficiencies of ER chaperones. Here we present evidence that the principle of disturbed ER processing represents a more general concept in ECM disorders. We report on six patients with a novel congenital myopathy Ehlers-Danlos overlap syndrome caused by autosomal-recessive mutations in an ER enzyme. Key clinical features include severe muscular hypotonia at birth, delayed motor development, muscular weakness, severe progressive scoliosis, joint hypermobility, sensorineural deafness, and normal intelligence. The candidate gene was identified by linkage analysis; truncating mutations were detected in all patients. The protein localises to the ER and is predicted to function as a protein folding catalyst. Western blot analysis and immunocytochemistry of cultivated fibroblasts of patients showed reduced amounts and/or disturbed organization of fibronectin and other ECM proteins. Deficient protein processing in the ER may not only cause autosomal recessive osteogenesis imperfecta but also other ECM disorders.

P-472

TWO NOVEL NORWEGIAN MUTATIONS OF HYDROXYMETHYLBILANE SYNTHASE CHARACTERIZATION OF THE MOLECULAR BASIS OF ACUTE INTERMITTENT PORPHYRIA

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Mutations in hydroxymethylbilane synthase (HMBS), the third enzyme of the haem synthesis, may induce acute intermittent porphyria (AIP). The molecular basis of the disease remains unclear and valuable information about phenotype-genotype correlations can be obtained by functional studies on the wild-type (wt) and mutants. Five mutations selected for this study (R116W, K132N, R167W, R173W and V215E) represent: i) mutations around the active site and/or interfering with binding of the dipyrromethane-cofactor, expected to have catalytic, misfolding and/or destabilization effects and ii) mutations far from the active site predicted to affect overall folding and flexibility. Characterization of K132N and V215E has not been reported previously. We have characterized the steady-state kinetic parameters of the mutants comparative to the wt, and investigated the conformational stability by fluorescence and circular dichroism. K132N and V215E possess 87% and 54% activity, respectively, compared to wt. R116W and R167W show activity <20%, while R173W shows no activity, as expected from previous reports. Wt-HMBS shows a high thermostability (Tm=79°C). K132N, R167W and V215E reveal a wt-like conformation and stability, whereas R116W and R173W show decreased thermostability (Tm=54°C). These results together with on-going investigations will provide insights in the pathogenic mechanisms in AIP and help selecting potential therapeutic strategies.

P-473

ANALYSIS OF THE GIANT AXONAL NEUROPATHY FIBROBLASTS PROTEOME

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Background: Giant axonal neuropathy (GAN) is a hereditary disease characterized by severe loss of mental and motor functions. This disease is morphologically characterized by aggregation of intermediate filaments, a part of the cytoskeleton. Underlying mutations are found in the GAN gene encoding the protein gigaxonin which is involved in clearance of misfolded or damaged proteins by means of the ubiquitin-proteasome system, but the underlying mechanism still remains elusive.
Methods: We compared the proteome of four control fibroblasts with that of four GAN patients by use of the quantitative iTRAQ 8-plex labeling method and mass spectrometry to test the hypothesis of accumulation of gigaxonin binding microtubule associated proteins (MAP's) in cultured skin fibroblasts and provide additional proteomics based insight into the cellular impact of gigaxonin mutations.
Results: Among the differentially expressed proteins in GAN fibroblasts no upregulation of known gigaxonin binding MAP proteins could be demonstrated and no up or downregulation of cytoskeletal proteins in general was detected. Differentially expressed proteins were mainly associated with transcription/translation and splicing and extracellular/membrane signaling pathways.
Conclusions: Differentially expressed proteins indicated that the cytoskeleton organization was disturbed via several ways. Galectin-1 might have a central role is reorganization of the cytoskeleton.

P-474

INTERACTIVE 3D VISUALISATIONS OF PROTEIN STRUCTURES TO AID THE STUDY OF INBORN ERRORS OF METABOLISM

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The Structural Genomics Consortium (SGC) spearheads the concept of open-source medical research by delivering structural biology knowledge on human proteins into the public domain. This wealth of 3D structures includes human enzymes implicated in inborn errors of metabolism. Understanding the effects of disease-associated mutations at the protein level is key to establish a causal relationship among sequence, 3D structure and patient phenotypes. To disseminate this structure-mutation data in an intuitive and approachable manner to the metabolic disease community, we are working with the Society for the Study of Inborn Errors of Metabolism (SSIEM) and Journal of Inherited Metabolic Disease (JIMD) to publish a collection of structure-mutation reports. Each article provides a jargon-free description of a protein structure and a table summary of associated missense mutations. Importantly the article is available online in an interactive visualisation format, where the reader can access each mutation to display a pre-programmed 3D scene, explore at will the molecular landscape in atomic detail and interpret the structural environment around the residue of interest. We hope that the interactive articles, the first of which is published in the 2011 June issue on fumarate hydratase deficiency, will close the knowledge gap between research communities and promote collaborations.

O-066

THE FACTORS THAT INFLUENCE DIETARY COMPLIANCE IN THE MANAGEMENT OF METABOLIC DISEASES

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Background: We performed a study to test the hypothesis that psycho-education of patients and parents has an influence on compliance for patients with a metabolic disease.
Methods: Thirty-five patients with different metabolic diseases were enrolled in the study. During an interview the following information was recorded: background of the patient, perception and knowledge of the disease and diet. Knowledge about disease, diet and consequences of non-compliance were scored on a Likert scale (1 to 5). Compliance was obtained by summing the scores, 0 (insufficient), 1 (acceptable) or 2 (preferable) of four items: 'number of contacts with the dietician (CD)', 'frequency of control of biochemical parameters (FC)', 'results of controls (RC)' and 'quantity of ordering diet products (QP)'.
Results: General knowledge about the diet and the disease was very good. In contrast, only a small majority showed good compliance, especially only moderate results for 'RC', 'QP' was achieved. No significant correlations were found between the degree of knowledge, the four individual compliance-scores, the total compliance-score and follow-up analyses of metabolites. An individualized approach by co-consultations with dietitian/psychologist is organized for "problem-cases".
Conclusion: Good knowledge of a disease and the treatment does not necessarily imply good compliance especially in adolescents and adults.

O-065

NUTRITIONAL ASSESSMENT OF PATIENTS WITH METABOLIC DISORDERS

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Background: Patients with metabolic disorders may have inadequate, excessive or unbalanced micronutrient intake. A protocol assessing biochemical markers for 15 micronutrients was implemented in 2009.
Methods: A chart review of 67 patients, fully or partially tested per protocol, compared blood results to normal ranges. Forty-six (69%) were on protein-restricted diets, of which 35 (52% total) were supplemented with amino acid medical food. Ten (15%) were on unrestricted diets and 11 (16%) on fat modified diets.
Results: The most common deficiency across diet groups was docosahexaenoic acid (DHA) (76% of 58 tested). Two patients on protein-restricted diets had low plasma vitamin B12. Low plasma levels of magnesium, zinc and vitamins D and E were more common when a medical food was not prescribed. Plasma folate was elevated in 93% patients on medical foods; elevated levels of vitamin A, E and riboflavin were also reported. Low levels of plasma manganese were found across all diet groups. Vitamin D status was of concern in unrestricted diets.
Discussion: Both elevated and low levels of micronutrients and long chain fatty acids were found in metabolic patients, despite regular assessment of nutrient intake and advice on suitable supplements. Regular monitoring under a revised protocol is recommended.

O-067

DIETARY CONSIDERATIONS IN CHILDREN WITH METHYLMALONIC ACIDAEMIA (MMA) AND CHRONIC KIDNEY DISEASE (CKD)

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Background: low protein diet, avoidance of fasting and emergency regimen is the primary treatment of MMA. CKD as a complication necessitates dietary adaptation.
Objectives: review of dietary manipulations in children with decreasing glomerular filtration rates (GFR), routinely measured from age 2y.
Case series: MMAVitamin B12 non-responsive n=14; age at presentation, neonatal (n=9) to 1.5y, current ages: 1mth to 16y (median 7.8y). MMA B12 responsive n=12; age at presentation, neonatal (n=2) to 4y, current ages: 2mths to 16y (median 6.3y).
Results: Cross section of GFR from last 2y. Non B12 responsive: mild CKD, GFR 60–89 ml/min/1.73 m squared n=3; moderate or severe CKD, GFR <59 n=9, aged 2 to 12y. Four children with moderate CKD, aged 2 to 5y. Vomiting, reflux, repeated illness and poor fluid intake cause episodic dehydration. Fluid intake is increased above normal as CKD progresses/ during illness. If renal bone disease, phosphate restricted further and vitamin D analogues/phosphate binders given. Normal calcium intake unless hypercalcaemic. Potassium restriction rarely necessary. B12 responsive: mild CKD n=7, others normal. No further dietary manipulations.
Conclusion: Early monitoring of kidney function is indicated. Episodes of dehydration contribute to deterioration in kidney function, regular dietary assessment of fluid, electrolytes and energy intake are therefore essential.

O-068

APPRAISING DIETARY KNOWLEDGE OF CAREGIVERS IN CHILDREN WITH PKU

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Introduction: In PKU, it is established that optimal blood phenylalanine control is dependent on adequate dietary knowledge of caregivers/patients. In practice, dietitians spend substantial time on caregiver education, in order to improve their diet understanding and treatment adherence.
Aim: an audit to assess caregiver's practical knowledge and understanding of a low phenylalanine diet.
Methods/Subjects: 26 caregivers of 32 children (median age 7.4y: range 10 months—16y) were visited at home. They completed a multiple choice questionnaires and a series of practical tests to review their understanding of dietary management of PKU.
Results: 40% were unable to calculate all 1 g protein (50 mg phenylalanine) exchanges from food labels; 30% failed to identify the amount of natural protein in one phenylalanine exchange; 50% were unable to correctly estimate by 'eye' the number of phenylalanine exchanges in common foods; 40% and 80% respectively, were unable to correctly define a low phenylalanine 'free' food or the protein content of 'free' vegetable sauces. 80% reported they weighed phenylalanine exchanges >50% of time.
Conclusion: This audit identified a number of gaps in caregiver knowledge and awareness about diet and PKU. It is essential that caregiver understanding is regularly tested and reviewed with education updates given.

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OXIDATIVE STRESS IN CHILEAN PATIENTS WITH PKU AND HPHE

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Objective: Oxidative stress (OS) has been associated with chronic diseases. The concomitance of phenylketonuria (PKU) and hiperphenylalaninemia (Hphe) could increase the OS risk and its consequences. The aim waw to describe and compare oxidant and antioxidant biomarkers in individuals with PKU in adequate metabolic control, Hphe in follow-up and healthy controls.
Design: Forty-five individuals (15 PKU, 15 Hphe and 15 controls) between 6 and 12-years-old participated; family smoking habits; anthropometric measurements; selenium (Se) and tocopherol intake along with thiobarbituric acid reactive species (TBARS), total antioxidant capacity (TAC), glutathione peroxidase (GSH-Px) activity and plasmatic tocopherol were assessed.
Results: Tocopherol intake was significantly higher in PKU individuals ($p<0.0001$); however, plasmatic measurement did not differ between groups. Se intake had a tendency to be higher in PKU ($p=0.0573$) nonetheless the GSH-Px activity was significantly lower in comparison to Hphe and controls ($p=0.0056$) as well as TAC ($p<0.0001$). Nevertheless TBARS did not show any difference ($p=0.5803$).
Conclusions: Even though TBARS is not affected, the antioxidant capacity is lower in PKU patients. The elevated Se intake in PKU did not correlate with low GSH-Px activity: thus, Se nutritional status must be studied. Hphe individuals should be monitored closely throughout life hence their nutritional risk.

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MORE PHE, MORE CHOICES: THINK HEALTHY

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The availability of medications and new metabolic formulas for the management of PKU in some cases has permitted the liberalization of the PKU diet. During this transition we recognized a need for nutrition focused educational material.
We analyzed 3 day diet records to assess macronutrients and evaluated BMI's at baseline and post diet modifications.The diet analysis of >40% of our patient population that fell within this group revealed poor macronutrient profiles.
In response to these analyses, we developed a series of educational modules to assist patients in making healthy, nutrient dense choices while maintaining their restricted and liberalized diets. Initially, a Foundations module was created to provide a base of information on healthy eating and became the template for the following modules: Early Childhood, Teenager, Adult, Pregnancy and Keeping it Simple (Low Literacy). These modules emphasized the importance of incorporating colorful fruits/vegetables, healthy fats and fiber in the diet. Recipes, menus, and snack suggestions provided guidance for patients appropriate for any level of dietary restriction.
3 day diet records post introduction of these modules revealed an improvement in nutrient profiles and a decrease in percent of weight gain.

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AN INTERNATIONAL SURVEY ON EDUCATIONAL TOOLS USED IN PKU MANAGEMENT

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An international survey was developed to examine educational tools available for patients with Phenylketonuria (PKU), ages 3–21. This survey represented participants from the United States, Canada, Europe, Central America, South America and Australia. Dietitians and physicians were asked to define the term dietary compliance for their individual practices. Phenylalanine levels "within treatment range" were chosen by 92.1% as a definition of compliance. A questionnaire was distributed through the Metabolic Dietitians list serve (PNO-Metab-L@listserv.cc.EMORY.EDU). Ninety anonymous responses were analyzed for data. A majority of those responses (80.9%) offer PKU nutrition education for patients and families. It was determined that one-on-one counseling (97.8%) is the most commonly used method for education with printed material at 84.4%. Nutrition education is provided every six months by over half (52.3%) of the respondents with 54% starting at ages 3–5. Diet management appears to decline during adolescents, when one-on-one counseling is the primary tool. In addition, parents as role models fall from the 70th to the 17th percentile. Group clinics are the least utilized educational tool. Despite education, factors that play a role in dietary compliance are embarrassment and frustration (90%) and poor family cohesion (83.3%).

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WEANING IN PKU TIME TO REVIEW THE EVIDENCE

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Weaning is a crucial stage of development and ideally, in PKU it should reflect the model for healthy infants. If weaning is sub-optimal, it potentially may indirectly affect short and even long-term quality of phenylalanine control. In PKU, weaning is associated with many additional components: administration of adequate protein substitute (PS), restricting phenylalanine intake and use of low protein foods.

No observational studies have examined weaning in PKU and there are no international weaning guidelines. In fact, national weaning practices are rarely reported. Any research should consider maternal and infant behaviour and social factors. Inconsistent and over-controlling caregiver behaviour may lead to unpleasant mealtimes with poor feeding progress. Introducing solids before 6-months may be advantageous; there is less persistent neophobic food response, possibly leading to better food acceptance. Between 6–12 months, a second concentrated source of phenylalanine-free PS is normally required; it is commonly given as a liquid, but the prescribed volume may affect appetite. Its administration as a paste/gel format may better suit feeding development but it has not been evaluated.

Overall, there is a need to evaluate, define and reappraise the weaning process in PKU so that this important developmental stage reflects that recommended for non-PKU infants.

Conflict of Interest declared.

P-479

OVERWEIGHT AND OBESITY IN PHENYLKETONURIC PATIENTS FROM THE NORTH OF PORTUGAL

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Background: When protein substitutes largely replace natural protein, the complete profile of nutritional intake changes. Therefore, the question is whether overweight and obesity are a problem or not in diseases necessitating protein substitutes such as PKU.

Objective: to study the prevalence of overweight and obesity in continuously treated PKU patients in Portugal.

Methods: Anthropometry was studied in 123 patients. Body mass index was transformed in z-scores according to the WHO standard growth charts. Results: No overweight or obesity was found in patients <5 years. The prevalences of overweight and obesity between 5 and 10 years were 10 and 10%, between 10 to 15 years 20.8% and 12.5%, between 15 and 19 years 25% and 0%, and in adults 35% and 10%, respectively.

Conclusion: Overweight and obesity does not seem to be a real problem in our population till late adolescence. In adults, the prevalence of overweight and obesity seems to be comparable to the general population. Clear data on control population in Portugal for younger individuals is lacking. Body composition studies of both general population and PKU patients are needed to compare the fat mass of overweight and obese patients with and without PKU.

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THE MICRONUTRIENT STATUS OF PATIENTS WITH PKU ON DIETARY TREATMENT: AN ONGOING AUDIT

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Phenylalanine-free protein substitutes in PKU are the major source of micronutrients for patients on diet. Deficiencies have been reported for iron, selenium, zinc, and Vitamin B12.

Aim: to assess the micronutrient status of patients aged 1—17y (median age 9y) with PKU on dietary treatment only.

Methods/subjects: Annual/bi-annual blood samples for nutritional markers were taken on a group of 73 patients (37 girls). The median number of blood samples per patient was 5 (range 1–14); in total 468 samples were analysed. Biochemical/haematological markers assessed were haemoglobin, mean corpuscular volume (MCV), plasma zinc and selenium, and serum vitamin B12. C-reactive protein (CRP) was also analysed in 57% of samples.

Results: The following percentage of results were below lower reference range: haemoglobin 14%; MCV, 6%; zinc, 25%; and selenium 6%. Percentage of results above reference range were haemoglobin, 23%; MCV, 3%; selenium, 6%; and vitamin B12, 23%. CRP was high in 3% of samples.

Conclusions: The majority of micronutrient concentrations were within reference range. Zinc status is of most concern although it is recognised that plasma zinc is not the best measure of zinc status. In PKU, interpreting micronutrient status is difficult and defining optimal nutritional profile of protein substitutes remains challenging.

Conflict of Interest declared.

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PROPOSAL OF A NEW EDUCATION SYSTEM FOR THE PKU DIET

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Background: Joyful and healthy eating is not in the focus in dietary treatment of PKU but rather the controlled intake of natural protein or phenylalanine. This is a burden on the families and the social life of the patients. Even relaxed diets due to sapropterin therapy are still complex. A working group of German, Austrian and Swiss Dieticians for relaxed PKU diet developed a new patient education system for strict and relaxed PKU diet.

Results: The system indicates graphically in pyramidal form food categories that should be consumed during a day, e.g. foods from plant sources (fruit, vegetables, cereal based foods), foods from animal sources replaced by the corresponding amount of protein supplement and a few phenylalanine-rich extras. Portion sizes using a hand model for each food group are specified. The pyramid system emphasizes analogies to recommendations for healthy children. Patients are enabled to control their diet proactively and prospectively with regard to portions still allowed for the day instead of recording yet consumed and metabolized foods. The system was hitherto successfully applied in two metabolic centres.

Conflict of Interest declared.

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EVALUATION OF CALCIUM-PHOSPHATE HOMEOSTASIS AND BONE DENSITY IN PATIENTS WITH INBORN ERRORS OF AMINO ACID METABOLISM, ON LOW-PROTEIN DIET

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Background: Exclusion of diary products in the treatment of inborn errors of amino acid (AA) metabolism results in a risk of deficit of absorbable calcium (Ca), phosphate (P) and vitamin D with consequences of decreased bone mineral density (BMD).

Patients and Methods: The following data from 28 patients (9 MSUD, 8 HT1, 4 MMA, 3 IVA, 2 MCC and 2 HOGA), in the age range 4–23 yrs: biochemical parameters of Ca-P metabolism, anthropometry, whole skeleton and lumbar spine BMD by DXA and nutritional status (based on diet records) were analyzed.

Results: Body weight and height were within ±2SD in 90% of patients. Laboratory Ca-P markers were normal except decreased 25OHD3 (below 20 ng/ml) in 35% of patients. DXA examination revealed physiological results in 48% of patients, Z-score for whole skeleton BMD was below normal value in 32%, at lumbar spine low-normal/below normal BMD in 20% of patients. Analysis of diet records showed decreased vitamin D intake (mean 41% of RDA) with unfavourable Ca/P ratio.

Conclusion: Our study shows that low-protein diet may result in insufficient vitamin D status, what influence adequate bone mineralization in the patients with inborn errors of AA metabolism.

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DIETARY MANAGEMENT OF NON-PYRIDOXINE RESPONSIVE HOMOCYSTINURIA

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Introduction: There is little information about dietary practices in non-pyridoxine responsive homocystinuria (NPR-HCU) across Europe.
Methods: 29 NPR-HCU dietary management questionnaires were returned from the SSIEM D-G network: (14 UK, 5 Germany, 3 Netherlands, 2 Switzerland, 2 Portugal, 1 France, 1 Norway, 1 Belgium).
Results: 182 patients with NPR-HCU (57% (n=103) on a methionine/intact protein restriction) were identified. Age distribution was: <1y, n=1; 1–10y, n−20; 11–16y, n=29; >16y, n=132. The use of diet with betaine increased with age: <1y, <1%; 1–10y, 10%; 11–16y, 12%; and >16y, 34%. The median intact protein intake (g\d) on diet only was <1y, 8 g; 1–10y, 12 g; 11–16y, 12 g; and >16y, 27 g. With diet and betaine, median intact protein intake (g\d) was: 1–10y, 14 g; 11–16y, 18 g; and >16y, 38 g. 41% (n=12) of centres used food methionine analysis for intact protein allocation; methionine exchange systems (primarily10mg or 20 mg) were common. 94% of patients on diet were prescribed methionine-free protein substitute. 48% of centres recommended cystine supplements for low plasma concentrations. Target concentrations for homocystine/homocysteine (free/total) and frequency of monitoring was inconsistent.
Conclusion: In NPR-HCU, the use of dietary restriction declined with age. There is a need for European consensus guidelines on the management of NPR-HCU.
Conflict of Interest declared.

P-484

DOCOHEXAENOIC ACID SUPPLEMENTATION IN CHILDREN WITH INBORN ERRORS OF AMINOACID CATABOLISM

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Background: Patients submitted to low protein diet (LPD) are susceptible to develop nutritional deficits, namely of vitamins, minerals and essential fatty acids, like docosahexaenoic acid (DHA). DHA is found mostly in fish, which isn't available in these patients' diet.
Objectives: evaluate plasma DHA concentration in individuals submitted to LPD due to an inherited disorder, comparing with a control group and assess evolution after DHA supplementation.
Methods: 20 patients (2 organic aciduria, 2 UCD, 10 PKU, 2 homocystinuria, 3 MSUD and 1 tyrosinemia type I), aged less than 18 years were submitted to analytic determination of plasma DHA in three moments (T0, T1 and T2), with 60 days intervals. In this period, patients were submitted to DHA supplementation (15 mg/Kg/day). Control group was randomly constituted by 33 individuals, aged matched and under normal diet.
Results: in T0, patients' group presented a significantly lower DHA value than controls (27,65±13,69 μg/ml vs 55,23±21,84 μg/ml). Between T0 and T2, there was a significant improvement of patients' plasma values to 59,71±22,61 μg/ml, similar to control group.
Conclusion: Daily supplementation of DHA allows plasma levels normalization and should be included in the nutritional schedule of these patients, in order to avoid deficits and optimize their growth and neurodevelopment.

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DEVELOPMENT OF A ROBUST STABLE ISOTOPE MASS SPECTROMETRY METHOD FOR THE QUANTIFICATION OF DOCOSAHEXAENOIC ACID IN PLASMA AND APPLICATION TO ASSESS DHA STATUS IN A COHORT OF PKU PATIENTS

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Background: Docosahexaenoic acid (DHA) is an omega-3 fatty acid which is essential for neurological development and function. Endogenous DHA contributes little to plasma concentration which is primarily determined by dietary DHA. Treatment of PKU involves administration of a protein restricted diet; therefore patients receive insufficient dietary DHA. At the present time, there is no service in the UK to quantitate DHA in plasma.
Aims: 1. To develop and validate a robust assay to quantitate DHA in plasma. 2. To establish a reference range and investigate plasma concentration in patients with PKU.
Methods: An LCMSMS method for quantification of DHAwas developed and fully validated. The assay was applied to establish a reference range (n=40), and to assess plasma DHA in 22 PKU patients.
Results: DHA reference range was 131 μM–589 μM. PKU patients who were following a protein restricted diet demonstrated significantly lower plasma DHA than both the reference group and those PKU patients who were on a restricted diet and receiving DHA supplements ($p<0.05$).
Conclusion: The method developed here is clinically useful in identifying patients deficient in DHA, and will be useful to clinicians who wish to supplement and monitor the DHA status of their patients in the future.

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DIETARY THERAPY IN PROPIONIC ACIDEMIA: DO ISOLEUCINE CONCENTRATIONS CORRELATE WITH PROTEIN INTAKE?

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Introduction: Propionic acidemia (PA) is caused by a congenital deficiency of propionyl-CoA carboxylase. Therapy includes a protein modified diet (with or without substitution of a precursor free amino acid mixture).
Aim: Aim of this retrospective study was to investigate a possible correlation between protein intake (total and natural) and isoleucine concentrations in patients with PA.
Methods: Laboratory parameters (e.g. concentrations of amino acids, n=280) and up to 4 nutritional records (period of time 01/01–07/10, 3 days record estimated or weighted by parents, 2 days telephone record by a dietitian) of 7 patients with PA (diagnosis molecularly and enzymatically confirmed) were evaluated.
Results: Isoleucine concentrations were in low or below reference range in all patients (mean: 33 μmol/l; median: 31 μmol/l, (range 7–67) n=25). They did not depend on total protein intake (mean: 1.27; median: 0.98 g/kg bw/d, (range 0.63–1.94) n=25).
Conclusion: Low isoleucine concentrations in patients with PA are not only caused by low protein intake.

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DIETARY MANAGEMENT OF FEEDING PROBLEMS, GASTROINTESTINAL SYMPTOMS AND PANCREATITIS IN CHILDREN WITH METHYLMALONIC ACIDAEMIA

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Background: Gastrointestinal symptoms and pancreatitis are recognised complications of Methylmalonic Acidaemia (MMA).
Objective: Investigate incidence and review management strategies of feeding problems, gastrointestinal symptoms and pancreatitis.
Case series: Fourteen patients with vitamin B12 non-responsive MMA. Age at presentation: neonatal (n=9) to 1.5y.
Twelve patients with vitamin B12 responsive MMA. Age at presentation: neonatal (n=2) to 4y.
Results: B12 non-responsive: 12 have feeding problems. Eleven are long term tube fed (from presentation n=4, by 6 months from diagnosis n=3 and all by 3 years) due to inadequate oral intake. 50% of those with feeding problems are on anti-emetics/anti-reflux medications and need feed slowly administered via feeding pump. Four have chronic diarrhoea and receive hydrolysed/elemental feeds. Four patients have presented with pancreatitis; isolated episodes n=3, acute on chronic n=1. Latter is now on anti-emetics and elemental feed with MCT via gastrostomy. During acute illness regimen is changed to slow jejunal feeds with increased fluid volume.
B12 responsive group: one is tube fed (elemental).
Conclusion: Feeding problems are frequent, presenting from diagnosis or acquired. Tube feeding becomes essential to meet prescribed diet and fluid requirements. Modifying feed rate, mode and 'type' may reduce gastrointestinal symptoms and enable enteral feeding in acute pancreatitis.

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THE SAFETY OF A NEW MCT BASED FORMULA IN THE DIETARY TREATMENT OF LONG CHAIN FATTY ACID DISORDERS: A PHASE 2 STUDY

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Treatment of long chain fatty acid oxidation disorders (LCFAOD) is primarily by diet, but defining the ideal nutritional composition of a generic formula to treat all conditions remains challenging.
Aim: To investigate the safety of a new MCT-containing (MCTF), nutritionally complete formula (Lipistart: Vitaflo International) in children with LCFAOD.
Methods/Subjects: 5 well-controlled children (VLCADD, n=2; LCHADD, n=1; CACTD, n=2; median age 9y; range 7–14y; 3 boys), who take daily MCT oil, completed a 21 day, phase 2 trial, on the safety of MCTF. MCTF (per 100 ml) contained 3.3% fat (2.6 g MCT); it replaced their usual formula (median volume 720 ml/d; range 500 ml–1900 ml/d). Their usual formula was given on day −7 to 0 and 8 to 21; MCTF was given day 0–7. Blood samples were taken day −7, 0, 2, 7 and 14 (analytes included liver function tests, creatine kinase, glucose, acyl carnitines, free fatty acids and 3-hydroxybutyrates). ECG was monitored day 0 and 7.
Results: There was no significant difference in biochemical control or ECG, and no child developed symptoms of metabolic decompensation on MCTF. Tolerance was good, although one subject developed constipation. MCTF was well accepted.
Conclusions: The new MCTF was safe and well tolerated in these LCFAOD patients.
Conflict of Interest declared.

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NUTRITION MANAGEMENT OF CLASSICAL AND DUARTE GALACTOSEMIA: RESULTS FROM AN INTERNATIONAL SURVEY

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A survey to assess current policies for dietary management of Classical and Duarte Galactosemia was posted on the Metabolic Dietitians list serve (PNO-Metab-L@listserv.cc.EMORY.EDU). 102 responses were collected. There was consensus among clinics to eliminate dairy-based foods and ingredients, but a variation in restriction of more minor sources of galactose. Thirty-three percent of clinics routinely eliminate fruits and vegetables, such as tomatoes and blueberries, with a free galactose content >20 mg/100 g of food. Twenty percent restrict these foods only during infancy and early childhood, but allow them for older individuals. Another 30% of clinics do not restrict any fruits and vegetables. Similar variation in clinic policies was found for other foods and ingredients. Policies also vary regarding use of liquid soy-based formulas containing carrageenan and use of elemental galactose-free formulas for infants with slowly decreasing red cell galactose-1-phosphate concentrations. For infants with the Duarte variant, 43% of clinics treat with a soy formula for the first year while 27% of clinics do not treat this form of galactosemia. Results from this survey demonstrate a need for evidence-based recommendations to better standardize treatment for this disorder.

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SIMPLIFYING THE KETOGENIC DIET EXPERIENCE IN PYRUVATE DEHYDROGENASE COMPLEX DEFICIENCY

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Background: Pyruvate dehydrogenase complex deficiency (PDHCD) comprises a range of phenotypes and may be treated with ketogenic diet. We present 4 patients with enzymatically confirmed PDHCD treated with a modified ketogenic diet similar to the modified Atkins diet. In all families there were barriers of language, geographical distance and/or social circumstances to hospital admission and/or diet calculations, required by the traditional ketogenic diet.

Case Reports: Three patients (3–5 years) commenced the diet at home with some or all of their usual milk drinks substituted with Ketocal.. Patient 1 was admitted to ensure safety of enteral feeding and parental understanding; 2 families made gradual changes at home to their child's usual eating plan whilst testing urinary ketones twice daily. All were advised on management strategies should urinary ketones exceed 16 mmol/l. All patients are maintaining urinary ketones between 1.5–8 mmol/l and report clinical improvement (less irritability, improved motor function, less hospital admissions). A 17 year old previously on a high fat diet, commenced carbohydrate restriction due to worsening lower limb pain. The diet is well tolerated with some reported clinical improvement.

Conclusion: A simplified diet to induce ketosis, commenced at home, may be less of a burden in PDHCD.

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GROWTH OF CHILDREN ON KETOGENIC DIET: DOES THE PROTEIN TO ENERGY RATIO MATTER?

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Ketogenic Diet (KD) is an effective treatment for children with intractable epilepsy. Despite energy and protein prescription fulfilling the Recommended Dietary Intake for ideal body weight and normal growth, some patients fail to grow while on the diet.

We hypothesised that growth failure in patients on a KD is due to an inadequate Protein:Energy (P:E) ratio.

Retrospective dietary details (protein and energy intake; P:E ratio), growth parameters and biochemical markers were collected on all patients who had been on KD for >6 m since October 2002, excluding those with a metabolic disorder (n=39; male:female: 21:18; age range 10 m–16.5 yrs. Information was incomplete for 2 patients.

Twenty-four children failed to grow: 10 had a P:E intake <1.3gm/100 kcal (n=5; age1–3y and>10y) or <1.5gm/100 kcal (n=5; age3–10y). Eight had selenium deficiency (or negligible intake). Appropriate selenium intake/blood level was confirmed in all 10 patients with low P:E ratio. Two had severe iron deficiency. Four had low energy and protein intake. Two did not grow for unknown reasons. Surprisingly, three of those who grew well (age3–8 years) had <1.3 g protein/100 kcal, were not selenium deficient and had adequate selenium intake.

P:E ratio and selenium deficiency are pivotal in the growth of children on a KD.

P-492

SPECIALIST FEED PRODUCTION FOR PATIENTS WITH INHERITED METABOLIC DISORDERS

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Introduction: Accurate and safe feed production is challenging for caregivers of children with IMD requiring specialist feeds with multiple ingredients.

Aim: To examine the accuracy of modular feed preparation.

Methods/subjects: 52 subjects (38 mothers, 13 fathers, 1 patient; 67% Asian, 29% Caucasian; 4% Afro-Caribbean) with IMD children requiring specialist feeds were studied. Two feeds, with the same nutrient composition, were made with either 2 or 6 ingredients. Following a demonstration, caregivers of children with IMD prepared both feeds under controlled conditions. Ingredients were measured by digital scales, syringe and measuring jugs. Prepared feeds were analysed for nutrient content to determine preparation accuracy.

Results: The nutrient composition of both feeds was inaccurate. Nutrients within 20% of the calculated amount for the 6 ingredient feed were: carbohydrate 92%; fat 2%; sodium 33%; potassium 79%; and zinc 23%. For the 2 ingredient feed: carbohydrate was 83%; fat 65%; sodium 4%; potassium 25% and zinc 76%. Fat was calculated as a % of expected minimum value. 6 ingredients feeds were more likely to be under concentrated and 2 ingredients feeds over concentrated.

Conclusion: Preparation of specialist feeds is inaccurate and there is a need to simplify feed production for caregivers of IMD children.

Conflict of Interest declared.

P-493

HOME ENTERAL TUBE FEEDING IN IMD CHILDREN: A REVIEW OF CARER KNOWLEDGE AND TECHNIQUE

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Home enteral tube feeding (HETF) is commonly used in children with inherited metabolic disorders (IMD). It is unclear how caregiver knowledge and their safety in using feeding techniques change over time.

Aim: To assess the long-term HETF knowledge and safety technique of caregivers with IMD children on HETF.

Methods: 32 caregivers of IMD children (median age 5.3 yrs: range 0.3–13.6 yrs) had annual interviews over 3 years using a structured questionnaire.

Results: At the study start, 34% (n=11) of subjects had been on HETF for >5 years and 22% (n=7) <1 yr. Over 3 years, caregivers' ability to change tubes increased (57% to 100% nasogastric; 50% to 74% gastrostomy). Fewer family members helped with HETF. Carer knowledge improved e.g. they could better explain how to clear tube blockages (19% to 69%); identify signs the NGT may be in the trachea (79% to 100%); identify correct position for night feeding (38% to 56%). Their attention in performing techniques declined with time: inaccurate feed ingredient measurement (64% to 89%); correct flushing of tubes (56% to 44%); infrequently checking tube position (28% to 41%); correct handwashing (38% to 25%).

Conclusions: Caregivers correct use of HETF techniques declined over time. They would benefit from annual training updates.

Conflict of Interest declared.

P-494

TOLERANCE AND ACCEPTABILITY OF A NEW READY TO USE NOVEL PROTEIN SUBSTITUTE DEVELOPED FOR OLDER INFANTS AND YOUNG CHILDREN

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In the UK, in young children >6 months of age with PKU, the most common way of administering phenylalanine-free protein substitute (PS) is in the form of a gel/paste given from a spoon pre-meals. It is prepared by adding water to powder. Disadvantages are associated with preparation inconvenience and changeable consistency, which are prevented with a ready-to-use (RTF) preparation.

Aim: To study the efficacy, acceptability and tolerance of a RTF PS gel (PKU Squeezy; Vitaflo International) in young children with PKU.

Methods/subjects: In a 7 day, observational trial, 10 subjects (9 PKU; 1 DHPR deficiency; 8 boys), median age 3y (1–10y), took the RTF, nutritionally fortified, PS gel at least once daily. Caregivers completed a daily diary examining tolerance, acceptability and ease of administration.

Results: Appearance and tolerance were very similar between usual PS and RTF gel. However, caregivers rated the RTF as easy to use/prepare (100% vs. 50% usual PS); easy to administer outside the home (75% vs. 40% usual PS); and easy for others to prepare and administer (100% vs. 50% usual PS). Blood phenylalanine control remained excellent.

Conclusions: This RTF PS gel developed for younger PKU children was successfully adopted by patients and more convenient for caregivers.

Conflict of Interest declared.

P-495

THE RELATION BETWEEN VITAMIN D, ADIPONECTIN AND INSULIN SENSITIVITY IN OBESE CHILDREN AND ADOLESCENTS

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Background: Previous studies have suggested vitamin D insufficiency is associated with increased obesity and metabolic risk factors. However data on the relation between vitamin D status, adiponectin, and insulin sensitivity among obese children and adolescents are lacking. The aim of this study is to investigate the relation between serum 25(OH)D, adiponectin, and insulin sensitivity in obese and non-obese children and adolescents.

Patients and Methods: Data from 40 obese and 40 non-obese children and adolescents were obtained. In every participants, anthropometric variables were recorded, fasting blood was assayed for 25(OH)D, glucose, adiponectin, insulin levels and insulin resistance was estimated by homeostasis model assessment (HOMA index)

Results: The mean (±SD) age of participants was 13.2±3,6. Insulin resistance was obtained in 15 of obese patients (37.5%). In the overall population 25(OH)D was significantly inversely correlated with body mass index (BMI), insulin levels and HOMA index ($p<0.05$), and positively correlated with adiponectin ($p<0.05$). Adiponectin levels were negatively correlated with HOMA index in obese group ($p<0.05$). Adiponectin levels were lower in obese group ($p<0.05$).

Conclusions: We observed realtionships between adiponectin, vitamin D, and insulin sensitivity in obese children and adolescents.

A-026

FOOD IS MY MEDICINE: TRANSLATING COMPLEX DIETARY THERAPY INTO THE LANGUAGE OF FOOD

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Dietary therapy is the major treatment of urea cycle disorders. The aim is to describe the practical aspects of dietary management of a toddler with citrullinemia where education and compliance with dietary therapy has been challenging due to language and parental education level.

A variety of repeated educational approaches have been used to manage this child including: visual resources, quizzes, supermarket tours, and inpatient bedside signs to prevent feeding of inappropriate foods. Dietary analysis of protein and nutritional adequacy is limited by the parent's inability to recall food intake and count protein. All modifications to the diet must be made in person. There have been four admissions in 20 months with hyperammonemia, necessitating formal education to nursing staff. Total dietetic time spent directly with this family has been 62 hours, plus preparation time for the resources above.

Inborn errors of metabolism present a challenging case for dietitians: to translate their dietary therapy into food-based therapy for families. This case demonstrates dietary education can be a lengthy, time consuming, and ongoing process due to the barriers created by language and parental education. Despite the time and resources invested, understanding of the disorder by the family is still very limited.

A-027

OPTIMISING GROWTH IN PHENYLKETONURIA: CURRENT STATE OF THE CLINICAL EVIDENCE BASE

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Introduction: The relationship between diet and growth in PKU has been under reported. We have reviewed publications on diet and growth in PKU.
Methods: A Pubmed search has highlighted<20 published research papers on growth outcome with PKU.
Results: The majority of papers have recruited study populations consisting mostly of children aged 12 years or lower. Most of the data originated in a small number of countries, particularly the USA, The Netherlands, France and Germany. There were differences between studies in the anthropometric parameters considered, although most included measurements of height/length, weight and/or body mass index, and head circumference. Few studies measured parental height. Some studies employed longitudinal measurements of parameters related to growth (follow-up periods ranged between <1 y and 10 y), while others had a cross-sectional or retrospective design. Most of the studies were not recent, with publication dates of 1998 or earlier for almost 70% of papers. Some studies detected differences in parameters related to physical development between these populations, but there were differing growth outcomes.
Conclusions: There is little recent, long-term growth outcome data throughout childhood and adolescence in PKU. In PKU, further prospective, multicentre studies on growth in PKU on current treatments are necessary.
Conflict of Interest declared.

A-028

NUTRITIONAL ASSESSMENT OF TETRAHYDROBIOPTERIN (BH4) TREATED PATIENTS WITH PHENYLKETONURIA (PKU)

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Background: In BH4-treated patients the primary outcome measure has been improvements in plasma phenylalanine (phe) concentrations, while the effect of dietary intake and nutritional status, have not been sufficiently elucidated.
Objectives: To assess the nutritional status of PKU patients undergoing BH4 therapy.
Methods: Six weeks before and during the treatment, phe tolerance were evaluated twice a week to fortnightly. Weight and height Z-scores; daily consumption of macro and micronutrients including phe, tyrosine and protein were calculated.
Results: Five BH4 responsive (≥ 30% reduction in phe levels) patients were followed for 7,3±5 months. The patients received sapropterin 15± 6,5 mg/kg/day. The increase in phe tolerance was 217%. Vitamin A, E, B1, B6, folate and iron consumptions supplied the RDA. Vitamin B12 consumption decreased from 96% to 86% RDA due to reduction of special formula consumption. Calcium, phosphorus and zinc intakes were increased to 87%, 92%, 62,8% of RDA, respectively.
Conclusions: Long-term dietary guidance and monitoring of the nutritional status of patients with PKU should be part of a follow-up programme in BH4 treatment.

A-029

AN AUDIT OF THE CURRENT DIETETIC LED CLINICS FOR PATIENTS WITH PHENYLKETONURIA (PKU)

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Background: Paediatric dietetic led clinics were set up for our patients with Phenylketonuria. Patients/carers see the dietitian in the dietetic led clinics which are supported by the metabolic nurse specialist, a dietetic assistant and the metabolic product company representatives.
Objective: To review and evaluate the effectiveness of the dietetic led PKU clinics
Methodology: A questionnaire was given to all carers of patients with PKU who attended the PKU dietetic clinics.
Results: A total of 22 questionnaires were given out of which 15/22 (68%) were returned. The questionnaire was designed to determine families thoughts on the new service, ways in which the service could be improved and areas in which further support is needed. 12/15 patients found attending the dietetic clinics more beneficial than seeing us within the multi-disciplinary clinics. The following suggestions were made by the patients/families on how the service could be improved:
- To consider group sessions
- To continue to invite the company representatives

Conclusion: We can conclude that the dietetic led PKU clinics were successful in improving the quality of service provided for our patients. We plan to develop the clinics further to address the areas in which patients felt they needed further information.

P-496

ACUTE KIDNEY INJURY IN TWO CHILDREN CAUSED BY RENAL HYPOURICEMIA TYPE 2

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Renal hypouricemia is a heterogeneous inherited disorder characterized by impaired tubular uric acid transport, reabsorption insufficiency and/or acceleration of secretion with severe complications, such as acute kidney injury, renal failure and nephrolithiasis. Diagnosis is based on biochemical markers: hypouricemia and increased fractional excretion of uric acid. Therapy is not available, however avoiding dehydration, vigorous exercise and alkalization of urine will minimise renal impairment. More than one hundred cases with a loss-of-function mutation in the SLC22A12 gene have been found, most of the described patients are Japanese (OMIM #220150, type 1). Four patients with renal hypouricemia caused by defects in the SLC2A9 gene have been described (OMIM #612076, type 2).

We describe the findings of 12 and 14 year old nonconsanguinous boys with acute kidney injury from UK; the concentrations of serum uric acid were 0.03 and 0.04 mmol/l and expressed as an increase in the fractional excretion of uric acid (46 and 93%). A diagnosis of renal hypouricemia type 2 was made on the bases of finding two novel missence transitions resulting in amino acid substitutions p.G216R and p.N333S.

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P-497

FAMILIAL JUVENILE HYPERURICEMIC NEPHROPATHY CASE REPORT

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Familial juvenile hyperuricemic nephropathy (FJHN, OMIM #162000) is an autosomal dominant inherited disorder characterized by hyperuricemia with decreased renal excretion of uric acid, gout and progressive renal failure. FJHN is caused by mutation in the UMOD gene (16p12.3) coding uromodulin.

In the family examined by us, the father of the patient was treated with gout, he died of chronic renal failure. The patient has been monitored since her 27 of age with hyperuricemia; gradually she developed a chronic renal insufficiency in the course of 20 years. Hyperuricemia and borderline creatinine values were also observed with the patient´s son also at the age of 27. The FJHN was diagnosed on the basis of the family medical history, examination of creatinine in serum, uric acid in serum and in urine, determination of the value of extraction fraction of uric acid and uromodulin in urine. Results of biochemical examinations were subsequently confirmed by molecular genetic analysis of UMOD gene (c.334 T>C, p.C112R) with the two abovementioned family members.

Allopurinol treatment leads to normalization of serum levels of uric acid and prevents gout attacks. Regular monitoring of renal functions is necessary, in case of renal failure hemodialysis or peritoneal dialysis is recommended, and kidney transplantation.

P-498

HPRT DEFICIENCY: IDENTIFICATION OF TWENTY FOUR NOVEL VARIANTS INCLUDING AN UNUSUAL DEEP INTRONIC MUTATION

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Background: Hypoxanthine phosphoribosyltranferase (HPRT) deficiency is an X-linked disorder of purine salvage. Clinical manifestations range from uric acid over production with hyperuricaemia and gout to severe neurological impairment, self-mutilation and dystonia in Lesch-Nyhan Syndrome. Molecular testing is necessary to identify female carriers within families as a prelude to prenatal diagnosis. During the period 1999–2010 the Purine Research Laboratory studied 106 patients from 68 different families. We report the spectrum of mutations identified to date.

Methods: Sequencing of genomic DNA or cDNA was used to define mutations in exons and flanking intronic regions.

Results: Genomic sequencing revealed mutations in 60/68 families (88%). Overall, 24 of these mutations have not been previously reported. In 8 patients, exon sequencing was not informative. Copy-DNA analysis in one patient revealed an insertion derived from a deep intronic sequence with a genomic mutation flanking this region created a false exon. Carrier testing was performed in 21 mothers of affected patients, of these 81% (17) were found to be carriers of the disease associated mutation.

Conclusions: Our results confirm the extraordinary variety and complexity of mutations in HPRT deficiency. A combination of genomic and cDNA sequencing may be necessary to define mutations.

P-499

PURINE NUCLEOSIDE PHOSPHORYLASE DEFICIENCY: A MUTATION UPDATE

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Background: Purine nucleoside phosphorylase (PNP) deficiency is an autosomal recessive disorder affecting purine degradation and salvage pathways. Clinically, patients typically present with severe immunodeficiency, neurological dysfunction and autoimmunity. Biochemically, PNP deficiency may be suspected in the presence of hypouricaemia. We present clinical, biochemical and genetic data on 8 patients from 7 families identified as PNP deficient.

Methods: Urine metabolites were separated and quantified on a Waters UPLC. PNP activity was measured in erythrocyte lysates. Identification of the underlying mutations was determined by DNA sequencing.

Results: In seven of eight patients purine nucleoside excretion was massively elevated in urine. However, in one patient multiple blood transfusions resulted in normal PNP activity and barely detectable urine metabolites. Seven different mutations were characterised of which two were novel, c.257A>G (p.H86R) and c.770A>G (p.H257R). Interestingly, four out of eight patients diagnosed with PNP deficiency had normal serum levels of uric acid.

Conclusions: To date 66 patients with PNP deficiency have been reported and 24 disease causing mutations identified. PNP deficiency should not be ruled out due to the presence of normal serum uric acid.

P-500

THE PATHOLOGICAL R326Q MUTATION CAUSING BETA-UREIDOPROPIONASE DEFICIENCY IS COMMON IN THE JAPANESE POPULATION

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Background: beta-ureidopropionase (beta-UP) is the third enzyme of the pyrimidine degradation pathway and its deficiency shows unspecific symptoms such as developmental delay, convulsion and hypotonia. We presented 12 Chinese cases in SSIEM 2010 who were all homozygous for the R326Q mutation.

Objectives: To investigate whether the R326Q mutation is also common in the Japanese population, we have analyzed the mutation in a Japanese patient with chemically diagnosed beta-UP deficiency and 100 anonymous Japanese volunteers.

Material and methods: Sequence analyses was performed of all exons of the UPB1 gene of the patient and exon 9 of UPB1 gene was investigated in one hundred samples from Japanese anonymous volunteers.

Results: Homozygosity for the mutation R326Q was detected in the Japanese patient. In the 100 Japanese volunteers, we detected two heterozygous carrier of R326Q mutation (two in 200 alleles).

Conclusion/discussion: A high frequency of the R326Q mutation was observed in the Japanese population. Therefore, beta-UP deficiency may not be a rare disease in the Japanese and east Asian population. Because the symptoms of beta-UP deficiency are not specific, many patients may be undiagnosed. Patients with unexplained neurological symptoms should be tested for this disease.

P-501

ß-UREIDOPROPIONASE DEFICIENCY: PHENOTYPE, GENOTYPE AND PROTEIN STRUCTURAL CONSEQUENCES IN 16 PATIENTS

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Background: ß-Ureidopropionase catalyses the conversion of N-carbamyl-ß-alanine and N-carbamyl-ß-aminoisobutyric acid to ß-alanine and ß-aminoisobutyric acid, ammonia and CO2. Five genetically confirmed patients with a complete ß-ureidopropionase deficiency have been reported thus far.

Method: We report on the clinical, biochemical and molecular findings of 11 newly identified ß-ureidopropionase deficient patients and compared these to the earlier reported patients.

Results: Patients presented mainly with neurological abnormalities (intellectual disabilities, seizures, abnormal tonus regulation, microcephaly, and malformations on neuro-imaging) and markedly elevated levels of N-carbamyl-ß-alanine and N-carbamyl-ß-aminoisobutyric acid in urine and plasma. Analysis of UPB1, encoding ß-ureidopropionase, showed 6 novel missense mutations and one novel splice-site mutation. Heterologous expression of the 6 mutant enzymes in Escherichia coli showed that all mutations yielded mutant ß-ureidopropionase proteins without significant activity. Analysis of a homology model indicated that the point mutations p.G235R, p.R236W and p.S264R lead to amino acid exchanges in the active site and therefore affect substrate binding and catalysis. The mutations L13S, R326Q and T359M resulted most likely in global folding defects and structural instability.

Conclusions: Two mutations were identified in several unrelated ß-ureidopropionase patients, indicating that ß-ureidopropionase deficiency may be more common than anticipated.

P-502

NUCLEOTIDE PRECURSORS IN NEONATAL AND ADULT SALIVA

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Background: Human saliva has been extensively described for its composition of major proteins, electrolytes, cortisol, melatonin and some metabolites such as amino acids. Little is known, however, about nucleotide precursors in human saliva. Nucleotides are essential for synthesis of DNA/RNA, supplying energy, regulating G-protein signalling, and biosynthesis.

Methods: Saliva samples were collected from full-term neonates, aged 1–3 days, using cotton swabs. Unstimulated fasting (morning) saliva samples were collected directly from adults. The samples were extracted and ultrafiltered, then nucleotide precursors were analysed by reversed-phase HPLC with UV-detection and mass spectrometry (with stable-isotope internal standards).

Results: Concentrations of salivary nucleobases and nucleosides as μM± SEM for Neonates\Adults respectively were: Pseudouridine 2±0.4\0.27± 0.07, Uracil 15±4\4.4±1.5, Thymine 0.2±0.1\0.35±0.16, Dihydrouracil 8±1\1.1±0.21, Dihydrothymine 2±02\0.43±0.1, Uridine 19±4\1.7±0.7, Urate 157±23\203±24, Hypoxanthine 41±9\3.8±1.5, Xanthine 29±7\4.9± 2.3, Adenosine 13±3\0.04±0.02, Inosine 22±7\0.43±0.13, Guanosine 11±3\0.22±0.05, Succinyladenosine 6±2\0.63±0.25. Deoxynucleosides and dihydroxy-adenine were negligible.

Discussion: Salivary concentrations of purine nucleotide precursors such as hypoxanthine, xanthine, adenosine, inosine and guanosine are surprisingly high in neonates and much higher than in plasma. Transition to lower adult levels appears to occur during the first year. These precursors in saliva may be useful biomarkers for diagnosis of inborn errors of nucleotide metabolism and the investigation of some pharmacogenetic disorders.

P-503

SIX YEARS EXPERIENCE WITH SELECTIVE SCREENING FOR DISORDERS IN PURINE AND PYRIMIDINE METABOLISM

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Purine and pyrimidine disorders (PPD) represent a heterogeneous group with variable clinical signs and symptoms. Testing for PPD should ideally be done in all urine specimens investigated for inborn errors of metabolism (IEM), but this is seldom done due to the low prevalence. Thus, metabolic laboratories face the challenge of selecting the samples for determination of purines and pyrimidines (PP).

In our laboratory, which is responsible for selective screening for IEM in Denmark, urine samples are analysed for PP in case of 1) increased/decreased uric acid excretion or 2) if the patient has symptoms of an immunological disease, intractable seizures, self-mutilation, hypertonicity, autism or any symptoms that may be related to kidney stone formation or nephrolitiasis.

Since 2005 approximately 20 % (in total 1601 samples) of all urine specimens received for metabolic screening has been evaluated for PPD. Using our selection approach we have diagnosed seven patients, not previously suspected to have a PPD. These include: Hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency; Adenylosuccinate lyase (ADSL) deficiency; Adenosine deaminase (ADA) deficiency and Dihydropyrimidine dehydrogenase (DPD) deficiency. Our findings highlight the importance of a selective screening approach for PPD.

P-504

DETERMINATION OF PURINES AND PYRIMIDINES IN DRIED BLOOD SPOTS

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New therapeutic options, e.g. cPMP substitution in molybdenum cofactor deficiency type A (MoCD), raised interest in early detection of affected infants with inherited defects of purine and pyrimidine metabolism. Established methods of selective screening, e.g. HPLC with UV- or MS/MS detection, use urine as matrix, not a common material in newborn material. In addition the cycle times are not suitable for high throughput screening. Therefore, a flow injection—MS/MS method with negative electrospray ionization was developed for fast screening of purines and pyrimidines in dried blood spots (DBS).

The identical sample preparation for analysing amino acids and acylcarnitines in DBS can be used for determination of purines and pyrimidines, too. Analysis of purines includes the measurement of xanthine, uric acid, and sulfocysteine for MoCD, analysis of pyrimidines includes the measurement of orotic acid, ureidopropionic and ureidoisobutyric acid for urea cycle disorders. Thus, a single extract can be used for simultaneous measurement of amino acids, acylcarnitines, purines and pyrimidines in DBS. This approach allows the inclusion of purine and pyrimidine disorders into the newborn screening panel without extensive additional efforts.

cPMP: cyclic Pyranopterinmonophosphate

P-505

PROLINE/CITRULLINE RATIO AS A BIOMARKER FOR OAT DEFICIENCY IN EARLY INFANCY

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Deficiency of ornithine-δ-aminotransferase (OAT) in humans results in gyrate atrophy of the choroid and retina. Early diagnosis may allow initiation of treatment before irreversible damage has occurred. However, diagnosis is commonly delayed well into adulthood. Here, we report findings in a neonate who was evaluated because of a positive family history of OAT deficiency. The reversed enzymatic flux in early infancy resulted in borderline low ornithine concentrations—evoking urea cycle disturbances—an increased proline and low plasma citrulline concentration.

Consequently, the proline/citrulline ratio was increased compared to controls. To find out whether newbornscreening (NBS) is suitable to detect this disorder, we performed aminoacid analysis on the original dried blood spot (from NBS) and compared it with >450.000 NBS data documented in the Minnesota database. The proline concentration (777 μmol/L) was just above the 99%ile (776 μmol/L), and citrulline concentration (4.5 μmol/L) was just above the 1%ile (4.37 μmol). The proline/citrulline ratio (172.9) was far above the 99% ile (97.6). Applying this ratio to NBS will lead to early and specific detection of neonatal OAT deficiency, with no additional expense for NBS laboratories quantifying aminoacids. Additional NBS samples from proven OAT deficient patients are required to provide disease reference ranges.

P-506

NEWBORN SCREENING FOR SUCCINYLACETONE, A PATHOGNOMONIC MARKER FOR TYROSINEMIA TYPE I

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Individuals with hereditary tyrosinemia type I(HT-1) are homozygous for one of several fumarylacetoacetate hydrolase (FAH)-mutations and are gradually deficient for the enzyme activity. As a result, the precursors of FAH accumulate and are metabolised to succinylacetone (SUAC).These precursors and SUAC have toxic effects.

Affected individuals show hepatic and renal diseases and also neurologic manifestations. Untreated, the disease might lead to premature death. HT-1 has a general incidence of 1:100,000 with a higher occurrence in particular ethnic groups.

Using general elevated tyrosine concentration as a marker for HT-1 has limited value because it is also common for tyrosinemia type II and III and other liver diseases. However, an increase in SUAC is pathognomonic for HT-1.

The objective of this study was to evaluate a validated commercial LC-MS/MS-test assaying newborn blood spots for SUAC among other markers for inborn errors of metabolism. Dried blood spots of 5,000 newborns were screened using the new method based on a simple extraction and derivatisation step. We compared the results with those obtained in parallel using our own in-house LC-MS/MS-method established at the Department of Pediatrics and Adolescent Medicine, Medical University of Vienna. Our data showed that the new method reliably identified all patients with HT-1.

P-507

RE-EVALUATION OF NEWBORN SCREENING FOR CBLC AND CBLD DEFECTS

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Background: Nine defects of intracellular cobalamin metabolism have been defined by means of somatic complementation analysis. Two of these defects cblC/D defects can cause combined methylmalonic-aciduria and homocystinuria.
Objectives: Re-evaluation of the newborn screening for cblC/D defects based on the analysis of false positive and negative rates.
Methods: Extended newborn screening was implemented in 2004 and 544,675 newborns were screened by MS/MS. Sequence analysis of genomic DNA was performed to identify disease-causing mutations in MMACHC and MMADHC.
Results: We identified five true-positive cblC defect cases(1/108,935), one false-negative cblD defect case (1/544,675) and 248 false-positives. All cblC patients showed the most common MMACHC mutation-c.271dupA. The molecular study of cblD patient, a girl identified at 8-month-old, revealed p.R250X mutation in MMADHC gene. At screening time, acylcarnitines presented C3=6.1 μM (cut-off >6.0); Met=15.2 μM (cut-off>12) and C3/Met=0.40; C3/C2=0.35; and C3/C16=2.0.
Discussion/Conclusion: Our results revealed that cblC/D defects are important conditions to be screened in our population. At sampling day, normal Met value associated with a borderline C3 value, lead to the misclassification of the cblD-patient as a normal-case. Until now, it was not possible to implement the second-tier tests, but in the future we intent to use this strategy to achieve a better assessment in the screening of these defects.

P-508

REDUCED RECALLS USING 2ND TIER TANDEM MASS SPECTROMETRY METHYLMALONIC ACID TESTING FOR NEWBORN SCREENING SAMPLES WITH ELEVATED C3 CARNITINE

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Aim: to evaluate the effect of second tier methylmalonic acid (MMA) testing on positive propionyl (C3) carnitine newborn screening tests.
Methods: From June 2007 MMA testing was performed on residual samples from the top 1% of C3 carnitines or ratios. After elution from a 3 mm blood-spot according to the non-derivatised method of La Marca et al, MMA was determined against (d3)-MMA. Data analysis used MRM pairs of 117.1/73.1 & 120.1/76.1
Results: From 1999 to 2007 our recall rate for elevated C3-carnitine or ratio was 217 of 154,000 neonates (0.13%). Retrospective MMA testing of residual samples from these infants detected 19 with elevated MMA, reducing the predicted recall rate to 0.06%. To date 1,200 further MMA tests have been performed, and 48 neonates recalled for a second sample if MMA was above the 99th centile (recall rate below 0.025). 12 had persistent elevated MMA and were referred for clinical assessment. Of these, 11 infants were diagnosed with B12 deficiency and 1 was confirmed to have Cobalamin A deficiency (Dr B Fowler).
Conclusion: The incorporation of second tier MMA determination into our routine newborn screening program continues to significantly reduce the false positive rate associated with the measurement of C3-carnitine and ratios.

P-509

DETECTION OF AN ASYMPTOMATIC MOTHER WITH GLUTARIC ACIDURIA TYPE 1 ASCERTAINED BY NEWBORN SCREENING FOR CARNITINE UPTAKE DEFECT

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Glutaric Aciduria type 1 (GA1) is a rare disease affecting lysine, hydroxylysine, and tryptophan metabolism. Classically reported presentations include acute childhood encephalopathy precipitated by metabolic stress. However, there is a subset of individuals with GA1 who may remain asymptomatic into adulthood.
A 33 year old woman had an uneventful pregnancy and delivery. Newborn screening in her daughter was positive for Carnitine Uptake Defect (CUD). Investigations in mother revealed low total blood carnitine of 2.5 μM and a free carnitine of <1 μM. Medical history and physical examination were unremarkable. Investigations for secondary causes of hypocarnitinemia included an acylcarnitine profile noting an elevation in Glutaryl-carnitine at 1.06 μM (<0.06) and urine organic acids revealing elevations in glutaric acid and 3-hydroxyglutaric acids, thereby securing a diagnosis of GA1. Carnitine supplementation was initiated at 70 mg/kg/day. Molecular analysis of the GCDH gene is underway with results pending.
This is the first reported case of an asymptomatic mother with GA1 ascertained by newborn screening of CUD in her unaffected child. Newborn screening has allowed us to detect asymptomatic mothers affected by inborn errors of metabolism, thereby forcing us to shift our paradigms with regards to the pathophysiology and management of these conditions.

P-510

QUANTIFICATION OF GLYCOSAMINOGLYICANS USING TANDEMMASSSPECTROMETRY FOR NEWBORN SCREENING OF MUCOPOLYSACCARIDOSES

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Background: Mucopolysaccharidoses (MPSs) are a deficiency of lysosomal enzymes to digest Glycosaminoglycans (GAGs), such as dermatan sulfate (DS), heparan sulfate (HS), and keratan sulfate (KS). The accumulation of GAGs into lysosome induces various symptoms. As enzyme replacement therapy (ERT) and bone marrow transplantation bring successful result for patients in early stage of MPSs, we need a suitable method for newborn screening (NBS) for MPSs using. dried blood spot samples (DBS) on Guthrie cards.
Material and Methods: We used a highly sensitive liquid chromatography tandem mass spectrometry (LC/MS/MS) to analyze the disaccharides digested from GAGs in DBS. We measured GAGs in Guthrie paper from normal control newborn and adult samples (n=200), newborn and several types of MPS patients (I: 2; II:5; III:2, IV:2, and VI:2).
Results: The results showed about 2–5 fold elevation of GAGs levels compared with those in normal control, and these levels decreased in ERT-treated MPS patients, suggesting this method is available for biomarker for monitoring ERT as well as NBS. Unexpectedly, KS levels were elevated in all MPS types.
Conclusion: Method for early detection of patients with MPSs has established. Pilot NBS study is now under planning.

P-511

ECONOMIC EVALUATION OF NEWBORN SCREENING FOR INFANTILE-ONSET POMPE DISEASE

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Background: Newborn screening prompts early detection of infantile-onset Pompe disease and allows initiation of enzyme replacement therapy (ERT) before irreversible muscle damage. The incidence of infantile-onset Pompe disease is around 1 in 50 000 in Taiwan. Starting from 2009, newborns could be screened for Pompe disease on a self-paid basis. Recently the government is considering including Pompe disease as one of the universal newborn screening items. Therefore the purpose of this study was to conduct an economic evaluation of different screening strategies from the societal perspective.
Study design: We used the modeling technique to perform a cost-effectiveness analysis comparing two screening strategies, namely universal screening and self-paid screening.
Results: Since over 90% of parents joined the screening program now on voluntary basis, from the point view of the society, a universal screening program would save USD$133 for every quality-adjusted life-year saved.
Conclusions: Compared to current screening a self-paid basis, a universal screening for infantile-onset Pompe disease could be cost-effective in spite of the low incidence rate. However, owning to the huge amount of treatment cost and the detection of late-onset patients as well, society may need more discussion about Pompe newborn screening in addition to cost-effectiveness only.
Conflict of Interest declared.

P-512

PURSUING OBJECTIVE PERFORMANCE METRICS OF NEWBORN SCREENING (NBS) TESTS FOR LYSOSOMAL STORAGE DISORDERS (LSD)

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NBS for one or more LSD has been implemented in New York, Illinois, and Taiwan by enzyme assay using either MS/MS or fluorometry. Other assays have also been proposed. To prevent a recurrence of the significant variability in NBS performance that characterized the application of MS/MS in NBS, a web site has been created to provide reference and disease ranges for all assays considered for LSD screening (www.nbstrn.org). Similar to the worldwide MS/MS collaborative project (McHugh DMS et al. Genet Med 2011;13:230–54), the goal is to collect assay-specific data sets of at least 50 true positive cases per LSD to establish disease ranges that will allow provision of cutoffs resulting in optimal analytical performance (high sensitivity and positive predictive value, low false positive rate). To achieve this goal, participation by screening laboratories and LSD specialists is crucial. All participants will have access to pages unique to their regional NBS program for data submission and comparison tools and to common pages inclusive of project tools and reports. LSD specialists are encouraged to facilitate the retrieval of any residual NBS samples of their patients.

P-513

EXPANDED NEONATAL SCREENING SINCE 2007: BIOTINIDASE DEFICIENCY IN THE NETHERLANDS. DO WE NEED TO TREAT PARTIAL BIOTINIDASE DEFICIENCY?

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Background: The incidence of profound (< 10 %) Biotinidase Deficiency (BD)(OMIM 254260) is about 1 in 112000 of partial BD (10%–30%) deficiency about 1 in 129000). In the Netherlands per year 28 BD deficient newborns were found versus 3 expected.
Objectives: Determine parameters for treatment of partial BD.
Patients: Newborns (from 2007–2010) with biotinidase activity (BA)<31 % and their parents were analysed: BA and mutations were determined. Plasma free biotin levels are pending. Patients with partial BD were treated with 5 mg of biotin weekly versus 5 or 10 mg daily in other centers.
Results: In EMC in three years 24 BD patients were found. Twenty patients were partial BD. Mean partial BA was 20.6 % (median 21 %). In the majority D444H/Q456H heterozygocity was found (EMC n=7). In The Netherlands in 2008 and 2009, 31 cases with D444H and another mutation were found. The D444H mutation is well known in partial BD. The BA ranged from 17 to 30 % (mean 22.4) compared to 14–30 % (mean 22 %) in patients with other mutations. No symptoms were seen in all BD patients.
Conclusion: It is not necessary to treat partial BD when BA is>15 %.

P-514

MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION ASSAY FOR DIAGNOSIS OF CONGENITAL ADRENAL HYPERPLASIA

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Background: Congenital adrenal hyperplasia (CAH) is a common autosomal recessive disorder and more than 90% of CAH cases are caused by a defect in 21-hydroxylase, which is encoded by the CYP21A2 gene. We describe the use of the multiplex ligation-dependent probe amplification (MLPA) method for convenient and rapid detection of deletions/duplications in the CYP21A2 gene.
Methods: We used MLPA to analyze the gene dose of CYP21A2 in 13 Korean patients who previously underwent direct sequencing for the molecular diagnosis of CAH. Genomic DNA was isolated from peripheral blood samples and the SALSA MLPA kit was used for MLPA analysis.
Results: MLPA confirmed the complete deletion of one CYP21A2 allele in 5 of 12 patients (Patients 1, 3, 4, 5, and 6). These 5 patients carried a single mutant allele peak in sequence analysis. We did not detect copy-number variation in Patient 2, who carries compound heterozygous mutations. No copy number variations were observed in seven patients (Patients 7 to 13) who presented with pigmentation or elevated 17-OHP alone, without clinical problems.
Conclusions: These results demonstrate the diagnostic usefulness of MLPA to detect copy number variation of CYP21A2 in CAH patients.

P-515

EVALUATION OF THE DEVELOPMENTAL OUTCOME OF NEWBORNS DIAGNOSED BY THE SCREENING PROGRAM (IN QATAR) FOR METABOLIC AND ENDOCRINE DISEASES UTILIZING PARENTS QUESTIONNAIRE VS PHYSICIANS EVALUATION

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Background: The newborn screening program in Qatar was launched in Dec 2003 in collaboration with University Children Hospital Heidelberg Germany

Parent were asked on phone (structured questionnaire) to evaluate the developmental status of their children

The treating physicians developmental rating was recorded The two ratings were compared

Methods: Between Dec 2003 and Dec 2010 99575 newborns were screened. In 137 babies metabolic or endocrine disease was confirmed 6 died

72/ 131 parents responded (55 %)

70/ 131 (53%) agreed to participate in the survey

In 10 cases (15%) parents judged their child as "abnormal" , 60 cases(85%) the children were reported normal

120 cases were evaluated by Physicians 111 were rated normal(92.5 %)

Discussion: 85% of parents whereas 92.5 % of physicians reported normal development

Comparing these 2 ratings (n=70) reveals the following:

Ratings were congruent in 62/70 cases (88%)

In one CH developmental delay was due non compliance

None of the babies rated normal by parents was rated abnormal by physicians

Recommendations: The Qatari program was effective in saving85% of affected children

Non compliance caused mental handicap in one CH

There is great need for more, effective education

and recall system for non compliant parents

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RARE DISEASE DETECTION PLATFORM: LEARNING BASED ICT FOR EARLY DIAGNOSIS OF METABOLIC DISEASES

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Background: Participation in an intern-ship project with IBM constituted an opportunity to work out and evaluate different possible platforms being possible candidates for supporting and improving the process of detection and diagnosing of Rare Diseases, Metabolic Diseases in particular.

Objective and methods: The pilot study was focused on Medium Chain AcylcoA Dehydrogenase deficiency (MCADD). A dataset of 120,000 laboratory analysis results were obtained as part of a systematic newborn screening panel during the year 2009. ICT methods used were a rule-based expert system, data mining and statistics.

Results:All MCADD cases were identified with a sensitivity of 1 and a specificity of 0,9994. Even a slight increase of the specificity up to 0.9998 was shown after taking into consideration other parameters such as ratio's of C8 and C2 acylcarnitines values. It is interesting to notice that all these results were obtained out of the raw data after a knowledge discovery process without making use of an initial learning set.

Conclusion: The unique integration of scientific medical knowledge and data mining capabilities is achieved and shows how ICT can support smarter and faster diagnosis for better health care.

P-517

EXPANDED NEONATAL SCREENING OF INHERITED METABOLIC DISORDERS BY TANDEM MASS SPECTROMETRY IN THE CZECH REPUBLIC: RESULTS OF A 18 MONTHS PERIOD IN ONE CENTER

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Background: To evaluate the performance of the screening program for 10 inherited metabolic diseases (IMD) using criteria recommended by the international project on neonatal screening- Region4Genetics (R4G). According to the R4G detection rate for 30 IMDs should be better than 1:3,000 and false positive rate below 0.3%.

Methods: The metabolites were extracted from blood spots using butylation kit and analysed by MS/MS profiling on API 2000 or API 3200.

Results: Between 1st October 2009 and 31st March 2011 we have analysed samples from 123 959 newborns, which covered about 2/3 of the Czech population. We have detected 32 neonates with subsequently confirmed IMD, i.e. 19 patients with PKU/HPA, 8 patients with MCAD deficiency, 3 patients with LCHAD deficiency and 2 patients with hydroxyprolinemia. The detection rate for these 10 IMDs was 1:3,873.

The false positive rate was 0.55% and 0.034% among neonates with birth weight<2500 g and≥2500 g, respectively; the total false positive rate was 0.10%.

Conclusions: The first eighteen months of expanded neonatal screening for 10 IMDs in our laboratory exhibited performance compatible with recommendations of the international R4G project.

The work was supported by the Grant MZ0VFN2005 by Ministry of Health of the Czech Republic.

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EXPANDED NEWBORN SCREENING PROGRAM WITH TANDEM MASS SPECTROMETRY (2002–2010): RESULTS OF A SINGLE CENTER IN TURKEY

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Background: Early detection of inborn metabolic disorders (IMD) by tandem mass spectrometry (MS/MS) enables early diagnosis and treatment to minimize morbidity and mortality.
Objectives: To evaluate expanded newborn screening (ENS) results retrospectively and to identify the incidence of IMD in Turkey with our laboratory's data.
Material and Methods: 107463 neonates were screened for IMD by tandem MS/MS from 2002 to 2010. Blood spots were collected between 2nd–7th days after birth. Acylcarnitine and aminoacid concentrations were determined by using tandem MS/MS. Further diagnostic tests were performed on newborns with elevated screening results by metabolic diseases centers and our laboratory.
Results: We detected a total of 170 patients with IMD; 87 patients with aminoacid metabolism defects, 26 patients with fatty acid oxidation defects and 57 patients with organic acidemia. The overall incidence of these diseases was 1:632. Defects in aminoacid metabolism were the most common disorders.
Conclusion: Our study showed that the incidence of IMD detected by ENS is very high in Turkey. The distribution of patients according to years revealed an increased incidence at recent years. This finding may be explained with the more widespread use of the ENS by tandem MS/MS.

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HOW TO OPERATE NEWBORN SCREENING HANDILY AND TO MANAGE THE DATA FREELY? TWO YEARS PRACTICE EXPERIENCE USING A NEW NSIS IN GUANGZHOU, CHINA

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Background: Many Steps, much more links and mass data make it difficult to be operated and to manage its data in neonatal screening. [Objective] To study and set up a new newborn screening information system (NSIS) as one of the basic platforms for neonatal screening of inherited metabolic disorders.
Methods: The new NSIS-part A: was used to collect the information all of the newborn which collected DBSS(dried blood spots samples), and to deal out the screened results after laboratory test. The new NSIS-part B: to service laboratory and treatment for neonatal screening of inherited metabolic disorders. [Results] All the data and information of total 343757 newborn screened from April of 2009 to March of 2011 include the information of newborn and their DBSS data, the laboratory test and its control data, the screening results and positive, the diagnosis and treatment information of babies with IMD can be managed freely. Parents can inquire about the baby' screening result on the appointed website by parents themselves.
Discussion: NSIS is absolutely necessary. Appropriate NSIS make newborn screening to be high efficiency, high accuracy and high safety. NSIS should be improved and be perfected continually.

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URINE BASED NEWBORN SCREENING STUDY APPLYING HIGH-RESOLUTION NMR SPECTROSCOPY IN TURKEY

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Background: Approximately 1:1000 neonates are affected by congenital metabolic diseases in central Europe and 1:500 in Turkey. Undetected and untreated these diseases can lead to irreversible organ failures invalidity or death. Fully automated NMR spectroscopy of body fluids are used an analytical approach for diagnosis known, but also as yet unknown inborn errors of metabolism.
Objectives: Primary objective of the study was to explore the range of variation (concentration and chemical shifts) of specific metabolites without clinically relevant findings. Secondary objective was the integration of the results from a healthy population of neonates into in NMR-knowledge base to perform routine and completely automatic screening for congenital metabolic diseases using targeted and untargeted approaches out of one measurement per sample.
Patients and methods: Urine samples of 512 neonates from 8 centers in Turkey were investigated by using fully automatic 500/600 MHz NMR spectroscopies.
Results and Conclusion: In the urine of 20 neonates we found different pathological metabolites in high concentration. We present and discuss the NMR and clinical data of these children. The statistical analysis and quantification of metabolites allow developing a normal model in specific population also allowing a general assessment of the health state of newborn children.

P-521

A UPLC-MS/MS METHOD IN THE FOLLOW-UP OF POSITIVE CASES FOUND BY MS/MS ON NEWBORN SCREENING IN DENMARK IN THE PERIOD 2006–2011

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In the period January 2006–April 2011 ~300,000 newborns in Denmark were screened by acylcarnitine and carnitine analysis on dried blodspots (DBS). 136 of these screened initially were found positive for one of the 8 diseases: CTD, GA1, HLCSD, LCHADD, MCADD, MMA, PA, VLCADD.

Confirmatory analyses were performed on plasma samples where underivatized acylcarnitines and carnitine were analysed using UPLC-MS/MS. Molecular genetic analyses were also performed.

The following number of cases of true positives/negatives were found: CTD: 8/28, GA1: 6/3, HLCSD: 0/6, MCADD: 34/5, MMA/PA: 1/25, VLCADD: 2/15 LCHADD: 3/0.

The following diagnostic concentration ranges in DBS/plasma were found: CTD(C0): 1.7–4.8 μM /4–6 μM; GA1(C5DC): 0.28–5.60/0.12–2.05 μM; HLCSD(C5OH): N/A; LCHADD(C18:1-OH): 0.46–1.42/0.68–1.78 μM; MCADD(C8): 0.58–14.02/0.15–14.00 μM; MMA(C3): 11.0/18.1 μM; PA (C3): N/A; VLCADD(C14:1): 1.41–1.93/3.39–5.86 μM.

The UPLC-MS/MS method provided improved confirmation of analyte identity by the addition of a chromatographic separation step before mass spectrometric analysis. It decreased ion suppression from co-eluting substances, resulting in improved sensitivity that made it easier to distinguish between pathological and normal values. The method was in all cases able to distinguish between true and false positives. However, the positives were only considered really true positives when two disease-causing mutations had been identified by mutation analysis.

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COMPARISON BETWEEN EXPECTED AND REAL FALSE-POSITIVE RESULTS IN EXPANDED NEWBORN SCREENING

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Background: Tandem mass spectrometry has allowed newborn screening programs to increase the number of disorders studied although more infants receive false-positive results. The number of these depends on the number of disorders screened and on the scenario(highly selected laboratories to real-world settings).

Objective: To compare the expected number of false-positive results using available population-based studies with the real rate of these in our laboratory.

Method:33.941 newborns were screened in 2010 from which we obtained the number of true-positives, false-positives, false-negatives and true-negatives, and calculated the sensitivity, specificity, PPV, NPV, prevalence and false-positive rate. We used the following equation(1):no. of false-positives=(1-(1-(1-prevalence)(1-specificity))k)*(no. of births), where k represents the number of tests performed with tandem mass spectrometry (24 primary targets) and prevalence refers to the average prevalence for an individual disorder screened (1 case per 111.997)(2).

Results: The estimated number of false-positive results was 34 for an expected specificity for an individual test of 99.995%(best-case scenario), 305 for a specificity of 99.95%(intermediate-case scenario)and 807 for a specificity of 99.9%(worst-case scenario). The real number of false-positive results was 653 and the average specificity for each disease was 99.92%.

Discussion: This estimation of specificities compares to real-world settings but all laboratories should document both their true-positive and false-positive results.

(1)Tarini.Pediatrics2006.(2)ZytkoviczClinChem2001.

P-523

RESIDUAL NEWBORN SCREENING BLOOD SPOTS (RNBSBS): AN IMPORTANT TOOL FOR RETRSOPECTIVE DIAGNOSIS OF INBORN ERRORS OF METABOLISM (IEM)

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Argentinean newborn screening panel does not include the analysis of acylcarnitines and aminoacids by tandem mass spectrometry. There are currently no legislation regarding the use and conservation of RNBSBS.
The aim of this work is to show how useful could be the storage of blood spots for retrospective diagnosis.
A 4-month pregnant woman came to metabolic evaluation. Two previous children had died from severe liver failure of unknown cause. Autopsy was not performed; the only biological sample available was the RNBSBS from the last child. Acylcarnitines and aminoacids were analyzed on that sample. High levels of tyrosin(TYR), tyrosine/valine(TYR/VAL) ratio and succynil acetone(SUA) were found suggested Tyrosimenia type I. After birth samples were obtained from cord blood, 12, 24 and 48 hs of life. All the samples presented elevation of SUA, the 12hs sample also had a high TYR/VAL ratio with normal levels of TYR, and in the 24hs sample, TYR was also high. The newborn was put on NTBC treatment from day 4. After 1 and half year of follow-up the patient has a good evolution and better prognosis according the early onset of treatment.
We demonstrate how important and helpful can be keeping RNBSBS for retrospective diagnosis of IEM.

P-524

FROM NEWBORN SCREENING TO OUTCOME EVALUATION: EXPERIENCE, CHALLENGES, AND OPPORTUNITIES FOR LONG TERM FOLLOW-UP OF METABOLIC DISORDERS.

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Background: Systematic collection of outcome data and public health benefits after expanded newborn screening is urgently needed, but scarce. One option is to enhance infrastructures of existing population-based birth defect surveillance programs.
Methods: We expanded two such programs in the US (Utah and Iowa) to conduct surveillance for 19 selected metabolic conditions diagnosed by MS-MS. We developed case definitions and data dictionaries to track diagnoses, treatment, morbidity, mortality, and services used. Metabolic physicians assisted in case review.
Results: The two-state cohort included 52 affected infants (cases) from among 229,353 live births delivered from 2005–2007. MCAD, PKU, and 3MCC accounted for 46 cases. Most cases had follow-up through age 2 years. 3MCC accounted for one-fifth of cases but one-half of those lost to follow-up. By age 2, one-fourth of children had an emergency room visit and one-third a hospitalization; such visits and hospitalizations decreased after age 1 year. Overall mortality was low (n=1, VLCAD deficiency). In terms of workload, these metabolic conditions added about 1% of cases to the surveillance programs.
Discussion: Enhancing birth defect surveillance programs can be an efficient strategy to develop and quickly deploy long-term follow-up of metabolic conditions, provided a systematic approach and best practices are followed.

P-525

NEWBORN SCREENING FOR UREA CYCLE DISORDERS BY MS/MS IN AUSTRIA CLINICAL EXPERIENCE AND OUTCOME

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Background: Newborn screening using tandem mass spectrometry for some urea cycle disorders (UCD), like citrullinemia type I and argininosuccinate lyase deficiency (ASLD), was introduced in Austria in 2003. This brought new challenges for follow up and treatment in patients with mostly mild variants.
Patients: 13 patients with persistent hypercitrullinemia (Cit >50 μmol/l) were detected. Initially measured mean citrulline for citrullinemia was 214 μmol/l; range 72–704; for ASLD 85 μmol/l; range 60–96.
Results: ASLD was confirmed by enzyme activity measurement in erythrocytes in three children. One of these patients had already developed a severe hyperammonemic crisis prior to arrival of the screening result; the others were asymptomatic in the neonatal period. In the other children citrullinemia type I was confirmed by ASS gene mutation analysis. One patient was symptomatic with classical neonatal-onset citrullinemia type I prior to the screening result.
Conclusion: Newborn screening for UCD may not prevent severe hyperammonemic crisis in early-onset forms as the results is available too late. Screening identifies many individuals with attenuated citrullinemia type I and ASLD of uncertain clinical relevance. Further studies of long-term outcome and phenotype/ genotype correlations are necessary to determine the value of newborn screening for these conditions.

A-030

TANDEM MASS SPECTROMETRY: A TOOL FOR RAPID DETECTION OF AMINO AND FATTY ACIDS DISORDERS

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Background: Tandem mass spectrometry (LCMS/MS) is rapidly being adopted to screen dried blood spots for many of metabolic diseases in a single assay. Limited information is available for setting the cutoffs of particular marker and for the resulting positive predictive values.
Methods: We screened>450 subjects by LCMS/MS. The markers were extracted from blood spots into an extraction solution with internal standards and then were derivatized before analysis by LCMS/MS. Cutoffs for each marker were set at 6–14 SD above the population mean.
Results: We identified 32 babies with amino acid disorders (4 phenylketonuria, 1 hyperphenylalaninemia, 5 maple syrup urine disease, 2 hypermethioninemia, 6 hypergylsenimia, 5 tyrosinemia, 2 citrullinimia, 3 with raised ornithine, 2 alanine and 2 argininemia) and 42 babies with fatty acids disorders (1 medium-chain acyl-CoA dehydrogenase deficiency, and 1 presumptive very-long chain acyl-CoA dehydrogenase deficiency,11Carnitine palmitoyl-transferase Ia deficiency,5 Carnitine palmitoyl-transferase II deficiency , 6 Methylmalonic acedimia,13 Malonic aciduria,4Glutaric academia type I and 1 3-Methyl crotonyl-CoA carboxylase deficiency).
Conclusions: In screening for 75 metabolic disorders by LCMS/MS, a mean positive predictive value of 8% can be achieved when using cutoffs for individual markers determined empirically on babies.

A-031

IMMEDIATE AND LATE FOLLOW-UP OF INFANTS WITH CONGENITAL HYPOTHYROIDISM DETECTED THROUGH NEWBORN SCREENING IN INDIA.

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Objectives: Newborns with Congenital hypothyroidism were evaluated by immediate and late follow up to ensure normal growth and development and determine to transient hypothyroidism.
Results: Of 21979 newborns screened in the 1st week of life, fifteen were retested with-in two weeks and. confirmed the diagnosis. Replacement therapy was started by the end of 3 rd week and evaluated at two weeks, six weeks, and three months in the first year. Late follow up after one year included developmental evaluation and thyroid function once in six months. At three years, re-evaluation done after withholding treatment.
Conclusion: Incidence of CHT was 1:1465. 40% of the cases had demonstrable thyroid gland abnormalities such as aplasia, hypoplasia and dyshormonogenesis. Treatment initiated by third week of life and at one year the developmental assessment and cognitive functions were normal in all the children.Long term follow up. Two of the children at 3 years of age were off the treatment and on follow up, one child had normal thyroid function and remained normal after 3 months of cessation of treatment indicating transient hypothyroidism. The other child showed elevation in TSH requiring restarting of thyroxin.

A-032

EXPANDED NEWBORN SCREENING: CHALLENGES IN MALAYSIA

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NBS for screening of metabolic disorders in Malaysia is carried out mainly in two centres, namely Institute for Medical Research (IMR) and Doping Control Centre (DCC). The former is under the auspices of Ministry of Health, Malaysia while DCC is set-up under Universiti Sains Malaysia. To our knowledge, a pilot study by IMR was completed in 2009. The outcome of this study has been published elsewhere and the impact of the findings has yet to trigger serious involvement from the Malaysian government. Initiation of NBS in Malaysia has been slow because of variety of factors and internal barriers. We believe it is important that healthcare authorities understand the rational for NBS existence and disseminate this information to the public. We also have difficulties integrating the NBS into public health care system due to the lack of support from the policy makers. We have now developed media based campaigns and the initial finding is positive towards accelerating community support for NBS. To ensure the entire population can have access to NBS in the near future, we will continue to educate the public who will in turn become the pressure group to the government for the implementation of NBS in the country.

A-033

ACRODERMATITIS ACIDAEMICA DUE TO FALSE POSITIVE NEWBORN SCREENING

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Background: Tandem mass spectrometry (MS/MS) based newborn screening programs (NBS) allow early diagnosis and treatment for some inborn metabolic disorders (IMD). Sometimes TM have false positives. We report two cases with acrodermatitis acidaemica (AA) due to false positive newborn screening (FPNBS) of MS/MS.
Patients: In a cohort of 272 patients with IMD, diagnosticated from January 2004 to December 2010 we have detected 4 patients with AA and 2 with of false positive of MS/MS.
Results: All patients with AA had low levels of isoleucine and normal levels of zinc, but the two AA with FPNBS began AA 24 hours after of start restricted protein diet, much earlier than the other AA case whom started during intercurrent infectous process.
Conclusions: When patients began an AA cutaneous rash 24 hours after of start restricted protein diet, we must think in a FPNBS in theses patients.

O-069

FOLLOW UP USING NMR SPECTROSCOPY OF 13 ADULT NIEMANN PICK C PATIENTS TREATED WITH ZAVESCA

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Niemann-Pick C (NPC) is a fatal progressive neurolipidosis. Miglustat, an inhibitor of glycosphingolipid synthesis, has been approved for the treatment of progressive neurological manifestations in children and adults with NPC. A preliminary study in three patients suggested that brain magnetic resonance spectroscopy (MRS) could be used routinely as a non invasive surrogate marker of treatment efficacy (Galanaud et al., 2009). Here we report additional evidence that treatment with Miglustat improves the Choline/Creatine (Cho/Cr) and Choline/NAA (Cho/NAA) ratios in the white matter of NPC patients. Among 13 adult NPC patients treated with Miglustat, 11 exhibited a decrease in their Cho/NAA ratio over the first 6–12 months of treatment. Two patients showed an increase of these ratios during the first 6 months but longer follow up was not available. Follow up of one untreated patient showed stable increased Cho/NAA values after 4 years. Values of Cho/NAA ratios did not correlate with clinical severity scores. Only one patient showed clear clinical progression despite improvement of NMRS. Overall, these results confirm improvement of MRS in NPC patients treated with Miglustat. This improvement is only visible after a year in some patients. Further work is necessary to link MRS ratios to clinical parameters.
Conflict of Interest declared.

P-526

LESSONS FROM PRACTICE : UNRAVELLING THE INBORN ERRORS OF METABOLISM IN ADULTS

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Background: With more than 350 different diseases identified to date, IEMs represent about one-third of genetic diseases, nevertheless they are overlooked as cause of neurologic disorders in adults.
Methods: Our study involved 192 patients evaluated in the neurogenetics clinics by geneticists and neurologists in a two-year period. Clinical anamnesis, physical exam, neuroimaging studies , ophtalmological and auditory evaluations, neurophysiological studies, biochemical tests, muscle biopsy with respiratory chain mitochondrial enzymes, lysosomal and peroxisomal studies and, when indicated, nerve/skin biopsy for EM studies and karyotype were performed in the course of the investigation.
Results: IEMs were identified in 62 patients. Adrenomyeloneuropathy, AVED, abetalipoproteinemia, Fabry disease, Niemann-Pick type C, OTC deficiency, intermitent acute porphyria and mitochondrial disorders were the commonest IEMs diagnosed. Lesch-Nyhan syndrome, AMACR deficiency, cerebrotendineous xanthomatosis and coenzyme Q10 deficiency were other rare IEMs identified. In 93 patients, it was possible to rule out an IEM as cause of the neurological syndrome; 37 patients are still under investigation.
Conclusion: Hereditary metabolic disorders can be an important cause of neurological disorders in adults and should not be missed, specially because many IEMs are often treatable diseases and, even the absence of a specific treatment, genetic counselling can be offered to the families.

P-527

TRANSITION OF YOUNG PATIENTS WITH INHERITED METABOLIC DISEASES (IMD) FROM PEDIATRIC TO ADULT CARE

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Objectives: The aim of our study was to evaluate IMD patients' satisfaction with the transition process and detect how it could be ameliorated.
Methods: A group of 93 adult patients with amino acids, carbohydrate, urea cycle defects, organic acidurias and lysosomal disorders were included. The adult outpatient clinic is located at the Division of Neurology of the St. Bassiano Hospital. The transition process considered the last visit in the pediatric setting and the first two visits in the adult one. Perceived satisfaction was evaluated with a questionnaire-interview through three main domains: patients'care, psychological aspects, logistics.
Results: The majority of patients were satisfied (90%). Positive features reported were: perception of independence and greater sense of control; the presence of new adult specialists required for adult care (i.e. vulnologist, cardiologist, gynecologist); regular clinical contacts with the adult metabolic expert, adult specialists and metabolic pediatricians; the neuropsychologist serves as the transition process reference. Major complaints were: lack of dietitian in the adult center; distance between the centers (50 km).
Conclusions: The transition process for IMD patients is a fundamental process that cannot be deferred. The major achievement for the patients is an improved self-consciousness of their disease and increase personal management of therapy.

P-528

PARENTAL VIEWS ON CONFIDENTIALITY WHEN ADOLESCENTS ARE SEEN ON THEIR OWN IN THE METABOLIC CLINIC

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Clinical guidelines suggest that adolescents should be seen on their own for part of clinic consultation. However, little is known of parental attitudes on this issue. We explored the views of parents of adolescents in our Metabolic Clinic, in which this is standard practice.
Of 40 eligible parents, 33 completed a questionnaire handed to them in clinic, seeking parental understanding of confidentiality, their views about when information should be shared with them, and their perception of benefits/concerns of consulting with young people alone.
The main advantages parents identified in adolescents seeing clinicians alone were: practicing talking to the doctor alone; taking responsibility for their own health; help them become more mature. Concerns identified included the possibility of not being informed about important issues (including eating disorders, cigarette smoking, alcohol and illicit drug use, pregnancy, and problems with parents); not being informed of the treatment plan, and concerns about their child possibly not understanding the issues or not remembering the treatment plan. Parental understanding of a confidential consultation with young people alone was remarkably similar to that in a general adolescent clinic (N=86).

P-529

ADULT ONSET ORNITHINE TRANSCARBAMYLASE DEFICIENCY: A RARE CAUSE OF LIFE-THREATENING HYPERAMMONEMIA IN ADULTS

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Background: Ornithine transcarbamylase (OTC) deficiency is an inherited metabolic disorder, better known to pediatrician because it is usually diagnosed during the neonatal period. Descriptions of late-onset forms are scarce but they are often fatal due to hyperammonemia. We report five adults who presented with various OTC deficiency symptoms after the age of 25 years.
Patients and Methods: We retrospectively studied five patients (three males, two females), and collected clinical, biochemical and molecular features.
Results: A 65-year old man, a 57-year old and a 43-year old female presented with acute neurological symptoms after unusually high protein intake. Ammonemia was high (78 to 327 μmol/L), associated with high orotic aciduria excretion. They were successfully treated with protein restriction and high caloric diet, a supply of ammonia scavenging medication, and hemodialysis in two cases. Two other asymptomatic brothers were diagnosed at 36 and 26 years of age after systematic screening owing to two fatal cases in their family. OTC deficiency was confirmed by DNA sequencing in four cases.
Conclusion: OTC deficiency can present with a large spectrum of clinical characteristics. We report five cases of late-onset presentation of OCT deficiency, including the oldest one to our knowledge in the literature.

P-530

PHENOTYPIC VARIABILITY IN UREA CYCLE DEFECTS(UCDS): 20 PATIENTS(PTS) DIAGNOSED IN ADULTHOOD IN ONE CENTER ONLY

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14 females (F) and 3 males (M) were affected by ornithine transcarbamylase deficiency (OTC), 2 F by citrullinemia, 1 F by argininosuccinic aciduria(AAS). Mean age at diagnosis 38 years (y), median 36y, range 17–82y. Most of them reported proteins avoidance since childhood. Diagnosis of OTC deficiency in 1 F and 2 M was made in peri-or post-mortem hyperammonemic coma (1 F 27y in the post-partum; 1 M 21y after a road accident; 1 M 45y after chronic steroid therapy and reduced caloric intake); 13 F and 1 M (56y) were diagnosed for family history. 2 citrullinemia F during the hyperammonemic coma in the postpartum (36 and 39y), one reported in Haberle et al, 2010. The AAS F following a post-surgery metabolic decompensation (32y).
16pts are alive (mean age 40y), 4 OTC (2 M and 2 F) died. One OTC F underwent liver transplantation and later had a pregnancy. The AAS pt has mental retardation, optic sub-atrophy and psychiatric problems. 1 M and 9 OTC F are asymptomatic with free diet. 3 OTC, 2 citrullinemia and 1 AAS pts have metabolic treatment and low protein diet.
UCDs must be included in the differential diagnosis of hyperammonemia at any age.

P-531

BODY COMPOSITION IN ADULTS WITH UREA CYCLE DISORDERS

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Urea cycle disorders (UCD), are associated with protein aversion and managed by low-protein diet, avoidance of catabolism and supplementation with specific amino acids and nitrogen-scavenging drugs where necessary. These dietary modifications may influence body composition.
We measured body composition in a group of UCD adults (12 F, 5 M; 2 F, 1 M non-OTC), aged 33.6±14.7 years (mean±SD) and body mass index 26.3±8.9 kg/m2 using prompt gamma neutron activation analysis (IVNAA) for total body protein (TBP), and total body dual-energy x-ray absorptiometry (DEXA) for bone mineral density (BMD), skeletal muscle mass (SMM) and total body fat (TBF).
In females, %TBF was 36.3±10.4, TBP expressed as an age- and sex-adjusted nitrogen index (normal NI=1.00±0.10) was 0.92±0.13, and SMM measured as height-adjusted appendicular lean tissue mass (ALTM) was 5.76±1.33 kg/m2. BMD z-scores were −0.61±1.60 (lumbar spine), -0.43±1.59 (femoral neck) and −0.47±1.62 (total body). In affected males, %TBF was 27.4±12.0, NI was 1.04±0.13, and ALTM was 8.10±0.23 kg/m2. BMD z-scores were −0.47±0.97 (lumbar spine), 0.00±0.64 (femoral neck) and −0.41±1.01 (total body).
UCD adult females with lifelong dietary protein aversion have reduced TBP and SMM as well as increased adiposity. Adult male survivors in this small sample do not differ significantly from healthy adults.

P-532

HYPERAMMONEMIA IN STARVATION FOLLOWING BARIATRIC SURGERY

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Hyperammonemia complicating bariatric surgery has been only rarely described1-3. After exclusion of a urea cycle defect, the mechanism underlying this remains unclear. We describe a 52 year old woman with a BMI of 31 who underwent billary pancreatic bypass (duodenal switch) Following operation she had several months of very poor oral intake and developed recurrent episodes of hyperammonemic encephalopathy. Empirical antibiotic therapy for bacterial overgrowth was not beneficial. Amino acid profile showed uniformly very low levels of amino acids including glutamine. Plasma acylcarnitines were normal. Urinary orotic acid profile was below detection limit on a number of occasions. Gene analysis for possible CPS and NAGS deficiency was undertaken with no pathogenic mutations identified. Because of the persistence of her symptoms she underwent reversal of her gastric surgery and had a dramatic improvement, with a normalized amino acid profile and an improved appetite. There have been no further hyperammonenic episodes. We hypothesize that this nutritionally deficient patient developed substrate deprivation of urea cycle intermediates and tissue depletion of glutamine, impairing its use as an ammonia shuttle to the liver, both leading to decreased urea cycle activity.

P-533

BODY COMPOSITION IN ADULTS WITH PHENYLKETONURIA

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Phenylketonuria (PKU) adults modify diet by phenylalanine restriction, protein restriction, or relaxation of diet, potentially affecting body composition.
We measured body composition in PKU adults (33 F, 9 M) aged 32.2±9.5 years (mean±SD), and body mass index (BMI) 27.9±6.4 kg/m2 using air-displacement plethysmography (ADP) for total body fat (TBF), prompt gamma neutron activation analysis (IVNAA) for total body protein (TBP), and total body dual-energy x-ray absorptiometry (DEXA) for both bone mineral density (BMD) and skeletal muscle mass (SMM).
In females, %TBF was 37.1±9.1, TBP using age and sex adjusted nitrogen index (normal NI=1.00±0.10) was 0.98±0.12, and SMM measured as height-adjusted appendicular lean tissue mass (ALTM) was 5.77±2.35 kg/m2. BMD z-scores were −0.27±1.26 (lumbar spine), -0.01±1.01 (femoral neck) and +0.07±1.15 (total body). In males, %TBF was 20.3±8.2, NI was 1.13±0.13, and ALTM was 8.24±0.45 kg/m2. BMD z-scores were −0.75±1.22 (lumbar spine), -0.23±1.47 (femoral neck) and −0.09±1.32 (total body).
PKU adults treated longterm with protein-modified but often high-energy diets have normal TBP and BMD, but adiposity is increased in women.

P-534

GLYCOGEN STORAGE DISEASE TYPE I IN WOMEN: FERTILITY, PREGNANCY AND QUALITY OF LIFE. RESULTS OF A MULTICENTER ITALIAN STUDY.

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Background: life expectancy of patients with glycogen storage disease (GSD) type I has improved considerably, opening new problems correlated with adult age. Fertility and pregnancy represent a big issue for women. Reduced quality of life has been described in children, but no data are available for adults.
Patients and methods: a total of 31 female with GSD I who were≥ 16 years (mean age 27±7) were included. Data about fertility and pregnancies were obtained from clinical records and interviews. 23 patients completed the questionnaire SF36 for quality of life assessment.
Results: 25.8% of patients had delayed menarca; 16.1% of patients had documented polycystic ovaries. 5 successful spontaneous pregnancies in 4 patients with GSD Ia and 2 in a woman with GSD Ib were reported. The latter had development and enlargement of hepatic adenomas during pregnancies. While standardized physical and mental component scales were near normality in GSD Ia patients, they were lower in GSD Ib and inversely correlated with the number of drugs taken.
Conclusion: successful pregnancies are possible in women with GSD Ia and Ib, but monitoring for adenomas is mandatory. Impaired quality of life is evident especially in patients with GSD Ib.

P-535

PREVALENCE OF FABRY DISEASE IN A FRENCH COHORT OF 900 YOUNG PATIENTS WITH ISCHEMIC STROKE: THE FIND STUDY.

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Background: Fabry disease is a X-linked lysosomal storage disorder leading to multi-systemic life-threatening complications including an increased risk of stroke in young adults.
Methods: The FIND ("Fabry: Initiative Nationale de Dépistage") was conducted between 2007 and 2009. Cases were men, aged 28 days to 55 years, with a first or recurrent ischemic stroke (50 adult neurology centers and 8 neuropediatric departments). Enzymatic activity of α-galactosidase A was measured by dried blood spots (DBS) using a filter-paper test. When activity was below a threshold a second visit was scheduled to answer a questionnaire oriented to confirm FD diagnosis using the gold standard leucocyte enzyme activity assay.
Results: The study sample consisted of 902 men with a mean age of 43 years old. Low plasma α-galactosidase A activity was detected in 3 patients but enzyme activity didn't confirm FD diagnosis. A 59-year-old man, who was wrongly included , also showed a reduced activity of α-Gal A. FD diagnosis was confirmed by a reduced enzyme activity.
Conclusions: Specific populations may be screened systematically for FD with a simple method using DBS. Our results suggest that the yield for FD screening in young patients with ischemic stroke is low.

P-536

OPTIMIZATION OF PRE-EXERCISE MEDIUM-CHAIN TRIGLYCERIDES SUPPLEMENTATION BY REAL-TIME MONITORING IN PATIENTS WITH LCHAD

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Supplementation of medium-chain triglycerides prior to exercise has been shown to reduce the biochemical abnormalities of long-chain fatty acid oxidation in individuals with long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency. It has also been shown to increase levels of β-hydroxybutyrate and lower heart rate during exercise. Titrating the patient's dose to their metabolic needs may optimize the therapeutic benefit. By optimizing the dose of pre-exercise MCT oil, we hope to avoid exercise-induced rhabdomyolysis and reduce the risk for arrhythmia by eliminating muscle catabolism and break down of endogenous long-chain fatty acids. In order to test this in the clinic setting, we designed a real-time protocol that could be implemented by the patient, without supervision, using facilities readily available in the community. We focused on monitoring CK, β-hydroxybutyrate, lactate and acylcarnitines for potentially toxic long-chain species. Monitoring was by serum samples and blood dot cards performed at measured time points, pre- and post-MCT oil supplementation and prescribed exercise. Preliminary results suggest that real-time monitoring of patients with LCHAD is feasible and allows determination of clinically relevant parameters; enabling fine tailoring of management plans to suit the patient's individual and potentially changing needs.

P-537

3-PHOSPHOGLYCERATE DEHYDROGENASE DEFICIENCY PRESENTING WITH A CHARCOT-MARIE-TOOTH-LIKE POLYNEUROPATHY IN AN ADULT

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Serine synthesis defect caused by 3-Phosphoglycerate dehydrogenase (3-PGDH) deficiency is considered to be a rare but treatable cause of epileptic encephalopathy in children. Two clinical forms of PGDH deficiency have been described until now, that differ mostly by their degree of severity. The infantile form associates congenital microcephaly, severe psychomotor retardation and intractable seizures, inconstantly associated with cataracts, nystagmus, and spastic tetraparesis. A milder form has been reported in two siblings who presented with absence seizures of juvenile onset and mild developmental delay. Here, we report the case of genetically confirmed 3-PGDH deficiency in an adult who presented with congenital cataract, mild psychomotor retardation, and chronic axonal sensorimotor polyneuropathy mimicking Charcot-Marie-Tooth (CMT) disease. Amino acid analysis showed low serine levels in plasma and Cerebrospinal fluid (CSF). Treatment with high dose of serine resulted in normalization of plasma serine values and subjective functional improvement.

P-538

MATERNAL PROPIONIC AND METHYLMALONIC ACIDEMIA. HOW SHOULD FOLLOW UP BE DURING PREGNANCY?

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Background: Present experience in diagnosis and treatment of the organic acidemias and all Inherited Metabolic Diseases, has allowed that women, with these pathologies, consider being mothers. Fear of possible descompensations during pregnancy and/or childbirth, and the ignorance of sanitary personnel involved in the care of the patient, generates great anxiety to the patient in consdiering having a child. We report 3 successful pregnancies in women with organic acidemias.

Methods: During each pregnancy profiles of amino acids, acylcarnitines, carnitine in plasma and organic acids in urine were performed. Patients sent samples of dry blood weekly for determination of amino acids and acylcarnitines and monthly for obstetric and diet evaluation and determination of urine organic acids.

Results: We observed a great decrease of propionylcarnitine and total canitine. Oral carnitine supplement was required in three pregnancies. We also observed a decrease in maternal essential amino acids, but restricted protein diet was necessary all through the pregnancy. Changes in organic acids were not observed.

Conclusions: We have observed a distinctive but common metabolic profile in 3 pregnancies. An exhaustive control of amino acids, acylcarnitines, carnitine and organic acids and diet management is essential for a good outcome in maternal organic acidemias.

P-539

A CASE OF RAPID DETERIORATION OF A PATIENT WITH ASYMPTOMATIC MOLYBDENUM CO FACTOR DEFICIENCY AFTER 12 YEARS OF PRESUMED NORMAL AND HEALTHY LIFE

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Molybdenum cofactor is essential for sulphite oxidase and xanthine dehydrogenase activities; and its deficiency is usually associated with devastating neurological manifestations. Although milder cases with later onset have been identified, the classical presentations are often fatal in the early age. We present a follow-up case of 23-year-old Caucasian female who was diagnosed at 11 years of age following similar diagnosis made in her younger sibling [1]. Biochemical profiles of her blood and fibroblast were consistent with the diagnosis. This was subsequently confirmed with MOCS2 gene analysis which showed compound heterozygous mutations. Despite a strikingly abnormal initial brain imaging, she was otherwise asymptomatic apart for mild lenses dislocation. Up until recently, she led a normal healthy life with stable employment and fully independence. Following recent serial family bereavements, she presented to a local neurology department with dramatic deterioration. She has marked apathy, dysarthria, dysphagia, associated with generalised dystonia but preserved comprehension. Serial brain imaging reveals significant progression in basal ganglia abnormalities and white matter changes. This case illustrates the dilemma in managing asymptomatic individuals over a prolonged period in this extremely rare condition.

P-540

SEARCHING FOR THE GOOD DOSAGE OF PYRIMETHAMINE IN PATIENTS WITH LATE ONSET TAY SACHS: SEQUENTIAL TREATMENT SEEMS THE BEST

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Late-onset Tay-Sachs (LOTS) is an autosomal recessive, neurodegenerative, lysosomal storage disease, caused by deficiency of ß-hexosaminidase A (Hex A). In vitro and in vivo studies suggest that Hex A activity can be partially rescued by the drug pyrimethamine. Here, four adult patients with LOTS were treated with different regimen of pyrimethamine for a total of 24 months. In vitro tests in patient fibroblasts prior to treatment shown a good response with 79 to 189% increase in basal Hex A activity. In vivo treatment response was assessed by repeated measures of Hex A activity in leukocytes. Although Hex A increased in all patients during the first four months of treatment, mirrored by some clinical improvement, prolonged inhibitory effect was observed in all patients after this initial period of time. This inhibitory effect was not reversed by decreasing the dosage of pyrimethamine, but only when the drug was reintroduced after at least two weeks of wash out. Attempts to reintroduce the drug at lower dosage (25 mg/d) resulted in the same inhibitory effect after 2–3 months. Finally, a cycle with continuous treatment for two months (25 mg/d) followed by two weeks of wash out resulted in the most sustained response.

P-541

PROTOPORPHYRINOGEN OXIDASE GENE ANALYSIS IN JEWISH FAMILIES WITH PORPHYRIA VARIEGATA

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Porphyria variegata (PV, OMIM 176200), is caused by a partial deficiency of protoporphyrinogen oxidase (PPOX, E.C.1.3.3.4.) and its clinical manifestations include acute neurovisceral attacks and/or cutaneous photosensitivity. Biochemical diagnosis of PV is based on abnormal fecal porphyrin profile, fluorometric plasma scan, and increased urinary levels of porphyrin precursors during an acute attack. In this study, the PV diagnosis was determined in index patients from 9 unrelated Jewish families on the basis of clinical symptoms and biochemical findings. DNA analysis revealed 5 mutations in the PPOX gene, four novel (p.Trp42X, p. Gly187Arg, p.His333Arg, p.Ala466Glu), mutation p.Val84Gly has been previously described. Mutation p.His333Arg was found in five unrelated families, the remaining mutations were specific only for the individual family. All families with mutation p.His333Arg are from Morocco, the mutation might be a founder one in Moroccan Jewish population. This mutation was not found in DNA samples of healthy unrelated controls of Moroccan Jewish origin (n=280) and Ashkenazi Jewish origin (n=330). The identification of the asymptomatic family members will reduce risk of PVonset by avoiding triggering factors. (Supported by grants 1 M0520 and MSM0021620806 from MSMT CR)

P-542

MOLECULAR PATHOLOGY OF ERYTHROPOIETIC PROTOPORPHYRIA IN A CZECH FAMILY

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Erythropoietic protoporphyria (EPP) is characterized by excess accumulation of protoporphyrin, particularly in the erythroid cells. EPP inheritance is complex, almost always associated with two molecular defects. In majority of EPP patients, clinical expression requires coinheritance of a private FECH mutation trans to a hypomorphic FECH*IVS3-48 C allele. This leads to decrease of FECH activity below threshold of cca 35%. Clinical manifestations of the disease are characterized by cutaneous photosensitivity in early childhood (burning, itching, swelling, and redness) in sun-exposed areas. Hepatic failure occurs in some patients (about 1-10% of EPP patients) which may necessitate liver transplantation (www.porphyria-europe.com; Lancet, 375: 924–937, 2010). We investigated mother and son of Czech origin with manifest EPP and 7 members of their family in 4 generations and found a novel mutation in the FECH gene in 4 individuals including probands (G→A transition at position 84 in exon 2; W28X, located in a mitochondrial targeting sequence). Both clinically manifest probands (in son, recently, a liver transplantation was performed) inherited a hypomorphic allele as well, while two clinically latent individuals with FECH mutation did not. (Supported by grants # 1 M0520 and MSM 0021 620806 from MSMT of Czech Republic)

P-543

CEREBROTENDINOUS XANTHOMATOSIS: TREATMENT MONITORING IN 7 PATIENTS USING BRAIN MAGNETIC RESONANCE SPECTROSCOPY

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Cerebrotendinous xanthomatosis (CTX) is a progressive neurodegenerative disorder caused by mutations in CYP27A1, the gene encoding sterol 27 hydroxylase. The enzymatic block leads to abnormal production of cholestanol, a toxic cholesterol derivative that accumulates in peripheral tissues and brain. Although treatment with chenodeoxycholic acid (CDCA) has been used for more than 25 years to stabilize or improve clinical manifestations, evidence of efficacy comes solely from isolated case reports and small clinical studies. Here, by using magnetic resonance spectroscopy (MRS), we show unambiguously from the follow up of 7 patients only, that treatment with CDCA improves brain function, as reflected by a significant and gradual decrease in the white-matter choline/creatine and choline/NAA ratios. This improvement in spectroscopic parameters was accompanied by a significant improvement in the Mini-Mental State Examination (MMSE) score and in some nerve conduction velocities. These findings indicate that MRS can be used as an objective quantitative method for monitoring treatment efficacy in small groups of patients with rare neurological disorders.

P-544

A MITOCHONDRIAL T-RNA MUTATION LYSINE CAUSING ISOLATED SEVERE MITOCHONDRIAL MYOPATHY

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Several mutations in mitochondrial transfer RNA genes cause mitochondrial myopathy. The mtDNA gene MT-TK encoding tRNA lysine is commonly associated with MERRF. Common mutation is A-to-G transition at nucleotide 8344. This mutation can also be associated with isolated “limb-girdle” myopathy.

We describe a 35-year-old caucasian female with a history of progressive exercise intolerance and increased CK. Physical examination showed a limb girdle weakness, facial diparesis, limitation of abduction on eye movements without ptosis and arreflexia. Lung function studies showed a restrictive pulmonary syndrome. The muscle biopsy revealed numerous ragged red fibbers. An A-to-G transition at nucleotide position 8344 in tRNA lysine gene was detected in 71% of the patient´s lymphocytes. At the age of 36 she gave birth to a male child with high plasma lactate levels and normal development. After she developed symptoms of nocturnal hypoventilation and non-invasive ventilation was initiated. Six years after the initial diagnosis she needed help to get up, but walked alone and needed nocturnal non-invasive ventilation.

In our patient symptoms were limited to a skeletal myopathy, “limb-girdle” distribution and respiratory involvement. Isolated mitochondrial myopathy is a rare form of presentation of the mutation tRNA Lys A8344G. Severe cases like this have been rarely described

P-545

MELAS SYNDROME IN ADULT NEUROLOGIC DEPARTMENT

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Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke (MELAS) syndrome is a rare multisystemic disorder with typical onset in childhood caused by mutations in mitochondrial DNA.
Cases reports
1: 28-year-old man, presenting with 2 acute episodes of behavioural and speech disorder with focal deficits and parieto-temporal lesion on MRI. The diagnosis of MELAS was based on his phenotype and history of hypogonadism and hypothyroidsm. MELAS was confirmed with 3243A>G mut.
2: A low stature boy beginning with seizures at 13 years. The MRI revealed multiple "vascular" lesions (thalamic and occipital bilaterally). Over the time, seizures increased in frequency and developed other neurological signs in relation with news lesions. The diagnosis was suggested by muscle biopsy. The respiratory chain was deficient in complex I. He died at age of 21.
3: 47-year-old man, presented at 45Y with partial seizure. CT appeared an extensive pseudo-stroke. About 7 months later he developed right homonymous hemianopsia and aphasia secondary a new left occipito-temporal lesion. Myoclonic epilepsy and metabolic acidosis began. Muscular biopsies suggest MELAS.
We present 3 MELAS, age at onset 13–45, with stroke-like episodes and associated seizures. The diagnosis was obtained by typical MRI, muscular biopsy and positive genetic analysis in one of them.

P-546

LEIGH4S SYNDROME AND IT'S PHENOTYPIC VARIABILITY IN THE ADULT METABOLIC POPULATION

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Leigh's syndrome is a mitochondrial disease related to various enzymatic defects that affect the oxidative metabolism and may occur in atypical way.
Cases:
1: 34 years old male begins ataxia, gait impairment and frequent falls at 3 years old. Later develops uncontrollable vomiting, ophthalmoplegia, hemiparesis and right hemidystonia. Laboratory tests show mutation of the gene SURF1.
2: Male, 22 years old, with depressed mood at the age of 10 and behavioral changes associated with auditory-verbal hallucinations at 19 years old.
3: 22 years old lady, with hypertrophic cardiomyopathy diagnosed at 3 years old. She started loss of coordination, at 8 years, progressing to a lower limb dystonia, involuntary choreo-dystonic movements and cognitive deterioration with increasing learning disability.
4: Female who started at the age of 13, after a flu syndrome, a "Guillain-Barré syndrome like" with generalized hypotonia, distal tetraparesis and a bilateral
hypoesthesia in glove and half. She died at 20 years old after development of neurologic deteriorations.
All this 4 patients have lactic acidosis and typical Brain MRI.
We bring 4 patients with Leigh's syndrome, who have distinct age or mode of presentation and clinical course. We intended to enhance the great phenotypic variability of this neurometabolic disease.

P-547

MUSCLE BIOPSY FINDINGS IN ADULT MITOCHONDRIAL DISORDERS

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Mitochondrial respiratory chain disorders (MRC), defined as primary diseases of the oxidative phosphorylation system are difficult to diagnose and classify.
The aim is to characterize primary findings on histopathology of muscle biopsies performed in patients with definitive MRC.
The study included patients of our neurometabolic adult consultation that fulfilled Walker's criteria for definitive MRC and had had a muscle biopsy performed. A total of 48 patients were assigned. 37.5% of the biopsies were considered normal. In the group with clinically predominant muscle involvement (group 1), the biopsy showed changes consistent with mitochondrial disease in 82% of cases against 37,5% on the other patients (group 2). COX-negative fibres were found in 64% of group 1 and 37,5% of group 2. In the first group, "red-ragged fibres" were seen in 71,4%, but only in sufficient number to be considered pathologic in 46,4%, while in the second group these numbers decreased to 50 and 25% respectively. Some other minor changes were seen: atrophy, blue-ragged fibres and increase in the variability of fibres diameter.
Muscle biopsy is an important study in MRC disorders, and there appears to be good correlation between changes in it and a diagnosis of MRC with predominant involvement of muscle.

P-548

MULTIPLE SYMMETRIC LIPOMATOSIS (MADELUNG DISEASE): THINK MERRF!

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Multiple symmetric lipomatosis (MSL), also called Madelung disease, is a disorder characterized by non encapsulated lipomas and fat accumulation around the posterior cervical region and upper trunk. It has been described in patients with myoclonic epilepsy with ragged-red fibers (MERRF) syndrome.
We report a 73-year-old woman with MERRF syndrome presenting with MSL. First symptoms consisted of progressive muscular fatigability of lower limbs at the age of 69 years. There was no history of myoclonus, epilepsy or ataxia. Physical examination showed an abnormal fat distribution and giant lipomas of shoulders and neck. There was no amyotrophy and muscular strength of limbs was normal.
Serum CK was 207 U/l, lactate 3.2 mmol/l and pyruvate 135 μmol/l (lactate/pyruvate ratio 23.7). Urinary organic acids were normal. EMG disclosed myopathic changes in the upper limb-girdle muscles. Histology of quadriceps muscle revealed lipid accumulation and ragged-red fibers. Blood DNA analysis identified a heteroplasmic m.8344A>G mutation of the tRNA-lysine gene.
Although a rare initial presentation, MERRF should be sought for in all patients with MSL given its potential severity and the implications for genetic counselling. As the mitochondrial content of adipocytes is high, it is thought that the 8344A>G mutation interferes with the maturation of fat cells.

A-034

HELPING TREATED ADULTS WITH PHENYLKETONURIA (PKU) RETURN TO DIET ISSUES TO CONSIDER

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Background: A key issue around management of adults with PKU is whether lifelong dietary treatment is essential in all patients to maintain neurological well being. The documented complications of PKU in adulthood are neurological and neuropsychological complications, dietary deficiencies and risks to the foetus in maternal PKU.
Method: Literature was reviewed. A questionnaire about practical aspects of managing adults with PKU was developed for clinicians.
Results: Common reasons for adults with PKU returning to diet include: Planning for pregnancy, negative 'symptoms' when off diet and to improve sperm motility in males.
Common problems faced by adults with PKU returning to diet include: Practicalities of diet and lifestyle, lack of cooking skills and equipment, lack of support, lack of dietary knowledge, motivation, past experience of diet and lack of adult services. Health professionals working with adults with PKU are using a variety of innovative strategies to meet the needs of their patient group.
Conclusion: Adults with PKU commonly face several problems when returning to diet. Practical solutions can be offered to address issues. The need for advocacy to encourage government agencies to provide funding for adequate on going care for this patient group was identified as a world wide issue.

A-035

A LATE ONSET METHYLMALONIC ACIDURIA WITH HOMOCYSTINURIA

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Male,63 years(y) old, normal weight and height, cognitively adequate. From 30 to 53y he had: membranoproliferative glomerulonephritis, atrial fibrillation, tibial vein thrombosis, polyneuropathy, pulmonary thromboembolism and a diagnosis of homocystinuria (plasma homocysteine 268 μmol/L) and antiphospholipid syndrome. He started oral anticoagulant, pyridoxine, folic acid, vitamin B12 therapy without benefit. From 56 to 62y: he had: transient ischemic attacks with following dizziness, subarachnoid hemorrhage, partial renal artery thrombosis.
The analysis of cystathionine beta synthase, methylenetetrahydrofolate and Transcobalamin II genes did not show mutations. At 63y, urine and plasma analysis showed increased urinary methylmalonic acid (ur.MMA) (2706 mmol/mol creatinine) associated to normal vitaminB12 (789 pg/ml) and hyperhomocysteinemia (357 μmol/L) and a diagnosis of methylmalonic aciduria (MMA) with homocystinuria was done. He started treatment with betaine (up to 21gr/day), hydroxocobalamin (OHCbl; 1 mg/day orally) and a low-protein diet (40 g/day) decreasing dizziness, plasmatic homocysteine (latest value 107 μmol/L) and ur.MMA (latest value 259 mmol/mol creat ur). MMACHC gene analysis showed only one mutation (p.Arg2106Gln), the [1–14 C] propionate uptake in fibroblasts was reduced and normalized in OHCbl-supplemented medium. MMADHC gene analysis is ongoing.
Before undertaking expensive genetic testing, a dosage of ur.MMA should be performed when homocystinuria is not associated to a vitamin B12 reduction

O-070

SYNDROME OF HYPERMANGANESAEMIA, DYSTONIA AND LIVER CIRRHOSIS—PHENOTYPES OF 14 NEW PATIENTS

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Since our publication (Tuschl et al, JIMD, 2008) describing two siblings with inherited hypermanganesaemia, dystonia and liver cirrhosis, we have collected data on additional fourteen patients from seven unrelated families. All of these patients have sequence changes in the same gene; expression studies are in progress to confirm they disrupt the function of the gene product.
Following normal initial development, patients typically present at the age of two to fourteen years, with gait disturbance and increased muscle tone in the lower limbs. Some patients also have upper limb involvement causing difficulties with fine motor movements. MRI imaging is characteristic with hyperintensity of the basal ganglia on T1 weighted scans and no corresponding abnormality on T2-weighted scans. Laboratory investigations show polycythaemia (Hb 16–22 g/dL) and whole blood manganese level above 2000 nmol/L. The degree of abnormality of liver function varies quite considerably—we have identified two further patients who died of the complications of liver cirrhosis. Chelation therapy plus iron supplementation, as used in the patient under our care, seems to halt progression of liver cirrhosis as well as improve dystonia.
This treatable disorder of Mn metabolism needs to be considered in any patient presenting with dystonia or Parkinsonian symptoms.

O-071

A NOVEL BIOTIN-SENSITIVE LEUKODYSTROPHY (BSL)

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Biotin (vitamin H) is a water soluble vitamin which serves as a cofactor for (1) pyruvate carboxylase (gluconeogenesis), (2) 3-methylcrotonyl CoA carboxylase, (3) propionyl CoA carboxylase and (4) acetyl CoA carboxylase. In addition, biotin regulates in vitro and in vivo the expression of numerous genes. In humans, two inherited metabolic diseases directly affect the metabolism of biotin: biotinidase and holocarboxylase synthetase deficiencies. A third disease, Biotin responsive basal ganglia disease (BBGD) was identified in patients from the Middle East presenting with encephalopathies responding to high doses of biotin. The gene responsible for the disease, SLC19A3, encodes a second thiamine transporter (ThTr-2). Here we report the identification of a novel biotin-responsive disease in four unrelated individuals, three of them originated from North Algeria. All patients display a characteristic leukodystrophy that involves the periventricular white matter, cortico-spinal tracts, cerebellar peduncles, and optic radiations. Clinical features encompass relapsing episodes of cerebellar ataxia and optic neuropathy. In between episodes, patients may present psychiatric problems, cerebellar ataxia and/or optic atrophy. Visual evoked potentials show absence of P100 or increased latency of the P100, consistent with involvement of optic nerves. Clinical, electrophysiological, radiological and spectroscopic parameters improve after treatment with very high doses of biotin.

O-072

SPASTIC PARAPLEGIA DUE TO CYP7B1 MUTATIONS (SPG5) : WHAT CAN WE LEARN ABOUT 27-HYDROXYCHOLESTEROL METABOLISM?

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Background: CYP7B1 encodes the cytochrome P450 alpha-hydroxylase and plays a role in the alternate/acidic pathway for primary bile acid production. Mutations in CYP7B1 were identified in children with severe liver disease and, recently, in adult SPG5 patients presenting with hereditary spastic paraplegias (HSP) (Tsaousidou, 2008). Increased levels of 27-hydroxycholesterol (27-OHC) were reported in the plasma of 4 SPG5 patients (Schüle, 2010). Besides its role in hepatobiliary metabolism, in vitro studies suggest that 27-OHC may decrease bone mineral density, inhibit the cardiovascular protective effects of estrogens and increase oxidative stress in retinal pigment epithelial cells.
Methods: We investigated 9 SPG5 patients from 6 families in order (i) to determine whether plasma 27-OHC can be used as a biomarker to screen HSP patients, and (ii) to explore the non-neurological manifestations that may result from the altered oxysterols metabolism in SPG5 patients—i.e. hepatobiliary and cardiovascular functions, bone homeostasis and retina.
Results: The marked elevation of plasma 27-OHC was associated with altered liver functions, reduced bone density and optic atrophy in SPG5 patients.
Conclusion: Following these investigations, we are now designing a clinical trial to determine the best candidate drugs—and doses—to lower 27-OHC in SPG5 patients.

P-549

HIGH EFFECTIVITY INDEX IN AN INTERNATIONAL COOPERATION FOR SELECTIVE SCREENING FOR INBORN ERRORS OF METABOLISM

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Background: Availability of sophisticated techniques for selective screening for inborn errors of metabolism is not available in Guatemala. Since 2002 the authors have a close collaboration between Guatemala and Germany/Switzerland.
Methods: Aminoacids and Acylcarnitines were extracted from dried blood spots (DBS), and measured by MS/MS. If necessary for confirmatory diagnostics, organic acids were determined after extraction of urine dried on filterpaper.
Results: Between 2002 and 2011 we have analysed 444 DBS samples from Guatemalan children. So far we found 11 children with a confirmed inborn error of metabolism (1*PKU; 1*GA-I; 1*Cit-type 2; 1*MCADD; 2*Arginase-def.; 1*VLCADD; 1*PA; 2*MSUD; 1*CAH), and 5 further cases with a presumptive, not yet confirmed, diagnosis (1*MADD; 2*OCTN2; 1*LCHADD, 1*CPT-II). The calculated effectivity index (confirmed cases only) is 2.4% (95% C.I.:1.2–4.4%) or 1 case in about 40 requests.
Conclusions: Although the total number of samples is still quite low, the high rate of positive cases indicates that: (1) The overall incidence of inborn errors of metabolism is (at least) as high as in other countries. (2) The preselection of patients to be investigated, is at least as good as in other countries. International cooperation is a simple and effective way to provide state-of-the-art diagnostics for developing countries.

P-550

MUTATION/ VARIATION DATABASES IN INBORN ERRORS OF METABOLISM

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Delivery of the best possible genetic healthcare to those with genetic disease requires access to all data of all individuals with their mutations and phenotype. The reasons and the utility of such data has been published recently (Cotton et al Genetics in Medicine, 11:843–9, 2009). Collection of such data has been suggested to be best by two complementary but partially redundant routes: (a) collection of all instances of each disease in each country and (b) collection of this data into gene or disease specific databases. There are examples of each of these: country (Australia) at www.hvpaustralia.org and colon cancer www.insight-group.org (Kohonen Corish et al Hum Mutat 2010; 31: 1374–81). The latter is curated by representatives of a society, InSiGHT (www.insight-group.org). A similar grouping is emerging in inherited neurological disease (Haworth A et al Neurogenetics, 2011; in press) and being initiated in Mitochondrial disease. Such groupings have advantages such as load sharing, more capacity to raise funds, etc. There is a case for a similar initiative in Inborn Errors of Metabolism supported by the Human Variome Project. Those interested could contact Nenad Blau at: nenad.blau@kispi.uzh.ch

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GUIA METABOLICA: EMPOWERMENT THROUGH HEALTH 2.0 TOOLS IN INBORN ERRORS OF METABOLISM

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Background: Empowerment is the "process of increasing capacity of individuals/groups to make choices and to transform them into outcomes and actions". Empowerment is essential for patients with rare diseases due to their infrequency, difficult management, and the disregarding by the research or medical community.
We aim to create an interface for people interested in inborn errors of metabolism (IEM), mainly parents, trying to facilitate the access to information, and the development of supporting groups as helpful tools to improve this empowerment process.
Methods: We developed an interactive website in Spanish offering updated scientific information in a comprehensible manner and general recommendations. "www.guiametabolica.com" offers the possibility to ask medical/nutritional questions, and provides the opportunity of share experiences between families.
Results: In 6 months, we obtained more than 30,000 website visits coming from more than 65 countries, most of them Spanish speaking countries. Twenty-two percent of the received comments were medical/nutritional questions, and a similar percentage represents responses from our nutritionists/medical staff. Appeals to contact with other parents represent 13%. Responses, recipes and medical information in an easy jargon were viewed positively by visitors.
Conclusions: Poor communication contributes to disparities in understanding of disease and health status interfering with the empowerment process.

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INBORN ERRORS OF METABOLISM BRAZILIAN CALL- FREE SERVICE (SIEM): 9 YEAR FOLLOW-UP

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SIEM is a call-free Brazilian service(covering all country)which provides information to physicians and healthcare professionals involved on diagnosis and management of patients with suspicion of an IEM,which are very important to allow an appropriate care of these cases.We present the results obtained since SIEM started operations(October 2001),with a total of 1877 records: in 56% of the cases the contact was made by a neonatologist or child neurologist; in most cases the professional was looking for support for diagnosis and initial management; if we exclude 134 calls intended to obtain IEM general information, a total of 1229 (70.5%)cases had a complete follow-up; on these cases, we confirmed 187 (15.2%)as IEM, 477(38.8%)as non-IEM, 307(25%)without a clear conclusion and in 258(21%)not enough information was provided. Among the IEM cases, amino acid disorders (20.6%), organic acidemias (19%) and LSDs (17,3%) were the most important groups. We found a high rate of consanguinity, early death and familial recurrence in the positive cases. Although these results indicate that it is still difficult to complete an investigation for IEM in Brazil, we show that SIEM is playing an important role to improve diagnosis and management of these diseases, and providing also data for research projects.

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BIOETHICAL ASPECTS RELATED TO RESEARCH AND TREATMENT IN CHILDREN AND OTHER VULNERABLE COMMUNITIES. THE NEED FOR A WORKING GROUP WITHIN SSIEM

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Since the number of patients for each rare disease worldwide is low, to meet technical and regulatory agencies requirements for clinical trials, children and adults from many underdeveloped and developed countries are being recruited worldwide. With the advances in pharmacogenomics, and the needs for early diagnoses and treatment even before birth, much more research is needed in pursue of effective diagnoses and therapies. Many potential participants are children, from economically and socially deprived communities even in developed countries and may be enrolled in clinical trials to get medical care, food, monetary compensation and hospitalization benefits they may not get otherwise. Most population is concentrated in the third world nations, (TWN) for which the rate of population growth is higher, allowing prediction that TWN may provide more patients for clinical trial than developed countries. Given the importance for: patients, researchers and the future of research on aspects such as new therapies, prenatal diagnosis and treatment on rare diseases, in this presentation arguments will be presented from the genetic, social, national legislation and world agreements, to support the convenience of establishing a working group within SSIEM, on bioethical issues, to build consensus and recommendations around these and other related topics.

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DIAGNOSIS OF INHERITED METABOLIC DISORDERS USING A TARGETED METABOLOMIC APPROACH

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Background: Metabolomics becomes an important tool in clinical research and diagnosing human diseases. In this work we focused on diagnosing of inborn errors of metabolism (IEMs) in plasma samples using a targeted metabolomic approach.
Methods: Plasma samples were analysed using the AbsoluteIDQ p 150 Kit (BIOCRATES Life Sciences AG, Austria). The standard flow injection method of the kit comprising two subsequent 10 ul injections (one for positive and one for negative detection mode) was applied for all measurements. All experiments were performed on an QTRAP 5500 tandem mass spectrometer (AB SCIEX, USA) with electrospray ionization. Multiple reaction monitoring detection was used for quantification.
Results: We analyzed 50 control samples and 34 samples with amino acids defects (phenylketonuria, maple syrup urine disease, tyrosinemia I, argininemia, homocystinuria, carbamoyl phosphate synthetase deficiency, ornithine transcarbamylase deficiency, non-ketotic hyperglycinemia) and with acylcarnitine defects (methylmalonic acidemia, propionic acidemia, glutaric aciduria I, 3-hydroxy-3-methylglutaric aciduria, isovaleric acidemia, medium-chain acyl-coenzyme A dehydrogenase deficiency and carnitine palmitoyltransferase II deficiency). Control samples were distinguished from patient samples by principle component analysis and hierarchical clustering.
Conclusions: This study shows that targeted metabolomics can be applied for diagnosing of various IEMs.
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UHPLC-TMS METHOD FOR SCREENING OF MULTIPLE INHERITED METABOLIC DISORDERS

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A screening method for 36 metabolites related to inborn errors of metabolism (IEMs) of purines, pyrimidines, creatine, organic acid and galactose metabolism is presented. The method utilises ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-TMS) for analysis of the compounds in urine.
Selected compounds were separated on a Phenomenex Luna 3 μ NH2 column in hydrophilic interaction mode with gradient elution. Mobile phase consisted of 20 mM ammonium acetate, pH 9.45 (A) and acetonitrile (B). Total analysis time was 13 min. Detection was performed by multiple reaction monitoring (MRM) in positive and/or negative mode.Two MRM transitions were chosen for each compound (with the exception of four compounds) and retention times were used for peak confirmation.
Detector response was linear in the range of expected concentrations, except for pyruvate and uric acid. Limits of detection (LOD) lie below or within the normal concentration range of the metabolites. The chosen metabolites were analysed in normal urine, elevated concentrations of typical metabolites were found in urines of patients with IEMs. The presented UHPLC-TMS method is fast, sensitive and requires only minimal sample preparation.
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1H NMR-BASED METABOLOMICS: CHEMOMETRIC METHODS FOR THE DIAGNOSIS OF INBORN ERRORS OF METABOLISM

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Background: 1H NMR spectroscopy has successfully been applied to the field of inborn errors of metabolism (IEM). However, body fluid NMR spectra lead to a wealth of data and it can be a daunting task to find the relevant information. In this study, chemometric methods were used to analyze urine NMR data.
Objectives: To demonstrate the use of chemometric methods in NMR-based urine analyses to diagnose patients with IEM. Alkaptonuria (ALK) and methylmalonic aciduria (MMA) will be used as examples.
Material and Methods: 1H NMR spectroscopy was performed on urine samples from 58 healthy volunteers, 5 patients with ALK and 6 patients with MMA. Principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were applied in order to establish models for discrimination between diseased and normal urine samples.
Results: Discrimination of ALK and MMA urine from healthy urine was achieved by PLS-DA with excellent results. Not only is the technique able to find perfect separation of the groups, also the resonances of the diagnostic metabolites for both diseases can easily be found.
Conclusion/Discussion: Chemometrics analyses can assist the interpretation of complex body fluid NMR data and may be useful in the rapid screening of patients with IEM.

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METABOLITE PATTERN ON IN VIVO 1H-MAGNETIC RESONANCE SPECTROSCOPY (MRS) OF THE BRAIN IN CHILDREN WITH METABOLIC DISEASES

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Introduction: Identifying MRS metabolite patterns that are diagnostic of metabolic diseases is a powerful tool in inborn errors of metabolism (IEM).
Methods: We analyzed brain 1H-MRS data from 1843 children from the basal ganglia (BG) and periventricular white matter (PVW). Our study involved measurements from two voxel localizations (BG and PVW) and two echo times (35 ms and 144 ms), and compared data from the 1.5 T and 3 T Philips Achieva clinical MRI scanners. Point-resolved spectroscopy (PRESS) acquisitions were recorded. Post-processing of MRS data and analysis was performed LC Model software. Retrospective analysis of MR spectra and quantitative information on all detected metabolites was done in children with 21 different metabolic diseases including MMA, Citrullinemia, Arginase deficiency, SSADH deficiency and GA type I.
Results: We identified a number of metabolite pattern including low total choline (tCho) in MMA, Arginase deficiency and SSADH deficiency. High Inositol (Ins) was observed in Citrullinemia. Low total creatine (tCr) was seen in Citrullinemia and Arginase deficiency.
Conclusions: Our retrospective analysis revealed a number of MRS metabolite pattern in children with various metabolic diseases. Further data exploration and prospective studies will identify novel metabolite patterns that are diagnostic for IEM and elucidate the neurometabolic mechanisms of disease.

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NMR SPECTROSCOPY FOR DIAGNOSIS AND MONITORING OF METABOLITES IN SOME INBORN ERRORS OF METABOLISM: OTC DEFICIENCY, GALACTOSEMIA AND ALKAPTONURIA

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The emerging field of metabolomics, in which a large number of small-molecule metabolites are detected quantitatively in a single step, promises immense potential for early-diagnosis, monitoring, and understanding the pathogenesis of many diseases. The clinical/ biochemical findings in some inborn errors of metabolism (IEM) are often nonspecific; an early differential diagnosis made in a single urinary sample it gives an important advantage. We present the spectrum of uriny metabolites from a 1 year-old girl with stroke-like episode, elevated transaminases, coagulopathy, being first interpreted as encephalitis. Beside the clinical presentation, the fast results gave by urinary NMR-spectrum showing a high concentration of orotic acid indicates the OTCD diagnosis. Beside this, we present our results and the utility of this method for rapid diagnosis and monitoring steps for galactosemia and alkaptonuria. The level of excretion of the metabolites in these three IEM has been well within the range of NMR detection.
In the critical care setting, IEM that were not diagnosed through the neonatal screening should be considered as cause of acute neurologic, hepatic/ renal decline, rapid diagnosis being essential. We demonstrate the effective use of NMR-spectroscopic-profiles of urine in differential diagnosis for UCD and the possibility of management in other IEM.

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URINARY PORPHYRINS AND BIOMARKERS OF OXIDATIVE STRESS IN TURKISH AUTISTIC CHILDREN

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Background: Autism or autism spectrum disorder is a quite common neurodevelopmental disorder presenting in childhood. Although genetic factors play an important role in the etiology of autism, environmental toxicities, such as metal or xenobiotic toxicity are also important factors in this disorder among some of these children. The aim of this study is to examine the relationship between urinary porphyrins, biomarkers of oxidative stress and heavy metals in Turkish autistic children.
Material and Methods: Urinary porphyrins were measured by an HPLC method; malondialdehide, arylesterase were assessed by colorimetric methods. Also, Pb, As, Cd, Hg were measured by AAS methods in the first morning urine samples of 36 autistic children and 26 age-matched control subjects.
Results: Compared to the control group while there was no difference in the other urine parameters, urine total coproporphyrin and coproporphyrin-III levels were increased statistically significant ($p<0.05$) in autistic children (Patient group; copro-III:109.2 ±62.7, total copro: 176.5 ±146.9; and control group; copro-III:41.5 ±11.9, total copro:72.7 ±17.0 nmol/L respectively). There was no significant correlation between urinary porphyrin levels in urine and other parameters.
Conclusion: Increased levels of urinary coproporphyrin may reflect the effect of environmental toxicity in Turkish autistic children

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RECESSIVE HEREDITARY METHEMOGLOBINEMIA TYPE 2 DUE TO DEFICIENCY OF CYTOCHROME B5 REDUCTASE WITH TWO NOVEL MUTATIONS LEADING TO SEVERE NEUROLOGICAL IMPAIRMENT

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Recessive hereditary methemoglobinemia (RHM) type 2 is an autosomal recessive metabolic disorder due to generalized deficiency of cytochrome b5 reductase (cytb5r). In contrast to RHM type 1, an almost benign disorder where cytb5r-deficiency is restricted to erythrocytes, a severe clinical picture with microcephaly, psychomotor retardation and dystonia develops. Cyanosis may be mild with methemoglobinemia values ranging between 10–42%.
We hereby present a female patient, cyanosis noted immediately after birth, showing elevated methemoglobin values (8–19%). Erythrocyte cytb5r-activity was severely reduced leading to diagnosis of congenital methemoglobinemia. Excessive crying was observed from the sixth week of life, followed by vomiting and dystrophy. In the twelfth week of life deceleration of head growth, neurological impairment with no fixation and smiling, poor head control and opisthotonous were noted. Fibroblast cytb5r-activity was markedly reduced proving RHM type 2. Two novel mutations in the CYB5R3 gene were detected (maternal point mutation and paternal deletion) resulting in a premature stop codon. At 2,5 years the patient shows severe psychomotor retardation and mean methemoglobin values of 12%. Unexplained lactate elevation occurred intermittently. Medication with ascorbic acid, riboflavin and toluidine blue had no clear clinical benefit. We hereby draw attention to this rare disorder which is probably underdiagnosed.

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IS HUTCHISON-GILFORD PROGERIA SYNDROME, A FARNESYLATION DEFECT, A TREATABLE METABOLIC DEFECT?

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Introduction: Hutchison-Gilford progeria syndrome (HGPS), an extremely rare disorder, is a premature ageing syndrome caused by a de novo mutation in the LMNA gene. The most frequent mutation is p.G608G, causing an aberrant splicing of exon 11. More recently it is known that in this disorder results in intracellular accumulation of an abnormal farnesylated form of lamin A (progerin) producing premature cellular senescence. Intelligence is normal. Treatment until recently was largely supportive and death usually occurred at an average age of 13 years. As the metabolic pathway of the formation of lamin A became known the possibilities of treatment also improved.

Case Report: We describe an eight year old child with failure to thrive and short stature, the characteristic facies of HGPS, alopecia, joint deformities and severe osteoporosis.

Our patient is medicated with calcium, vitamin D, low dose of aspirin and is also currently integrated in an international clinical trial with a combination of pravastatin, zoledronic acid and lonafarnib (a farnesyltransferase inhibitor) drugs that act at different points of the pathway.

Comments: Although the efficacy of this treatment is still currently being assessed an evaluation after one year shows some improvement of weight and hair growth.

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DEFECTS OF RED CELL LONG-CHAIN FATTY ACID METABOLISM IN AUTISTIC PATIENTS POSSIBLE DIAGNOSTIC TEST

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We measured the concentrations of 26 [C12-C24] long-chain fatty acids [PUFAs] in the red cells of 10 male patients with autism and 48 normal controls using the same methods of separation of their fatty acids by GC analysis of their methyl esters on a free fatty acid phase column [Dacremont and Vincent, 1995]. We also used innovative non-parametric statistical methods appropriate to the nature of the data.

We have thus demonstrated that in 16 of these 26 fatty acids that this group of ten autistic patients had highly statistically significant reduced concentrations of their [C12–C24] Long Chain Fatty Acids in their red cells by comparison with those of a group of 48 normals

Also using the known normal metabolic pathways, it was possible to locate and delineate defects in elongase and desaturase systems that normally lead to the formation of these fatty acids, ultimately leading to defects in the formation and incorporation of phospholipids sphingomyelin and sphingolipids in cellular membranes of affected patients. We speculate that this might constitute a diagnostic test.

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DISCRIMINATIVE KEYS IN THE METABOLIC DIAGNOSIS OF CUTIS LAXA SYNDROMES

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Cutis laxa is a disorder in which patients have wrinkled, abundant skin with abnormal elasticity. Skin symptoms may be associated with systemic involvement. Several new genetic defects have been discovered to cause cutis laxa. Surprisingly, a number of these syndromes are inborn errors of metabolism. These include disorders of glycosylation: COG7-CDG, ATP6V0A2-CDG as well as deficiencies in mitochondrial enzymes (P5CS and PYCR1).

Despite these different etiologies, discriminating between ATP6V0A2-CDG and PYCR1-deficiency remains challenging due to overlapping features: cutis laxa, dysmorphisms, joint hyperlaxity, cognitive deficits, growth delay and late closing of the fontanel.

We report on the metabolic and clinical features of eight patients with cutis laxa, diagnosed with a novel genetic defect. The four PYCR1 patients had a progeroid appearance and arthrogryposis. Two patients showed athetoid movements. MRI detected hypoplastic corpus callosum in one patient. Serum lactate and alanine were increased in two patients. The four patients with ATP6V0A2-CDG had glycosylation abnormalities and typical facial features. MRI revealed neuronal migration defects in three patients. Awareness of differences between these patient groups should lead to a timely diagnosis. We therefore suggest to measure lactate and alanine and perform cerebral MRI additional to screening for glycosylation in patients with cutis laxa.

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NEURONAL CEROID LIPOFUSCINOSES 6 PRESENTING AS EARLY ONSET PARKINSONISM

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The Neuronal Ceroid Lipofuscinoses (NCL) are the most common autosomal recessive neurodegenerative disorders in childhood. Ten different subtypes are classified based on age at onset, clinical features, biochemical aspects and genetic background.

NCL6 (vLINCL gene on chromosome 15 q21–q23) has been associated with the Late-Infantile variant form and presents with epileptic seizures, myoclonus, speech delay, progressive mental and motor deterioration, and visual failure.

We report a case of a boy of 13 years who present at the age of 5 with speech delay and mild coordination disorder. In the following few years he developed parkinsonism, ataxia, neuromotor regression, dysarthria with severe palylalia and generalized epilepsy. The mental functioning was relatively preserved. Brain MRI was initially normal and the possibility of a Dopa-responsive disorder was considered. However, CSF examination showed normal neurotransmitters and pterins. Afterwards a progressive cerebral and cerebellar atrophy became evident associated with a severe decline of white and gray matter NAA on H-MRS. In contrast there was no evidence of retinopathy with loss of vision. Genetic analysis of the vLINCL 6 gene revealed that the patient was homozygous for the F234L mutation.

This case suggests that NCL6 should be considered in the differential diagnosis of early onset parkinsonism.

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A NEW DIAGNOSIS OF INFANTILE FORM OF ALEXANDER DISEASE WITH A NOVEL DNA MUTATION OF THE GLIAL FIBRILLARY ACID PROTEIN GENE (GFAP)

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We describe a 4 year old female patient born healthy and naturally, after normal pregnancy course. Neurophysiological development and anthropometric parameters were reported as adequate until 2=years old, when she showed episodes of acute vomiting, with concomitant language and neurologic disturbance and macrocephaly.

The girl was diagnosed with a phonological disorder with specific language impairment. The following investigations were done when she came to our department, at 4 years of age: aminoacids, screening for congenital disorders of glycosylation (CDG), galactocerebrosidase, arylsulfatase A, VLCFA, N-acetylaspartate, karyotype, telomeres, urinary aminoacids, oligosaccharides, mucopolysaccharides and organic acids, electroencephalogram, were all normal. MRI showed frontal lobe white matter abnormalities. Mutation analysis for the GFAP gene was positive. Novel DNA mutations in exon 4: c.715 C>T (p.R239C) of the glial fibrillary acidic protein gene (GFAP), underlie the infantile form of Alexander disease, a rare autosomal recessive disorder (1:100.000) of the central nervous system of unknown etiology, characterized by the accumulation of Rosenthal fibers. Some authors have reported the p. R239C mutation in 4 patients with Alexander disease, 3 of them died before 4 years old and 1 before 6. So far this mutation was reported in patient with hereditary spastic paraplegia.

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NEUROPATHOLOGICAL PHENOTYPE-GENOTYPE RELATION IN A SERIES OF 65 FETAL CASES OF LISSENCEPHALY TYPE II

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Background: Lissencephaly type II (LISII) is a brain malformation associating smooth and/or cobblestoned brain surface to hydrocephalus. LISII is considered as pathognomonic of the Cerebro-Oculo-Muscular dystrophies, a group of recessive autosomal disorders with a large clinical spectrum. Part of these syndromes has been linked to mutations in 6 genes involved in post-translational modifications of α-Dystroglycan.

Objectives: to establish a genotype-phenotype in foetal LISII

Material (Patients) and Methods: Neuropathological study and molecular study were performed in a series of 65 foetuses (SOFFOET centers) affected with LISII.

Results: After sequencing the 6 genes, a causal mutation was observed in 68% of families. Neuropathological studies (histological identification of neuroglial ectopia in the arachnoid space and cerebellar dysplasia) showed3 subtypes with a good genetic correlation. The severest type named as LISII-Awas linked to mutations in POMT1/POMT2 (34%/9%) and FKRP (2%) while the least severe type, LISII-C, was exclusively linked to POMGNT1 (19%). An intermediary type, LISII-B, was linked to LARGE mutations (4%).

Conclusion/Discussion: This first large neuropathological and molecular survey of a series of LISII foetal cases led us to the distinction of three neuropathological subtypes with good genetic correlations. This is instrumental for rapid molecular study and prenatal diagnosis.

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SAFETY AND RESPONSE TO BILATERAL PALLIDAL DEEP BRAIN STIMULATION (DBS) IN SEVERE CHILDOOD DYSTONIA SECONDARY TO INBORN ERRORS OF METABOLISM.

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Objectives: Reporting safety and response to DBS in four severe childhood dystonias secondary to glutaric aciduria type 1 (GA1) and complex I deficiency.

Background: DBS is recognised for treatment of primary and secondary dystonia. Little is known about its use in metabolic conditions.

Methods: DBS was implanted as a one stage procedure under general anaesthesia.

Case 1,2,3: Patients with GA1, received DBS at 15, 11 and 10 years respectively using our typical GA1 protocol for surgery.

Case 4: complex I deficiency (MTND6 gene) with rapidly progressive dystonia, received DBS at 5.5 years.

Follow up was at 36, 12, 18 and 30 months.

Response to DBS was measured with the Burke-Fahn-Marsden Dystonia Rating Scale (BFMDRS). The Paediatric-Pain-Profile (PPP) was used in cases 1,2.

Results: No acute decompensation during or immediately post surgery occurred. BFMDRS motor score improved from 5–18% and PPP from 40–100% in case 1; 2–7%, and 100% respectively in case 2.

BFMDRS improved 11–15 % in case 3. Case 4 experienced rapid BFMDRS deterioration of 52% two months preceding DBS and has been stable since surgery.

Conclusions: DBS does not worsen metabolic conditions. DBS improves dystonia and comfort in GA1 and may slow motor progression in certain mitochondrial disorders.

Conflict of Interest declared.

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THE CLINICAL CASE OF RARE FORM ORGANIC ACIDURIA ACCOMPANIED WITH DEFICIENCY OF FOLATE CYCLE ENZYME

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Background: Diagnosis and treatment of hereditary diseases of metabolism (HDM)—one of the biggest challenges for the paediatrician. A variety of metabolic disorders in the same age group may have similar clinical manifestations.
Case report: Patient E., 3 years, preliminary diagnosis: the delay of kinetic and psycho-speech development. Complaints: muscle weakness, features of autism. Phenotype: dolichocephaly, protruding forehead, underdevelopment of the subcutaneous fat. Neurological status: diffuse muscle hypotonia. Karyotype 46 XX. US: diffuse changes of liver, pancreatopathy. Blood biochemistry: increased activity of lactate dehydrogenize. At the molecular study of gene polymorphisms of folate cycle detected polymorphisms MTHFR 667 C/T heterozygous and MTRR 66A/G in the homozygous state. The child suffered an episode of ketosis vomiting later. Gas chromatography of organic acids: increase of 3-hydroxy-3-mehtilgutaric acid to 15.59 (N=0–1,09), which is associated with developmental delay, anxiety and other neurological disorders.
Conclusions: The observation of the child in the dynamics and study of the level of organic acids due of metabolic crisis allowed to establish diagnosis: rare form organic aciduria (3-hydroxy-3-mehtilgutaric) with deficiency of folate cycle enzymes. The correct understanding of the pathogenesis of HDM helps ensure optimal neuro-psychological development of children and to his family members timely specialist medical genetic care.

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PREIMPLANTATION GENETIC DIAGNOSIS FOR FAMILIES WITH INHERITED METABOLIC DISORDERS IN THE CZECH REPUBLIC

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Background: Preimplantation genetic diagnosis (PGD) as a part of in vitro fertilisation (IVF) provides an alternative to current postconception diagnostic procedures (chorionic villus sampling or amniocentesis) in families, where a child with an inherited metabolic disorder (IMD) was born, and enables to avoid pregnancy termination if the embryo is affected. The main premise for PGD is precise diagnosis of IMD of the proband on molecular genetic level.
Method: PGD is based on DNA analysis in one cell from every embryo in early stage of development before transfer to the uterus for implantation. Method principle is preimplantation genetic haplotypisation, i.e. indirect linkage analysis of polymorphic microsatellite DNA markers in the defective gene predetermined in the proband and his parents.
Results: In 2007–2010 PGD protocols have been prepared for 13 couples with 11 IMD: SCID, erythropoetic protoporphyria, propionic acidemia, Fabry disease, LCHAD deficiency, adenylosuccinatelyase deficiency, adenosindeaminase deficiency, mucopolysaccharidosis type I, ornithine-transcarbamoylase deficiency, congenital adrenal hyperplasia and Smith-Lemli-Opitz syndrome. Seven couples underwent IVF. Four pregnancies are in progress or successfully finished.
Discussion: IVF with PGD is available to families with IMD in the Czech Republic. Although PGD remains rather controversial, information about its possibility should be a part of pre-conception genetic counseling.

A-037

INCIDENTAL FINDING OF A BEAKED VERTEBRA

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Case: A 14 month old boy was referred for investigation of possible dysostosis multiplex associated with a storage disorder. Having presented with pectus carinatum, a lateral chest x-ray showed abnormal anterior "beaking" of the 2nd lumbar vertebral body (L2), confirmed on lateral spinal view (figure 1). He was the first child to non-consanguineous parents. He was otherwise well with normal growth and development. Examination showed pectus carinatum but no other abnormalities were found. There was no coarsening of features; skin, hair, joints, hands and spine appeared normal.
Skeletal survey did not reveal other skeletal abnormalities. Ophthalmology assessment was normal. Urinary mucopolysaccharides and oligosaccharides were normal, as were leukocyte α-iduronidase, iduronate-2-sulphatase, galactose-6-sulphatase, α-mannosidase, and β-galactosidase.
Discussion: The finding of a beaked vertebra is rare and may be associated with lysosomal storage disorders (in particular the mucopolysaccharidoses), bone dysplasias, and neuromuscular conditions. Pectus carinatum, while occasionally the presenting feature of Morquio syndrome, is usually an incidental finding in healthy children. Increased prevalence in families suggests a hereditary origin.
Our case is unusual given the strong suggestion of initial radiographs, which self resolved. We hypothesise that delayed maturation of a growth centre may have lead to a temporary minor modelling abnormality in L2.

A-038

HALLERMANN-STREIFF SYNDROME WITH HYPERPHENYLALANINEMIA: CASE REPORT

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Hallerman-Streiff (HSS) is a rare syndrome primarily affecting the head and the face. This entity has a typical physical appearence: bird-like facies, abnormal dentition, hypotrichosis, skin atrophy, proportionate dwarfism and ophthalmic features such as congenital cataracts and microphthalmia. Although the gene defect of this syndrome has been described on choromosome 6 (6q21–q23.2), the patients are generally diagnosed with their physical appearence.
Hyperphenylalaninemia (HPA) is a disorder due to phenylalanine hydroxylase (PAH) gene defect which is located on chromosome 12.
The co-existence of these two disorders has not been reported in the literature before.
Case Report: A three months old girl patient has been referred to our clinic to investigate etiology of congenital cataract. She was born to consanguineous parents after uneventful pregnancy. Her cousin has been diagnosed with phenylketonuria before and followed-up by our clinic on phenylalanine free diet without any complications. The case had typical physical appearence of HSS with bird-like facies, hypotrichosis, atrophy of skin, proportionate dwarfism, congenital cataract, bilateral microophthalmia. Additionally HPA had been diagnosed by neonatal screening.
Conclusion: The patients with the diagnosis of dysmorphic syndromes should also be evalueted in terms of metabolic disorders, which are commonly encountered in countries where consangineous marriages are common.

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