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001-P

SULPHITE OXIDASE DEFICIENCY IN THREE MALAYSIAN PATIENTS: CLINICAL, BIOCHEMICAL AND MOLECULAR FINDINGS

Chen BC[1], Shanti B[1], Chng GS[1], Norsiah MD[2], Vigneswari G[3], Keng WT[1], Ngu LH[1]
[1]Dept Genetics, Kuala Lumpur Hospital, Kuala Lumpur, Malaysia
[2]Molecular & Diagnostic Protein Unit, IMR, Kuala Lumpur, Malaysia
[3]Dept Ped Neuro, Penang Hospital, Penang, Malaysia

Introduction/Objective: Sulphite oxidase deficiency (SOD) is a rare inborn error disorder of sulphur metabolism. This defect is characterized by progressive neurological abnormalities, ectopia lentis and premature death. We report here the first three Malaysian patients with SOD.
Materials and Methods: We screened patients with unexplained encephalopathy and intractable seizure for excretion of urinary sulphite metabolites. SUOX gene analysis was performed in two patients.
Results: Three patients of Chinese ethnicity were identified. All of them were born healthy without any medical problem initially. Two patients developed early-onset encephalopathy with multi-organ system dysfunction requiring intensive life support within first week of life. They subsequently had intractable seizures, microcephaly, feeding difficulties and severe psychomotor retardation. Another patient developed epilepticus at 6 weeks old. One patient had subluxated lens noticed at 7 months. Cranial MRI of all patients showed profound cerebral atrophy and diffuse cystic white matter changes. All were initially diagnosed as cerebral palsy secondary to hypoxic-ischemic encephalopathy. Diagnosis was made on the basis of increased levels of urinary sulphite, sulphocysteine, normal plasma uric acid, normal urine xanthine and hypoxanthine. SUOX gene analysis showed homozygous 1029C>G (Y343X) mutation in one patient, and two heterozygous mutations [1029C>G (Y343X) and 478G>A (R160Q)] in another patient.
Conclusions: Our report highlights the importance of considering SOD in infants with unexplained neurological illness. Urinary screening for sulphite metabolites will help to differentiate SOD from hypoxic-ischemic condition and improved diagnosis.

002-P

ACUTE TYROSINE ADMINISTRATION INHIBITED MITOCHONDRIAL ENERGY METABOLISM IN CEREBRAL CORTEX AND LIVER OF YOUNG RATS

Streck EL[1], Scaini G[1], Ferreira GK[1], Rochi N[1], Benedet J[1], Ferreira GC[2], Schuck PF[1]
[1]Universidade do Extremo Sul Catarinense, Criciúma, Brazil
[2]Universidade do Sul de Santa Catarina, Tubarão, Brazil

Background: Tyrosine (Tyr) levels are abnormally elevated in tissues and body fluids of patients with inborn errors of Tyr metabolism. Tyrosinemia type II, which is caused by tyrosine aminotransferase deficiency, provokes eyes, skin and central nervous system disturbances in the affected patients. Considering that the mechanisms of brain damage in this disorder are poorly known, we investigated the in vivo effect of Tyr on various parameters of energy metabolism in cerebral cortex and liver of young rats.
Methods: Thirty-day-old Wistar rats were killed one hour after a single intraperitoneal injection of Tyr (500 mg/kg) or saline (control group). The activities of citrate synthase, malate dehydrogenase, succinate dehydrogenase and complexes I, II, III and IV were evaluated in cerebral cortex and liver homogenates.
Results: We observed that Tyr administration inhibits the respiratory chain complexes, citrate synthase, succinate dehydrogenase and malate dehydrogenase activities in cerebral cortex, as compared to control group. Furthermore, complexes II and II-III of the respiratory chain, citrate synthase and malate dehydrogenase activities were also inhibited in liver.
Conclusions: These results suggest that acute Tyr administration causes energy deficit in young rats. These findings may contribute to the pathophysiology of brain and liver damage found in hypertyrosinemic patients.

003-P

IN VITRO EFFECT OF TYROSINE ON ENERGY METABOLISM PARAMETERS IN CEREBRAL CORTEX AND LIVER OF YOUNG RATS

Ferreira GK[1], Scaini G[1], Ferreira GC[2], Schuck PF[1], Streck EL[1]
[1]FISIOPAT, UNESC, Criciúma, Brazil
[2]PPGCS, UNISUL, Tubarão, Brazil

Background: Tyrosinemia type II is caused by a deficiency of the enzyme tyrosine transaminase. Affected patients present alterations in the skin, the ocular cornea, and the central nervous system. Considering that the pathophysiology of tyrosinemia type II is poorly known, we investigated the in vitro effect of L-tyrosine on bioenergetic parameters in cerebral cortex and liver of young rats.
Methods: Thirty-day-old Wistar rats were killed by decapitation and the cerebral cortex and liver were used. Tyrosine (Tyr; 0.1, 1.0, 2.0 or 4.0 mM) was added to the reaction medium and the activities of citrate synthase, malate dehydrogenase and respiratory chain complexes I, II, II-III and IV were evaluated.
Results: Tyr, at 1.0 mM and higher concentrations, inhibited citrate synthase and respiratory chain complex IV activities only in cerebral cortex. Furthermore, complex II activity was inhibited by Tyr (1.0 mM and higher) in cerebral cortex and liver. On the other hand, malate dehydrogenase and complexes I and II-III activities were unaffected in both structures evaluated.
Conclusions: Our results show that Tyr in vitro impairs energy metabolism in cerebral cortex and liver of young rats, which may be related to the pathophysiology of tyrosinemia type II.

004-P

A RAPID, SENSITIVE GAS CHROMATOGRAPHY—MASS SPECTROMETRY METHOD FOR QUANTITATION OF LEUKOCYTE CYSTINE

Van der Watt GF[1], Bahar B[2], Omar F[1], Bergstedt B[1]
[1]Div Chem Path, Univ of Cape Town, Cape Town, South Africa
[2]Dept Biochem, Gazi Univ, Ankara, Turkey

Background: Nephropathic cystinosis is the commonest identifiable cause of paediatric renal Fanconi syndrome, and diagnosis depends on leukocyte cystine (LC) quantitation. Current methods for LC are slow and require large sample volumes
Objectives: To develop a sensitive, fast GC-MS method for LC quantitation.
Methods: L-Cystine[15 N] and norvaline internal standards (ISTDs) were added to the supernatant of a leukocyte protein precipitate before solid-phase extraction and propyl-chloroformate derivatization followed by injection onto an Agilent 7890A/5975C GC-MS system. Norvaline was used as ISTD in the range 0.24–6 nmol/aliquot and L-Cystine[15 N] in the range 0.02–0.24 nmol/aliquot. Total GC preparation and run time was 25 minutes per sample.
Results: The method was linear in the range 0.02–6 nmol/aliquot ($r = 0.99$) with a limit of detection of 0.02 nmol/aliquot. Within-run and total imprecision, was 12.7 and 16.9% at 0.15 nmol/aliquot and 4.5 and 10.2% at 2.5 nmol/aliquot, respectively. Linear regression of 15 LC specific quality assurance samples run over two years against 24 laboratories using HPLC, MS-MS and cystine binding methods yielded the equation $y = 1.136x + 0.068$ nmol/aliquot ($r = 0.99$, $Sy/x = 0.22$), where y = GC-MS cystine and x = the median cystine value obtained in the scheme. Improved sensitivity allowed determination of an adult LC reference interval of 0–0.11 nmol cystine/mg protein.
Conclusions: Chloroformate-derivatisation of amino acids for GC-MS quantitation with dual ISTDs enables accurate rapid quantitation of LC over a wide concentration range and with sufficient sensitivity to diagnose cystinosis on 1 mL of whole blood.

005-O

FATAL CEREBRAL EDEMA ASSOCIATED WITH SERINE DEFICIENCY IN CSF

Keularts IMLW[1], Leroy PLJM[2], Rubio-Gozalbo ME[3], Spaapen LJM[1], Weber J[4], Dorland L[1], de Koning TJ[5], Verhoeven-Duif NM[5]
[1]*Lab Bioch Gen, Dept Clin Gen, Univ Hosp, Maastricht, Netherlands*
[2]*Dept Pediatr, Maastr Univ Med Center, Maastricht, Netherlands*
[3]*Dept Clin Gen & Pediatr, Univ Med Center, Maastricht, Netherlands*
[4]*Dept Child Neur, Maastr Univ Med Center, Maastricht, Netherlands*
[5]*Dept Pediatr, Wilhelmina's Child Hosp, Utrecht, Netherlands*

Background: Two young females without a notable medical history except for asthma presented with a rapidly progressive toxic encephalopathy and brain edema causing death within 24 hours after the first symptoms.
Methods: Selective metabolic screening in plasma, urine and CSF as well as measurement of enzyme activity of 3-phosphoglycerate dehydrogenase (3-PGDH) in cultured fibroblasts.
Results: We measured plasma serine concentrations of 31 and 33 μmol/l (ref. 95–116 μmol/l) and CSF serine concentrations of 8 and 7 μmol/l (19–38 μmol/l). The concentrations of the other amino acids were normal or decreased according to a non-specific pattern. Deficiencies of one of the serine biosynthesis enzymes were unlikely on clinical grounds. Normal enzyme activity of 3-PGDH was measured in one of the patients. Further metabolic screening revealed increased ketone bodies and lactate in urine, plasma and CSF. The plasma acylcarnitine profile showed an elevated concentration of OH-C4-carnitine. A fatty acid oxidation defect was excluded.
Discussion: Two young female patients with extremely low serine concentrations in CSF and plasma comparable to 3-PGDH deficient patients without clinical features suggestive for a serine biosynthesis defect. On basis of the fasting state, ketone bodies and lactate in plasma, urine and CSF, we speculate that reduced serine levels were due to its use as gluconeogenic substrate, conversion to pyruvate by brain serine racemase or decreased L-serine production because of a lack of glucose.
Conclusion: These are the first strikingly similar cases of patients with a clear secondary serine deficiency associated with a toxic encephalopathy.

006-P

COEXISTENCE OF MOLYBDENUM COFACTOR DEFICIENCY AND PYLORIC STENOSIS

Giżewska M[1], Romanowska H[1], Sass JO[2], Walter M[2], Sykut-Cegielska J[3], Hnatyszyn G[1], Krzywińska-Zdeb E[1], Gawrych E[1], Walecka A[1], Tuziak M[1]
[1]*Pomeranian Medical Univ, Szczecin, Poland*
[2]*Lab Clin Biochem Metab Univ Child Hosp, Freiburg, Germany*
[3]*Child Memorial Health Inst, Warsaw, Poland*

Molybdenum cofactor deficiency (MoCD) is a rare metabolic disorder characterized by severe and progressive neurological damage caused by loss of sulfite oxidase (SO) activity. As the result of accumulation of toxic metabolites such as S-sulfocysteine, most patients present with severe intractable seizures within the first days of life. Among many other symptoms the main are dysmorphic facial features and progressive neurodegeneration leading to severe cerebral palsy and early death. Substitution therapy with cyclic pyranopterin monophosphate, precursor of the molybdenum cofactor, has been reported recently to be beneficial to early diagnosed patients with MoCD type A.
In the literature there are two reports on coexistence of MoCD and SO deficiency (SOD) with pyloric stenosis (Parini et al. 1997, Currie and Gains 2009). Multifactoral toxicity to the nervous system with involvement of the myenteric plexus in the stomach and pylorus has been postulated as one of the explanations. We report a girl diagnosed with MoCD at the age of 4= months based on the clinical presentation, typical results of biochemical tests (increased urinary excretion of xanthine, hypoxanthine, sulfocysteine and hypouricaemia with undetectable uric acid excretion) as well as undetectable activity of SO in cultivated fibroblasts. At the age of 1 month she was operated for hypertrophic pyloric stenosis. Although the fatal course of MoCD and SOD is generally known to be complicated by severe feeding problems, this should not prevent from considering a diagnosis of pyloric stenosis as a possible cause of gastroenterological problems.

007-P

IN VIVO INTRACEREBROVENTRICULAR ADMINISTRATION OF ORNITHINE AND HOMOCITRULLINE INHIBITS MITOCHONDRIAL ENERGY PRODUCTION AND INDUCE OXIDATIVE STRESS IN CEREBRAL CORTEX OF YOUNG RATS

Viegas CM[1], Busanello EN[1], Ferreira GC[1], Moura AP[1], Tonin AM[1], Grings M[1], Ritter L[1], Schuck PF[2], Wyse ATS[1], Wajner M[3]
[1]*Departamento de Bioquímica, ICBS, UFRGS, Porto Alegre RS, Brazil*
[2]*Lab Fisiopatologia Experimental Unesc, Criciúma SC, Brazil*
[3]*Servico de Genética Médica, HCPA, Porto Alegre, RS, Brazil*

Background: Hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome is a genetic disorder biochemically characterized by tissue accumulation of ornithine (Orn), ammonia and homocitrulline (Hcit). Affected patients present lethargy, ataxia, delayed development and severe mental retardation whose pathogenesis is poorly understood.
Objective: We investigated the effects of intracerebroventricular (icv) administration of Orn and Hcit on important parameters of energy metabolism and oxidative stress in cerebral cortex of rats.
Material and methods: A single icv injection of Orn or Hcit was given to 30-day-old male Wistar rats. Animals were killed 30 minutes after the injection, the cerebral cortex was isolated and used for the biochemical assays.
Results: Orn and Hcit inhibited CO2 production (citric acid cycle activity) and the activity of the respiratory chain complex I-III (oxidative phosphorylation). Furthermore, Orn and Hcit induced lipid peroxidation, whereas Hcit also diminished reduced glutathione concentrations and the activity of the antioxidant enzymes catalase and glutathione peroxidase.
Conclusion: These results indicate an impairment of energy production and induction of oxidative stress caused by Orn and Hcit in vivo. It is presumed that these mechanisms may be involved in the pathophysiology of the neurological dysfunction characteristic of HHH syndrome.
Financial Support: CNPq, FINEP research grants from Instituto Brasileiro de Neurociência (IBN-Net) #01.06.0842-00 and INCT-EN.

008-P

INTRASTRIATAL ADMINISTRATION OF LYSINE INDUCES OXIDATIVE AND BIOENERGETICS DAMAGE IN STRIATUM OF DEVELOPING RATS

Seminotti B[1], Fernandes CG[1], Amaral AU[1], Zanatta A[1], Leipnitz G[1], Dutra Filho CS[1], Wajner M[2]
[1]*Servico de Genética Médica, HCPA, Porto Alegre, RS, Brazil*

Background: Familial hyperlysinemia (FH) is an inherited metabolic disease characterized by tissue accumulation of lysine (Lys). Although many patients are asymptomatic, a considerable number of affected individuals present neurological dysfunction.
Objectives: The present work investigated the ex vivo effects of Lys on various parameters of energy metabolism and oxidative stress in striatum of young rats.
Methods: The activities of Na+, K±ATPase, creatine kinase (CK), glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT), as well as lipid and protein oxidative damage and GSH concentrations were determined after a single intrastriatal administration of Lys (4 μmol).
Results: Our results demonstrated that intrastriatal administration of Lys inhibited the activities of Na+, K±ATPase and GPx, but did not alter the activities of CK, SOD and CAT. Lys also induced lipid peroxidation and diminished the major non-enzymatic cerebral antioxidant defense GSH. Furthermore, the combination of vitamin C and E and melatonin prevented the induction of lipid oxidative damage and decrease of GSH levels caused by Lys.
Conclusions: The biochemical deleterious alterations here observed due to in vivo administration of Lys may increase the vulnerability of the central nervous system to other insults such as systemic infections in symptomatic patients affected by FH leading these patients to central nervous symptoms especially during crises of metabolic decompensation in which tissue concentrations of Lys dramatically increase.
Financial support: Research grants from CNPq, PROPESq/UFRGS, FAPERGS, PRONEX, FINEP Rede Instituto Brasileiro de Neurociência (IBN-Net) # 01.06.0842-00 and INCT-EN.

009-P

NEUROCHEMICAL EVIDENCE THAT THE MAJOR METABOLITES ACCUMULATING IN MAPLE SYRUP URINE DISEASE DISTURB MITOCHONDRIAL BIOENERGETIC IN BRAIN OF YOUNG RATS

Amaral AU[1], Leipnitz G[1], Fernandes CG[1], Seminotti B[1], Schuck PF[2], Zanatta A[1], Eichler P[1], Cecatto C[1], Dutra Filho CS[1], Wajner M[3]
[1]*Lab Fisiopatologia Experimental Unesc, Criciúma SC, Brazil*
[2]*Servico de Genética Médica, HCPA, Porto Alegre, RS, Brazil*

Background: Maple syrup urine disease (MSUD) is a neurometabolic disorder caused by a severe deficiency of branched-chain L-2-keto acid dehydrogenase complex activity, leading to accumulation of branched-chain amino acids, keto acids and hydroxyl acids. The compounds that most accumulate in this disorder are leucine (Leu), α-ketoisocaproic acid (KIC) and α-hydroxyisovaleric acid (HIV).
Objectives: In the present study we investigated the in vitro effects of Leu, KIC and HIV on bioenergetics homeostasis in mitochondrial preparations from brain of 30-day-old rats.
Methods: States 3 and 4 respiration, respiratory control ratio (RCR), ADP/O ratio, NAD(P)H levels, activities of citric acid cycle enzymes, membrane potential (ΔΨm) and mitochondrial swelling were determined. Glutamate/malate, succinate and α-ketoglutarate were used as substrates.
Results: KIC increased state 4 respirations and decreased the RCR with all substrates used. Furthermore, KIC and Leu decreased state 3 respirations and the ΔΨm using α-ketoglutarate as substrate. In addition, KIC provoked an inhibition of α-ketoglutarate dehydrogenase activity and reduced the ADP/O ratio and the NAD(P)H levels. Otherwise, HIV did not affect any of these parameters.
Conclusion/Discussion: The present data indicate that KIC acts as an uncoupler of oxidative phosphorylation and as a metabolic inhibitor, while Leu behaves as a metabolic inhibitor. It is suggested that impairment of mitochondrial homeostasis caused by the major metabolites accumulating in MSUD may be involved in the neuropathology of this disease.
Financial Support: Research grants from CNPq, PROPESq/UFRGS, FAPERGS, PRONEX, FINEP Rede Instituto Brasileiro de Neurociência (IBN-Net) # 01.06.0842-00 and INCT-EN.

010-P

NA+,K+-ATPase ACTIVITY AND GENE EXPRESSION IN RATS SUBJECTED TO EXPERIMENTAL HYPERPROLINEMIA

Ferreira AGK[1], Stefanello FM[1], Cunha AA[1], da Cunha MJ[1], Pereira TCB[2], Bonan CD[2], Bogo MR[2], Netto CA[1], Wajner M[3], Wyse ATS[1]
[1]*Dep Biol Cel Molec, PUCRS, Porto Alegre, Brazil*
[2]*Servico de Genética Médica, HCPA, Porto Alegre, RS, Brazil*

Background: Hyperprolinemia is a metabolic disease whose symptoms include neurological manifestations. Na+, K±ATPase is a membrane-bound enzyme that plays an essential role in controlling neuronal excitability.
Objectives: To investigate the effect of chronic proline (Pro) administration on thiobarbituric acid-reactive substances (TBARS), as well as the influence of antioxidant vitamins E plus C on the effects Pro-mediated on Na+, K±ATPase activity and expression of catalytic subunits in cerebral cortex of rats.
Materials and Methods: Wistar rats were Pro administered (12.8–18.2 μmol/g) subcutaneously twice a day, from the 6th to the 28th day of age. Concomitantly, vitamin E (40 mg/kg) plus C (100 mg/kg) were injected intraperitoneally once a day. Rats were sacrificed 12 h after the last injection. Synaptic plasma membrane was prepared and Na+, K±ATPase activity assay were performed (Wyse et al. 2000). TBARS were determined according to Ohkawa (1979). The analysis of Na+, K±ATPase catalytic subunits expression was carried out by a semi quantitative reverse transcriptase polymerase chain reaction (RT-PCR) assay.
Results: Pro increased TBARS levels, suggesting lipid peroxidation. Concomitant administration of vitamins significantly prevented the inhibition of Na+, K±ATPase activity caused by Pro, suggesting the involvement of oxidative stress in such effect. We did not observe any change in levels of Na+, K±ATPase mRNA transcripts after treatments.
Conclusion: These findings provide insights into the mechanisms by which Pro exerts its effects on brain function and suggest that treatment with antioxidants may be beneficial in neurological dysfunctions observed in hyperprolinemic patients.
Technical Support: CNPQ, FINEP (IBN-Net–01.06.0842-00)

011-P

FOLLOW UP OF PATIENTS TREATED FOR TYROSINEMIA TYPE I: BLOOD SPOT ANALYSIS OF NITISINONE AND SUCCINYLACETONE

Sander J[1], Janzen N[2], Peter M[1], Gokcay G[3], Demirkol M[3], Ozer I[3], Das AM[2]
[1]*Screening-Laboratory Hannover, Ronnenberg, Germany*
[2]*Paediatric Metabolic Medicine, Hannover, Hannover, Germany*
[3]*Children's Hospital, Istanbul University, Istanbul, Turkey*

Background: Nitisinone (2-(nitro-4-trifluoromethylbenzoyl)1,3-cyclohexanedione) is the treatment of choice in tyrosinemia type I (OMIM 276700). For monitoring therapy, nitisinone and succinylacetone were measured in dried blood spots, together with tyrosine, methionine and partly bile acids.
Methods: Discs of filter paper spotted with blood from tyrosinemia patients were extracted with methanolic standard solution as described earlier. Nitisinone was analyzed by UPLC-MS/MS on a Xevo mass spectrometer, other substances were quantified by LC-MS/MS. The calibration curves were linear. Recovery exceeded 70%, CV intraday and interday were below 13%.
Results: Nitisinone concentrations varied enormously. Whilst some patients showed blood levels above 30 μM, others presented with concentrations near 10 μM. Succinylacetone was not detected as long as therapy was continued. In cases of temporary unavailability of the drug or of poor compliance, levels of nitisinone fell below 3 μM and succinylacetone reappeared. Depending on dietary regimen and liver function great differences were observed in concentrations of amino acids and bile acids. Extremely elevated levels of nitisinone were found in a patient who died from liver failure.
Conclusion: Quantification of nitisinone, succinylacetone, amino acids and bile acids is possible and an easy and handy tool to survey nitisinone treatment in tyrosinaemia type I.

012-P

MUTATIONS IN FUMARYLACETOACETATE HYDROLASE GENE AND GENOTYPE-PHENOTYPE RELATION

Ozgul RK[1], Guzel A[2], Mesci L[1], Sivri HS[1], Kilic M[1], Ozcay F[3], Gunduz M[4], Aydin HI[5], Aliefendioglu D[6], Coskun T[1], Dursun A[1]
[1]*Dept of Ped, Metab Uni, Hacettepe Univ, Ankara, Turkey*
[2]*Dept of Biology, Hacettepe Univ, Ankara, Turkey*
[3]*Dept of Ped, Baskent Univ, Ankara, Turkey*
[4]*Diskapi Research and Training Hosp, Ankara, Turkey*
[5]*Metabolism Unit, GATA, Ankara, Turkey*
[6]*Dept of Ped, Kirikkale Univ, Kirikkale, Turkey*

Tyrosinemia type I is a rare autosomal recessive inherited disorder caused by deficiency of fumarylacetoacetate hydrolase (FAH) enzyme. In this study, 32 patients with tyrosinemia type I were studied for mutations in FAH gene. In addition, clinical and biochemical findings were evaluated to establish a genotype and phenotype relation in the patients. The mutation screenings were performed by a 50 K custom resequencing microarray chip and sequencing analysis. Of 12 mutations detected, six (V166G, R174X, D233V, R237X, IVS6-1G>A, and IVS12+5G>A) were reported previously in the literature, others (E201K, N232K, V259D, c.191delA, IVS3-3C>G, and IVS9+2 T>C) were designated as novel. Biochemical findings showed that altered levels of coagulation parameters (PT/PTT), and AST are remarkable as much as alpha fetoprotein which is a nonspecific biomarker in the disease. This study also strengthens that D233V mutation is specific for Turkish population and suggests that this mutation has a potentially strong influence to develop liver cancer compared to other mutations even if the patients have been under NTBC treatment. The study was supported by State Planning Organization of TURKEY (Project No: DPT 2006 K120640).

013-P

MUTATION PROFILE OF BCKDHA, BCKDHB AND DBT GENES FOR MAPLE SYRUP URINE DISEASE IN TURKEY

Ozgul RK[1], Guzel A[2], Dundar H[1], Yucel D[2], Yilmaz A[2], Unal O[1], Tokatli A[1], Sivri HS[1], Coskun T[1], Dursun A[1]
[1]*Dept of Biology, Hacettepe Univ, Ankara, Turkey*

Maple syrup urine disease (MSUD) is an inherited metabolic disorder caused by mutations in the branched chain alpha-keto acid dehydrogenase complex which impair the degradation of the branched-chain amino acids (leucine, isoleucine and valine). Alpha-keto acid dehydrogenase complex consists of E1α, E1β and E2 subunits which are encoded by BCKDHA, BCKDHB, DBT genes, respectively. MSUD can be caused by defects in any of these three genes. In this study, 26 unrelated Turkish patients with MSUD were analyzed with custom designed resequencing microarray. Four different mutations were found in BCKDHA gene, two missenses (C258Y, A253T), one nonsense (C258X) and one novel deletion (c.703delT-p.Y235TfsX95). Five different mutations were found in BCKDHB gene, A91V, S339L were known mutations and G159P, R170C mutations were novel mutations of four missense changes and a known nonsense (Y383X) mutation. In DBT gene, one previously described missense (I413T) and one novel duplication (c.773_801–4dup31) type of mutations were identified. As a result of mutation screening in three different genes, 46% of mutations were located in BCKDHA gene,46% in BCKDHB gene and 8% in DBT gene. The study was supported by State Planning Organization of TURKEY (Project No: DPT 2006 K120640)

014-A

MOLYBDENUM COFACTOR DEFICIENCY IN TUNISIAN PATIENTS

Hammami MB[1], Nasrallah F[1], Hadj Taieb S[1], Omar S[1], Sanheji H[1], Tebib N[2], Ben Dridi MF[2], Feki M[1], Kaabachi N[1]
[1]*Laboratory of Biochemistry, Rabta Hosp, Tunis, Tunisia*
[2]*Paediatric department, Rabta Hosp, Tunis, Tunisia*

Background: Molybdenum is the cofactor of xanthine oxidase (XO), sulfite oxidase (SO) and aldehyde oxidase. Accordingly, the molybdenum cofactor deficiency (MoCD) results in a deficit of these enzymes activities. XO is essential for purine degradation and its deficiency causes lens dislocation and urinary calculi. SO allows the detoxification of sulfite, a neurotoxic metabolite resulting from the sulfur amino acid metabolism pathway. SO deficiency manifests early in infancy mainly with seizures, dystonia and developmental delay. The presence of sulfites in urine and the low serum and urinary uric acid are the laboratory hallmark of MoCD. We report the clinical and biochemical features in Tunisian patients.
Materials and methods: The study included 5 patients diagnosed between 1990 and 2009. Quantitative amino acids were performed by an amino acids chromatography analyzer (Beckman). Serum and urinary uric acid were assessed by enzymatic methods.
Results: The age of onset was below three months for 4 patients and 3 years for one patient. The sex ratio was of 4. First degree consanguinity was noted in 3 cases. Significant developmental delay was the prominent abnormality (80%). Three patients had seizures. The fourth had hypotonia and the fifth had abnormal movements. In all cases, the levels of urinary thiosulfate and sulfocysteine were increased. Serum and urinary uric acid levels are drastically reduced.
Conclusion: Although there is no effective treatment for isolated SO deficiency, a cofactor supplementation could provide a better prognosis in MoCD. Quantification of uric acid is very interesting for the differentiation between these diseases.

015-P

NONKETOTIC HYPERGLYCINEMIA IN TUNISIA: ABOUT 34 PATIENTS

Hadj Taieb S[1], Nasrallah F[1], Hammami MB[1], Romdhane M[1], Elasmi M[1], Sanheji H[1], Omar S[1], Feki M[1], Kaabachi N[1]
[1]*Laboratory of Biochemistry, Rabta Hosp, Tunis, Tunisia*

Background and Aim: Nonketotic hyperglycinemia (NKH) is an autosomal recessive disorder caused by a defect in the glycin cleavage system. The objective of this retrospective study was to determine the profile and to estimate the frequency of NKH in Tunisia.
Material and Methods: During the period from1987 to 2009, and following clinical orientation, 34 patients were diagnosed. Ion exchange chromatography of free amino acids in plasma and cerebrospinal fluid was performed on an amino acids analyzer.
Results: The age of patients at diagnosis ranged from 1 day to 17 months. The sex ratio was 2,4 (24 boys and 10 girls). Consanguinity was observed in 85 % of patients .The main clinical symptoms were hypotonia, lethargy, myoclonic seizures (100 %); coma, feeding difficulties (94%). 32 patients died during the neonatal period. Using the Hardy Weinberg formula, the incidence was estimated to be 1/21088.
Conclusion: NKH seem to be frequent in Tunisia, because of a high rate of consanguinity. Antenatal diagnosis, will avoid the recurrence of this disorder.

016-P

PROFILE OF INHERITED DISORDERS OF AMINOACIDS METABOLISM OTHER THAN PHENYLKETONURIA IN TUNISIA

Hadj Taieb S[1], Romdhane M[1], Nasrallah F[1], Hammami MB[1], Elasmi M[1], Tebib N[2], Omar S[1], Sanheji H[1], Ben Dridi MF[2], Feki M[1], Kaabachi N[1]
[1]*Paediatric department, Rabta Hosp, Tunis, Tunisia*

Background and Aim: The objective of this retrospective study was to determine the profile of aminoacidopathies (AA) other than phenylketonuria (PKU) and to estimate their incidence in Tunisia.
Material and Methods: During the period from1987 to 2009, the laboratory archived 13171 analysis for patients with symptoms suggestive of AA.
Results: After excluding PKU, 243 patients were diagnosed with 12 different AA.
The most frequent AA were maple syrup urine disease(MSUD):(28.3%), tyrosinemia type I (HTI):(25%) and nonketotic hyperglycinemia (NKH): (14%).The sex ratio was 0, 56.The age ranged from 1 day to 30 years with a mean age at diagnosis of 29.6 months. Consanguinity was observed in 60.5 % of patients and 44.4% have had familial cases previous. The main clinical symptoms were hypotonia (35%), hepatomegaly (30%), seizures (29.6%), failure to feed (23.9%) and coma (22.2%). Using the Hardy-Weinberg formula, the incidence was estimated to be 1/13716 for MSUD, 1/14804 for HTI and 1/21088 for NKH.
Conclusion: AA seems to be highly frequent in Tunisia. After PKU; MSUD is the major AA, suggesting that the establishment of systematic neonatal screening is urgent for these two diseases in our country.

017-P

FAMILIAL HYPERLYSINAEMIA WITH PROGRESSIVE SPASTIC QUADRIPLEGIA—HYPERLYSINAEMIA TYPE 2

Tuschl K[1], Mills PB[1], Clayton PT[1]
[1]UCL Institute of Child Health, London, United Kingdom

A 17 year old girl of Pakistani origin born to first cousin parents presented to us with progressive spastic tetraplegia, dysarthria and global developmental delay. Her problems became apparent at two years with difficulties walking and a scissoring gait. Over the years she lost motor skills and became wheelchair bound in her teens. The family history was suggestive of an autosomal recessive disorder with two of six siblings and three cousins affected by a similar condition. Clinical investigations revealed significant hyperlysinaemia (lysine 624 μmol/L, normal 100–300), mildly raised plasma threonine (274 μmol/L, normal 70–220) and hyperlysinuria (lysine/creatinine ratio 841 μmol/mmol). Other causes of hereditary spastic paraparesis were excluded.

Two further cases of hereditary hyperlysinaemia, progressive spastic paraparesis and developmental delay have been described (Yiannikas et al 1996, Kobayashi et al 1977). Benign familial hyperlysinaemia (type 1) caused by a deficiency of the alpha-aminoadipic semialdehyde (α-AASA) synthase is not associated with a clinical phenotype. We therefore surmised that a different defect in lysine catabolism is responsible for hyperlysinaemia (type 2) combined with neurological problems.

Patients with pyridoxine-dependent epilepsy caused by deficiency of α-AASA dehydrogenase have normal lysine levels suggesting that the major pathway for catabolism of lysine may involve a different dehydrogenase. In yeast, Lys2 and Lys5 are required for the synthesis of α-aminoadipic acid from α-AASA. We hypothesized that one of their homologues in human, U26 or mLys5, was affected in our patient. However, sequencing of both genes did not reveal a mutation, excluding their involvement in the disease pathology in these patients.

018-P

MANAGEMENT OF TYPE I TYROSINEMIA: A TUNISIAN EXPERIENCE

Azzouz H[1], Ben Chehida A[1], Ben Romdhane M[1], Ben Turkia H[1], Ben Abdelaziz R[1], Nasrallah F[2], Chouchene N[3], Monastiri K[3], Tebib N[1], Kaabachi N[2], Abdelmoula MS[1], Ben Dridi MF[1]
[1]Dep Pediatr and Metab Dis, Rabta Hosp, Tunis, Tunisia
[2]Biochemestry labo, Rabta Hosp, Tunis, Tunisia
[3]Dep Pediatrics, Monastir Hosp, Monastir, Tunisia

Background: Type I Tyrosinemia is an inherited metabolic disease, severely affecting liver and kidney, associated with tyrosine catabolic pathway and causing early death if left untreated. NTBC changed its prognosis radically.

Purpose: To evaluate the evolution of children affected by type I Tyrosinemia treated with NTBC in Tunisia.

Patients and Methods: All Tunisian children affected by type I Tyrosinemia treated with NTBC between 1990 and 2010 were studied.

Result: 19 patients were enrolled. The median age at diagnosis was: 3.5 months for acute form (10 cases) versus 10.5 months for sub-acute form (6 cases). Two children had a chronic form (34 and 60 months) and a child was identified by prenatal diagnosis. NTBC was started after a median delay of 1 month (0–5) for the acute and sub-acute forms and respectively after 4.5 and 10 years for the two children having the chronic form. Except one child, all had dietary treatment (controlled in Tyrosine and phenylalanine). Precocious death was observed in 4 cases. Hepatocellular carcinoma occurred in two cases: one had favorable outcome after liver transplantation and the second died. Thirteen patients had a favorable outcome: hepatic tests normalized within the first month in half of the cases and 7/10 normalized their rates of alpha foetoprotein within the first year of evolution.

Conclusion: type I tyrosinemia is a serious illness. Its prognosis improved after the discovery of NTBC. This treatment must rather be instituted as early as possible in order to avoid hepatocellular carcinoma.

019-P

ATTENTION DEFICIT IN THE PATIENTS WITH TYROSINEMIA TYPE 1

Pohorecka M[1], Jakubowska-Winecka A[1], Biernacka M[1], Biernacki M[2], Kusmierska K[1], Kowalik A[1], Wolanczyk T[3], Sykut-Cegielska J[1]
[1]The Children's Memorial Health Institute, Warszawa, Poland
[2]University of Warsaw, Warszawa, Poland
[3]Medical University of Warsaw, Warszawa, Poland

Background: The patients with tyrosinemia type 1 are treated with Orfadin, which inhibits the tyrosine metabolism at the stage of 4-hydroxyphenylpyruvate dioxygenase, blocking accumulation of toxic metabolites, but causing increase of plasma tyrosine level. The recent reports suggest possible disorders of cognitive development.

Objectives: The aim of the study was to evaluate if the children treated by Orfadin (together with the phenylalanine- and tyrosine-restricted diet) present with psycho-motor development retardation and/or cognitive problems and to find out whether plasma tyrosine and phenylalanine levels may impact on this.

Material and Methods: Cognitive development and behavior, and plasma tyrosine and phenylalanine levels were analyzed in eight patients during their first five years of Orfadin treatment. The Child Behavior Checklist CBCL/4–18 and the Wechsler Intelligence Scale for Children (WISC-R) were used. The results were compared by correlation analysis and repeated measures analysis of variance.

Results: 1) In all patients IQ score was in normal range. 2) In four children a significant deficit of attention without any other psychological problems was detected. 3) Plasma tyrosine level in patients with attention deficit was more variable $F(4,12)=9{,}249 p<001 \eta 2=.755$ compared to children with no such problems $F(4,12)=1{,}009 p=$n.s. 4) There was no correlation between attention deficit and plasma phenylalanine level. 5) There was reverse correlation between attention deficit and results from verbal scale (WISC-R)$r=-.884, p<.01$, what may suggest decreased ability to memorize.

Conclusion: The study results showed that in the patients with tyrosinemia type1, attention deficit is not rare, and may be connected with variable plasma tyrosine level.

020-P

INTERMITTENT CHOREOATHETOSIS IN A 9-YEAR-OLD BOY WITH LATE ONSET NKH CAUSED BY A NOVEL HOMOZYGOUS MISSENSE MUTATION IN THE GLDC GENE

Brunel-Guitton C[1], Casey B[2], Hewes D[3], Vallance H[2], Stockler-Ipsiroglu S[1], Mercimek-Mahmutoglu S[1]
[1]Div Biochemical Dis, Dep Ped, Univ BC, Vancouver, Canada
[2]Dep Path, Univ BC, Vancouver, Canada
[3]Abbotsford Hospital, Vancouver, Canada

Background: Nonketotic hyperglycinemia (NKH) is an autosomal recessive disorder of glycine degradation. Clinical subtypes include neonatal, infantile and late onset. Late onset NKH patients can present with a progressive spastic paraplegia, choreoathetosis, behavioral problems, cognitive impairment, optic atrophy and epilepsy.

Case Presentation and Results: This 9-year-old boy with normal cognition presented with choreoathetosis during a febrile illness at age 3 years. Following this episode he had 4 more episodes between 4 and 9 years of age. Plasma amino acids showed 4 times elevated glycine levels during his last episode. His past medical history and family history were unremarkable. In his neurological examination he had brisk muscle stretch reflexes, mildly abnormal cerebellar tests, intentional tremor and myoclonic jerks. His CSF glycine was 7 times elevated. CSF/plasma glycine ratio was 0.044 (reference range<0.02). A novel homozygous missense mutation, (c.605C>T; p.Ala202Val) was identified in the GLDC gene. Alanine at 202 is predicted to be highly conserved amino acid by three separate algorithms to damage the protein function. His parents were carrier for the same mutation. He had no optic atrophy, no peripheral neuropathy and no intellectual disability. His cranial MRI and MR-spectroscopy were normal.

Conclusion: We report a 9-year-old boy with late onset NKH and normal neurocognitive function who presented with a non-progressive intermittent choreoathetosis during febrile illnesses. This is the first report of late onset NKH with molecular confirmation. Late onset NKH should be in differential diagnosis of intermittent choreoathetosis secondary to infections.

021-P

EXPERIENCE WITH AN LC-MS/MS METHOD FOR ANALYSIS OF BRANCHED CHAIN AMINO ACIDS IN DRIED BLOOD SPOTS

Alodaib A[1], Wiley V[2], Sim K[3], Carpenter K[3], Wilcken B[1]
[1]*Disc Paeds & Child Health, Sydney Univ, Sydney, Australia*
[2]*NSW Newborn Screening Prog, Sydney, Australia*
[3]*Biochem Genetics, Children's Hosp, Sydney, Australia*

Analysis of branched chain amino acids is required for diagnosis and management of patients with Maple Syrup Urine Disease. Tandem mass spectrometry (MS/MS) based newborn screening is unable to distinguish between isobaric leucine species so identification of the pathognomonic marker, alloisoleucine, requires further testing. Traditional ion exchange chromatography is time consuming and plasma assays require venipuncture and transport of samples to the reference laboratory. Second tier testing of the initial dried blood spot (DBS) combining liquid chromatography with MS/MS has been used to improve specificity for MSUD newborn screening. We report our experience in using such a method and highlight the utility of the method for monitoring patients from home using dried blood spots and as a backup for traditional plasma assays.

The method is based on that of Oglesbee et al and utilises stable isotope labelled internal standards for valine, alloisoleucine and leucine. Analytes are separated using reverse phase chromatography with gradient elution with a total run time of 15 minutes. DBS standard values were assigned to give optimal correlation for DBS results with plasma samples taken at the same time and analysed by amino acid analyser (Biochrom 30).

Comparison of 60 plasma values with DBS taken at the same time showed excellent correlation for all analytes, ($r2 > 0.97$). Within batch precision ranged from 3% for Leucine to 10.67% for Isoleucine, between batch precision is typically around 10%.

We hope to achieve further reductions in analysis time by moving to a UPLC platform in the near future.

022-P

PULMONARY HYPERTENSION ASSOCIATED WITH INBORN ERRORS OF METABOLISM: REPORT OF 7 CASES

Del Toro M[1], Arranz A[2], Riudor E[2], Moreno A[3], Ribes A[4], Briones P[4], Armengué T[3], Roig M[1]
[1]*Pediatric Neurology, Hosp Vall d'Hebron, Barcelona, Spain*
[2]*Metabolic Laboratory, Hosp Vall d'Hebron, Barcelona, Spain*
[3]*Pediatric Pneumology, Hosp Vall d'Hebron, Barcelona, Spain*
[4]*Institut de Bioquimica Clinica, Barcelona, Spain*

Background: Inborn errors of metabolism (IEM) and primary pulmonary hypertension (PPH) have been occasionally associated, especially with glycine and mitochondrial disorders.

Objectives: To report 7 infants diagnosed of PPT and IEM related to different glycine pathways.

Patients: Retrospective review of 3 boys and 4 girls diagnosed of PPH and IEM between 1992 and 2008.

Results: All 7 infants were diagnosed of PPH(mean age 4 months (2–16)) and of an IEM (mean age 5 months (2–13)). In 4, diagnosis of PPH preceded the diagnosis of IEM, in 2 of them it was simultaneous and in 1 IEM was first detected. Diagnosis of PPH was established in basis of echocardiogram in all patients and was confirmed by catheterisation in 5 of them.

Regarding to clinical presentation we can distinguish two groups: a) progressive vacuolating leukoencaphalopathy with a rapid neurological deterioration (4 patients), b) hypoxemia, pulmonary haemorrhage and metabolic acidosis (3 patients). Age of death was under 18 months in all patients.

Metabolic diagnosis was of atypical NKH in the first group. GCS deficiency confirmed by enzymatic studies in frozen liver but no mutations found in candidate genes (GLDC, GCST and GCSH). The second group showed a profile compatible with dihydrolypoil-dehydrogenase deficiency (dysfunction in glycine, pyruvate and lysin degradation). Genetic studies of involved genes are not conclusive.

Conclusion: Etiological study of PPH must always include screening for IEM, especially those related with glycine and mitochondria. When glycine metabolism is involved, in our experience, it leads to death before the age of 18 months.

023-P

ADENINE PHOSPHORIBOSYLTRANSFERASE (APRT) DEFICIENCY: PHENOTYPE AND GENOTYPE CHARACTERIZATION OF A LARGE COHORT

Bollée G.[1], Dollinger C.[2], Boutaud L.[2], Bensman A.[3], Harambat J.[4], Deteix P.[5], Daudon M.[2], Knebelmann B.[1], Ceballos-Picot I.[1]
[1]*1- Paris Descartes University, Paris, France*
[2]*Necker-Enfants Malades Hospital, APHP, Paris, France*
[3]*Université Paris 6, Paris, France*
[4]*Bordeaux Universitary Hospital, Bordeaux, France*
[5]*Clermont-Ferrand Universitary Hospital, Clermont-Ferrand, France*

Background: Adenine phosphoribosyltransférase (APRT) deficiency is a rare autosomal recessive disorder causing 2,8 dihydroxyadénine (2,8-DHA) stones and renal failure secondary to intratubular crystalline precipitation. The disease can be efficiently treated by allopurinol. Data on clinical presentation of APRT deficiency are very limited, especially in Caucasian population.

Methods: We retrospectively reviewed genotype and phenotype of all cases of APRT deficiency identified at Necker Hospital, Paris, France, between 1978 and 2009.

Results: Diagnosis of complete APRT deficiency was made in 53 patients from 43 families, at a median age of 28.9 (5.6–51) years. Full clinical data were available in 40 patients from 33 families. A striking finding was that diagnosis was delayed for years from onset of symptoms in many patients. Fourteen (35%) patients had decreased renal function at diagnosis. Six (15%) patients had reached end stage renal disease (ESRD) and APRT deficiency was detected once disease recurred in renal transplant. Eight patients (20%) reached ESRD over follow-up (median duration 74 (14–112) months). Sequencing of aprt gene was performed in 31 families. Fifty-four mutated chromosomes were found on the 62 chromosomes analyzed (87%). Eighteen different mutations were identified, 14 of which being novel. A single T insertion at the intron 4 splice donor site (IVS4+2insT) leading to a truncated protein, accounted for 40.3% of mutations.

Conclusion: Our report, the largest series reported, highlights the under-diagnosis and the potential severity of APRT deficiency. Early recognition of the disease is crucial to prompt treatment and prevent renal complications.

024-P

IMPROVING PATIENT EXPERIENCE IN CYSTINURIA: CAN SERIAL URINE PROFILES REPLACE TIMED URINE COLLECTIONS IN THE FOLLOW-UP OF PATIENTS WITH CYSTINURIA?

Lopez B[1], Tomson C[2], De Hora M[1], Kemp H[1]
[1]*Dept Clin Biochem; Southmead Hospital, Bristol, United Kingdom*
[2]*Dept Renal Med; Southmead Hospital, Bristol, United Kingdom*

Background: Treatment strategies in cystinuria include hyperdiuresis, urinary alkalinisation and thiol-binding drugs. Patients are followed up by measurements of urinary cystine concentration and volume in day/night or 24 hour urine collections. Urine is collected directly into acid to ensure that all excreted cystine remains in solution and to prevent bacterial degradation of cystine. Acid also inhibits thiol-disulphide exchange reactions in patients on these drugs, preventing changes to free and complexed levels in vitro. Timed collections can, however, be very inconvenient to patients and prone to errors of over and under-collection. Furthermore, as urinary cystine concentration varies over 24 hours, pooling of urine may mask periods of cystine super-saturation. We tested an alternative involving the collection of small aliquots of urine at each void.

Methods: The stability of urinary cystine, when acidification was delayed at time of collection, in the presence and absence of Penicillamine was investigated. The clinical utility and acceptability of serial urine profiles versus timed urine collections were assessed in five patients. An in-house UPLC-HILIC MS/MS method was used.

Results: Urinary cystine concentrations were stable for up to 72 hours, with and without Penicillamine, in the presence of Thymol ($p=0.419$; $p=0.500$ respectively by Factorial ANOVA). Serial urine profiles detected transient episodes of cystine supersaturation which would have evaded detection by timed urine collections and patients found these collections as easy but more convenient as assessed by questionnaire (n=3).

Conclusions: Serial urine profiles provide additional clinical information and are more acceptable than timed urine collections to patients with cystinuria.

025-P

A NOVEL RAPID UPLC/MSMS METHOD FOR THE QUANTITATION OF CYSTINE IN URINE

Lopez B[1], DeHora M[1], Williams M[1], Kemp H[1]
[1]Biochemical Genetics, Southmead Hospital, Bristol, United Kingdom

Background: Measurement of urinary cystine concentration is useful to optimize treatment in patients with cystinuria. However, the most established method, the dedicated amino-acid analyser based on ion-exchange HPLC, is time-consuming, labour intensive and expensive. Furthermore, the steps involved in sample preparation may disrupt thiol drug-cystine complexes in patients on these drugs such that the true level of unbound cystine may not be determined.
Methods: A rapid, robust, urinary cystine assay requiring minimum sample preparation was developed using UPLC-HILIC-MS/MS (Ultra-Performance Hydrophilic Interaction Liquid Chromatography).
Results: The method was linear up to 2000 μmol/L. The mean recovery of cystine was 112.3%. The limit of quantification was 7.8 μmol/L. Within and between batch precision was less than 10% at the medically relevant levels in cystinuria. Comparison with the amino-acid analyzer showed that the method displayed a small negative bias (-4.0%; 95% CI -8.6 to 0.6%) but a small positive bias (3.2%; 95% CI -5.3 to 11.6%) in patients on thiol-binding drugs. A negative bias was anticipated as MS/MS methods are characterized by high specificity. However, the positive bias suggests that the amino-acid analyzer method may under-estimate cystine concentration in patients on thiol-binding drugs.
Conclusion: The analytical performance of the method will allow direct analysis of underivatized cystine with the advantages of simplicity, faster throughput, cost-efficiency and minimal risk of analyte loss or alteration during analysis.

026-P

CEREBRAL ACCUMULATION OF 3-HYDROXYISOVALERIC ACID IN ADULTS UNTIL RECENTLY UNAWARE OF HAVING 3-METHYLCROTONYL-COA CARBOXYLASE (MCC) DEFICIENCY

Van der Graaf M.[1], Engelke U.F.H.[1], Morava E.[1], Janssen M.C.H.[1], de Vries M.C.[1], Kluijtmans L.A.J.[1], Góraj B.[1], Heerschap A.[1], Wevers R.A.[1]
[1]Radboud University Nijmegen Med. Centre, Nijmegen, Netherlands

Background: Recently, our group showed for the first time by in vivo MR spectroscopy (MRS) cerebral accumulation of 3-hydroxyisovaleric acid (3HIVA) in a pediatric patient with 3-Methylcrotonyl-CoA Carboxylase (MCC; EC 6.4.1.4) deficiency. 3HIVA is considered to be neurotoxic, but this is under debate.
Objective: To investigate whether 3HIVA is increased in adult asymptomatic MCCD patients to gain information about its neurotoxicity.
Case report: Two female subjects (30 and 31 yrs) with MCCD, whose deficiency was discovered by a positive neonatal screening of their healthy new-born babies. Diagnoses in the mothers were established by demonstration of high 3-methylcrotonylglycine and C5-OH-carnitine, and in one subject confirmed by enzyme measurement. The 31-yr old subject had no health complaints at all and the other suffered from a depression.
Methods: Measurement of 3HIVA concentrations by in vitro NMR spectroscopy of urine samples and in vivo MRS of the brain.
Results: 3HIVA concentrations in urine were 1400 micromol/mmol creatinine for the 31-yr old subject without complaints and 1990 micromol/mmol creatinine for the other. In vivo MRS showed predominantly in white matter a clear single resonance of 3HIVA at 1.28 ppm, in the order of magnitude of 1.1–1.4 mmol/L in the 31-yr old subject and 0.7–1.0 mmol/L in the other woman. Fot both subjects, other brain metabolite concentrations were in the normal range.
Conclusion: As relatively high levels of cerebral 3HIVA were found in adult MCCD patients with no or minor complaints, our results corroborate the disbelief about the neurotoxicity of 3HIVA.

027-O

PYRROLINE-5-CARBOXYLATE (P5C) SYNTHASE DEFICIENCY: NOVEL CLINICAL AND BIOCHEMICAL INSIGHTS

Martinelli D[1], Haeberle J[2], Colafati S[1], Giunta C[2], Hausser I[3], Goffredo BM[1], Carrozzo R[1], Meschini MC[1], Bevivino E[1], Boenzi S[1], Baumgartner M[2], Dionisi-Vici C[1]
[1]Div. Metabolism, Bambino Gesù Hospital, Rome, Italy
[2]Div. Metabolism, Children's Hospital, Zurich, Switzerland
[3]EM Lab., Dept Dermatol, Un. Heidelberg, Heidelberg, Germany

Background: P5C-synthase catalyzes the synthesis of proline from glutamate and plays a pivotal role in ornithine and arginine metabolism. P5C-synthase deficiency has been so far reported only in 6 patients from two families who showed neurodevelopmental delay (6/6), joint laxity (6/6), cutis laxa (6/6) and cataract (3/6). Two patients (R84Q/R84Q) displayed fasting hyperammonemia, reduced plasma proline, arginine, ornithine and citrulline, whereas patients from the second family (H784Y/H784Y) had no metabolic abnormalities.
Case Report and Results: A 1 year-old patient, compound heterozygous for the G93R/T297I mutations, presented macrocephaly, sparse hair, mild developmental delay, cataract, visible veins, joint and skin laxity, reduced plasma proline, arginine, ornithine, citrulline and normal ammonia levels. Brain MRI showed cortical atrophy, vessels ectasia and tortuosity; MRS showed reduced creatine peak with reversed NAA/creatine and CHO/creatine ratios. Ultrastructural analysis of a skin biopsy revealed abnormal collagen fibrils with irregular contours and variable sized diameters. Elastic fibers and morphology of mitochondria were normal. A normal mitochondrial network was confirmed by mitotracker probe.
Discussion: Reduced brain creatine probably reflects limited availability of its substrate arginine. Similarly to skin and joint laxity, brain vessel dysplasia may be caused by impaired collagen synthesis due to low proline levels. Despite phenotypic similarities with cutis laxa type-2, caused by P5C-reductase mutations, morphological studies in our patient displayed a different pattern of abnormalities. Our observation adds novel insights into this very rare metabolic disease.

028-P

TYROSINEMIA TYPE 1- EFFECT OF METABOLITES ON FIVE DIFFERENT DNA- REPAIR ENZYMES

Bliksrud YT[1], Ellingsen A[1], Bjxrås M[1]
[1]Dep Med Bioch, Oslo Univ Hosp, Oslo, Norway

Background: Hereditary Tyrosinemia type I (HT1) is a disease of autosomal recessive inheritance and associated with a high risk of hepatocellular carcinoma. It is caused by deficiency of fumarylacetoacetase (FAH), the last enzyme of tyrosine degradation which mainly takes place in the liver. The condition leads to accumulation of the metabolites fumarylacetoacetate (FAA) and succinylacetone (SA). The hypothesis of increased mutagenesis in hepatocytes of HT1 patients is supported both by the high incidence of hepatocellular carcinoma and by the frequent phenomenon of point mutation reversion in liver nodules. FAA is shown to produce glutathione adducts, and increased oxidative stress in the HT1 hepatocytes is thought to contribute to the DNA instability. Less is known about the influence of FAA and SA on DNA repair enzymes that initiate the major pathway for mutagenic DNA base lesions.
Methods: The activities of the purified human DNA repair enzymes Ogg1, Neil1, Neil2, UNG and O6-methylguanine-DNA methyltransferase were measured under influence of different concentrations of FAA and SA. The substrates were radio labeled 8oxoG:C, 5-OH-cytosine, uracil and N-metylnitrosurea respectively.
Results: We showed that the enzyme activities of the glycosylases Ogg1, Neil1 and Neil2 are impaired when exposed to FAA. The same enzymes seem not to be impaired by SA-exposure. The activity of UNG and O6-methylguanine-DNA methyltransferase seem not to be impaired by either of the metabolites.
Conclusion: Impaired repair of oxidative DNA base lesions may contribute to fixation of mutations, increased genome instability and tumor development in liver of HT1 patients.

029-P

HEREDITARY TYROSINAEMIA TYPE I: DATA OF A SPANISH REGISTRY

Del Toro M[1], Couce ML[2], Aldamiz L[3], Dalmau J[4], Sánchez F[5], Pintos G[6], Manzanares J[7], Bueno M[8], Gil D[9], Gil M[10], Gomez L[11], Lopez E[12], Navas V[13]
[1]*Pediatric Neurology, Hosp Vall d'Hebron, Barcelona, Spain*
[2]*Pediatric Dep, Hosp Clin Univeristario, Santiago de Compostela, Spain*
[3]*Metabolism Unit, Hosp Cruces, Barakaldo, Spain*
[4]*Pediatric Nutrition, Hosp La Fe, Valencia, Spain*
[5]*Pediatric Nutrition, Hosp Virgen Camino, Pamplona, Spain*
[6]*Pediatric Dep, Hosp Germans Trias Pujol, Badalona, Spain*
[7]*Pediatric Nutrition, Hosp 12 Octubre, Madrid, Spain*
[8]*Pediatric Nutrition, Hosp Virgen Rocio, Sevilla, Spain*
[9]*Ped Gastroenterol Dep, Hosp V. Arrixaca, Murcia, Spain*
[10]*Ped Gastroenterol Dep, Hosp Reina Sofia, Cordoba, Spain*
[11]*Pediatric Nutrition, Hosp Sant Joan Deu, Barcelona, Spain*
[12]*PedGastroenterol Dep, Hosp Torrecardenas, Almeria, Spain*
[13]*Pediatric Dep, Hosp Carlos Haya, Malaga, Spain*

Background: Hereditary tyrosinaemia type I (HT1) is an autosomal recessive inborn error of tyrosine catabolism caused by the deficiency of fumarilacetoacetase which leads to hepatic failure, hepatocellular carcinoma, renal tubular dysfunction and acute porfiria.
Objective: To present the results of Spanish registry of pediatric patients affected with HT1 and treated with NTBC.
Methods: Retrospective review of patients diagnosed of HT1 in Spain since 1996.
Results: 36 patients (18 male, 18 female) with a mean age at diagnosis of 4,2 months (1–12 months). Consanguinity was reported in 12/36 and affected siblings in 10/36.
Genetic testing in 25 cases showed IVS6-1(g>t) as the most common mutation in 49% (35% in homozygosis). Acute hepatic failure was the initial symptom in 65% and diagnosis by screening was made in11.8%. Mean tyrosine levels before treatment were of 424,5 mcmol/L, urine succinilacetone 203 mmol/mol de creat, alphaphetoprotein 125.300 mg/dl. Ultrasound imaging showed hepatomegaly in 44% of cases, with nodules in 38% and nephrocalcinosis in 28%.
All patients received treatment with NTBC (mean dose of 0.87 mg/kg/day) with good response and tolerance (minimal side effects in 11%). NTBC levels were maintained at a mean of 45.67 mmol/L. Diet adherence was not optimal in 33% of cases. 2 patients underwent liver transplant, one because of an acute decompensation and another one because of hepatocarcinoma.
Conclusions: In our series, acute hepatic failure is the most frequent clinical presentation, often with some degree of renal tubulopathy. NTBC treatment has improved considerably the prognosis of HT1.

030-P

DEFECT IN PROLINE SYNTHESIS: PYRROLINE-5-CARBOXYLATE REDUCTASE 1 DEFICIENCY IN PATIENTS WITH CUTIS LAXA AND MENTAL RETARDATION

Kretz R[1], Kariminejad A[2], Rohrbach M[1], Bartholdi D[3], Bozorgmehr B[2], Baumgartner M[3], Hausser I[4], Giunta C[1], Häberle J[1]
[1]*University Children's Hospital, Zurich, Switzerland*
[2]*Kariminejad Pathology & Genetics Center, Tehran, Islamic Republic of Iran*
[3]*Institute of Medical Genetics, Zurich, Switzerland*
[4]*Institute of Dermatology, Heidelberg, Germany*

Pyrroline-5-carboxylate reductase 1 (PYCR1) catalyzes the last step in proline synthesis. PYCR1 deficiency, caused by a defect in PYCR1, was recently described in patients with cutis laxa, intrauterine growth retardation, bilateral congenital hip dislocation, and mental retardation. The pathogenesis of this new disorder and the role of proline for the development of the clinical phenotype are still unclear. Herein, we describe the work-up in two patients referred because of cutis laxa.
Both patients were of consanguineous Iranian origin and presented with moderate cutis laxa, bilateral congenital hip dislocation, mild facial dysmorphism, and mental retardation. Plasma proline levels were normal. We performed genetic investigations of the PYCR1 gene as well as skin electron microscopy studies.
Genetic analysis revealed homozygous mutations, c.616G>A (p.G206R) in patient 1 and c.89 T>A (p.I30K) in patient 2, respectively, in PYCR1. Investigations of skin biopsies by electron microscopy showed smaller and fragmented elastic fibres, abnormal morphology of the mitochondria and their cristae, and slightly abnormal collagen fibril diameters with irregular outline and variable size within the dermal connective tissue.
PYCR1 deficiency belongs to the differential diagnosis of autosomal-recessive cutis laxa and results in characteristic changes of skin electron microscopy. However, it remains difficult to reconcile the metabolic defect of proline synthesis with the clinical phenotype of PYCR1 deficiency and the morphological changes particularly those seen in mitochondria.

031-P

MAPLE SYRUP URINE DISEASE (MSUD) IN BRAZIL: A CROSS-SECTIONAL STUDY OF 41 PATIENTS

Herber S[1], Bittar C[2], Netto CBO[2], De Barba ML[2], Schwartz I[2], Giugliani R[2], Souza CFM[2]
[1]*UFRGS, Postgraduation, Brazil*
[2]*Medical Genetics Service, HCPA, Porto Alegre, Brazil*

MSUD is an inborn error of metabolism that leads to a buildup of leucine, isoleucine and valine. These aminoacids are toxic to the central nervous system, leading to acute and chronic sequelae. The world incidence is around 1:185.000 but in Brazil the data is unknown. Early diagnosis and correct treatment may guarantee a normal development. This was an observational, cross-sectional study and data were collected through interview with doctors assisting patients with MSUD. Sixty-four patients were identified and complete data for 41 cases were obtained. In the first 10 days of life 54% presented symptoms, 46% were diagnosed before the age of 30 mo and special metabolic formula was only promptly available in 16% of the cases. Nowadays, 50% of the patients are getting the formula regularly, 92% present DNPD, 61% have convulsions, 54% respiratory abnormalities and 34% have the characteristic MSUD odor. There was familiar recurrence in 17% and 15% of the cases died before 10 months of age. The patients that had normal development received early diagnosis and treatment, regular supply of the metabolic formula and correct medical and biochemical monitoring. This work will contribute to the elaboration of new programs to better assist patients with MSUD.

032-P

NEW METHOD TO MEASURE CYSTINE IN GRANULOCYTES USING LIQUID CHROMATOGRAHY-TANDEM MASS SPECTROMETRY

García-Villoria J[1], Hernández JM[1], Arias A[1], Ribes A[1]
[1]Dp Biochem and Mol Genet.Hospital Clinic, Barcelona, Spain

Background: Cystinosis is a rare autosomal recessive disorder characterized by an accumulation of intralysosomal cystine due to a defect in the transport of free cystine out of lysosomes. Accurate measurement of intracellular cystine is necessary both, for the diagnosis and monitoring of treatment with cysteamine. We describe a new method using liquid chromatography-electrospray-tandem mass spectrometry (HPLC-ESI-MS/MS).
Method: Cells were isolated and lysed in the presence of N-ethyl-maleimide. After deproteinization, 20 μl of sample and 10 μl of 25 μM d6-cystine, as internal standard, were butylated and finally reconstituted in 100 μl of mobile phase: water acidified with formic acid (0.5 mL/L)/ acetonitrile (80/20); 50 μL were injected into the HPLC-ESI-MS/MS with a flow rate of 0.2 mL/min, using a 3.5-μm, 2.1 x 50 mm Symmetry C18. column. Run time was 5 minutes. The detection was performed in positive mode using MRM; the transitions (m/z) were 353 > 130 and 359 > 131 for cystine and d6-cystine di-butyl ester, respectively.
Results: HPLC-ESI-MS/MS assay was linear up to 50 μmol/L. The lower limit of quantification was 100 nmol/L. Within-run and between-run precision coefficients of variation were 4% and 6%, respectively and the analytical recovery was 101%.
Conclusions: Measurement of cystine in granulocytes by the present method results in good analytical performance that is useful for the measurement of low concentrations of intracellular cystine. In addition, this method is faster than other HPLC-ESI-MS/MS method previously reported (Chabli A et al. Clin Biochem 2007; 40: 692–698).

033-O

GLUTAMINE SYNTHETASE DEFICIENCY IN A 3 YEAR OLD WITH SEVERE NEUROLOGICAL DISEASE

Häberle J[1], Paesold P[1], Kolker S[2], Hoffman G[2], Shahbeck N[3], Ben-Omran T[3]
[1]University Children's Hospital, Zurich, Switzerland
[2]University Children's Hospital, Heidelberg, Germany
[3]Department of Pediatrics, Doha, Qatar

Glutamine synthetase (GS) is ubiquitously expressed and catalyzes the conversion of glutamate and ammonia to glutamine. It is the only known enzyme capable of glutamine synthesis and has many roles in the human organism. A defect in GLUL, encoding for GS, was so far described in only two patients who suffered from an early lethal multisystemic disorder. Here, we describe a third patient with GS deficiency that is still alive at 3 years of age.
The patient was born to healthy first cousin Arab parents from Sudan. He presented in early infancy and developed chronic encephalopathy and seizures. Based on low levels of plasma glutamine (179 μmol/l) and mild hyperammonemia (values ranging from 70 to 150 μmol/l), GS deficiency was investigated.
The patient was found to be homozygous for the mutation c.970C>A (p.R324S) of the GLUL gene. Western Blotting showed a strong GS upregulation in fibroblasts in accordance with previous findings in GS deficiency. Brain MRI revealed atrophic changes with white matter hypomyelination.
GS deficiency is not always an early lethal disorder. If residual enzyme activity allows for some degree of glutamine synthesis, patients can survive beyond neonatal age but will still be severely affected mainly by neurological disease.

034-P

CHRONIC FORM OF TYROSINEMIA TYPE 1 PRESENTED WITH RICKETS SIGNS

Dikme G[1], Soyucen E[2], Zorer G[3], Canpolat N[4], Aydin A[2], Tuysuz B[1]
[1]Dep Ped Genet, Ist. Uni Cerrahpasa Med, Istanbul, Turkey
[2]Dep Ped Metab, Ist Uni Cerrahpasa Med, Istanbul, Turkey
[3]Ped Ortop, Istanbul Hospital, Istanbul, Turkey
[4]Dep Ped Nephrol, Ist Uni, Cerrahpasa Med, Istanbul, Turkey

Tyrosinemia is an autosomal recessive metabolic disorder characterized by increased tyrosine and its metabolites in blood and other body fluids. Three types are defined according to the enzyme deficiency. The most common type is tyrosinemia type 1 which is caused by fumaryloacetoacetate hydrolase deficiency. 8 year-old girl was admitted with X bain deformities. The clinical findings were enlargement of wrist, X bain deformity and hepatosplenomegaly. The height, weight and head circumference were less than 3th percentile. The laboratory findings were hypophosphatemia, hypokalemia, elevated alkaline phosphates and parathormone levels. Urine pH was 8. Generalized aminoaciduria and glucosuria were detected in urine analysis. Tubular phosphate reabsorbtion was 6.2% (N:80–96). The blood gases analysis showed compensated metabolic acidosis. Osteopenia, irregularity and enlargement of metaphysis were detected in skeletal radiographs. The patient was diagnosed as renal Fanconi syndrome. Galactosemia, fructosemia, Dent disease, Lowe syndrome, cystinosis, Wilson disease, Fanconi Bickel syndrome and tyrosinemia were considered in differential diagnosis. Tyrosine was slightly elevated in blood and succinylacetone, 4-OH- phenyl pyruvic acid and 4–OH-phenyl lactic acid were elevated in urine organic acid analysis. She was diagnosed as tyrosinemia type 1. Acute form of the disease is characterized by liver failure in infancy, whereas growth retardation, tubular dysfunction and mild liver involvement are seen in chronic form. In conclusion, patients with rickets who are unresponsive to treatment, tyrosinemia type 1, especially rare chronic form should be considered.

035-O

D-AMINO ACIDS AS DIAGNOSTIC MARKERS FOR INBORN ERRORS OF METABOLISM

Visser WF[1], LWJ Klomp[1], Albersen M[1], Verhoeven-Duif NM[1], de Koning TJ[1]
[1]Dpt Metab Endcrn Dis, UMC Utrecht., Utrecht, Netherlands

Background: It has recently been discovered that humans synthesize specific D-amino acids, including D-serine and D-aspartate by the action of the enzymes serine racemase (SRR) and aspartate racemase (DR) respectively. Only two human enzymes are known to degrade D-amino acids: D-amino acid oxidase (DAO) and D-aspartate oxidase (DDO), both of which are located in peroxisomes.
Interestingly, several reports suggest that D-amino acid levels may be altered in neurodegenerative disorders, which could allow them to be used for diagnostic purposes. Inactivating mutations in the gene encoding DAO have recently been shown to cause Amyotrophic Lateral Sclerosis (ALS). DAO is presumably also inactivated in Zellweger syndrome, which is caused by defects in peroxisomal biogenesis.
Objectives: To study the levels of D-amino acids in human biological fluids in order to evaluate the possibility of using them as diagnostic markers for human disease.
Materials and Methods: We have developed a sensitive UPLC-MS/MS based method for stereoselective amino acid quantification. Using this method, we have measured D-amino acid levels in human biological fluids.
Results and Discussion: We successfully measured endogenous levels of different D-amino acids in human plasma, urine, sputum and cell culture medium, which enables us to implement this method in future diagnostics. We have measured plasma and CSF samples from healthy controls and ALS patients, and found significant differences in several D-amino acids. Moreover, D-serine was also elevated in CSF from patients with Zellweger syndrome. We are currently examining diagnostic patterns in peroxisomal disease.

036-P

EXPANDED NEWBORN SCREENING IN THE NICU POPULATION

Porta F[1], Tortorelli S[1], Gavrilov D[1], Oglesbee D[1], Raymond K[1], Matern D[1], Rinaldo P[1]
[1]*BGL, Mayo Clinic College of Medicine, Rochester, MN, United States*

Background: Cut-off values for expanded newborn screening can lead to unreliable results in NICU newborns, given the peculiar biology and the intensive care needs of this population.
Objectives: To assess the percentile distributions for 59 analytes in NICU newborns screened in Minnesota between January 2006 and December 2009.
Materials and Methods: The screening outcomes at 24–72 hours, 15, and 30 days of life in NICU newborns have been analyzed and compared to the Region4 Genetics collaborative MS/MS data project (http://region4genetics.org/msms_data_project/priority1/).
Results: The comparison between the 99 percentiles of selected analytes (microMol/L) is shown in the table.

Region 4 Project* (n=4,652)	NICU 24–72 h (n=4,090)	NICU 15 d (n=3,485)	NICU 30 d
Amino Acids			
Ile-Leu	230	376	280 253
Met	45	143	186 87
Cit	29	25	32 34
Phe	97	173	132 110
Arg	31	46	44 40
Tyr	205	379	356 293
Acylcarnitines			
C3	4.68	6.74	3.04 4.87
C5	0.39	0.98	1.32 0.82
C8	0.20	0.22	0.56 0.39
C14:1	0.35	0.44	0.34 0.32
C16	5.87	4.16	2.32 2.09
C18-OH	0.07	0.03	0.03 0.03

* Cumulative values from 94 laboratories worldwide
Conclusion: The application of cut-off values derived from the normal neonatal population to NICU newborns is prone to raise the false positive rate for some amino acid disorders, unless additional samples during the first month of life are collected. Any specific abnormality of medium- or long-chain acylcarnitines detected in NICU newborns at 24–72 hours of life must be regarded as a possible fatty acid oxidation disorder.

037-P

SUCCESSFUL TREATMENT OF TWO NEONATES WITH MOLYBDENUM COFACTOR DEFICIENCY (MOCD) TYPE A, USING CYCLIC PYRANOPTERINE MONOPHOSPHATE (CPMP)

Schwahn BC[1], Galloway PG[2], Bowhay S[3], Veldman A[4], Santamaria JA[5], Schwarz G[5], Belaidi AA[5]
[1]*Dept Metabolic Medicine RHSC, Glasgow, United Kingdom*
[2]*Dept Biochemistry RHSC, Glasgow, United Kingdom*
[3]*Dept Pharmacy RHSC, Glasgow, United Kingdom*
[4]*Monash Newborn, Monash Medical Centre, Melbourne, Australia*
[5]*Inst Biochemistry, Univ Cologne, Cologne, Germany*

Background: MOCD type A is a rare metabolic disorder that is associated with neonatal-onset encephalopathy due to sulfite accumulation, usually leading to death in early infancy. There has been no effective treatment until recently when the molybdenum cofactor precursor, cPMP, became available in purified form. We present two unrelated cases of neonatal-onset MOCD type A with favorable response to cPMP.
Patients: Both children are term AGA babies born to consanguineous parents. Diagnosis was made on day 1 of life in Baby 1 due to a previous fatally affected sibling. Baby 2 was diagnosed on day 4 of life by selective metabolic screening due to intractable seizures from day 1.
Treatment: Experimental treatment with intravenous cPMP supplementation was started on compassionate grounds on day 7 of life in Baby 1 and on day 5 of life in Baby 2, respectively, after consideration of clinical and research ethics and having obtained permission from parents and the Health Board. Treatment efficacy and safety were monitored using a study protocol provided by the manufacturer.
Results: Both neonates tolerated treatment without adverse reactions for 5 and 2 months, respectively. We observed a quick and sustainable biochemical response. A clinical response could be recognised after 2 days. Both babies are now seizure-free off anticonvulsants, self-feeding and thriving. Head growth in Baby 1 has been normal for 5 months. Both exhibit a dyskinetic movement disorder.
Conclusion: Our observations thus far indicate that early treatment of MOCD type A with cPMP supplementation is very effective.

038-P

AMINO ACID ANALYSIS IN DRIED PLASMA SPOTS FOR THE DIAGNOSIS AND MONITORING OF AMINO ACID DISORDERS

Prunty H[1], Krywawych S[1]
[1]*Enz & Metabolic Unit, Gt Ormond St Hosp, London, United Kingdom*

Plasma amino acid analysis is essential for the diagnosis and monitoring of patients with inherited amino acid disorders. Prompt separation from red blood cells and storage at -20:C prior to analysis is necessary to prevent amino acid degradation. We present a method for the analysis of amino acids in dried plasma spots providing increased stability at room temperature, thereby enabling specimens to be sent easily over long distances.
Patient plasma is dried onto #903. paper (Whatman). The method comprises elution of the amino acids from the punched spots in 70% methanol containing the internal standard and analysis as phenylisothiocyanate derivatives by reversed phase partition liquid chromatography, using pH 6.5 sodium acetate mobile phase with an increasing gradient of organic solvent (acetonitrile, methanol); and detection by UV absorbance at 254 nm.
The average difference in amino acid results in dried plasma spots compared to plasma was +5% (range: +15% for taurine to –6% for isoleucine) (n = 46). The intra-batch CV for each amino acid measured ranged from 0–3% (overall mean 3%) (n=10) and the inter-batch CV ranged from 0–8% (overall mean 5%) (n=6). No appreciable change in amino acid concentrations was seen in samples stored for several months at –20:C. Stability studies at room temperature are to be performed.
In conclusion, an accurate and reproducible method for the analysis of amino acids in dried plasma spots has been developed, allowing for easier and more convenient sample transportation.

039-P

MOLYBDENUM COFACTOR TYPE A DEFICIENCY (MoCD-A) MAY RESULT IN FETAL CHANGES IN LATE PREGNANCY, WHICH CAN BE SUCCESSFULLY REVERSED WITH cPMP

Van Spronsen Fj[1], Schwarz G[2], Meiners Lc[3], Lunsing I[4], Bouman K[5], Erwich Jj[6], Heiner-Fokkema R[7], Boersma Hh[8], Veldman A[9], Bergman K[1]
[1]*Beatrix Child Hosp, Univ Med Center, Groningen, Netherlands*
[2]*Dept Chem Centre Mol Med, Univ Cologne, Cologne, Germany*
[3]*Dept Radiol, Univ Med Center, Groningen, Netherlands*
[4]*Dept Ped Neurol, Univ Med Center, Groningen, Netherlands*
[5]*Dept Clin Genet, Univ Med Center, Groningen, Netherlands*
[6]*Dept Obstetr, Univ Med Center, Groningen, Netherlands*
[7]*Lab Metab Dis, Lab Cent, Univ Med Center, Groningen, Netherlands*
[8]*Pharmacy, Univ Med Center, Groningen, Netherlands*
[9]*Monash Newborn, Monash Univ, Melbourne, Australia*

Background: MoCD-A patients carry mutations in MOCS1 causing a block in synthesis of cyclic pyranopterin monophosphate (cPMP; first intermediate Moco pathway). The mother of a MoCD-A patient (death at day 9, brain edema and atrophy, 418+1G->A in MOCS1) reported hiccups during last week pregnancy. The parents wanted their next child to be treated.
Aim: To study antenatal course MoCD-A and outcome after very early start treatment.
Methods: In antenatal diagnosed child (418+1G->A), fetal MRI and ultrasound were performed at 20–30–34–36 weeks, and intravenous cPMP was started within 4 hours postnatally.
Results: Fetal growth showed some increased growth at 20–30 weeks but stabilised afterwards. Fetal ultrasound was normal at 20–30–34 weeks. At 34–36 weeks, extra tissue was found around the legs. Fetal cerebral MRI at 20–30–34 weeks showed no abnormalities, at 36 weeks there was some edema and some temperofrontal atrophy. After induction of labour at 36 weeks, seizures were observed within 1 hour postnatally. Cerebral MRI at 5 hours postnatally showed diffuse edema and minor atrophy especially fronto-temporally. After start of cPMP, only one short single seizure was observed. Clinical examination at 3 weeks of age showed some delay in development. EEG abnormalities disappeared largely, MRI at 3 weeks possibly showed recovery of loss of brain tissue.
Conclusions: MoCD-A may cause antenatal damage in late pregnancy in some patients, but early start treatment resulted in spectacular improvement. In affected families close monitoring and early induction of labour to start treatment in selected cases might be considered.

040-P

OUTCOME OF MAPLE SYRUP URINE DISEASE (MSUD) IN INDIA

Bijarnia S[1] , Puri RD[1] , Verma J[1] , Shigematsu Y[2] , Yamaguchi S[3], Singh N[1] , Verma IC[1]
[1]*Center Med Gen, Sir Ganga Ram Hosp, New Delhi, India*
[2]*Dept Health Sci, Fac Med Sc, Univ Fukui, 23Matsuoka-Shimoaizuki Eiheiji-cho Fukui, Japan*
[3]*Dept Ped, Shimane Univ Sch ool Medicine, 89–1 En-ya, Izumo, Shimane, Japan*

Background: Prevalence of MSUD is high in India, due to huge number of births (27 million/year), and high degree of consanguinity.
Objectives: To evaluate outcome of MSUD in India, and develop a strategy for its management.
Material (Patients) and Methods: Fifteen patients diagnosed at our center with follow up information were analyzed. Diagnosis was based on plasma amino acids, MS-MS on dried blood spots and GC-MS on urine. After emergency management, treatment with protein restricted diet and branched chain amino acid free supplement was carried out.
Results: Seven patients presented in neonatal period, 4 each in infancy and post-infancy. Five (5/7)children with neonatal presentation and 1/4 children presenting in infancy died. Of those surviving, 5/9 received treatment including MSUD supplement at diagnosis. Three (3/9)were managed with protein restriction alone. One (1/9) child underwent liver transplantation at 22 months and is doing well. Two (2/9)children stopped supplement due to financial constraints, and continue to have developmental delay. Two children (2/9)diagnosed after infancy is on supplement; their development has improved to the satisfaction of the parents. One child (1/9)presenting at 9 years remains normal without treatment. Seven prenatal diagnoses were performed in families.
Conclusions: Treatment of MSUD remains difficult in Indian patients. Training to diagnose and manage these disorders in neonatal period is needed, cost of metabolic supplements should be borne by the State, as in Gujarat, and liver transplant be considered as a viable option. Prenatal diagnosis is vital to prevent recurrences and reduce the number for whom therapy is required.

041-P

TYROSINEMIA TYPE I IN SOUTHERN BRAZIL: EXPERIENCE, LIMITATIONS AND OUTCOMES

Vairo F.[1], Netto C.B.O.[2], Bittar C.M.[1], Vieira S.[1], Schwartz I.V.D.[1], Souza C.F.M.[1]
[1]*Medical Genetics Service, HCPA, Porto Alegre, Brazil*
[2]*Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil*

Introduction: Tyrosinemia type I is a disorder of childhood that causes liver failure, rickets, and hepatocarcinoma. If untreated, death typically occurs before the age of 2 years . Its prevalence is about 1 in 100,000 newborns.
Case 1: 14 year-old boy diagnosed at 10 years presenting with benign hepatic nodules, jaundice, and rickets. Treatment was started with free-tyrosine formula and nitisinone (NTBC). Liver transplant was performed at 12 years of age. He is presenting adequate metabolic control.
Case 2: 8 year-old boy diagnosed by neonatal screening, maintaining adequate metabolic control with NTBC and low-tyrosine diet.
Case 3: 30 months-old boy diagnosed due to benign hepatic nodules, hepatic failure, rickets and renal tubulopathy. He has received NTBC and free-tyrosine formula for 9 months. Liver transplant was performed 27 months of age. He is mantaining adequate metabolic control with protein restrict diet and NTBC.
Case 4: 20 months-old boy diagnosed due to epistaxis and thrombocytopenia at 7 months-old. He has received NTBC and free-tyrosine formula till 18 months, when liver transplant was performed. Nowadays, maintaining adequate metabolic control with protein restricts diet and NTBC. Case 5: 24 months-old boy evaluated just after birth due to history of 2 siblings deceased with tyrosinemia. At 8 days of life, he started on free-tyrosine formula and NTBC. He is maintaining adequate metabolic control with no target organ impairment.
Conclusion: This report emphasizes the importance of early diagnosis and rigorous treatment of tyrosinemia type I patients and shows the success of liver transplant for this disorder.

042-P

DIAGNOSTIC RELEVANCE OF PHENYLALANINE/TYROSINE RATIOS (PHE/TYR)

Scholl-Burgi S[1], Fuchs D[2], Haberlandt E[1], Rostásy K[1], Karall D[1]
[1]Med Univ Innsbruck, Dep Ped IV, Innsbruck, Austria
[2]Med Univ Innsbruck, Div Biol Chem, Innsbruck, Austria

Introduction: Phenylalanine (4)-hydroxylase (PAH) converts phenylalanine (phe) to tyrosine (tyr). An altered phe to tyr ratio (phe/tyr) indicates disturbed hydroxylation reaction which can be detected by parallel measurements of phe and tyr [1]. 5,6,7,8-Tetrahydrobiopterin (BH4), cofactor of PAH, is easily oxidized. In situations with oxidative stress this will lead to a decreased hydroxylation of phe. Aim of this retrospective study is to investigate, if neuroinflammatory disorders are indicated by changes of phe/tyr.
Methods: Phe/tyr was determined in 210 simultaneous venous and lumbar punctures from children/juveniles with neurologic symptoms (acute or chronic) or inborn errors of metabolism. The CSF samples were obtained in a standardized manner [2].
Results: The cut-off value for phe/tyr obtained in 26 individuals without proven neuroinflammatory or inherited disorders was 1.15 in CSF and plasma [3]. Phe/tyr was elevated in both, plasma and CSF in 21 individuals (group 1). Additionally, phe/tyr was elevated in 13 CSF samples (group 2) and in 8 plasma samples (group 3). Neuroinflammatory disorders (including e.g. ADEM, MS) were diagnosed in 6/21 patients (29%) from group 1, in 4/13 (31%) of group 2, and 1/8 (13%) of group 3 but less often in the remaining patients (6/142 = 4%; group 1/2 $p < 0.05$, Chi2-test).
Conclusion: Neuroinflammatory disorders were more often observed in patients with elevated phe/tyr indicating disturbance of PAH. We conclude that this ratio may support the diagnosis of neuroinflammatory disorders.
References: [1] Neurauter et al (2008) Curr Drug Metabol; [2] Scholl-Burgi et al (2008), Pediatrics; [3] Scholl-Burgi et al (2010), Pteridines

043-O

SUCCESSFUL TREATMENT OF MOLYBDENUM COFACTOR DEFICIENCY TYPE A WITH CYCLIC PYRANOPTERIN MONOPHOSPHATE (cPMP) IN FIVE PATIENTS

Veldman A[1], Schwahn B[2], Galloway P[2], van Spronsen F[3], Bergman K[3], Weis I[4], Nuesslein T[4], Gianello R[5], Sass JO[6], Beleidi AA[7], Santamaria-Araujo JA[7], Schwarz G[7]
[1]Monash Inst Med Research, Monash Univ, Melbourne, Australia
[2]Royal Hospital for Sick Children, Glasgow, United Kingdom
[3]University Medical Center Groningen, Groningen, Netherlands
[4]Klinik fur Kinder- und Jugendmedizin, Koblenz, Germany
[5]Orphatec Pharmaceuticals GmbH, Niederkassel, Germany
[6]Clin Biochem & Metab, Univ Hospital Freiburg, Germany
[7]Inst Biochem & Dept Chem, Univ Cologne, Cologne, Germany

Background: Molybdenum cofactor (Moco) 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 is the usual outcome.
Objectives: MoCD type A patients carries mutations in MOCS1 causing a block in the synthesis of cyclic pyranopterin monophosphate (cPMP), the first intermediate in the Moco pathway. Experimental substitution therapy with cPMP has been started in five patients, one of which being recently published (Veldman et al. 2010, Pediatrics). Patients: Except one, all patients developed typical symptoms of MoCD, including severe seizures. Patients were diagnosed prenatally (418+1G>A), day 1 and 4 (R319Q), 6 (G175R) and 24 (G126D).
Results: cPMP treatment was started with daily i.v. administration of 80–240 μg/kg body weight on day 0–36. A change in urinary biomarkers of sulfite oxidase deficiency (sulfite, S-sulfocysteine, thiosulfate) was observed after 24 hours of treatment start, with sulfite being completely depleted within 3–4 days. S-sulfocysteine, which returned to 10–30 mmol/mol creatinine within 1–2 weeks, was a reliable marker in monitoring cPMP treatment efficacy and guided dose adjustment. Biomarkers of xanthine oxidase deficiency (xanthine, uric acid) returned to almost normal values within 2–3 weeks and stayed also constant throughout the treatment. Clinically, all infants became alert, convulsions disappeared rapidly and EEG showed nearly normalization.
Conclusion: Substitution of cPMP represents the first causative therapy available for MoCD patients and early diagnosis will optimize clinical outcome. cPMP is currently developed by Orphatec Pharmaceuticals.

044-P

KETOGENIC DIET IN NONKETOTIC HYPERGLYCINEMIA

Bzduch V[1], Behulova D[2], Kolnikova M[3], Payerova J[3], Fabriciova K[1]
[1]First Dept Pediat, Univ Child Hosp, Bratislava, Slovakia
[2]Dept Labor Med Univ Child Hosp, Bratislava, Slovakia
[3]Dept Pediat Neurol, Univ Child Hosp, Bratislava, Slovakia

Background: Nonketotic hyperglycinemia (NKH) is a devastating neurometabolic disorder, leading to early death or severe disability. No effective treatment is available. Ketogenic diet is known to have beneficial effect on many diseases with brain involvement and seizures, so we used it in infant with classical neonatal form of NKH.
Case report: Female proband developed lethargy, muscular hypotonia, myoclonic seizures, and respiratory insufficiency on the second day of life. Before the correct diagnosis, phenobarbital and valproate treatment was indicated.
At the age of 6 weeks, aminoacid analysis showed highly elevated concentrations of glycine in plasma (768 μmol/L, normal < 370), cerebrospinal fluid (CSF) (124 μmol/L, normal < 10,1) and urine (3580,8 mmol/mol creat.). CSF/plasma glycine ratio was markedly elevated (0,16). Treatment with sodium benzoate (500 mg/kg/day) dextromethorphan (5 mg/kg/day) and L-carnitine (50 mg/kg/day) was started. Despite such treatment the child was still extremely hypotonic. At the age of 18 months, after informed consent of parents, ketogenic diet was used. Shortly after its institution, clinical state promptly improved. 3 months after classical treatment of NKH and ketogenic diet, glycine level in CSF remained unaltered (115,9 μmol/L). Frequent vomiting was severe complication of treatment, so sodium benzoate and dextromethorphan was gradually discontinued. The only treatment is now ketogenic diet and L-carnitine. After one year of such treatment, levels of plasma glycine are surprisingly at reference range (358 μmol/L).
Conclusion: Ketogenic diet improved clinical state and some laboratory findings in child with NKH.

045-P

REVERSIBLE CAUSE OF MYOPATHY IN A CASE OF LYSINURIC PROTEIN INTOLERANCE

Wood M[1], Harmar S[1], McSweeney M[1], Abulhoul L[1]
[1]Great Ormond St. Hospital for Children, London, United Kingdom

Background: Lysinuric protein intolerance (LPI) is caused by defective cationic amino acid transport at the basolateral membrane of epithelial cells in kidney and intestine. Muscle weakness has been previously described in this condition.
Case Report: A 15 year old girl with LPI presented with increasing weakness, anterior knee pain and altered gait pattern. She had been treated since diagnosis at the age of 11 years with a low protein diet, vitamin and mineral supplementation and Citrulline; however her compliance was poor. She had evidence of a proximal myopathy with an abnormal gait. Vitamin D deficiency was confirmed biochemically. The patient was treated with ergocalciferol and her medical management was optimised; this included physiotherapy and hydrotherapy. Gaitrite (electronic pressure sensitive walkway) was used to assess any changes to gait pattern following treatment.
Results: The proximal myopathy improved dramatically with treatment. The GaitRite was found to identify positive changes in velocity and step length and functional abilities improved.
Conclusion: Vitamin D deficiency is increasingly common in UK and should be considered as a treatable cause of myopathy in patients with co-existing metabolic disease. The Gaitrite is an accessible way of providing quantitative analysis of gait in the clinical environment. It proved to be an excellent monitoring tool to identify changes in gait parameters following medical and physiotherapy treatment of vitamin D myopathy.

046-P

SAMPLE STABILITY AND REPRODUCIBILITY OF WHITE CELL CYSTINE MEASUREMENT IN CYSTINOSIS

Turner C[1], Dalton RN[2]
[1]WellChild Lab, Evelina Children's Hosp, London, United Kingdom
[2]Wellchild Lab, King's College London, London, United Kingdom

Background: Mixed leucocyte cystine measurement (WCC) is required for diagnosis and treatment monitoring of patients with nephropathic cystinosis. Based on our initial method evaluation (van't Hoff 1992), we prepare leucocytes from heparinised blood samples after storage or shipping at room temperature up to 24 h after sampling. It has recently been suggested, based on a small study (n=3), that any delay in preparation generates erroneous results (Fidler et.al., PedNeph 2009). We have therefore re-investigated the stability and reproducibility of WCC.
Methods: We measured WCC in replicate samples from cystinotic patients using isotope dilution LC-MSMS (cystine) and Lowry's method (protein). Assay imprecision was assessed from pooled controls. Overall method imprecision was obtained from 14 paired samples prepared simultaneously. For sample stability, in 6 samples the first replicate was prepared within 4 h and the second after 24 h, in 6 further samples the second replicate was prepared after 48 h.
Results: Pooled control WCC inter-batch imprecision was 7.8% and intra-batch imprecision was 5.3%. (1.82 nmol1/2Cystine/mg protein, n=38). The overall method imprecision from simultaneously prepared samples was 14.4% (1.37 nmol1/2Cystine/mg protein, n=14, mean discrepancy 12.9%) There was no significant difference in the average WCC between samples prepared immediately or after 24 h (1.12v1.28 nmol1/2Cystine/mg protein, n=6, p=0.71) or 48 h (1.82v1.84 nmol1/2Cystine/mg protein, n=6, p=0.94). However, variability was 11.0% at 24 h and 22.5% at 48 h, with a mean discrepancy between replicates of 12.0% and 36.9% respectively.
Conclusions: WCC assay has significant variability. Samples can be stored at room temperature and prepared up to 24 h after sampling, however longer delay introduces unacceptable variation.

047-P

NATIONWIDE SURVEY OF EXTENDED NEWBORN SCREENING BY TANDEM MASS SPECTROMETRY IN TAIWAN

Chiang C[1], Ho H[2], Kao S[1], Niu D[3], Chien Y[4], Hwu W[4], Chiang S[5], Kao C[3], Liu T[6], Chiang H[2], Hsiao K[5]
[1]Chinese Foundation of Health, Taipei, Taiwan
[2]Taipei Institute of Pathology, Taipei, Taiwan
[3]Dep Pedia, Taipei Veterans General Hosp, Taipei, Taiwan
[4]Dep of med Gen, Natl Taiwan Univ Hosp, Taipei, Taiwan
[5]Med Res & Edu, Veterans General Hosp, Taipei, Taiwan
[6]Genome Res Ctr, Ntl Yang-Ming Univ, Taipei, Taiwan

In Taiwan, during the period March, 2000 to June, 2009, 1,495,132 neonates were screened for phenylketonuria (PKU) and homocystinuria (HCU) and 1,321,123 neonates were screened for maple syrup urine disease (MSUD), methylmalonic academia (MMA), medium-chain acyl-CoA dehydrogenase deficiency (MCAD), isovaleric academia (IVA) and glutaric aciduria type 1 (GA-1) using tandem mass spectrometry (MS/MS). In a pilot study, 592,717 neonates were screened for citrullinemia, 3-methylcrotonyl- CoA carboxylase deficiency (3-MCC) and other fatty acid oxidation defects in the MS/MS newborn screening. A total of 170 newborns and 4 mothers were confirmed to have inborn errors of metabolism. The overall incidence of the inborn errors of metabolism was approximately 1: 5882 (1:6219 without mothers). The most common inborn error found was a defect of phenylalanine metabolism (5 classical PKU, 20 mild PKU, 40 mild hyperphenylalaninaemia (HPA) and 13 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency). MUSD was the second most common amino acidopathy and, significantly, most of the MSUD patients (10 out of 13 patients) belonged to the Austronesian aboriginal tribes of southern Taiwan. 3-MCC deficiency was most frequently detected among the organic acid disorders (14 newborns and 4 mothers). GA-1 and MMA were the second most common organic acid disorders (GA-1: 13 and MMA: 13 newborns) In fatty acid disorders, 5 carnitine transport defect (CTD), 5 short chain acyl-CoA dehydrogenase deficiency (SCAD) and 2 medium chain acyl-CoA dehydrogenase deficiency (MCAD) were confirmed in this screening. This is the largest case of MS/MS newborn screening in the Chinese population, to date.

048-P

DEFICIENCY OF MOLYBDENUM COFACTOR BIOSYNTHESIS DUE TO A NOVEL MUTATION IN THE MOCS2 GENES IN A CHILD WITH SHORT STATURE

Arranz JA[1], Pintos G[2], Montlleó L[2], Gutiérrez M[1], Fernández P[1], Outeiral A[1], Riudor E[1]
[1]Lab Metab, Hosp Univ Vall d'Hebron, Barcelona, Spain
[2]Pediat Dpt Hosp Uni. Germans Trias i P, Badalona, Spain

Molybdenum cofactor (MoCo) is common to sulfite oxidase, xanthine dehydrogenase and aldehyde oxidase. Its deficiency usually leads to a very severe and progressive neonatal neurological disorder attributed to sulfite accumulation in CNS. Mutations in MOCS1 (mainly), in MOCS2 and in gephyrin genes (few cases) have been described.
A 2-y.old male patient was investigated due to marked short stature with normal psychomotor development. Dwarfism aetiology has still not been elucidated. Incidentally, a standard biochemical panel performed at 6 y. showed a decrease in plasma urate: 0.6 mg/dl (N:2.3–4.7). Metabolic work-up resulted in positive urine sulfite test, increased sulfocysteine: 595.0 (N<11.3 mmol/mol cr), xanthine: 187.0 (N:<68.0), hipoxanthine: 42.9 (N:<62.0) and taurine: 535.9 (N:9–180) pointing to MoCo deficiency. Mutational analysis of MOCS genes yielded a potentially pathogenic alteration in homozygosis in the overlapping region of the two open reading frames, c.246A>T/c.57A>T in the MOCS2A and B genes resulting in protein missense changes p.I82F in the MOCS2A protein and p.L19F in the MOCS2B one, which appear compatible with some residual enzymatic activities and mild clinical picture. A control allele study discarded a polymorphic change.
This would concurs with Johnson's prediction about the existence of a broad spectrum of mild manifestations owing to trace levels of MoCo are sufficient for human molybdoenzymes. The patient genotype appears to correlate with the clinical phenotype but not with the biochemical one (characteristic of the classical MoCo deficiency). The expected sulfite pathogenia in this and other mild cases is not well understood.

049-O

METABOLIC CORRECTION AND LONG-TERM RESCUE OF MURINE INTERMEDIATE MAPLE SYRUP URINE DISEASE (IMSUD) USING HUMAN AMNIOTIC EPITHELIAL CELL TRANSPLANTATION (hAEC-Tx)

Skvorak KJ[1], Dorko K[1], Hansel M[1], Marongiu F[1], Tahan V[1], Bottiglieri T[2], Sun Q[3], Gibson KM[4], Strom SC[1]
[1]Dept Pathol, Univ Pittsburgh Sch Med, Pittsburgh PA, United States
[2]Inst Metab Dis, Baylor Univ Med Ctr, Dallas TX, United States
[3]Biochem Genet Lab, Baylor Coll Med, Houston TX, United States
[4]Dept Biol Sci, Michigan Tech Univ, Houghton, MI, United States

Background: Skvorak and coworkers recently documented the efficacy of hepatocyte transplantation in newborn iMSUD mice. Extended lifespan was accompanied by reduced branched chain amino acids (BCAAs) in brain and blood, increased liver branched-chain ketoacid dehydrogenase (BCKDH) activity, correction of brain monoamine (dopamine, serotonin) abnormalities and ~3% engraftment of exogenous non-syngeneic hepatocytes. Long-term correction was likely impaired, however, by immunogenicity of exogenous cells. Conversely, hAECs (isolated from amnion tissue) represent a heterologous cell population positive for stem cell markers, with multilineage differentiation potential and potent immunosuppressive properties. With regard the latter, we have investigated hAEC-Tx in iMSUD animals.
Methods: Subjects were wild-type (WT; n=5), sham (iMSUD, PBS injected; n=7), and hAEC-Tx (n=7), employing two transabdominal injections (one million cells, day of life 1–10) and weekly booster injections (2 million cells).
Results: BCAA levels and alloisoleucine were partially or completely corrected (compared to WT and sham animals) in both blood and brain. Brain monoamine analysis revealed partial or complete correction of altered dopamine metabolism. Importantly, hAEC-Tx resulted in ~70% survival at 3 months of life (sham iMSUD animals=100% lethality by 1 month), with healthy appearance of treated animals.
Conclusions: Our data suggests that hAEC-Tx has therapeutic relevance for hepatic regeneration, and deserves further study as a treatment approach in murine MSUD.

050-P

RAPID ANALYSIS OF CYSTINE FOR THE DIAGNOSIS OF RENAL CALCULI

Veyssier JP[1], Tanyalcin T[2]
[1]Biochrom Ltd. 22 Cambridge Science Park, Cambridge CB4 0FJ, United Kingdom
[2]Tanyalcin Medical Laboratory, Izmir, Turkey

Classical cystinuria is the most common inborn error of amino acid transport. The fact that the cystine is the least soluble natural aminoacid, its overexcretion frequently results to the formation of cystine calculi in renal pelves, ureters and bladder along with infection, obstruction and with high risk of recurrent urinary stone formation that even may direct to renal failure. Cystine stones are difficult to treat and require lifelong therapy. Diagnosis by quantification of cystine in urine and monitoring the patient with cystine excretion has a great impact on this disorder. This study presents a rapid screening method for cystine measurement in urine with high peak resolution and accuracy and improved run time; 77 minutes per sample. The short program developed on Biochrom 30 Amino Acid analyzer will make it possible to run 16 samples per day. The separation is achieved using 20 X 4.6 mm physiological high resolution column using predominantly buffer CII (pH: 3.15). Norleucine is used as an internal standard. This short program will ease the work load of clinical laboratories that have a continuous requirement for the analysis of urine specimens from patients with renal calculi to screen for cytinuria or follow up the patients under treatment.

051-P

SIBLINGS WITH METHIONINE ADENOSYLTRANSFERASE (MAT) I/III DEFICIENCY, PRESENTING ELEVATION OF PLASMA TOTAL HOMOCYSTEINE AND TRANSIENT MRI ABNORMALITIES IN WHITE MATTER LESIONS

Yamamoto S[1], Ogawa A[2], Ogawa E[2]
[1]Dep Ped, Shimoshizu National Hosp, Yotsukaido, Japan
[2]Dept Ped, Chiba Univ Sch of Med, Chiba, Japan

We report male siblings with MAT I/III deficiency. Case1 was previously reported (American Journal of Neuroradiology 25:1843–1845, 2004). Hypermethioninemia was detected on neonatal screening. In addition to elevated plasma methionine (740 μmol/L), laboratory studies showed elevated total homocysteine (t-Hc) (37.4 μmol/L), leading to a tentative diagnosis of CBS deficiency. At 3 weeks, he began treatments with methionine-restricted diets and oral betaine; pyridoxine was started at age 2.5 years. His early development was normal, and his neurologic examination was unremarkable. MR imaging at 3 years, 10 months revealed abnormal finding of white matter, which demonstrating delay of myelination. Treatment of betaine and pyridoxine was discontinued at 3 years, 11 months. After discontinuance of the treatment, plasma t-Hc was elevated (13.9_24.7 μmol/L). The abnormal findings of MRI markedly improved at 5 years. Analysis of MAT 1A gene revealed a compound heterozygote: 274 T to C (Y92H) and 1067G to C (R356P), which led to a diagnosis of MAT I/III deficiency. Case2 is a younger brother of Case1. Hypermethioninemia was detected on neonatal screening. Plasma methionine (820–2250 μmol/L) and t-Hc (15.3_41.3 μmol/L) were elevated. Methionine restriction was started at 3 months, which reduced the plasma levels of methionine (790–1200 μmol/L) and of t-Hc (8.2_22.3 μmol/L). Analysis of MAT IA gene at 5 months revealed the same mutations. Methionine restriction was discontinued at 1 year, 10 months, which caused elevation of Met (900–1140 μmol/L) and of t-Hc (19.2_25.6 μmol/L). His development was normal, and his neurologic examination was unremarkable.

052-P

TRIMETHYLAMINURIA (TMAU) IN A PATIENT WITH HOMOCYSTINURIA ON BETAINE THERAPY—DETECTION OF A HOMOZYGOUS ALLELIC VARIANT IN THE FMO3 GENE AND SUBSEQUENT BENEFICIAL EFFECT OF RIBOFLAVIN

Manning NJ[1], Smith EJ[1], Sharrard MJ[1], Allen KE[2], Kirk RJ[2]
[1]Dept Clin Chem, Childrens Hospital, Sheffield, United Kingdom
[2]Dept Genetics, Childrens Hospital, Sheffield, United Kingdom

Background: Betaine (trimethylglycine) therapy for homocystinuria has been previously associated in some patients with body odour which may result in non-compliance. Fish Odour Syndrome due to TMAU may result due to a deficiency in the enzyme flavin containing mono-oxygenase type 3 (FMO3) but may also be caused by overproduction of intestinal trimethylamine (TMA). Mild TMAU due to homozygosity for polymorphic variants in the gene encoding FMO3 is known to be exacerbated by increased enterobacterial TMA production. Betaine degradation in the gut may result in TMAU when administered in pharmacologic doses, although this is rarely measured.

Case: A 7 year old girl diagnosed with partially pyridoxine responsive homcystinuria was treated with pyridoxine, folate, low protein diet and betaine. At 16 years of age, she was taking 16 g/day betaine but suffered with a strong 'fishy' odour detected by her family.

Results: Urinary TMA measurement showed markedly increased excretion at 392 μmol / mmol creatinine (ref. < 11). Betaine and diet were not changed but riboflavin was tried at 100 mg/day. Subsequently she reported that the odour was not detectable. TMA excretion was lowered to 77 after 4 weeks and 37 after 7 weeks. Dimethylglycine was consistently increased at more than 1,000 μmol/mmol creatinine (ref. < 16) demonstrating betaine compliance.

Mutation analysis showed our patient to be homozygous for the FMO3 allelic variant p.[Glu158Lys;Glu308Gly] which has been associated with a mild TMAU phenotype.

Conclusion: This patient has been significantly challenged with a high dose of betaine but with a satisfactory response to riboflavin.

053-P

END-STAGE RENAL FAILURE IN A YOUNG ADULT: AN UNUSUAL PRESENTATION OF LATE-ONSET COBALAMIN C DISEASE

Kern I[1], Bonafé L[2], Bourquin V[3], Girardin E[1], Boulat O[4], Baumgartner MR[5], Fowler B[6], Hadaya K[3]
[1]Nephr & Metab Dis Unit, Univ Child Hosp, Geneva, Switzerland
[2]Div Molecular Pediatrics, Univ Hosp, Lausanne, Switzerland
[3]Div Nephrology, University Hospital, Geneva, Switzerland
[4]Clin Chem Lab, Univ Hosp, Lausanne, Switzerland
[5]Div Metabolism, Univ Child Hosp, Zurich, Switzerland
[6]Metabolic Unit, Univ Child Hosp, Basel, Switzerland

The cblC type of methylmalonic aciduria and homocystinuria is a rare autosomal recessive disorder leading to defective intracellular cobalamin metabolism. Late-onset disease in adolescents and young adults has been reported in less than 20 cases, characterized by neuro-psychiatric and/or thrombo-embolic manifestations and a more favorable outcome than in early presenting cases.

In this report, we describe the clinical, biochemical and molecular findings in a 23-year-old man who was initially misdiagnosed as essential malignant hypertension with haemolytic uremic syndrome. He progressed to end-stage renal failure 15 days after admission. Thereafter, the finding of hyperhomocysteinemia prompted further studies in fibroblasts leading to the diagnosis of the cblC disorder. Sequencing of the MMACHC gene showed the homozygous missense mutation c.565C>A. Hyperhomocysteinemia decreased rapidly under intensive treatment with i.v. hydroxycobalamin, oral betaine, folate and carnitine, allowing the patient to undergo a living related renal transplantation. Eighteen months post-transplant, biochemical parameters had improved dramatically and kidney function is normal.

Renal complications such as thrombotic microangiopathy, proximal renal tubular acidosis, and chronic renal failure have been described mainly in early-onset cblC disease. To our knowledge, this is the first case of end-stage renal failure with haemolytic uremic syndrome in a young adult, presumably secondary to thrombotic microangiopathy, although this could not be confirmed since the hypertensive crisis precluded a diagnostic kidney biopsy.

CblC disease should be included in the differential diagnosis of haemolytic uremic syndrome and malignant hypertension in both children and adults.

054-P

IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF A NOVEL MUTATION IN CBS GENE

Mendes M[1], Salomons GS[2], Tavares de Almeida I[1], Blom H[2], Rivera I[1], Leandro P[1]
[1]*Met&Gen, iMed UL, Fac Pharm, Univ Lisbon, Lisbon, Portugal*
[2]*Metabolic Unit, Clin Chem, VUMC, Amsterdam, Netherlands*

Cystathionine beta-synthase (CBS; EC 4.2.1.22) catalyses the first step of the transsulfuration pathway of methionine metabolism, where serine and homocysteine are condensed to cystathionine. CBS deficiency results in classic homocystinuria (OMIM 236200), an inherited disease characterized by hyperhomocysteinemia and hyperhomocysteinuria. To date, more than 150 mutations have been identified in CBS gene. Here we describe and characterize a novel mutation found in a Portuguese patient diagnosed with classic homocystinuria. DNA obtained from peripheral blood of the index patient was used to amplify individual exons and flanking intronic regions of the CBS gene. Direct sequencing of the resulting fragments allowed the identification of c.1051G>T mutation (p.G351R) in exon 10. To characterize the p.G351R mutant form, CBS wild-type cDNA was cloned into pET28b vector (Clontech), 3' to the 6His peptide encoding sequence. The c.1051G>T mutation was introduced by site directed mutagenesis. Recombinant wild-type and mutant proteins were produced in E. coli, after IPTG induction, in LB supplemented with δ-aminolevulinic acid, and purified by affinity chromatography (IMAC/6His-tag). The enzymatic assay was performed according to standard procedures, with minor modifications. The produced cystathionine was quantified by capillary GC. Size exclusion chromatography was used to evaluate the oligomeric profile of the purified proteins. The developed expression system allowed us to obtain high yields of functional CBSwt mainly as tetramers. The p.G351R protein displayed lower expression levels and a decreased activity, when compared to CBSwt.

Taken together our data suggest that the novel c.1051G>T mutation (p.G351), here described, is a disease-causing mutation.

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055-P

ACUTE HYPERHOMOCYSTEINEMIA ALTERS PLATELETS COUNT AND BLOOD COAGULATION SYSTEM IN RATS

Cunha AA[1], da Cunha MJ[1], Machado FR[1], Wajner M[2], Wyse ATS[1]
[1]*Departamento de Bioquímica, ICBS, UFRGS, Porto Alegre RS, Brazil*
[2]*Servico de Genética Médica, HCPA, Porto Alegre, RS, Brazil*

Background: Hyperhomocysteinemia has been related to various diseases, including homocystinuria, atherosclerosis, thrombosis and other vascular disease.

Objectives: To investigate the effect of acute administration of homocysteine, similar to those found in homocystinuria, on parameters of coagulation system such as platelet count, prothrombin time, activated partial thromboplastin time and fibrinogen in blood and plasma of rats. Also, nitrite levels were evaluated.

Material and Methods: Wistar rats, 29 days of age, received a single subcutaneous dorsal injection of saline (control) or D,L-homocysteine (0.6 μmol/g body weight). Fifteen minutes, 1 h, 6 h or 12 h after the injection the rats were sacrificed and the blood was collected.

Results: Homocysteine significantly increased platelet count in blood and fibrinogen levels in plasma of rats at 15 min and 1 h, but not at 6 h and 12 h, when compared to the control group. Prothrombin time, activated partial thromboplastin time and nitrite levels significantly were decreased in plasma at 15 min and 1 h, but not at 6 h and 12 h, after homocysteine administration.

Conclusion: Our findings suggest that platelet activation, endothelial dysfunction and hypercoagulability can occur after acute homocysteine administration what might be associated, at least in part, with the vascular dysfunction and thromboembolic complications observed in homocystinuric patients.

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056-P

EFFECT OF CHRONIC HYPERHOMOCYSTEINEMIA ON Na+, K+-ATPaSE ACTIVITY AND GLUTAMATE UPTAKE IN HIPPOCAMPUS OF RATS

Machado FR[1], Ferreira AGK[1], Cunha AA[1], da Cunha MJ[1], Mussulini B[1], Wofchuk S[1], Wajner M[2], Wyse ATS[1]
[1]*Servico de Genética Médica, HCPA, Porto Alegre, RS, Brazil*

Introduction: Homocystinuria is an inborn error of metabolism caused by deficiency of the enzyme cystathionine-β-synthase leading to tissue accumulation of homocysteine (Hcy). Na+, K±ATPase is a membrane-bound enzyme responsible for generating the membrane potential of the cell through ion exchange. It has been demonstrated that Hcy might promote glutamatergic excitotoxicity due to overstimulation of NMDA receptors. Vit C is an important chain-breaking antioxidant responsible for scavenging free radicals and suppression of peroxidation in the cytosol and membrane of the cell.

Objectives: We analyzed the influence of Vit C on the effects elicited by hyperhomocysteinemia on the Na+, K±ATPase activity and glutamate uptake.

Material and Methods: Wistar rats received daily twice subcutaneous injection of D,L-Hcy (0.3–0.6 μmol/g body weight), and/or Vit C (100 mg/kg) from 6th to their 21th day of life. Twelve hours after the last injection the rats were sacrificed and hippocampus was dissected. Glutamate uptake was performed according to Frizzo et al., (2002) and the Na+,K±ATPase activity was quantified by the method described by Wyse et al., (2000).

Results: Results showed that chronic Hcy administration significantly inhibited the Na+, K±ATPase activity as well as the glutamate uptake in rat hippocampus. Furthermore, Vit C was able to prevent the effects caused by Hcy.

Conclusion: Our results suggest that the inhibition of Na+, K±ATPase as well as the impairment on the glutamate uptake promoted by hyperhomocysteinemia in the hippocampus of young rats, is probably mediated by oxidative stress, since the treatment with antioxidant Vit C prevented such effects.

057-P

EPIGENETIC SYNTROPY PHENOMENON ASSOCIATES WITH FOLATE CYCLE ENZYME DEFICIENCY (MTHFR, MTRR, MTR)

Grechanina O.Ya.[1], Gusar V.A.[1], Grechanina Yu.B.[1], Volobuyeva I.A.[2]
[1]*Ukr Institute Clin Genet KhNMU, Kharkov, Ukraine*
[2]*Specialized Med Genetic Cent, Kharkov, Ukraine*

The methionine conversion to S-adenosylmethionine in folate cycle seems to involve the syntropy phenomenon we encountered when studying role of the folate cycle enzyme deficiency and hypomethylation in manifestation of monogenic, chromosomal and multifactorial pathologies in patients.

Methods: A total sample of 1938 patients, including patients with monogenic (5.0%), chromosomal (6.0%) and multifactorial (24.0%) pathologies. The SNPs in MTHFR and MTRR were screened by PCR test-systems.

Results: C677T MTHFR and A66G MTRR polymorphisms were calculated in patients. The C677T MTHFR allele frequencies were as follows: CT – 43.3%, TT – 8.7%, CC – 48.0%. The 677T mutant allele frequency was 30.0%. The A66G MTRR allele frequencies were as follows: AG – 41.8%, GG – 37.0%, AA – 21.2%. The 66G mutant allele frequency was 58.0%. Compound frequency for C677T MTHFR/A66G MTRR polymorphisms were calculated in patients: Htzg/Htzg – 18.3%; Htzg/Hmzg – 16.3%; N/Htzg – 20.3%; N/Hmzg – 17.1%; Hmzg/Htzg –3.3%; Hmzg/Hmzg – 3.4%.

Carrying out phenotypic and genotypic correlations allowed to reveal the syntropy phenomenon for 18 syndromes (ex. Rett, Zellweger et cet.) and to suppose that folate cycle enzyme deficiency is accompanied by methylation disturbance which, in its turn, influences epigenetic status of a gene resulting in the launch of epimutations and, consequently, in manifestation of epigenetic and oncogenetic syndromes.

The phenotypic syntropy seem to result from the action of syntropic genes MTHFR/MTRR. This fact previously described by Puzyrev V.(2006–2008) has been confirmed in our studies. The treatment based on the stated hypothesis has shown positive results.

058-P

MOLECULAR ANALYSIS OF HOMOCYSTINURIA IN TURKISH PATIENTS

M Karaca[1], RK Ozgul[2], H Dundar[2], T Coskun[2], A Tokatli[2], S Sivri[2], A Dursun[2]
[1]*Faculty Sci&Arts, Dept.Bio, Aksaray Unv., Aksaray, Turkey*
[2]*Dept.Pediat.Metab.Unit.Fac.Med.Hac.Unv., Ankara, Turkey*

Homocystinuria (MIM # 236200) is an autosomal recessive disease most commonly caused by mutations in cystathionine β-synthase (CBS) gene. We investigated the mutation patterns of CBS gene in 13 homocystinuric Turkish patients with of 11 different families. Mutation analysis of the CBS gene was performed by direct DNA sequencing. A total of 12 different mutations were identified in patients with homocystinuria. Nine mutations have been described previously (p.P145L, IVS11 -2A>C, p.G259S, IVS12 +1G>A, p.S349N, p.G85R, p.T353M and p.I278T/844ins68) and three novel mutations were found (p.D281V, p.L251P, IVS7 -2A>T). All mutations were detected at homozygous state in patients with the exception of IVS11 -2 A>C and p.I278T/844ins68 mutations, detected in a patient as compound heterozygous state. According to the data obtained from this study, mutations have been frequently detected in exons 7, 8 and 10 of the CBS gene. All mutations are distributed in catalytic domain of CBS enzyme. In this present study, we introduce first time the characterization of CBS mutations at molecular level in Turkish patients with homocystinuria.

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059-P

EVALUATION OF THE USE OF DRIED BLOOD-SPOT HOMOCYSTEINE MEASUREMENT FOR NEONATAL DETECTION OF HOMOCYSTINURIAS

Alodaib A[1], Carpenter K[2], Wiley V[3], Wotton T[3], Wilcken B[1]
[1]*Disc Paeds & Child Health, Sydney Univ, Sydney, Australia*
[2]*Biochem Genetics, Children's Hosp, Sydney, Australia*
[3]*NSW Newborn Screening Prog, Sydney, Australia*

Newborn screening for the homocystinurias by tandem mass-spectrometry involves initial measurement of methionine to detect cystathionine β-synthase (CBS) deficiency, and propionyl and acetyl carnitines (C3, C2) to detect cobalamin C (CblC) defect. Sensitivity is poor for CBS deficiency and specificity somewhat poor for both. Measurement of total homocysteine in dried blood spots has been used as a second-tier test in screening for CBS deficiency, and recently as an initial test. We have measured homocysteine in dried blood spots in normal newborns, and retrospectively in newborns with established disorders, using an LC-MS/MS method. The reference range for current neonatal samples was 5.4–10.7 μmol/L, and for samples stored for 10 years was 1.7–5.5 μmol/L (n=50), markedly reduced. The neonatal dried-blood spot homocysteine, measured retrospectively in five patients with confirmed CBS deficiency, was 21.6 to 43.6 μmol/L, and that in four CblC deficient patients was 40.7, 58.5, 60.6, 68.5 μmol/L. Ages at sampling were 2–4 days, and sample ages at testing 1–12 years. All CBS patients were pyridoxine non-responsive, and initially detected by raised methionine levels at routine newborn screening. No newborns with pyridoxine-reponsive CBS deficiency have been detected. The cobalamin C deficient patients were detected because of elevated C3/C2 ratios, without elevated C3 alone.

We conclude that pyridoxine-nonresponsive CBS deficient patients have very clear elevations of homocysteine at 2–4 days of age. The sensitivity of this test for pyridoxine-responsive patients remains unknown. The newborn levels found in cobalamin C deficient babies were even higher, and seem fully discriminatory.

060-O

S-ADENOSYL HOMOCYSTEINE ACCUMULATION DECREASES GLOBAL PROTEIN ARGININE METHYLATION STATUS IN CULTURED HUMAN ENDOTHELIAL CELLS

Esse R[1], Rocha MS[1], Barroso M[1], Goncalves Jr I[1], Leandro P[1], Teerlink T[2], Jakobs C[2], Blom HJ[2], Castro R[1], Tavares de Almeida I[1]
[1]*Metabolism and Genetics, iMed.UL/FFUL, Lisbon, Portugal*
[2]*Dep. Clin. Chem., VU Medical Center, Amsterdam, Netherlands*

Background: Accumulation of the homocysteine (Hcy) precursor S-adenosyl homocysteine (SAH) may contribute to Hcy-related vascular disease due to its potent inhibitory effect on methyltransferases. The posttranslational methylation of arginine (Arg) residues is catalyzed by PRMTs (protein arginine methyltransferases) and results in asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) formation. It is unknown whether protein hypomethylation is implicated in Hcy-related vascular disease.

Methods: SAH intracellular accumulation was achieved through incubation of human cultured endothelial cells with increasing concentrations of adenosine-2.3-dialdehyde, an inhibitor of SAH hydrolase. SAH hydrolase reversibly converts SAH into Hcy. After 24 and 48 h of incubation, aliquots of culture medium were collected for tHcy, ADMA and SDMA determination by adequate HPLC methods. Protein-bound ADMA and SDMA were determined in acid-hydrolyzed protein fractions of whole cell lysates. PRMT1 expression was evaluated by Western blot analysis.

Results and Discussion: ADA elicited a significant decrease in extracellular tHcy concentrations ($p<0.05$; n=4), confirming the inhibition of SAH hydrolase activity and consequent intracellular SAH accumulation. Extracellular ADMA and SDMA concentrations also decreased significantly ($p<0.01$; n=7), in a dose-dependent manner. To ensure that these results were due to protein hypomethylation, protein-bound Arg methylated residues were quantified. Both protein-bound ADMA and SDMA concentrations decreased significantly ($p<0.01$; n=7) after 24 and 48 hours of incubation. PRMT1 expression was unaffected, ruling out the effect of differential abundance. Our results suggest that PRMTs are a target for SAH-mediated inhibition of methyltransferases and that SAH accumulation promotes protein Arg hypomethylation.

This study was supported by the F.C.T. (PTDC/SAU-GMG/68714/2006-SFRH/BD/48585/2008).

061-P

IMMUNOFLUORESCENCE MICROSCOPY IS A USEFUL TOOL FOR EVALUATING THE EXPRESSION OF MUTANT CBS PROTEINS IN EUKARYOTIC CELLS

Casique L[1], De Lucca M[1], Martínez JC[1], Rodríguez K[2], von Bergen A[3], Castillo C[1]
[1]*Instituto de Estudios Avanzados, Caracas, Venezuela*
[2]*Universidad Simón Bolívar, Caracas, Venezuela*
[3]*NYU School of Medicine, New York, United States*

Classic Homocystinuria (HCU, OMIM236200) is an inborn metabolic disease caused by deficiency of cystathionine beta synthase protein (CBS, EC4.2.1.22). CBS participates in the first step of the transsulfuration pathway in which cysteine is synthesized. The CBS gene is located on the 21q22.3 region. So far, more than 140 mutations on the CBS gene associated to HCU has been reported. We describe the study of two missense mutations, p.T87N (c.260C>A) and p.D234N (c.700G>A), and one nonsense mutation p.Q243X (c.727C>T) in the CBS gene, which were found in three Venezuelan HCU patients. Mutations were expressed in HEK-293 cells and their expression was evaluated by PAGE and Western blot under native and denaturing conditions. The missense mutations generated less soluble protein (60% T87N y 90% D234N) with respect to the wild type enzyme, but the Q243X form was not observed. Corrected assembled tetramers were only obtained for the mutant D234N protein, although half of the amount observed for the wild type. Immunofluorescence and confocal microscopy demonstrated that these observations were the result of restriction of mutant proteins to an intracellular compartment inside HEK-293 cells. T87N mutant was restricted probably to the lysosome, whereas native protein was homogeneously distributed inside the cells. The D234N protein showed both kind of distribution. Surprinsingly, around 1% of Q243X transfected cells expressed the protein in similar form to native CBS. Microscopic techniques contributed to demonstrate a mechanism through which these mutations might contribute with HCU phenotype, generating unstable proteins which are totally or partially degraded by the cells.

062-O

SEARCH FOR NOVEL THERAPEUTIC TARGETS IN CYSTATHIONINE BETA-SYNTHASE DEFICIENCY

Ko-ich V[1], Sokolová J[1], Kopecká J[1], Krijt J[1], Hnízda A[1], Raková K[1]
[1]*Inst IMD, 1st Fac. Med, Charles Univ, Prague, Czech Republic*

The pathogenesis of cystathionine beta-synthase (CBS) deficiency is only partially understood and consequently the therapy is mostly based on manipulating the fluxes of homocysteine.

To get an insight into the molecular pathology we firstly examined in a prokaryotic expression system the properties of a series of 27 mutant variants, which together represent 70% of known patient-derived alleles. The mutants formed on average only 12% of tetramers and their median activity reached only 3% of the wild type enzyme. In a subset of nine mutants we examined their structural flexibility and conformational stability by proteolytic techniques and we observed either a faster cleavage suggesting their tendency to unfold, or in superactive mutants a slower proteolysis suggesting their conformational rigidity. In the next step we showed that 14 mutants responded by at least 30 % increase in the amount of correctly assembled tetramers and enzymatic activity to the coexpressional presence of either δ-ALA—a heme precursor—or of the osmolytes betaine or glycerol. Topology of mutations appeared to determine in part the degree of their misfolding and residual activity as well as of their response to chaperones; buried mutations formed less tetramers, exhibited 23-times lower activity and responded less to chaperones than the solvent exposed mutations. In addition to conformational changes, about one half of mutants responded abnormally to S-adenosylmethionine or S-adenosylhomocysteine.

This study may serve as a basis for exploring pharmacological approaches aimed at correcting abnormal CBS function in homocystinuria.

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063-O

ALDEHYDE-ADDUCTION OF FIBRILLIN-1: A NOVEL PATHOGENIC MECHANISM TO EXPLAIN THE PRESENCE AND ABSENCE OF MARFANOID CONNECTIVE TISSUE DISTRUBANCES IN GENETIC HOMOCYTINURIAS

Maclean K.N.[1], Petersen D.R.[1], Rozen R[2], Van Hove J.L[1], Stabler S.P.[1], Allen R.H.[1], Jiang H.[1]
[1]*University of Colorado, Aurora, United States*
[2]*McGill University, Montreal, Canada*

Background: Cystathionine beta-synthase deficient homocystinuria (CBSDH) is unique among the homocystinurias in exhibiting marfanoid connective tissue disturbances. Marfan syndrome results from mutations in Fibrillin-1, a highly cysteine rich protein prone to chronic toxic insult. Of all the homocystinurias, CBSDH has the greatest potential for oxidative stress due to the abolition of endogenous synthesis of the cellular antioxidant cysteine. Oxidative stress induces lipid-peroxidation generating reactive aldehydes such as 4-hydroxynonenal and malonyldialdehyde that can impair protein folding by forming covalent adduct with cysteine, lysine and histidine residues. We investigated if aldehyde-adduction of fibrillin-1 constitutes a CBSDH-specific mechanism for marfanoid fibrillinopathy.

Results: We observed significantly elevated levels of reactive aldehydes in the plasma of both CBSDH mice and untreated CBSDH patients compared to normal controls and methylenetetrahydrofolate reductase (MTHFR) null mice. Immunohistochemical analysis revealed high levels of 4-hydroxynonenal and malonyldialdehyde adduction of proteins in tissues from CBSDH mice and untreated CBSDH patients. No adduction was observed in normal mice, human controls or in the liver of MTHFR null mice. Fluorescent double-staining using a anti-fibrillin-1 antibody and an anti-4-hydroxynonenal protein-adduct antibody revealed 4-hydroxynonenal adduction of Fibrillin-1 in dermal fibroblast lines from CBSDH patients (n=8). No adduction of fibrillin-1 was observed in either control (n=8) or human MTHFR homocystinuric (n=4) cell lines.

Conclusion: Our data indicates that oxidative stress induced aldehyde-adduction of fibrillin-1 constitutes a novel pathogenic mechanism offering an explanation for both the presence of marfanoid connective tissue disturbances in CBSDH and their absence in homocystinuria due to remethylation defects.

064-P

ROBIN SEQUENCE IN THE OFFSPRING OF AN UNTREATED CYSTATHIONINE B-SYNTHASE (CBS) DEFICIENT PREGNANCY

Augoustides-Savvopoulou P[1], Mayiovas P[2], Ioannou C[1], Pavlou E[3], Tsiounis S[2]
[1]*Univ 1st Pediatr Dept, Thessaloniki, Greece*
[2]*Univ 2nd Neurol Dept, Thessaloniki, Greece*
[3]*Univ 2nd Pediatr Dept, Thessaloniki, Greece*

Background: Maternal hyperhomocysteinemia has been associated with a variety of fetal defects but its role in the pathogenesis of fetal malformations remains unclear.

Objective: A neonate with Robin sequence whose mother was diagnosed with CBS deficiency postpartum is described with the objective of highlighting the possible deleterious effect of maternal hyperhomocysteinemia during embryogenesis and Robin sequence in this setting.

Case report: A postpartum thromboembolic stroke in a mentally normal 29 year old woman led to the presumptive diagnosis of CBS deficiency (Marfanoid habitus, lens ectopia, scoliosis, high plasma tHcy 300 μmol/L, n.v. 3–15, and methionine 165 μmol/l, n.v. 17–37). Homozygosity for the 677C>T polymorphism in the MTFHR gene was also present. A regimen with high doses of pyridoxine resulted in significant reduction of tHcy levels. Fibroblast enzyme and molecular analyses are pending. A prenatal ultrasound suggested facial malformations of the fetus which were confirmed by the presence of micrognathia, cleft lip and cleft palate in the neonate. According to the obstetric history this was her first pregnancy and she had not received any potentially teratogenic drugs.

Conclusions: This report strengthens the hypothesis that maternal hyperhomocysteinemia is a risk factor for defective embryogenesis. The particularly high levels of tHCy in this case (combined CBS deficiency and MTFHR 677C>T homozygosity) may have accentuated the possible teratogenic effect of Hcy. In view of the decreased clinical awareness for disorders pertaining to hyperhomocysteinemia and the potentially devastating effects, measurement of plasma tHcy should perhaps be included in the routine workup of pregnant women.

065-P

MOLECULAR ANALYSIS OF CYSTATIONINE BETA-SYNTHASE (CBS) GENE MUTATIONS IN SLOVENIAN PATIENTS WITH HOMOCYSTINURIA

Zerjav Tansek M[1], Hovnik T[1], Dolzan V[2], Repic-Lampret B[1], Battelino T[1]
[1]*Dept. Metab Dis, Univ Child Hosp Ljublja, Ljubljana, Slovenia*
[2]*Med University, Instit of Biochemistry, Ljubljana, Slovenia*

In the present study the molecular basis of CBS deficiency in Slovenian patients with classical clinical features of homocystinuria was determined. Analysis of the entire coding region of the CBS gene by direct sequencing was used in all 7 patients from 5 unrelated non- consanguineous families with homocystinuria from the Slovenian national registry. The association with pyridoxine responsiveness was determined.

The most prevalent mutation in our patients was surprisingly the splicing mutation c.828+1G>A. It was present in homozygous state in three patients (one pair of siblings) and in one compound heterozygous patient. The other pair of siblings was homozygous for the missense mutation c.442G>A. Mutation c.1224–2A>C was found in one compound heterozygous patient. The most frequent mutation in Caucasian population c.833 T>C was detected in one homozygous patient. Pyridoxine elicited no biochemical response in 6 patients which was in accordance with the predicted responsiveness of the identified mutations.

The calculated prevalence of CBS deficiency in Slovenia was estimated to 1/300 000. The most frequent mutation detected in Slovenia is described in Czech and Polish patients and may indicate a common Central European ancestry. Six Slovenian patients were pyridoxine non-responsive while responsiveness/semi-responsiveness is expected in half of the CBS deficient patients worldwide. It was assumed that the patients with milder phenotypes remained undiagnosed.

066-P

CBS GENE MUTATIONS IN FRENCH HOMOCYSTINURIC PATIENTS

Redonnet-Vernhet I[1], Colombies B[1], Mesli S[1], Bedel A[1], de Verneuil H[1], Ged C[1]
[1]*Laboratoire Biochimie CHU Bordeaux, BORDEAUX, France*

The molecular bases of cystathionine beta-synthase (CBS) deficiency have been studied in thirty-four independent French families coming from the whole country. The patients presented different phenotypes from a mild one (phlebitis), to a severe one (thrombo-embolic events associated with mental retardation and bone deformities). The amino acids profile and the total plasma homocysteine level were in agreement with the diagnosis of classical homocystinuria by transsulfuration defect. Each of the seventeen exons and their adjacent intronic sequences have been amplified from genomic DNA and sequenced.

On sixty-eight alleles we found thirty different mutations: twenty-six missenses, three small deletions resulting in frame shift, one splicing mutation. Ten mutations were novel: seven missenses (p.G153R, p.P375L, p.R413C, p.M458I, p.T460M, p.T495M, p.D538H) and three frame shift (p.I214S fsX220, p.L230R fsX268, p.S501R fsX538).

Additionally, the molecular studies showed: (1) fourteen (20%) p.I278T, the B6-responsive central European mutation, (2) eight (12%) p.T191M, the Iberian mutation, (3) only one allele with p.G307S, the common Celtic mutation, (4) absence of c.1224–2A>C, p.R114V, p.R125Q, p.E144K, p. R266K described as frequent mutations of CBS gene.

Conclusion: The French profile of CBS mutants differ from other European countries. Novel genotypes are described associated with a variety of clinical manifestations.

067-P

EFFECT OF INTRACELLULAR SAH ACCUMULATION AND DNA HYPOMETHYLATION ON DDAH ACTIVITY

Rocha MS[1], T Teerlink[1], de Jong S[1], Jakobs C[1], Tavares de Almeida I[2], Rivera I[2], Castro R[2], Blom HJ[1]
[1]*Dep Clin Chem, VU Univ Medical Center, Amsterdam, Netherlands*
[2]*Met&Gen Group, iMed.UL, Fac of Pharmacy, Lisbon, Portugal*

Background: The only source of intracellular homocysteine (Hcy) production results from S-adenosylhomocysteine (SAH) reversible hydrolysis, which is catalyzed by SAH hydrolase. Hcy is a risk for vascular disease but, the underlying pathogenic mechanism remains uncertain. Within this context, evidence has been gained about the Hcy ability, via accumulation of its precursor SAH, to affect DNA methylation. Recent observations suggest that asymmetric dimethylarginine (ADMA), an endogenous inhibitor of endothelial nitric oxide synthase, may play a role in endothelial dysfunction associated with hyperhomocysteinemia. Dimethylarginine dimethylaminohydrolase (DDAH) is the key enzyme for the degradation of ADMA to citrulline.

Aim: This study was designed to investigate the effect of intracellular SAH accumulation, and consequent global DNA hypomethylation, on DDAH activity.

Methods: Human vein endothelial cells were isolated from umbilical cords, cultured under standard conditions, and treated with or without adenosine-2,3-dialdehyde (ADA), an inhibitor of SAH hydrolase. Extracellular concentrations of ADMA were determined by HPLC. DDAH mRNA and protein expression levels were quantified by qRT-PCR and Western blot analysis, respectively. DDAH activity was assessed by measuring the amount of citrulline produced during 6 h.

Results and Conclusions: As expected, ADA resulted in an increased intracellular SAH and DNA global hypomethylation. In addition, we observed an increase on both mRNA levels and activity of DDAH, and a decrease in ADMA production, so disclosing an up-regulation of the DDAH gene (responsible for an increased ADMA degradation), and ruling out its causal role in the Hcy-induced endothelial dysfunction.

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068-P

CLINICAL AND LABORATORY FINDINGS AND OUT COME IN 17 PATIENTS AFFECTED OF HOMOCYSTEINURIA TYPE II IN IRAN

Zaman Talieh[1], Rahmanifar Ali[1]
[1]*Metab Unit,Tehran Univ, Tehran, Islamic Republic of Iran*

Background: Homocyteinuria type II is a common inborn error of vitamin B12 metabolism, The disease is due to adenosylcobalamine and methylcobalamine synthesis defect, causes methylmalonic acidemia as well as homocystinuria and has two distinct clinical phenotypes,early onset presents up to age of 12 months and late onset disease presents after age of 4 years .Symptoms including; seizure, apnea, anemia,irritability and poor feeding usually in early onset and psychomotor delay and learning disability in late onset form.

Objective: To determine clinical presentation, laboratory finding and outcome of homocysteinuria type II in Iranian patients.

Methods: A retrospective study of all patients diagnosed to have homocysteinuria type II at National institute of hereditary metabolic disease in Tehran between (2002 to 2009).

Results: The total of 17 patients of both sexes; 11(65%) male and 6(35%) female, non related 10, early onset ;11 patients. The most common presentation in early onset type; convulsion and acidosis and mean age at onset was 3.9 months (3.3 to11.3), but in late onset form were psychomotor problem and learning disability and mean age at onset was7.1 years Homocystien level;21–130, mean ;38uml/l (normal 3.3- 11.3) C3>o.47,C3/C2>0.27,meanC4DC level was 0.57um/l (normal; age depended)Urine MMA was high, serum B12was nomal. Treatment was started as soon as the diagnosis was confirmed by typical plasmatic and urinary metabolic profiles (GC MS/MS) In 8 years post treatment follow up acidosis and seizure were resolved in most cases and psychomotor disability improved markedly.

Conclusion: The study showed differences between clinical feature of early onset and late onset form, specific therapy is effective in HCUII, if started soon

069-O

3-METHYLGLUTACONIC ACIDURIA TYPE I REDEFINED, A SYNDROME WITH LATE ONSET LEUKOENCEPHALOPATHY

Wortmann S.B.[1], Kremer B.[2], Graham A.[3], Willemsen M.A.A.P.[4], Loupatty F.J.[5], Hogg S.L.[6], Engelke U.F.H.[7], Kluijtmans L.A.J.[7], Wanders R.J.[8], Illsinger S.[9], Wilcken B.[10], Cruysberg J.R.[11], Das A.M.[9], Morava E.[1], Wevers R.A.[7]
[1]*IGMD, Dep of Ped, RUNMC, Nijmegen, Netherlands*
[2]*Dep of Neuro, UMCG, Groningen, Netherlands*
[3]*Dep of Neuro, Ipswich Hospital, Ipswich, United Kingdom*
[4]*Dep of Neuro, RUNMC, Nijmegen, Netherlands*
[5]*Dep Clin Chem, Onze Lieve Vrouwen Hosp, Amsterdam, Netherlands*
[6]*Biochem Gen Unit, Cambridge Univ Hosp, Cambridge, United Kingdom*
[7]*Dep of Lab Med, RUNMC, Nijmegen, Netherlands*
[8]*Dep Clin Chem, AMC, Amsterdam, Netherlands*
[9]*Dep of Ped, Hannover Medical School, Hannover, Germany*
[10]*Dep Biochem Genet, Child Hosp Westmead, Sydney, Australia*
[11]*Dep of Ophthalmology, RUNMC, Nijmegen, Netherlands*

Background: 3-Methylglutaconic aciduria type I is a rare inborn error of leucine catabolism. Until now it is thought to present in childhood with unspecific symptoms; it was even speculated to be a non-disease. The natural course of disease is unknown.
Methods: This is a study on ten patients with 3-methylglutaconic aciduria type I. We present the clinical, neuroradiological, biochemical and genetic details on two new adult-onset patients and follow-up data on two patients from literature.
Results: Two unrelated patients with the characteristic biochemical findings of 3-methylglutaconic aciduria type I presented in adulthood with progressive ataxia. One patient additionally had optic atrophy, the other spasticity and dementia. Three novel mutations were found in conserved regions of the AUH gene. In both patients MRI revealed extensive white matter disease. Follow-up MRI in a ten year-old boy, who presented earlier with isolated febrile seizures, showed mild abnormalities in deep white matter.
Conclusion: We redefine 3-methylglutaconic aciduria type I as an inborn error of metabolism with slowly progressive leukoencephalopathy clinically presenting in adulthood. In contrast to the non-specific findings in pediatric cases, the clinical and neuroradiological pattern in adult patients is highly characteristic. White matter abnormalities may already develop in the first decades of life. The variable features found in affected children may be coincidental. Long term follow-up in children is essential to learn more about the natural course of this presumably slowly progressive disease. Dietary treatment with leucine restriction may be considered.

070-P

LIPOPEROXIDATION IN PATIENTS WITH DISORDERS OF PROPIONATE METABOLISM IS PREVENTED BY TREATMENT WITH L-CARNITINE

Ribas GS[1], Sitta A[1], Manfredini V[2], Wayhs CAY[1], Vanzin C[1], Biancini G[1], Wajner M[1], Vargas CR[1]
[1]*Univ Fed Rio Grande do Sul, Porto Alegre, Brazil*
[2]*Univ Reg Int Alto Uruguai Missões, Erechim, Brazil*

Background: The disorders of propionate metabolism, propionic (PA) and methylmalonic (MMA) acidemias are inborn errors of metabolism caused by a deficient activity of propionyl CoA carboxylase and methylmalonyl CoA mutase, respectively. The affected patients present severe metabolic acidosis in the neonatal period and long-term neurological deficits whose pathophysiology is not completely established. There are increasing evidences in literature demonstrating antioxidant properties for L-carnitine, which is used in the treatment of PA and MMA. So, the objective of this study was to investigate lipoperoxidation in patients with PA and MMA, before and during treatment with L-carnitine (100 mg/Kg/day).
Methods: We evaluated the levels of malondialdehyde, a final product of lipoperoxidation, and the concentrations of total, free and esterified L-carnitine in plasma of patients with PA and MMA, at the moment of diagnosis of these diseases and during treatment with L-carnitine.
Results: Our results showed a significant increase of MDA in plasma of patients at diagnosis in relation to controls of similar ages. On the other hand, patients under treatment presented a marked reduction of MDA content in relation to patients at diagnosis. In addition, we verified a significant negative correlation between MDA levels and plasma total and free L-carnitine concentrations in PA and MMA patients.
Conclusions: The present data indicate that treatment with L-carnitine may be involved in the reduction of lipoperoxidation in patients affected by disorders of propionate metabolism.

071-P

HIGH DISEASE PREVALENCE AND SINGLE MUTATION FREQUENCY FOR GLUTARYL-COA DEHYDROGENASE DEFICIENCY IN BLACK SOUTH AFRICANS

Van der Watt GF[1], Owen EP[1], Berman P[1], Watermeyer N[1], Olpin SE[2], Manning NJ[2], Baumgarten I[1], Leisegang F[1], Meldau S[1], Henderson H[1]
[1]*Div Chem Path, Univ of Cape Town, Cape Town, South Africa*
[2]*Dept Clin Chem, Sheffield Child Hosp, Sheffield, United Kingdom*

Background: Glutaric aciduria type 1 (GA-1) is generally considered a rare organic acidaemia that is usually associated with a severe irreversible neurological syndrome if not diagnosed early and treated preventatively.
Objectives: Following the recent new diagnosis of 12 cases of GA-1 within 25 months in our laboratory, we investigated the clinical, biochemical and molecular features of this disorder in South Africa.
Patients and Methods: One published, and all 12 new cases of GA-1, together with extracted DNA from 750 randomized dried blood spots derived from black South African newborns were studied. The glutaryl-CoA dehydrogenase (GCDH) gene was sequenced and urinary organic acid levels and skin fibroblast GCDH activities determined in the cases studied. Mutation carrier frequencies were estimated from the population DNA.
Results: Unrelated black South African children constituted 11 of the 13 known cases of GA-1 in South Africa and all of those sequenced (n=10), tested homozygous for the same A293T mutation in the GCDH gene. In addition, 21/750 blood spots tested heterozygous for the same A293T mutation giving an estimated newborn incidence of 1/5184 for GA-1 in this population. Urinary glutarate was not elevated in 4 of these cases despite absent fibroblast GCDH activity and elevated urinary 3-OH glutarate in all cases.
Conclusion: Glutaric aciduria type 1 is a treatable disorder with an unexpectedly high estimated incidence in the South African black population that would benefit enormously from an appropriate newborn screening program. Molecular screening may be more appropriate than conventional biochemical screening in this setting.

072-P

EFFECTS OF BLOOD AND BLOOD PRODUCT TRANSFUSION ON NEWBORN METABOLIC SCREENING FOR BIOTINIDASE DEFICIENCY

Bamforth F[1]
[1]Dept Lab Med & Path, Univ of Alberta, Edmonton, Canada

Background: Biotinidase deficiency screening is included in many newborn screening programs. Blood or blood products transfused prior to sample collection potentially may cause a false negative newborn screen.
Objectives: To evaluate biotinidase activity in blood products typically transfused in a neonate and to assess likelihood of a false negative newborn screen.
Materials and Methods: Biotinidase activity was measured semi-quantitatively by incubating dried blood spots overnight with N-biotinyl-PABA, and measuring the released PABA colorimetrically. Biotinidase activity was measured in the following: packed red cells, platelets (pooled and apheresis), human albumin 5% and 20% USP (Plasbumin.), frozen plasma. If biotinidase activity was present, the product was mixed with biotinidase deficient blood (prepared by heat inactivation of enzyme in serum and reconstituting with washed red blood cells) to simulate typical transfusion volumes for a 2.72 Kg neonate.
Results: Prepared biotinidase deficient blood showed no biotinidase activity. Biotinidase activity was absent in packed red cells and albumin (5% and 20%) but present in platelets and frozen plasma. The interpretation of the biotinidase screen after 'transfusion' of platelets and frozen plasma to biotinidase deficient blood was as follows: platelets =or<5 mL/Kg body weight: Profound deficiency; platelets =or>10 mL/Kg: Partial deficiency; frozen plasma =or<5 mL/Kg: Partial deficiency; frozen plasma =or>10 mL/Kg: Normal screen.
Conclusion: This study quantitates the extent to which transfusion of blood products may give a falsely normal biotinidase on the newborn screen. Transfusion of platelets or frozen plasma but not albumin or packed red cells may give a false negative screen.

073-P

IDENTIFICATION OF UROCANYLGLYCINE IN UROCANASE DEFICIENCY

Engelke U[1], Kwast H[1], Artuch R[2], Pineda M[2], Wevers R[1]
[1]Dep Lab Med, Radboud UMCN, Nijmegen, Netherlands
[2]Clin Biochem Dep, Hosp Sant Joan de Déu, Barcelona, Spain

Background: Urocanic aciduria (OMIM 276880) is caused by urocanase deficiency, a defect in the histidine pathway. Affected patients have an increased concentration of urocanic acid (UA) in their urine. UA and an unknown metabolite (U1) were observed in the urine from a girl with urocanic aciduria.
Objective: Isolation, identification and quantification of U1 in the urine from the patient with urocanic aciduria.
Case Report: Published in JMedGenet2009;46.
Methods: HPLC (reversed-phase column) of purines/pyrimidines and NMR spectroscopy (NMRS) were used to measure urine and CSF from the patient. For peak identification, fractions around the retention time in the chromatogram of peak U1 were collected and measured by NMRS. NMR spectra of urocanyl-conjugates were simulated and compared with the NMR spectrum of fraction U1.
Results: The NMR spectrum of fraction U1 showed detailed structural information of the unknown metabolite. The NMR pattern could be assigned as urocanylglycine (UG). In urine of the patient, the concentration of UG was 180 μmol/mmol creatinine (ref <1). The concentration of UA was 200 μmol/mmol creatinine. No UA and UG were found in the cerebrospinal fluid of the patient.
Conclusion: UG in urine is a novel and major hallmark in urocanic aciduria. As demonstrated here, the combination of HPLC with NMRS provides a powerful tool for the elucidation of the structure of compounds present in urine samples.

074-P

ISOVALERIC ACIDEMIA: A NOVEL MUTATION WITH MILD PHENOTYPE

Matalon K[1], Grady J[2], Matalon R[2]
[1]Dept. of Health & Human, Univ of Houston, Houston, United States
[2]Univ of Texas Medical Branch, Galveston, United States

Background & Objectives: Isovaleric Acidemia (IVA) is considered a severe metabolic disorder with significant morbidity and mortality. It is caused by deficiency of the enzyme Isovaleryl-CoA dehydrogenase (IVD). Early treatment reduces the episodes of the severe metabolic crises. With the advent of neonatal screening, IVD deficiency has shown a spectrum of serverity. We describe a novel homozygous mutation, R332L in the IVD enzyme with benign phenotype.
Case Report: A four month old female was seen in our center because of persistently marked elevation in urine of Isovaleryglycine, 150 mmol/mol creatinine. Blood Acylcarnitine was elevated, C5 2.31 μmol/L. The elevations in blood and urine have persisted on repeated tests. The baby had pneumonia as a newborn without metabolic decompensation and without the offensive odor characteristic for IVA.
Results: Sequence of the IVD gene was performed and homozygous mutation, G>T in exon 10 (c.995G>T), was found. This substitution resulted in changing of Arginine to Leucine, at position 332. No other sequence changes were found in the rest of the IVD gene.
Search of the literature did not reveal such mutation. In spite of the benign phenotype, treatment with carnitine and glycine was started to prevent the possibility of metabolic decompensation.
Discussion & Conclusion: Longer follow-up of patients with benign IVD mutations will be required to determine the need for treatment. We predict that more benign cases will be discovered due to universal newborn screening. We suggest that mutations registry of IVD be formed similar to registries of mutations for other diseases.

075-P

CLINICAL AND MOLECULAR STUDIES OF FIVE PATIENTS WITH SUCCINYL-CoA:3-KETOACID CoA TRANSFERASE DEFICIENCY

Fukao T[1], Sass JO[2], Thimm E[3], Wendel U[4], Ficicioglu C[5], Monastri C[6], Guffon N[7], Baric I[8], Zabot M-T[9], Kondo N[1]
[1]Dept Pediatr, Grad Sch of Med, Gifu Univ, Gifu, Japan
[2]Lab Clin Biochem Metab, Univ Child Hosp, Freiburug, Germany
[3]General Pediatr, Univ Child Hosp, Dusseldorf, Germany
[4]Metab unit, Univ Child Hosp, Dusseldorf, Germany
[5]Sect Metab Dis, Child Hosp Philadelphia, Philadelphia, United States
[6]11 Rue Manfalouti, Sousse, Tunisia
[7]Metab Dis, CERLYMM, HCL, Lyon, France
[8]Dept Pediat, Univ Hosp Cent & Sch of Med, Zagreb, Croatia
[9]Centre de Biotech Cell, GHE-HCL, Bron, France

Succinyl-CoA:3-ketoacid CoA transferase (SCOT) deficiency is an inborn error of ketone body metabolism and causes episodic ketoacidosis. We herein report clinical and molecular analysis of 5 cases of SCOT deficiency. All 5 patients developed their first severe ketoacidotic crises with blood gas pH <7.1, and experienced multiple ketoacidotic crises (twice~7 times). GS07 (France), GS13 (Croatia), and GS15 (Tunisia) are homozygotes of S405P, L327P, R468C, respectively. GS17 (USA) and GS18 (Germany) are compound heterozygotes of S226N and A215V, and V404F and E273X, respectively. Missense mutations were further characterized by transient expression analysis of mutant cDNAs. Among 6 missense mutations, L327P, R468C, and A215V mutants retained some residual activities and their mutant proteins were detected in immunoblot analysis in expression at 37 centigrade. Among 5 patients, hence, GS07 and GS18 had null mutations in both alleles and other three patients retained some residual SCOT activities. They are now more than 9 years old and their growth and development are within normal ranges.

076-P

SUBTLE ABNORMALITY IN URINARY ORGANIC ACID AND BLOOD ACYLCARNITINE PROFILES MAY RESULT IN MISSING THE DIAGNOSIS OF BETA-KETOTHIOLASE (T2) DEFICIENCY WITH MILD MUTATIONS

Fukao T[1], Maruyama S[2], Ohura T[3], Toyoshima M[2], Mushimoto Y[4], Kobayashi H[4], Hasegawa Y[4], Yamaguchi S[4], Kondo N[5]
[1]*Unit Grad Sch Drug Discov Med Inf Sci, Gifu, Japan*
[2]*Dept Pediatr, Kagoshima Univ, Kagoshima, Japan*
[3]*Dept of Pediatr, Sendai City Hosp, Sendai, Japan*
[4]*Dept Pediatr, Shimane Univ Fac Med, Izumo, Japan*
[5]*Dept Pediatr, Grad Sch of Med, Gifu Univ, Gifu, Japan*

T2 deficiency is characterized by intermittent ketoacidotic episodes. We recently experienced three Japanese patients. One patient (GK69) experienced two ketoacidotic episodes at the age of 9 months and 3 years, and no further episodes until the age of 25 years. GK69 was a compound heterozygote of H144P and S390P mutations. She was not suspected to have T2 deficiency by repeated urinary organic acid analysis during her childhood episodes, but she was diagnosed as T2 deficient at age of 25 years by re-evaluation using fibroblasts. GK77 and GK77b were identical twin siblings who presented the first ketoacidotic crisis simultaneously at the age of 3y4m. GK77b was died during the first crisis and GK77 achieved intact survival. They were homozygotes of H144P mutation. This H144P mutation retained significant residual T2 activity in the transient expression analysis of mutant cDNA. Even during severe crises, C5-OH and C5:1 were within normal ranges in their blood acylcarnitine profiles and no tiglylglycine and small amount of 2-methyl-3-hydroxybutyrate were detected in their urinary organic acid profiles. The "mild" H144P mutation may result in these atypical profiles. T2-deficient patients with "mild" mutations cause severe ketoacidotic crises but their chemical phenotypes may be subtle even during acute crises.

077-P

FUNCTIONAL ANALYSIS OF A NOVEL PCCA MUTATION WITH PARTIAL RESIDUAL ACTIVITY IDENTIFIED IN A LATE-INFANTILE ONSET PROPIONIC ACIDEMIA PATIENT

Gallego L[1], Lianou D[2], Michelakakis H[3], Pérez-Cerdá C[1], Pérez B[1], Ginis S[2], Jakobs C[4], Ugarte M[1], Desviat LR[1]
[1]*CEDEM, C. Biología Molecular CSIC-UAM, Madrid, Spain*
[2]*Ag. Sophia Childrens' Hospital, Athens, Greece*
[3]*Institute of Child Health, Athens, Greece*
[4]*VU University Medical Center, Amsterdam, Netherlands*

We report a Greek patient who presented symptoms at four months and was diagnosed as having propionic acidemia on the basis of biochemical and enzymatic analysis. Following the emergency treatment, the patient was started on the appropriate dietary management as well as drug therapy with L-carnitine, biotin and metronidazole. The response has been adequate with appropriate growth, lack of decompensation and slow but steady progress in his psychomotor development. Genetic analysis revealed that the patient is homozygous for a novel mutation (p.R288G, c.862A>G) in the PCCA gene. No other potentially pathogenic change was identified in the exons and flanking intronic sequences in either the PCCA or PCCB genes. The p.R288G mutation affects a well conserved arginine in the biotin carboxylase domain of the PCCA protein and is not directly involved in the active site. The functional significance of this novel change was investigated in a eukaryotic expression system revealing that the mutant protein is associated with partial enzyme deficiency (27% of wild-type level activity). In this case the genotype-phenotype correlation is clear, with the mutant protein with residual activity contributing to the milder phenotypic expression of the disease in the patient. In view of the high number of novel private mutations identified in patients with propionic acidemia the results emphasize the necessity of expressing the variant alleles identified to confirm their pathogenicity and to provide a rationale for the observed phenotype. In addition, early diagnosis and treatment implementation is important to promote an adequate psychomotor development.

078-O

POTENTIAL PHARMACOLOGYCAL CHAPERONE THERAPY FOR ISOLATED METHYLMALONIC ACIDURIA cblB TYPE

Jorge-Finnigan A[1], Underhaug J[2], Desviat LR[1], Martinez A[2], Ugarte M[1], Perez B[1]
[1]*CEDEM, C. Biología Molecular, CSIC UAM, Madrid, Spain*
[2]*Dpt. Biomedicine, University of Bergen, Bergen, Norway*

The present work describes the search for pharmacological chaperones as tailored-treatment for methylmalonic aciduria type B (OMIM#251110, MMA cblB type), based on previous structural and functional studies where we have described misfolding mutations affecting ATP:cob(I)alamin adenosyltransferase (ATR, E.C.2.5.1.17), the enzyme which converts reduced cob(I)alamin to adenosylcobalamin. Therefore, we have screened 2000 compounds from the MyriaScreen library using high-throughput ligand screening by differential scanning fluorimetry, obtaining 25 compounds that specifically increase ATR's Tm between 6–17:C. We have narrowed our selection of compounds down to 6 (I-VI) using thermal denaturation analyzed by circular dichroism as well as heat stress followed by size exclusion chromatography. The cytoxicity assay using fibroblast cell lines revealed that the compounds do not compromise cell viability in a wide concentration range (10–80 μM). Using a well-established prokaryotic expression system we have validated the effect of the six drugs (80 μM) by analyzing the increase in half life of the human wild-type ATR protein. In particular, compounds IV and VI increase the half life 3- and 6-fold, respectively (p-value<0.0005). In addition, the chaperones were tested on purified recombinant pI96T-ATR, previously characterized as a misfolding mutant which exhibited some residual activity and lower half-life than wild-type ATR. Using compounds III, IV, V and VI at 80 μM the half-life of pI96T was increased 2-fold (p-value<0.01 for III and VI; p-value<0.005 for IV and V). In conclusion, this work has revealed four different compounds (III, IV, V and VI) that specifically might act as pharmacological chaperones with potential for treatment of MMA cblB type.

079-P

THE NEUROPSYCHOLOGICAL PROFILE OF METHYLMALONIC ACIDAEMIA (MMA): A CASE NOTE REVIEW

Bond K[1], Rankin P[2], Grunewald S[1]
[1]*Metabolic Medicine, Great Ormond St Hosp, London, United Kingdom*
[2]*Institute of Child Health, UCL, London, United Kingdom*

Background: Methylmalonic Acidaemia (MMA) is an autosomal recessive inborn error of metabolism associated with metabolic decompensation in addition to renal, neurological and cognitive impairment.
Methods: A systematic review was completed of 78 MMA patients treated at Great Ormond Street Hospital 1970–2009. 27 children had died as a result of MMA, 10 had been transferred to adult services, 18 children transferred to other hospitals, with 21 current patients. The metabolic, renal, neurological and neuropsychological outcomes of current patients were analyzed.
Results: The majority of MMA cases (N= 60) were unresponsive to vitamin B12 treatment; unresponsive patients had significantly higher mortality rates, 21 versus 1 responsive (5 diagnosis unknown). Neuroimaging records were available for 23 patients (5 vitamin B12 responsive, 16 unresponsive, 2 unknown), only 6 presented with normal imaging; bilateral abnormality of the globus pallidus the most common deficit, occurring in 9 cases (7 of which were vitamin B12 unresponsive). Statistical analysis indicated that vitamin B12 non-responsive patients were diagnosed at a younger age, presented with more lifetime metabolic instability and renal dysfunction and demonstrated significantly poorer neuropsychological outcome in the domains of intellect (median Full Scale IQ 70), memory, numeracy, spelling and attention skills.
Discussion: This study challenges the assumption that vitamin B12 responsive MMA patients are cognitive 'spared' as intellect (median FSIQ 88.5), processing speed, memory, language and attention abilities fell below the normative interquartile range. Across both groups, the younger the age at diagnosis (measured in days) the poorer the medical and neuropsychological outcomes were.

080-P

CLINICAL AND GENETIC INVESTIGATION OF 19 JAPANESE CASES OF GLUTARIC ACIDEMIA TYPE 1

Mushimoto YM[1], Hasegawa YH[1], Kobayashi HK[1], Purevsuren JP[1], Li HL[1], Taketani TT[1], Fukuda SF[1], Yamaguchi SY[1]
[1]*Department of Pediatrics, Shimane Univer, Izumo, Japan*

Glutaric acidemia type1 (GA1) is an autosomal recessive metabolic disease due to a deficiency of glutaryl-CoA dehydrogenease, which is involved in the catabolic pathway of lysine, hydroxylysine, and tryptophan. Untreated patients develop severe striatal degeneration. More than 150 disease-causing mutations have been identified in the GCDH gene, and a few common mutations are known in specific ethnic groups. In Japan, however, genetic mutations are reported only in a few cases. Herein, we investigated clinical and genetic aspects of 19 Japanese patients with GA1, including 6 previously-reported patients.

Material and Methods: The diagnoses were made by urinary organic acid and/or blood acylcarnitine analysis. Genomic DNA was extracted from fibroblasts or peripheral blood lymphocytes.

Results and Discussion: All cases showed high urinary glutaric acid excretion. Eleven cases were severely impaired (three cases died), three had mild impairment, and five showed normal development. Four of 5 cases with normal development were detected by sibling or neonatal screening. Children with the mild impairment were diagnosed and treated earlier than severely impaired subjects. Interestingly, a sibling case with same mutations showed different outcome. Nineteen mutations in 26 alleles that we performed in this study were identified, and eight of them were novel. The S305L (12.1%, 4/33 alleles) and S139L, R355H (9.1 %, 3/33 alleles) were found in several cases, indicating that these mutations may be rather common mutations. Our results indicated that there is no correlation between genotype and clinical phenotype, and that early diagnosis and treatment are critical for a better outcome.

081-P

DIFFERENTIAL EXPRESSION OF GLUTARYL-CoA DEHYDROGENASE IN ADULT RAT CNS, PERIPHERAL TISSUES AND DURING EMBRYONIC DEVELOPMENT

Ballhausen D[1], Bonafé L[1], Braissant O[2]
[1]*Div Mol Ped, CHUV, Lausanne, Switzerland*
[2]*Clin Chem Lab, CHUV, Lausanne, Switzerland*

Glutaryl-CoA dehydrogenase (GCDH, EC 1.3.99.7) deficiency, known as glutaric acidemia type I, is one of the more common organic acidurias. To investigate the role of this pathway in different organs we studied the tissue-specific expression pattern of rat Gcdh.

The open reading frame cDNA of the rat Gcdh gene was cloned from rat brain mRNA by RT-PCR, allowing the synthesis of digoxigenin-labeled in situ hybridization (ISH) riboprobes. Gcdh mRNA expression was analyzed by ISH on cryosections of adult rat brain, kidney, liver, spleen and heart muscle, as well as on E15 and E18 rat embryos.

Gcdh was found expressed in the whole rat brain, almost exclusively in neurons. Gcdh was absent from astrocytes but expressed in rare oligodendrocytes. Strong Gcdh expression was found in liver and spleen, where expression appears predominant to lymphatic nodules. In kidney, the highest Gcdh expression is found in the juxtamedullar cortex (but not in glomerula), and at lower levels in medulla. Heart muscle was negative. During embryonic development, Gcdh was found well expressed in liver, intestinal mucosa and skin, as well as at lower levels in CNS.

Further studies are ongoing to provide evidence on the presence of the entire pathway in CNS in order to understand the mechanisms leading to neurotoxicity in glutaric aciduria. The high expression of Gcdh in kidney may explain why certain patients with residual enzyme activity are low excretors at the urine metabolite level.

082-O

FEASIBILITY OF AMINOGLYCOSIDE MEDIATED SUPPRESSION OF NONSENSE MUTATIONS AS A NOVEL THERAPEUTICAL APPROACH IN PROPIONIC ACIDEMIA

Sánchez-Alcudia R[1], Pérez B[1], Ugarte M[1], Desviat LR[1]
[1]*CEDEM, C.Biología Molecular CSIC-UAM, Madrid, Spain*

In propionic acidemia, caused by a defect of propionylCoA carboxylase (PCC) involved in the metabolism of several amino acids, odd-chain fatty acids and cholesterol, nonsense mutations constitute ~10% of the total alleles in both the PCCA and PCCB genes encoding both subunits of the PCC enzyme. Aminoglycosides and drugs such as Ataluren (PTC124) have been known to induce premature termination codon (PTC) readthrough constituting a potential therapy for nonsense mutations. Among these, those generating an UGA stop codon are most susceptible to readthrough so we have selected patients' fibroblasts available in the laboratory with this type of nonsense mutations. All have greatly decreased levels of both immunorreactive protein and mRNA, probably due to the nonsense mediated decay mechanism. The functional recovery of nonsense suppression is being assayed testing different concentrations of aminoglycosides gentamicin and geneticin, as well as by expression analysis of the putative missense mutations generated after aminoglycoside treatment. We have also tested in vitro suppression of 6 nonsense mutations in the PCCA and PCCB genes using a coupled transcription-translation assay (TNT). Our results show a dose-dependent effect for geneticin (0.1–2.5 μg/mL) resulting in up to 15 or 25% of full-length protein and for gentamicin (5–20 μg/mL) with up to 16 or 19% for the PCCA mutations R313X and S562X, respectively. However among the 4 PCCB mutations studied, only for G94X and R111X < 5% full-length protein is produced in the same conditions, suggesting each patient's genotype must be analyzed for its susceptibility to read-through before considering therapeutical applications.

083-P

NEUROCHEMICAL EVIDENCE THAT METHYLMALONIC ACID ELICITS LIPID AND PROTEIN OXIDATIVE DAMAGE IN VITRO AND EX VIVO IN BRAIN OF YOUNG RATS

Fernandes CG[1], Borges CG[1], Seminotti B[1], Amaral AU[1], Leipnitz G[1], Zanatta A[1], Wannmacher CMD[1], Wajner M[2]
[1]*Departamento de Bioquímica, ICBS, UFRGS, Porto Alegre RS, Brazil*
[2]*Servico de Genética Médica, HCPA, Porto Alegre, RS, Brazil*

Background: Methymalonic aciduria, one of the most frequent disorders of branched-chain organic acidurias, is biochemically characterized by a predominant tissue accumulation of methylmalonic acid (MMA). Although patients present severe neurological symptoms, the pathophysiology of the cerebral damage is not fully understood.

Objectives: The present study investigated the in vitro (in brain synaptosomes) and ex vivo (by intrastriatal administration) effects of MMA (0.2–10 mM) on important parameters of oxidative stress in brain of young rats.

Methods: Thiobarbituric acid-reactive substances levels (TBA-RS), carbonyl formation, sulfhydryl oxidation, dichloroflorescein diacetate (DCF-DA) oxidation and the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were determined.

Results: Our results demonstrate that MMA significantly increased TBA-RS levels in vitro and ex vivo, indicating that this metabolite induced lipid peroxidation. Furthermore, the addition of free radical scavengers prevented MMA-induced in vitro increase of TBA-RS levels, suggesting that free radicals were involved in this effect. It was also verified that MMA induced sulfhydryl group oxidation (ex vivo) and carbonyl formation (in vitro), suggesting that this organic acid provoked protein oxidative damage. Finally, intrastriatal injection of MMA significantly increased reactive oxygen species formation, as reflected by increased DCFH-DA oxidation. The activities of CAT and GPx were not altered by MMA, whereas SOD activity was increased.

Conclusion: It is presumed that MMA induces oxidative stress in brain, which may be associated with the neurologic damage found in patients affected by methylmalonic aciduria.

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084-P

GENETIC ANALYSIS OF MUT, MMAA, AND MMAB IN THAI PATIENTS WITH ISOLATED METHYLMALONIC ACIDEMIA

Vatanavicharn N[1], Champattanachai V[2], Liammongkolkul S[1], Sawangareetrakul P[2], Keeratichamroen S[2], Cairns JRK[2], Srisomsap C[2], Sathienkijkanchai A[1], Svasti J[3], Wasant P[1]
[1]*Dept Pediatrics, Siriraj Hosp, Bangkok, Thailand*
[2]*Lab Biochem, Chulabhorn Res Institute, Bangkok, Thailand*
[3]*Faculty of Science, Mahidol Univ, Bangkok, Thailand*

Background: Methylmalonic acidemia (MMA) is one of the most common organic acid disorders. Isolated MMA could be categorized by mutations in the MUT (mutase), MMAA (cblA), and MMAB (cblB) genes. The aim of this study was to delineate the genotypes and clinical phenotypes of Thai patients with MMA.
Materials and Methods: The clinical features of the 9 MMA patients with age of between 4 months and 12 years were retrospectively studied. They were characterized into 3 subgroups belonging to mut, cblA, or cblB defects.
Results: Five patients were classified as a cblB defect, two patients as a cblA defect, and two patients as a mut defect. Five patients had neonatal-onset and severe phenotype, and all had cblB defect. Interestingly, one of them had dilated cardiomyopathy. Patients belonging to mut and cblA subgroups had later-onset and milder phenotype, however, one patient with mut had early onset of renal complications at age of 2 years. Most patients older than 2 years (5/6 patients) had some types of renal involvement. Mutation analysis identified: IVS11–2A>G and R694W in the MUT gene; R98G and R145X in the MMAA gene; E152X, c.455delA, c.682delG, and c.563_577dup in the MMAB gene. The R98G, c.455delA, and c.682delG are novel mutations.
Conclusion: This is the largest series of MMA patients from Thailand. We found that cblB is the most common defect with most severe clinical phenotype. Renal complications are common and not associated with other clinical severity. Cardiomyopathy could be another complication especially in a severe case.

085-P

DETERMINATION OF CELL-SPECIFIC NEUROTOXICITY OF MALONATE, METHYLMALONATE AND PROPIONATE IN A 3D RAT BRAIN CELL AGGREGATE SYSTEM

Ballhausen D[1], Henry H[2], Bonafé L[1], Braissant O[2]
[1]*Div Mol Ped, CHUV, Lausanne, Switzerland*
[2]*Clin Chem Lab, CHUV, Lausanne, Switzerland*

Malonate, methylmalonate and propionate are potentially neurotoxic metabolites in branched-chain organic acidurias. Their effects were tested on cultured 3D rat brain cell aggregates, using dosages of 0.1, 1.0 and 10.0 mM with a short but intense (twice a day over 3 days) and a longer but less intense treatment (every 3 rd day over 9 days).
CNS cell-specific immunohistochemical stainings allowed the follow-up of neurons (axons, phosphorylated medium-weight neurofilament), astrocytes (glial fibrillary acidic protein) and oligodendrocytes (myelin basic protein). Methylmalonate and malonate were quantified by tandem mass spectrometry. Tandem mass spectrometry analysis of harvested brain cell aggregates revealed clear intracellular accumulation of methylmalonate and malonate. In immunohistochemical stainings oligodendrocytes appeared the most affected brain cells. The MBP signal disappeared already at 0.1 mM treatment with each metabolite. Mature astrocytes were not affected by propionate, while immature astrocytes on intense treatment with propionate developed cell swelling. 1 mM methylmalonate induced cell swelling of both immature and mature astrocytes , while 1 mM malonate only affected mature astrocytes. Neurons were not affected by methylmalonate, but 10.0 mM malonate on less intense treatment and 0.1, 1.0 and 10.0 mM propionate on intense treatment affected axonal growth.
Our study shows significant uptake and deleterious effects of these metabolites on brain cells, principally on astrocytes and oligodendrocytes. This may be explained by the absence of the pathway in glial cells, which thus are not able to degrade these metabolites. Further studies are ongoing to elucidate the underlying mechanisms of the observed neurotoxic effects.

086-P

MANAGEMENT AND MONITORING OF PREGNANCIES IN PATIENTS WITH ISOVALERIC ACIDEMIA: ESSENTIAL OR NOT?

Habets DDJ[1], Schaper NC[2], Rogozinski H[3], van Spronsen FJ[4], Bierau J[1], Bakker JA[1]
[1]*Lab of Bioch Gen, Maastr Univ Med Centre, Maastricht, Netherlands*
[2]*Dep of Med Endocr,Maastr Univ Med Centre, Maastricht, Netherlands*
[3]*Dep of Pediatr, Bradford Royal Infirmary, Bradford, United Kingdom*
[4]*Beatrix Chil Hosp, Univ Med Centre Gron, Groningen, Netherlands*

Background: Thanks to effective treatment isovaleric acidaemia (IVA) patients reach adolescence and may consider having children. Pregnancies in IVA are a challenge to the patients, physicians, dietitians and biochemists to maintain metabolic stability.
Case report: We present three cases of maternal IVA and their five single- and twin pregnancies, whose clinical condition was managed with contrasting approaches, aimed at maintaining metabolic stability of the patients. In the first case, two pregnancies were strictly managed and monitored by measuring acylcarnitine- and amino acid profiles and blood-gas analyses followed by adjustment of the diet, and L-carnitine and/or glycine supplementation. In addition, complications were prevented by intravenous glucose and L-carnitine during labour and post-partum period. In two other cases the metabolic condition of patients were less frequently controlled and additional treatment with intravenous L-carnitine and glucose/dextrose was only prescribed during periods of hyperemesis gravidarum. With respect for variance in management and monitoring of maternal IVA these five pregnancies were successful for mother and child.
Conclusion & Discussion: Additional treatment of maternal IVA during pregnancy results in a well-controlled condition of the patient. These cases suggest that individual monitoring and management of a pregnant patient with IVA is useful but not essential.

087-P

A PILOT STUDY OF NEONATAL METABOLIC SCREENING BY THE GC/MS METHOD USING URINE

Aoki K[1], Inokuchi T[1], Watanabe Y[1], Tashiro K[1], Inaba M[1], Inoue K[1], Matsuishi T[1]
[1]*Res Inst of GC/MS,Kurume Univ, Kurume, Japan*

We have performed a pilot study for neonatal mass screening using the GC/MS method since 1996. Thus far, we have screened urine samples from 92,788 neonates and found 78 cases of a total of 18 diseases, including 13 cases of MMA. The total incidence was extremely high at one in 1,200. During the study period, we experienced two cases of girls who had no abnormalities on GC/MS screening as neonates but developed OTC-deficiency with hyperammonemia and disturbance of consciousness at the age of five years. The incidence observed during 14 years of neonatal screening using the GC/MS method demonstrates the high sensitivity and diagnostic accuracy of this method and confirmed the effectiveness of screening. In particular, MMA can be detected and diagnosed only using GC/MS urine analysis, and it was also considered an extremely important disease to target in screening due to its frequency (1/7,100). The fact that two girls developed OTC-deficiency at age 5 confirmed that preclinical detection of OTC-deficiency is difficult with neonatal screening, and that OTC-deficiency is not a suitable target disease for screening. However, because GC/MS urine analysis is essential for chemical diagnosis of OTC-deficiency, and mild increases in urinary uracil have been observed prior to onset in some patients, its use can be considered sufficiently meaningful.

088-A

CLINICAL AND MRI FINDINGS IN A CASE OF D-2-HYDROXYGLUTARIC ACIDURIA

Mesli S[1], Villega F[2], Colombies B[1], Redonnet-Vernhet I[1], Lamireau D[2], Mansour R[3], Balestrat S[1], Bertuletti B[1], Espil-Taris C[2], de Verneuil H[1]
[1]*Laboratoire de Biochimie,CHU de Bordeaux, Bordeaux, France*
[2]*Département de Pédiatrie, CHU Bordeaux, Bordeaux, France*
[3]*Service de Pédiatrie, CHG Agen, Agen, France*

Introduction: D-2-hydroxyglutaric aciduria (D-2-HGA) is a rare autosomal recessive neurometabolic disorder. The severe phenotype is characterized by neonatal or early-infantile-onset epileptic encephalopathy with marked hypotonia, cerebral visual failure or delayed cerebral visual development and serious developmental delay. Developmental delay and hypotonia were the most frequent findings in the mild phenotype. Case report: Here we report a 11-year-old boy with a mild phenotype. He was born from non-consanguineous parents and present speech difficulty, psychomotor delay and difficulties in school apprenticeship. EEG recordings reveal paroxystics abnormalities in the right centro-temporal area. Physical examinations were normal and there were no dysmorphic features. There was no evidence of a cardiomyopathy on ultrasound. Magnetic resonance imaging (MRI) of the brain shows focal white-matter lesions which preferentially affect frontal and subcortical regions. No abnormalities were observed in basal ganglia and temporal lobes. Urine organic acid profile revealed massive excretion of 2-hydroxyglutaric acid (1077 μmol/mmol creatinine, control < 15 μmol/mmol creatinine). The 2-HG excreted by this patient was found to be of the D-configuration.
Conclusion: The present clinical picture in this newly detected patient confirms the most frequent findings reported in previously patients. On the other hand the MRI finding does not correspond to the most frequent findings which are regardless of the clinical phenotype enlargement of the lateral ventricles, occipital more than frontal. This report indicates the importance of routine examination of urinary organic acids in children presenting with psychomotor delay and abnormal brain MRI white matter lesions. D-2-HGA should by consider as a probable diagnostic.

089-P

EXPANDED NEONATAL SCREENING IN REGION OF MURCIA: VERY HIGH INCIDENCE OF METHYLMALONIC ACIDURIA

Juan Fita MJ[1], Egea Mellado JM[1], González Gallego C[1], Fernández Sánchez A[1]
[1]*Centro de Bioquímica y Genética Clínica, Murcia, Spain*

Introduction: Methylmalonic aciduria (MMA) is an autosomal recessive disorder resulted from deficient activity of methylmalonyl-CoA mutase or cobalamine cofactor synthesis. The early diagnosis is based on the increase of propionylcarnitine (C3) in blood spot samples quantified by tandem mass-spectrometry (MS/MS).
The aim of the study is the early detection of MMA patients by MS/MS in Region of Murcia (Southeast of Spain). In this area, immigrant population has grown significantly from 3.28% to 16.44% in the period between 2000–2008, mainly from North Africa and South America. Immigrant birth rate doubles that of Spaniards.
Material and Methods: Blood spot samples received from Neonatal Screening Programme since February 2007 until March 2010 were analysed by MS/MS for the early detection of metabolic diseases, including MMA.
Results: From the 56547 newborns analysed, 9 were diagnosed of MMA (total incidence: 1/6283) and 5 of them were immigrants (56.6% of the patients diagnosed of MMA). The incidence of MMA in analysed immigrant population is 1/3122 versus 1/10234 of native population.
One of the patients resulted in false negative as C3 and C3/C2 values were under the cutoff established (C3= 2.8 uM; C3/C2 = 0.13; normal values under 3.5 uM (p95.4) and 0.15 (p92), respectively.
Conclusion: The prevalence of MMA in Region of Murcia is one of the highest defined, probably due to high levels of immigration. The immigration is a factor to take into account to expand the neonatal screening panel.

090-P

cblE TYPE OF HOMOCYSTINURIA: POSSIBLE ROLE OF HOMOCYSTEINE IN STRESS OXIDATIVE AND APOPTOSIS PROCESSES

Richard E[1], Gallego L[1], Ugarte M[1], Pérez B[1]
[1]*CEDEM, C. Biología Molecular CSIC-UAM, Madrid, Spain*

Oxidative stress and apoptosis analysis using cell models of disease including fibroblasts from isolated methylmalonic aciduria (MMA), MMA combined with homocystinuria and homocystinuria patients has been our main focus in order to provide more insight into the physiopathology of these disorders. Previous results have shown that cblC fibroblasts present the highest ROS levels and apoptosis rate compared to other isolated MMA cells indicating that homocysteine might play an important role in ROS production. To validate this hypothesis, herein we present the analysis of several parameters related to oxidative stress and apoptosis in three patients with homocystinuria cblE type. 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. On the other hand, ROS content was 50% decreased in cell lines treated with antioxidants, such as Tiron. Additionally, oligomycin, an inhibitor of the H±ATP synthase, reduces ROS content indicating that the majority of ROS comes from mitochondria. Our results suggest that homocysteine may activate p38 and JNK MAPKs in a ROS-dependent manner which could be responsible for apoptosis activation. The toxic built-up of homocysteine in cblC and cblE patients could have an important role in ROS production which may represent a genetic modifier of the phenotype, supporting the potential of using antioxidants as novel therapeutic agents to improve the severe neurological outcome of these rare diseases.

091-P

NOVEL HOMOGENTISATE DIOXIGENASE (HGD) GENE MUTATIONS IN ALKAPTONURIA PATIENTS

Hatipoglu E[1], Ozgul RK[2], Sivri HS[2], Coskun T[2], Tokatli A[2], Karaca S[3], Kucuk O[1], Kilic M[2], Akcelik M[1], Dursun A[2]
[1]*Dept of Biology, Ankara Univ, Ankara, Turkey*
[2]*Dept of Ped, Metab Uni, Hacettepe Univ, Ankara, Turkey*
[3]*Dept of Biology, Aksaray University, Aksaray, Turkey*

Alkaptonuria is a rare hereditary metabolic disease which inherited as an autosomal recessive trait. Mutations occured in homogentisate 1,2-dioxygenase (HGD) gene cause alkaptonuria. In this study, mutation screening of all the nucleotide sequences of 14 protein encoding exons and the nucleotide sequences in exon-intron boundaries for HGD gene in 13 alkaptonuria patients were performed. The results of mutation screening study were evaluated, it can be grouped that six patients are carrying mutations as a homozygous state, while the other six patients are compound heterozygote for HGD gene. Mutation could not be identified only in one patient. As a result of classification of the mutations detected by DNA sequence analysis, five novel missense (p.N219S, p.R225C, p.P274L, p.G251D, p.V316I), one deletion (c.1115–1116delGAC) and one insertion type of mutations, (c.656–657insAATCAA) have been reported firstly by this study and one frame shift (c.342delA) and three missense mutations, (p.R330S, p.G161R, p.R53W) that have been identified previously by different groups, have been found. Absence of the novel mutant alleles was tested in 100 healthy chromosomes in control population. Moreover, known p.H80Q, IVS2 +35 T>A ve IVS5 +25 T>C polymorphisms has also been found in the patients.
The study was supported by State Planning Organization of TURKEY (Project No: DPT 2006 K120640)

092-P

SIX NOVEL MUTATIONS IN TURKISH PATIENTS WITH ISOVALERIC ACIDEMIA

Kucuk O[1], Ozgul RK[2], Karaca M[3], Sivri HS[2], Coskun T[2], Tokatli A[2], Hatipoglu E[1], Unal O[2], Akcelik M[1], Dursun A[2]
[1]*Dept of Ped, Metab Uni, Hacettepe Univ, Ankara, Turkey*
[2]*Dept of Biology, Aksaray University, Aksaray, Turkey*

Isovaleric acidemia (IVA), a rare autosomal recessive disorder, is caused by a deficiency of isovaleryl-CoA dehydrogenase (IVD) which catalyzes the third step of the leucine degradation pathway. In this study, 16 patients with IVA from 14 unrelated families have been investigated for mutations in IVD gene. Preliminary diagnosis of the patients was performed with amino acid and organic acid analysis. To further confirm the diagnosis, IVD gene was screened for nucleotide changes. The mutation screening strategy used is direct sequencing of all exons and exon-intron boundries of IVD gene in the patients. No disease causing nucleotide changes were detected in two patients and one patient carries only a nucleotide change as a heterozygous state. Of nine different mutations detected three mutations (p.R21H, p.R21P, and IVS4+2 T>C) were reported previously in different ethnic groups. Other six mutations (p.A268V, p.I297M, p.G346D, p.E379K, 506_507insT, and IVS2+3 T>C) were depicted as novel mutations because none of these nucleotide changes were not found in control individuals. Of three different polymorphisms detected two polymorphisms (IVS2+14 T>C, IVS10+41A>G) were reported previously and one polymorphism (IVS5+57A>G) was found as novel polymorphism. Although mutation spectrum for IVD gene in Turkish patients is different from other ethnic groups, larger cohort is needed to make reasonable genotype-phenotype relation. This study is first report on the molecular pathology of isovaleric acidemia from Turkey.

The study was supported by State Planning Organization of TURKEY (Project No: DPT 2006-K120640)

093-P

MUTATION DETECTION IN TURKISH PATIENTS WITH GLUTARIC ACIDURIA TYPE I

Guzel A[1], Ozgul RK[2], Yucel D[1], Karaca M[3], Kilic M[2], Coskun T[2], Tokatli A[2], Sivri HS[2], Dursun A[2]
[1]*Dept of Biology, Hacettepe Univ, Ankara, Turkey*
[2]*Dept of Ped, Metab Uni, Hacettepe Univ, Ankara, Turkey*
[3]*Dept of Biology, Aksaray University, Aksaray, Turkey*

Glutaric aciduria type 1 (GA1) is an autosomal recessive neurometabolic disorder caused by deficiency of the mitochondrial enzyme glutaryl-CoA dehydrogenase (GCDH). In GCDH mutation database, 150 disease-causing mutations have been described in the GCDH gene for GA1 patients. Certain mutations show predominance in specific populations. P.Arg402Trp mutation is identified as the most common Caucasian mutation. Mutation screening study was performed with a resequencing microarray designed originally by our group (TR_06_01r520489/Affymetrix). The 50 K custom resequencing microarray was designed to cover all exonic and their flanking intronic sequences of GCDH gene. All detected nucleotide changes was also confirmed by direct sequence analysis. Five different missense mutations were detected in GCDH gene in seven patients. Three of them (p.Pro248Leu, p.Arg402Trp, p.Gly185Arg) were reported previously. Other two mutations p.Arg88His and p.Glu181Gln was detected as novel mutations in this study.

The study was supported by State Planning Organization of TURKEY (Project No: DPT 2006 K120640)

094-P

SEVERE NEONATAL PRESENTATION OF THE COBALAMIN F (cblF) DEFECT IN A TWIN WITH A GOOD OUTCOME

Sharrard MJ[1], Manning NJ[2], Olipn SE[2], Clarke S[2], Scott CAB[2], Fowler B[3]
[1]*Paediatric Medicine, Children's Hospital, Sheffield, United Kingdom*
[2]*Clinical Chemistry, Children's Hospital, Sheffield, United Kingdom*
[3]*University Children's Hospital, Basel, Switzerland*

The cblF defect is a rare inborn error of cobalamin metabolism with variable presentation and outcome.

Case Report: A female twin born at 38 weeks gestation, weight 2.5 kg. developed poor feeding, vomiting and encepalopathy on day 3. Examination was unremarkable. Initial sepsis was treated with antibiotics and improved rapidly. Brain, chest and abdomen imaging was normal. At ten days, sepsis had resolved but she continued to require nasogastric feeds. Glossitis was observed at day 21. pH, ammonia and lactate were normal. Urinary methylmalonate excretion was increased (1.255 mol/mol creat, <0.008), plasma methionine decreased (12 μmol/L,15–65), homocysteine elevated (104 μmol/L, <16) and serum B12 normal (297 ng/L, 211–911). These all rapidly normalized with daily 1 mg IM hydroxycobalamin injections, as did her clinical condition.

Methionine synthesis measured by incorporation of label from [14C] formate in cultured fibroblasts was low at 0.58 nmol/16 h, control 1.0–4.0, and became normal with hydroxycobalamin supplementation, and serine synthesis low normal 0.41 nmol/16 h, control 0.38–3.7. There was relatively high uptake of [57Co] (88 pg/mg protein, control 40–156), with deficient production of methyl- and adenosyl- cobalamin (1.4 and 0.7 %, controls 40–76 and 14–28% of total). [14C] propionate incorporation was low at 1.32 nmol/mg.protein./16 h, control 3.5–24.4, correcting with hyroxycobalamin. Cells complemented cblC but not cblF mutant cell lines confirming the cblF defect.

She receives 1 mg B12 IM twice weekly and at 8 months is growing and developing normally.

The cblF defect may present as a severe neonatal encephalopathy in patients without physical defects and respond well to B12 with normal outcome.

095-P

INBORN ERRORS OF METABOLISM REVEALED BY ORGANIC ACID PROFILE ANALYSIS IN HIGH RISK EGYPTIAN PATIENTS: SIX YEARS EXPERIENCE

Gouda A[1], Fateen E[1], Boehles H[2], Sewell A[2]
[1]*Biochemical Genetics Department, NRC, Cairo, Egypt*
[2]*Child Hosp,Wolf Univ, Frankfurt, Germany*

Objective: To determine the prevalence and types of inborn errors of amino acid or organic acid metabolism in a group of high risk Egyptian children with clinical signs and symptoms suggestive of inherited metabolic diseases.

Material and Methods: 117 (79 males = 67.5 % and 38 females = 32.5 %) high risk patients with signs and symptoms of a metabolic disorder were studied, their ages ranged from 3 days to 12 years.

Analysis of urine organic acids by gas chromatography /mass spectrometry (GC/MS) was performed to all patients.

Results: 22(18.8 % of the total) cases were diagnosed with different types of aminoacidopathies or organic acidurias. The disease profile showed increased lactate in 12 cases (54 %) , glutaric aciduria type I 3cases (13 %), phenylketonuria 2 cases (9 %) , maple syrup urine disease 1 case (4.5 %), glutaric aciduria type II 1 case (4.5 %), methylmalonic aciduria 1 case (4.5 %) , Canavan disease 1 case(4.5 %) and non ketotic hyperglycemia 1 case (4.5 %).

Conclusion: The results demonstrate the importance of the organic acid profile in the diagnosis of high risk patients. The diagnosed organic acid pattern in this study showed that 10.2 % of the patients had a mitochondrial energy defect.

096-P

DYSMYELINATION IN EARLY-TREATED PATIENTS WITH GLUTARIC ACIDURIA TYPE I

Lee NC[1], Chien YH[1], Peng SF[1], Cheng PW[1], Chang LM[1], Huang AC[1], Hwu WL[1]
[1]*National Taiwan University Hospital, Taipei, Taiwan*

Background: Glutaric aciduria type I (GAI) is an inborn error of lysine and tryptophan metabolism. Patients with GAI usually present symptoms similar to dystonic or dyskinetic types of cerebral palsy, but at that time treatment is not effective. Newborn screening for GAI started in 2001 in Taiwan.
Objective: We wish to understand the neurological outcome of patients with GAI who were treated immediately after diagnosis brought by newborn screening.
Material and Methods: Serial brain magnetic resonance imaging (MRI) was performed for these patients. MRI findings were correlated to clinical outcome and biochemical parameters including blood spot glutaryl-carnitine (C5DC), and plasma lysine and tryptophan.
Results: Six patients were enrolled in the study. They were all on dietary control and there was no encephalopathic crisis occurred in any of the patients. Their mean C5DC level was 0.93±0.60 uM, mean plasma lysine level 99.19±38.44 uM, and mean plasma tryptophan level 47.58± 12.69 uM. However, late-onset dysmyelination characterized by high intensity on T2-weighted images over deep white matters was observed in four patients at a mean age of 3.91±1.34 years old. There was no basal ganglion damages noted on MRI. Occurrence of dysmyelination was correlated to plasma lysine and tryptophan levels but not to clinical outcome.
Conclusion/Discussion: Early treatment of GAI improves patients' outcome. Late-onset dysmyelination may be a warning sign for incomplete treatment effect, but will need further investigation.

097-P

ISOVALERIC ACIDURIA: TUNISIAN EXPERIENCE

Hammami MB[1], Nasrallah F[1], Hadj Taieb S[1], Omar S[1], Azzouz H[2], Sanheji H[1], Tebib N[2], Ben Dridi MF[2], Feki M[1], Kaabachi N[1]
[1]*Laboratory of Biochemistry, Rabta Hosp, Tunis, Tunisia*
[2]*Paediatric department, Rabta Hosp, Tunis, Tunisia*

Background: Isovaleric aciduria (IVA) is a rare autosomal recessive inborn error of leucine metabolism caused by a deficiency of the mitochondrial enzyme; isovaleryl-CoA dehydrogenase. It results in an accumulation of isovaléryl glycine in urine and blood. IVA is considered to be a potentially life-threatening disorder that manifests with encephalopathy, recurrent episodes of vomiting, lethargy, coma and varying degrees of developmental delay. This study was conducted to investigate clinical and biochemical characteristics about a series of 10 Tunisian patients.
Materials and Methods: Ten patients are diagnosed with IVA between 1988 and 2008. Clinical features were collected and urinary organic acids profiles were analysed by gas chromatography mass spectrometry.
Results: The average age of patients was 27 months, with a sex ratio of 4. Consanguinity was observed in 8 cases and was of first degree for four of them. IVA was dominated by metabolic acidosis (80%), hypotonia (70%) and anemia (70%). Other signs such as coma (30%), vomiting (40%), feeding and respiratory difficulties (10%) and ketosis (10%) were less frequent. The diagnosis of IVA was confirmed by the presence of isovaléryl glycine in urinary organic acids chromatography.
Conclusion: Despite IVA is clinically heterogeneous; the association, hypotonia, anemia and metabolic acidosis evoked this disease. An organic acids chromatography should be performed to search and confirm diagnosis of IVA allowing appropriate management.

098-P

IDENTIFICATION OF MUTATIONS IN THE PCCA AND PCCB GENES CAUSING PROPIONIC ACIDEMIA IN TURKISH PATIENTS

Ozgul RK[1], Yucel D[2], Hismi B[1], Karaca M[3], Sivri HS[1], Coskun T[1], Tokatli A[1], Dursun A[1]
[1]*Dept of Ped, Metab Uni, Hacettepe Univ, Ankara, Turkey*
[2]*Dept of Biology, Hacettepe Univ, Ankara, Turkey*
[3]*Dept of Biology, Aksaray University, Aksaray, Turkey*

Propionic acidemia (PA) is an autosomal recessive disorder of organic acid metabolism caused by deficiency of the propionyl-coenzyme A (CoA) carboxylase. The enzyme consists α and β subunits which are encoded by PCCA and PCCB genes, respectively. Until now, a greater heterogeneous mutation spectrum was observed in patients with PA and more than 50 different mutations were detected in PCCA and PCCB genes. In this study, disease causing mutations in PCCA and PCCB genes were screened by using custom resequencing microarray and direct sequence analysis in eight unrelated Turkish patients with PA. Five different types of mutations in PCCA gene (two splice site, two missense and one deletion) and two different types of mutations in PCCB gene (one nonsense, one missense) were detected. PCCA gene harbored for four novel mutations including IVS16;+7A>G, IVS13;+3A>G, S291L, c.1196–98delG and one known mutation, P423L. On the other hand, two novel mutations R113X and G202R detected in PCCB gene. Mutation screening of 13 patients in addition to these patient group in PCCA and PCCB genes are still continuing.
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099-P

A TURKISH PATIENT WITH LATE ONSET cblC DEFECT CAUSED BY c.394C>T MUTATION

Kilic M[1], Dursun A[1], Tokatli A[1], Sivri HS[1], Anlar B[2], Fowler B[3], Coskun T[1]
[1]*Pediatr Metab Dis, Hacettepe University, Ankara, Turkey*
[2]*Pediatr Neurol, Hacettepe University, Ankara, Turkey*
[3]*Div. Metab. Dis, UKBB, Basel, Switzerland*

The cobalamin C (cblC) defect is an error of intracellular cobalamin metabolism. A 4- year 4-month old girl, who had normal development milestones up to 4 months ago, was admitted to hospital for speaking difficulties, drowsiness, ataxia, and failure to swallow solid food. Physical examination showed a thin, feeble girl with ataxia, diminished eye contact, hypotonia and negative deep tendon reflexes. Laboratory investigations showed Hb 10 g/dl, MCV 83.9 fl, and massive methylmalonic aciduria (2080 μmol/mmol creatinine). Serum levels of B12, propionylcarnitine, methionine, and homocysteine were 695 pg/ml, 6.13 μmol/L, 5.4 μmol/L, and 232 μmol/L, respectively. Cranial MR imaging showed bilateral signal intensity changes in basal ganglia and cerebral white matter, diffuse atrophy of the corpus callosum and cerebrum. MR spectroscopy revealed lactate and myoinositol peaks in the basal ganglia. Complementation studies confirmed cblC defect and molecular studies of the MMACHC gene revealed c.394C>T (R132X) homozygous mutation, which is common in the Asiatic-Indian/Pakistani/Middle Eastern populations. Hydroxycobalamin, folic acid, and betain were started. After treatment, levels of plasma homocysteine, propionylcarnitine, and urine methymalonic acid decreased to 32 μmol/L, 3.2 μmol/L, and 195 μmol/L, respectively. Plasma methionine levels increased to 19 μmol/L. In about a year, clinical follow up of the patient showed improvement in perception, speech and walking. Apart from mild weakness in lower extremities and mild cognitive delay, nearly complete recovery was achieved.

100-A

ISOVALERIC ACIDEMIA: A CASE REPORT

Nurani N[1], Sjarif RD[2]
[1]Pediatric Dept. Gadjah Mada Univ, Yogyakarta, Indonesia
[2]Pediatric Dept, Univ of Indonesia, Jakarta, Indonesia

Background: Isovaleric Acidemia (IVA) due to isovaleryl-coenzyme A dehydrogenase deficiency is one of the branched-chain aminoacidopathies. Early diagnosis and treatment with a protein-restricted diet and supplementation with carnitine and glycine are effective in promoting normal development in severely affected patients. IVA may present in the neonatal and intermittent late-onset period.
Objective: To report a case of Isovaleric Acidemia due to isovaleryl-coenzyme A dehydrogenase deficiency.
Case Report: A 36–month-old child came to the emergency unit with profuse vomiting and decreased consciousness. His mother reported that he had been in good health until 2 days before admission. He had been hospitalized when he was 3 weeks due to septic condition. He was admitted to the Pediatric Intensive Care Unit and presented severe dehydration and intractable metabolic acidosis. Physical examination revealed hyperthermia, tachycardia, kussmaul respiration, and a prominent "sweaty feet odor", filled the examination room. Laboratory evaluation exhibited a very high anion gap level,and liver function test were within normal limits. Despite a normal glucose value, the tests for urine ketones were positive. Acylcarnitine analysis from genetic metabolic laboratory Amsterdam Medisch Centrum, suggested C5-carnitine was extremely high, together with a decreased level of free carnitine. Intravenous fluid therapy of 10% dextrose in normal saline, carnitine supplementation, and restricted protein diet especially leucine were given.
Conclusion: IVA due to isovaleryl-coenzyme A dehydrogenase deficiency could be managed optimally. Early detection became a necessity for the management.

101-P

NEUROLOGICAL DETERIORATION IN TWO PATIENTS WITH METHYLMALONIC ACIDURIA FOLLOWING LIVER TRANSPLANTATION AND SUBSEQUENT RELAXATION OF NATURAL PROTEIN INTAKE

Yoshino M[1], Oohira T[1], Watanabe Y[1], Okada J[1], ohya T[1], Matsuishi T[1]
[1]Dpt Pediatr, Kurume Univ Sch Med, Kurume, Japan

Background: Liver transplantation (LT) is a therapeutic option for methylmalonic aciduria (MMA) patients who poorly respond to conservative treatments. It is known that methylmalonyl-CoA mutase is expressed in neuronal cells.
Objective: To report outcome of relaxation of natural protein intake after LT in two patients with methylmalonyl-CoA mutase deficiency.
Case Reports: Patient 1 had grown on a dietary regimen that provided 0.5–0.7 g/kg/day of natural protein. She underwent LT at the age of 7 years and 3 months of age, after which natural protein intake was relaxed to 1.2 g/kg/day. Ninety days after LT, she began to develop episodes of quick torsional movements of the head and developed an episode of tonic seizure at 9 years and 10 months of age.
Patient 2. This boy had been fed 0.4–0.7 g/kg/day of natural protein until the age of 5 years and 2 months, when he underwent LT. Natural protein intake was then increased to 1.2 g/kg/day. At 7 years and 2 months of age, he exhibited weakness of the right extremities and flexion of the right upper extremity, which responded to trihexylphenidyl HCl .
Discussion: LT may alleviate distorted methylmalonate metabolism in the extra neuronal tissues, but not in the neuronal cells, in MMA patients. Relaxed intake of precursor amino acids subsequent to LT may cause buildup of methylmalonate and its precursors in neuronal cells to induce neuronal damage.
Conclusion: In MMA patients, relaxation of protein intake may aggravate neurological symptoms even if they undergo liver transplantation.

102-P

CLINICAL AND BIOCHEMICAL HETEROGENEITY ASSOCIATED WITH FUMARASE DEFICIENCY

Ottolenghi C[1], Hubert L[2], Allanore Y[2], Brassier A[2], Boddaert N[3], Valayannopoulos V[2], Slama A[4], de Keyzer Y[2], Toulhoat H[5], de Lonlay P[2]
[1]Metab Bioch, Necker H, AP-HP,Descartes U, Paris, France
[2]INSERM U781, Div Pediatr, Necker Hosp, Paris, France
[3]Pediat Radiol, Necker H, AP-HP, Paris, France
[4]Div Biochem, Bicêtre Hosp, AP-HP, Le Kremlin-Bicêtre, France
[5]Scient Dir, IFP, French Inst Petroleum, Rueil-Malmaison, France

Background: Fumarase deficiency is a rare metabolic disorder of the citric acid cycle that causes severe neurological impairment. Grossly increased levels of fumaric acid are a hallmark of fumarase deficiency. To date, about 20 unrelated cases of fumarase deficiency have been described. In this case study, we report two additional unrelated patients with dramatically different clinical presentation.
Methods: We compared organic acid profiles to previously published reports and to a large unselected cohort of patients investigated for known or suspected metabolic diseases. Enzymatic activity was measured in lymphocytes, skin fibroblasts, and muscle. Hypoxia inducible factor (HIF-alpha) activation was investigated in fibroblasts. In silico predictions of protein structural changes were compared to previously reported mutations.
Results: The two patients harbor novel missense mutations in the fumarase gene. One patient had severe neonatal encephalopathy and undetectable enzymatic activity, but only moderately increased levels of urinary fumaric acid. The second patient had moderate mental retardation and between 30 and 60% enzymatic activity in three cell types tested; however, urine contained massive amounts of fumaric acid. We were unable to detect alterations of the HIF-alpha pathway, involved in fumarase-related tumorigenesis. This does not rule out possible tissue-specific effects. Minor structural changes associated with either mutation were predicted for the encoded protein.
Conclusions: These results extend the range of clinical and biochemical variation associated with fumarase deficiency. They provide support for previous suggestions that patients showing moderate increases in fumarate excretion should be investigated for fumarase deficiency.

103-P

GLYCEROLURIA: CLUE TO POINT TO CHROMOSOMAL REGION OF GENETICAL DEFECT FOR PATIENTS WITH DIFFERENT CLINICAL PRESENTATION

Daneberga Z[1], Micule I[1], Locmele Dz[1], Krumina Z[1], Grinfelde I[1], Osipova O[1], Vevere P[1], Pronina N[1], Lugovska R[1]
[1]Medical Genetic Clinic, Univ Child Hosp, Riga, Latvia

Background: Glyceroluria is known as a feature of glycerol kinase deficiency (GKD), usually discovered by evaluation of organic acid profile by gas chromatography/mass spectrometry (GC/MS). The severity of the disease is highly variable. The severe infantile form results from a contiguous gene deletion syndrome involving loss of GK gene, DMD gene and AHC gene. The isolated GKD can lead to symptomatic juvenile form or benign adult form. In most cases of glyceroluria extensive deletion of Xp21.2–21.3 can be found.
Patients and methods: We present two patients with glyceroluria and different clinical symptoms.
Patient 1: 2 month old boy with feeding difficulties, dehydration, diffuse skin hyperpigmentation, impaired thermoregulation, hypotonia, primary adrenal insufficiency. Laboratory findings: karyotype 46,XY; organic acids in urine—massive glyceroluria; DNA analysis (multiplex PCR)—exon 60 deletion in DMD gene (analysed exons 1–60).
Patient 2: 10 years old boy with feeding difficulties in infancy, motor development delay—head control at 1,5 years, rolling over at 3 years, sitting at 6 years, walk with assistance at 8 years; severe mental retardation; almost no speech and seizures in anamnesis. Laboratory findings: karyotype 46, XY; organic acids in urine—massive glyceroluria; DNA analysis (multiplex PCR)—no deletion in DMD gene (analysed exons 1–60); without changes in SNRPN gene methylation status.
Discussion: We consider, that glyceroluria can not completely explain clinical presentation of patients, but can give a clue for chromosomal region of genetical defect. It can be helpful if availability of enzymatic testing and DNA diagnostic are limited.

104-P

INHIBITION OF Na+,K+-ATPase ACTIVITY IN SYNAPTOSOMES FROM CEREBRAL CORTEX OF YOUNG RATS BY 3-METHYLGLUTARIC ACID

Ribeiro CAJ[1], Hickmann FH[1], Vargas CR[2], Wyse ATS[1], Wajner M[3]
[1]*Departamento de Bioquímica, ICBS, UFRGS, Porto Alegre RS, Brazil*
[2]*Departamento de Análises Clínicas, UFRGS, Porto Alegre RS, Brazil*
[3]*Servico de Genética Médica, HCPA, Porto Alegre, RS, Brazil*

Background: 3-Methylglutaric acid (MGA) accumulates in 3-hydroxy-3-methylglutaric aciduria (3HMGA) and in a group of diseases known as 3-methylglutaconic aciduria (MGTA). Although neurological symptoms are common in these disorders, the underlying mechanisms of brain damage presented by the affected patients are poorly known.
Objective and Methods: The aim of the present study was to investigate the in vitro effect MGA on viability, Na+,K±ATPase activity and oxidative stress in synaptosomes isolated from cerebral cortex of young rats.
Results: MGA significantly reduced synaptosomal viability (25%) and inhibited the activity of Na+,K±ATPase (30 %). MGA also increased 2′,7′-dichlorofluorescein (DCFH) oxidation (30%) that reflects oxidative species production. Moreover, the inhibitory effected elicited by MGA on Na+, K±ATPase activity was totally prevented by co-incubation with the scavenging antioxidants creatine and melatonin, implying a role for reactive species in this effect.
Conclusions: Taken together, the present data indicate that MGA compromises the activity of an important enzyme for the maintenance of the membrane potential necessary for neurotransmission and, besides, elicits reactive species production. It is therefore presumed that these deleterious effects may be involved in the pathophysiology of brain damage observed in patients affected by disorders in which MGA accumulates.
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105-P

TWO INBORN ERRORS OF METABOLISM IN A NEWBORN: GLUTARIC ACIDURIA TYPE I COMBINED WITH ISOBUTYRYLGLYCINURIA

Popek M[1], Walter M[1], Fernando M[1], Lindner M[2], Schwab KO[3], Sass JO[1]
[1]*Lab Clin Biochem Metab, Univ Child Hosp, Freiburg, Germany*
[2]*Univ Child Hosp, Heidelberg, Germany*
[3]*Univ Child Hosp, Freiburg, Germany*

Background: Glutaric aciduria type 1 (GA1) is an inborn error in the metabolism of the amino acids tryptophan, lysine and hydroxylysine due to mutations in the GCDH gene coding for glutaryl-coenzyme A dehydrogenase. Isobutyryl-coenzyme A dehydrogenase (IBD) is an enzyme encoded by the ACAD8 gene and involved in the catabolism of the branched-chain amino acid valine. Both GA1 and IBD deficiency can be detected by expanded newborn screening using tandem-mass spectrometry, if they are considered screening targets.
Methods: Tandem-mass spectrometry and gas-chromatography were used for the assessment of key metabolites in body fluids of a patient with abnormal findings in newborn screening. Mutations were investigated by direct sequencing and by restriction fragment lengths analysis. Valine metabolism was studied in vitro in immortalized lymphocytes.
Results: Following accumulation of acylcarnitines C5DC and C4, of 3-hydroxyglutaric acid and isobutyrylglycine in body fluids, sequence analysis in the GCDH gene revealed homozygosity for a missense mutation, which has been reported in GA1 before. In the ACAD8 gene a novel mutation c. 841+3 G>C was identified, which results in loss of exon 7 and predicts a premature stop of translation. Impaired valine degradation was corroborated by the increased post-load level of acylcarnitine C4 in lymphocytes.
Conclusions: The molecular basis of two inborn errors of metabolism in a newborn was elucidated. The results of the metabolite studies underline the use of urinary C4 acylcarnitine as a sensitive marker of IBD deficiency. A functional test of IBD activity in lymphocytes may replace more invasive investigations with fibroblasts.

106-P

TWO DIFFERENT CLINICAL PHENOTYPE IN TWO SIBLINGS WITH 3-METHYLGLUTACONIC ACIDURIA TYPE I

Mercimek-Mahmutoglu S[1], Bhanji N[2], Waters PJ[3], Stockler-Ipsiroglu S[1]
[1]*Div Biochemical Dis, Dep Ped, Univ BC, Vancouver, Canada*
[2]*Dep of Ped, Univ BC, Vancouver, Canada*
[3]*Dep Path, Univ BC, Vancouver, Canada*

Background: 3-methylglutaconic aciduria type I (3-MGA-I) is an autosomal recessive organic aciduria in leucine degradation due to 3-methylglutaconyl-CoA hydratase (3-MG-CoA-H) enzyme deficiency. Less than 20 patients have been described. The clinical phenotype ranges from normal development to severe global developmental delay with progressive neurologic symptoms.
Case presentations and results: 12-year-old female was identified with markedly elevated 3-hydroxyisovalerate, 3-methylglutarate and 3-methylglutaconate in urine organic acid analysis by a selective screening of learning difficulties. Her acylcarnitine profile revealed markedly elevated 3-hydroxyisovalerylcarnitine. Her general clinical and neurological examinations were unremarkable. She had gross motor and fine motor delay as well as cognitive dysfunction. Her cranial MRI showed leukodystrophy in subcortical bilateral frontal and parietal lobes. Her cranial MR-spectroscopy showed elevated 3-hydroxyisovalerate. Her parents were first cousins of Pakistani ethnic background. Her family history was remarkable for an 8-year-old brother with severe expressive language delay. His general physical and neurological examinations were normal as well as his cognitive function. His urine organic acid analysis and acylcarnitine profile were similar to his older sister. 3-MG-CoA-H enzyme activity in cultured skin fibroblasts was not detectable confirming the diagnosis of 3-MGA-I in both of siblings. Both were started on l-carnitine supplementation therapy and sick day management protocol with increase calorie intake. There was no history of metabolic decompensation or encephalopathy in their past medical history.
Conclusion: We describe two siblings with 3-MGA-I with 2 different clinical phenotype. Despite high consanguinity, heterogeneous phenotype could indicate the contribution of other factors, such as alternative genomic loci containing modifier genes.

107-P

ACYLCARNITINE ANALYSIS IN SERUM AND URINE ON SEVERELY EPILEPTIC CHILDREN LONG-TERM TAKING VALPROIC ACID

Maeda Y[1], Ito T[2], Nakajima Y[2], Ichiki S[2], Kurono Y[1], Sugiyama N[3], Togari H[2]
[1]*Dept Hosp Pharm, Nagoya City Univ, Nagoya, Japan*
[2]*Dept Pediatr, Nagoya City Univ, Nagoya, Japan*
[3]*Dept Pediatr, Aichi-Gakuin Univ, Nagoya, Japan*

Background: A long-term administration of valproic acid would be high-risk of carnitine deficiency. We developed determination method of individual acylcarnitine isomers by HPLC-MS/MS. Valproylcarnitine concentration in blood and its excretion rate into urine on severely epileptic children long-term taking valproic acid were investigated to recognize the relation between carnitine deficiency and valproylcarnitine excretion.
Materials: Serum and urine were obtained from 14 severely epileptic children long-term taking valproic acid. The average age of these patients was 8.1.
Results: The concentration of free carnitine in blood was less than 30 μmol/L in 9 of 14 samples. Those of 3 patients were even less than 20 μmol/L. These values were lower than normal level. However, the concentration of valproylcarnitine was only 0.012 μmol/L. The ratio of the valproylcarnitine to total acylcarnitines is only 0.12%. The concentration of octanoylcarnitine which is isomer of valproylcarnitine is 0.08 μmol/L. Meanwhile, the concentration of valproylcarnitine in urine was 4.6 μmol/g7cre and that of octanoylcarnitine was 0.19 μmol/g7cre. Valproylcarnitine was excreted into urine more than octanoylcarnitine. The amount of valproylcarnitine excretion into urine was 4.5% of the whole amount of acylcarnitine excretion.
Discussion: Although the clear accumulation of valproylcarnitine in blood was not recognized, free carnitine concentration in blood seemed to become the low value in mentally and physically handicapped child. However, because excretion amount of valproylcarnitine into urine increased than that of octanoylcarnitine and about 4.5% of the total acylcarnitine excretion were valproylcarnitine, the carnitine deficiency would be due to the excretion into urine for the long-term.

108-P

N-CARBAMYLGLUTAMATE TREATMENT FOR ACUTE NEONATAL HYPERAMMONAEMIA IN ISOVALERIC ACIDAEMIA

Kasapkara CS[1], Ezgu FS[1], Tumer L[1], Biberoglu G[1], Okur I[1], Hasanoglu A[1]
[1]*Div Ped Metab Dis, Gazi University Hosp, Ankara, Turkey*

Hyperammonaemia occurs mainly in patients with branched chain organic acidaemias. Its pathophysiological process is mainly via the competitive inhibition of N-acetylglutamate synthetase. Oral Carglumic acid (N-Carbamylglutamate) administration has been demonstrated to correct hyperammonemia in neonates with propionic and methylmalonic acidemias and could avoid dialysis therapy. Isovaleric acidaemia is an autosomal recessive disease of leucine metabolism due to deficiency of isovaleryl-Coa dehydrogenase. For the first time, we report a neonate with isovaleric acidaemia, whose plasma ammonia concentration dropped dramatically after one oral load of carglumic acid.
Case Report: A full term male child of consanguineous parents manifested lethargy, refusal to feed, vomiting, jitterness, sweaty feet odor and dehydration at the third day of life. His chemical profile showed a plasma ammonium concentration of 568 ug/dl (Normal:31–123 ug/dl), urine gas chromatography revealed isovalerylglycine and blood acylcarnitines demonstrated an elevation of isovalerylcarnitine suggesting that the patient had isovaleric acidaemia. The baby was treated with intravenous infusions of glucose, lipids, carnitine (100 mg/kg per day). Single dose of Carglumic acid (150 mg/kg per day) was adminestered through a nasogastric tube and well tolerated. Over the following six hours, the plasma ammonium level dropped dramatically to 72 ug/dl and blood ammonia remained normal thereafter. At the age of one month he has been neurologically normal, receiving protein restricted diet, glycine and L- carnitine with good metabolic control. This experience suggests that carglumic acid could be considered for acute hyperammonemia resulting isovaleric acidemia. However further trials with more patients would certainly be needed.

109-P

CHARACTERIZATION OF MUTATIONS UNDERLYING AMINOACYLASE 1 DEFICIENCY

Sommer A[1], Sass JO[1]
[1]*Lab Clin Biochem Metab, Univ Child Hosp, Freiburg, Germany*

Aminoacylase 1 (ACY1) is a zinc binding enzyme which hydrolyzes N-acetyl amino acids into the free amino acids and acetic acid. Deficiency of ACY1 due to mutations in the ACY1 gene follows an autosomal recessive trait of inheritance and is characterized by accumulation of N-acetyl amino acids in the urine. In affected individuals neurological finding such as febrile seizures, delay of psychomotor development or moderate mental retardation have been reported. Except for one missense mutation which has been studied in E. coli, mutations underlying ACY1 deficiency have not been characterized so far. This has prompted us to approach expression studies of all mutations known to occur in ACY1 deficient individuals in a human cell line (HEK293), thus providing the authentic machinery for posttranslational modifications. Mutations were inserted using site directed mutagenesis. ACY1 enzyme activity was assessed in cells overexpressing ACY1, using N-acetyl methionine as a naturally occurring high-affinity substrate. Overexpression of the wild type enzyme in HEK293 cells resulted in an approximately 20-fold increase of the ACY1 activity of homogenized cells. Most mutations resulted in a nearly complete loss of enzyme function. Notably, the mutation R393H resulted in considerable residual activity of the enzyme, which is tentatively explained by its localization and molecular characteristics. Investigations of the molecular bases of additional cases of ACY1 deficiency should result in a better understanding of this inborn error of metabolism whose clinical significance and long-term consequences remain to be elucidated.

110-P

DOES A PRIMARY DEFECT IN 3-HYDROXYISOBUTYRATE DEHYDROGENASE EXIST? TWO CASES OF 3-HYDROXYIOSBUTYRIC ACIDURIA

Barski R.R.[1], Henderson M.J.[1], Olpin S.E.[2]
[1]*Biochem Genetics, St James's Univ Hosp, Leeds, United Kingdom*
[2]*Dept Clin Chem, Sheff Child Hosp, Sheffield, United Kingdom*

The presence of 3-hydroxyisobutyric aciduria is suggestive of a disorder in valine catabolism with a defect in methylmalonic semialdehyde dehydrogenase or 3-hydroxyisobutyrate dehydrogenase most likely. Cases of the former have tended to present with an increased urinary excretion of 3-hydroxyisobutyrate as well as increased levels of beta-aminoisobutyric acid, alpha amino adipic acid and β-alanine. Cases due 3-hydroxyisobutyrate dehydrogenase deficiency have presented without these amino acid abnormalities due to the proposed site of the metabolic block. Controversy remains, however, over the exact nature of the enzyme lesion in this latter disorder due to the inability to demonstrate a primary enzyme deficiency. Here we present two further cases of 3-hydroxyisobutyric aciduria. Case 1 presented aged four years with coarse facial features and chronic ataxia. Marked excretion of 3-hydroxyisobutyrate and increased excretion of 2-ethyl-3-hydroxypropionate have been observed repeatedly. β-alanine and alpha amino adipic acid were not elevated suggesting that 3-hydroxyiosbutyrate dehydrogenase deficiency is the more likely cause. Fibroblast studies measuring release of 14CO2 from [1–14C]β-alanine and [1–14C] isobutyric acid were normal therefore failing to elucidate the site of the metabolic block and suggesting that there is no primary defect in valine catabolism in our patient. These results provide further evidence that 3-hydroxyisobutyrate dehydrogenase deficiency is either not expressed in fibroblasts or may be a secondary consequence of a defect with an as yet unknown origin. Case 2 is the younger sister of Case 1. She has demonstrated a similar biochemical profile but has not yet developed the clinical problems associated with her brother.

111-O

NEWBORN SCREENING (NBS) FOR DISORDERS OF PROPIONATE, METHIONINE AND COBALAMIN METABOLISM USING SECOND TIER TESTING

Gavrilov D[1], Tortorelli S[1], Turgeon C[1], Oglesbee D[1], Raymond K[1], Rinaldo P[1], Matern D[1]
[1]*BGL, Mayo Clinic, Rochester, MN, United States*

Background: There are more than 10 disorders of propionate, methionine and cobalamin metabolism as well as nutritional deficiencies that can present with abnormal concentrations of methionine (Met) and/or propionylcarnitine (C3-AC). However, the specificity of these markers is low. To avoid a high false positive rate, we implemented a 2nd tier NBS test for methylcitric acid (MCA), methylmalonic acid (MMA) and total homocysteine (tHCY).
Objectives: To review the impact of the 2nd tier method on disease detection, false positive rate and positive predictive value of NBS for disorders of propionate, methionine and cobalamin metabolism.
Results: Cut off values for Met, C3-AC, tHCY, MMA and MCA were set based on the disease ranges as determined by the Region 4 Genetics Collaborative MS/MS data project (http://region4genetics.org/msms_data_project/). Prospectively applied, 2.3% of all NBS samples required 2nd tier analysis. The highest sensitivity and specificity were achieved when algorithms were developed and applied for samples with suspicious primary screening results. Since 2005, we prospectively identified 2 infants with β-cystathionine synthase deficiency, 1 with MTHFR deficiency, 1 with Cbl G deficiency, 2 with methylmalonyl-CoA mutase deficiency, 7 with Cbl C deficiency, and 8 vitamin B12 deficient mothers.
Conclusions: Implementation of a 2nd tier tHCY, MMA and MCA determination in DBS for samples with abnormal Met and/or C3-AC primary NBS results significantly improves the false positive rate and positive predictive value of NBS (0.009% and 44% respectively in our laboratory). With appropriate algorithms in place, such 2nd tier testing could minimize the associated costs for collaborating NBS programs.

112-P

MOLECULAR ANALYSIS OF TURKISH PATIENTS WITH METHYLMALONIC ACIDURIA

Liu MY[1], Tanyalcin T[2], Chang YC[3], Fan YL[4], Chiang SH[5], Hsiao KJ[5], Liu TT[3]
[1]*Inst of Genet, Natl Yang-Ming Univ, Taipei, Taiwan*
[2]*Tanyalcin Med Lab, Select Screen Metab U, Izmir, Turkey*
[3]*Genome Res Center, Natl Yang-Ming Univ, Taipei, Taiwan*
[4]*Dep Edu and Res, Taipei City Hosp, Taipei, Taiwan*
[5]*Dep Med Res and Edu, TPE Veter Gen Hosp, Taipei, Taiwan*

Background: Methylmalonic aciduria (MMA) is caused by defects in methylmalonyl CoA mutase (MCM, E.C. 5.4.99.2, MIM 251000, MCM, E.C.5.4.99.2; gene symbol: MUT) apoenzyme or other defects in the biosynthesis of adenosylcobalamin, leading to impairment of the MCM activity and accumulation of methylmalonic acid in body fluids.
Objectives: The aim is to uncover the molecular defects in Turkish patients clinically diagnosed by elevation of urinary methylmalonic acid.
Methods: Six patients diagnosed by urinary organic acid analysis with elevated methylmalonic acid were subjected to mutation analysis of the MUT gene. Four of the six patients were from consanguineous families. The entire coding region, 5′ and 3′ untranslated region sequences of the MUT gene were analyzed by PCR-based sequencing.
Results: A total of five mutations in the MUT gene, namely c.277C>T (p.R93C), c.360dupT, c.2020C>T (p.L674F), c.1561–1G>A and c.[1912 T>A+1918G>A] (p.[F638I+D640Y]), were identified. Of which, the c.277C>T, c.2020C>T and c.1561–1G>A, were newly identified. The allelic frequencies of the nucleotide variations among normal Turkish controls, linkage analysis in the families, recurrence of the mutations and/or conservation of amino acid affected were studies in order to verify the disease-causing alterations. None of these mutations were identified in 88 normal chromosomes. These data indicated that these alterations identified in Turkish patients might be disease-causing mutations in mut type MMA and thus confirmed as mut type MMA.
Conclusion: The identification of the disease-causing mutations in these Turkish MMA patients could be an aid to genetic counseling, carrier detection and prenatal diagnosis.

113-P

MUTATION PROFILES IN THE MUT GENE OF CHINESE METHYLMALONIC ACIDURIA PATIENTS

Liu TT[1], Liu MY[2], Yang YL[3], Chang YC[1], Fan YL[4], Chiang SH[5], Niu DM[6], Lin SP[7], Han LS[8], Qi Y[9], Hsiao KJ[5]
[1]*Genome Res Center, Natl Yang-Ming Univ, Taipei, Taiwan*
[2]*Inst of Genetics, Natl Yang-Ming Univ, Taipei, Taiwan*
[3]*Dept Pediatr, Peking Univ, First Hosp, Beijing, China*
[4]*Dept Edu and Res, Taipei City Hosp, Taipei, Taiwan*
[5]*Dept Med Res and Edu, TPE Veter Gen Hosp, Taipei, Taiwan*
[6]*Dept Pediatr, TPE Veter Gen Hosp, Taipei, Taiwan*
[7]*Dept Pediatr, Mackay Memorial Hosp, Taipei, Taiwan*
[8]*Dept Pediatr, Xinhua Hosp, Shanghai, China*
[9]*Central Lab, Peking Univ, First Hosp, Beijing, China*

Background: The mut type methylmalonic aciduria (MMA, MIM 251000) is caused by a deficiency of mitochondrial methylmalonyl CoA mutase (MCM, E.C. 5.4.99.2) activity, which results from defects in the MUT gene. The aim of this study is to elucidate the mutation spectrum of the MUT gene in Chinese patients diagnosed by elevated urinary methylmalonic acid.
Methods: Thirteen exons of the MUT gene, including the untranslated regions, were analyzed by PCR-based sequencing for 45 unrelated Chinese MMA patients. Disease-causing mutations were confirmed by studying the prevalence of these mutations among 100 normal Chinese alleles, linkage analysis in the families, recurrence of the mutations and/or the conservation of amino acid affected.
Results: A total of 41 mutations were identified. Of which, 19 were novel ones, including one nonsense mutation (c.103C>T), 11 missense mutations (c.316A>C, c.424A>G, c.494A>G, c.554C>T, c.599 T>C, c.919 T>C, c.1009 T>C, c.1061C>T, c.1141G>A, c.1267G>A and c.1295A>C), one duplication (c.755dupA), four deletions (c.398_399delGA, c.1046_1058del, c.1359delT and c.1835delG) and two mutations might affect mRNA splicing (c.754–1G>A and c.1084–10A>G). None of the 11 missense mutations were identified in 100 normal alleles. Among these mutations identified, the c.1280G>A, c.729_730insTT, c.1106G>A, c.1630_1631GG>TA and c.2080C>T accounted for 41% of the diseased alleles. The c.1280G>A and c.729_730insTT mutations were found to be the most frequent mutations in southern and northern Chinese, respectively.
Conclusion: The mutation analysis for gene responsible for mut type MMA may provide a molecular diagnostic aid for differential diagnosis of MMA and could be applied in carrier detection and prenatal diagnosis for the mut type MMA families.

114-P

THE REDUCTION OF PROPIONYLCARNITINE CUT OFF LEVELS ASSOCIATED TO A SECOND-TIER TEST FOR METHYLMALONIC ACID ALLOWS TO DECREASE FALSE NEGATIVES IN EXPANDED NEWBORN SCREENING

Malvagia S[1], Funghini S[1], Pasquini E[1], Cavicchi C[1], Morrone A[2], Zammarchi E[2], Donati MA[1], la Marca G[3]
[1]*Metab and Musc Unit, Meyer Hospital, Florence, Italy*
[2]*Dep Woman and Child Health Florence Univ, Florence, Italy*
[3]*Dept Pharmacology, Florence Univ, Florence, Italy*

Background: The expansion of diseases included in the newborn screening panel has led to an increase of false positives with associated parental stress. A high cutoff value reduces false positives but leads to an increase of false negatives.
Objectives: Introduction of a second-tier test, consisting of a more highly specific method. Second-tier test minimizes false-positive rate but not avoids the risk of false negatives because it is applied only for initial out-of-range screening results. -Reduction of cutoff value to decrease false negatives. Some methylmalonic and cobalamin disorders may be missed in the newborn screening for the initial low levels of propionylcarnitine (C3).
Methods: We previously proposed a second-tier test for methylmalonic and propionic acidurias. This method is able to reveal 3-OH-propionate and methylmalonate (MMA) on dried blood spots. Being the method robust, sensitive and rapid, and since it requires no sample preparation, we established a low cutoff for C3 (3.3 μmol/L) with the aim to identify those affected babies with low C3 levels at the newborn screening.
Results: We report an infant who showed a C3 value of 3.81 μmol/L, normal C3/C2 ratio and methionine of 12.2 μmol/L (n.v. 7–46) on newborn screening. The second-tier test revealed moderately elevated blood MMA levels. At her admission to the hospital, high levels of homocysteine and methylmalonic acid in plasma and urine were detected. Biochemical and molecular analysis is still in progress.
Conclusion: The availability of second-tier test for MMA allows to set low C3 cutoff reducing recall rate and false negatives.

115-P

SSADH DEFICIENCY: A NEW MUTATION ASSOCIATED TO A MILD PHENOTYPE IN AN ITALIAN GIRL

Casarano M[1], Alessandrì MG[1], Salomons G[2], Jakobs C[2], Tosetti M[1], Cioni G[3], Battini R[1]
[1]*Dpt Dev Neurosc, IRCCS Stella Maris, Pisa, Italy*
[2]*Metab Unit Univ Med Center, Amsterdam, Netherlands*
[3]*Div Child Neurol Psych Univ Pisa, Pisa, Italy*

Succinic semialdehyde dehydrogenase (SSADH) deficiency is a rare autosomal recessive disorder of the GABA pathway. Due to the heterogeneity of neurological clinical symptoms, including mental retardation, speech delay, seizures, ataxia, movement disorders, it is often undiagnosed unless organic acids analysis is performed. Vigabatrin may be helpful to reduce the amount of toxic metabolite (4-hydroxybutiric acid, GHB).
We describe a 6 years old girl affected by SSADH deficiency from an Italian family in which we found a novel mutation in ALDH5A1 gene. The child presented only mild mental retardation with severe speech delay. Basal neurometabolic work-up, usually performed in our Department for patients with similar picture, showed an increase of GHB excretion (311 μmol/mmol creatine; controls < 5), while the other exams were normal.
The diagnosis of SSADH deficiency was suspected and MRI-1H-MRS showed signal abnormalities involving globus pallidus, subcortical white matter and cerebellar dentate nuclei. SSADH activity in patient's lymphocytes was strongly decreased (48 pmol/min/mg pro; controls 1907–3901 pmol/min/mgpro); two pathogenic mutations have been identified in the ALDH5A1 gene: c.278G>T in exon 1 and a new mutation in exon 10 (c.1557 T>G) and in the pedigree. Treatment with Vigabatrin at low doses (25 mg/Kg/die) significantly reduced GHB levels in biological fluids including CSF, with a concomitant increase of GABA, and a clinical improvement throughout the two years of follow-up was observed.
In conclusion we suggest that SSADH deficiency should be considered in the differential diagnosis of patients with mental retardation and language delay being likely a presentation of mild phenotype.

116-O

EVIDENCE FOR GENETIC HETEROGENEITY IN D-2-HYDROXYGLUTARIC ACIDURIA

Kranendijk M[1], Struys EA[1], Salomons GS[1], Jakobs C[1]
[1]*Metabolic Unit, VU Univ Medical Center, Amsterdam, Netherlands*

Molecular, enzyme, and metabolic studies were performed in 50 patients with D-2-hydroxyglutaric aciduria (D-2-HGA)—a rare neurometabolic disorder—who accumulated D-2-hydroxyglutarate (D-2-HG) in physiological fluids. Presumed pathogenic mutations were detected in 24 of 50 patients in the D-2-hydroxyglutarate dehydrogenase (D2HGDH) gene, which encodes D-2-hydroxyglutarate dehydrogenase (D-2-HGDH). Enzyme assay of D-2-HGDH confirmed that all patients with mutations had impaired enzyme activity, whereas patients with D-2-HGA whose enzyme activity was normal did not have mutations. Significantly lower D-2-HG concentrations in body fluids were observed in mutation-positive D-2-HGA patients than in mutation-negative patients.
These results imply that multiple genetic loci may be associated with hyperexcretion of D-2-HG [1]. Accordingly, we have suggested a new classification: D-2-HGA Type I associates with D-2-HGDH deficiency, whereas idiopathic D-2-HGA manifests with normal D-2-HGDH activity and higher D-2-HG levels in body fluids compared with Type I patients. It remains possible that several causes for idiopathic D-2-HGA patients with diverse genetic loci will be revealed in future studies.
[1] Kranendijk M, Struys EA, et al. Evidence for genetic heterogeneity in D-2-hydroxyglutaric aciduria. Human Mutation 31, 279–283 (2010)

117-P

CIRRHOSIS ASSOCIATED WITH PROPIONATE METABOLISM

Dursun A[1], Dundar H[1], Ozgul RK[1], Talim B[2], Kale G[2], Demir H[3], Temizel SI[3], Tokatli A[1], Sivri HS[1], Coskun T[1]
[1]*Dept of Ped, Metab Uni, Hacettepe Univ, Ankara, Turkey*
[2]*Dept of Ped, Pathol Uni, Hacettepe Univ, Ankara, Turkey*
[3]*Dept of Ped, Gastro Uni, Hacettepe Univ, Ankara, Turkey*

Chronic liver disease is reported in many inborn errors of metabolism including galactosemia, tyrosinemia type I, and hereditary fructose intolerance and different organel dysfunctions. We report 4 patients with inborn errors of propionate metabolism associated with cirrhosis. Two of the patients had methylmalonic acidemia (MMA) due to Muto defect and the other two had propionic acidemia (PA) due to PCC deficiency. Two unrelated patients with MMA were diagnosed in the newborn period and the diagnosis was confirmed by enzyme analysis. Mutation analysis including all exon sequencing of MUT gene revealed that one patient was homozygous for c.1844 C>T (P615L) mutation and the other patient was heterozygous for c.983 C>T (L328F) mutation, which have been reported previously. Liver biopsies performed at 9 months and 2.5 years of age for hepatomegaly were compatible with micronodular cirrhosis. The patients with PA were siblings and diagnosed in the newborn period with urine organic acid and enzyme analysis. Mutation analysis of one sibling showed that the patient was homozygous for c.657insA (235fsxstop) mutation. While postmortem investigation of liver tissue of one of the siblings performed at 9 months of age revelaed micronodular cirhosis, liver biopsy of the other sibling at the age of 3 showed chronic hepatitis (HAI 5, stage 2). To our knowledge, there are no reports in the literature about propionate metabolism associated with cirrhosis. We want to stress that the liver should be followed up cautiously in inborn errors of propionate metabolism.
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118-P

GLUTARIC ACIDURIA TYPE I ASSOCIATED WITH HEMIHYPERTROPHY IN AN INFANT

Soyucen E[1], Cetin K[2], Aktuglu Zeybek C[3], Ozudogru SN[2], Cansever S[1], Aydin A[4]
[1]*Dep Ped Metab Dis, Cerrahpasa Med Fac, Istanbul, Turkey*
[2]*Cerrahpasa Med Fac, Istanbul, Turkey*
[3]*Dep Ped Metab Dis, Bilim Univ, Istanbul, Turkey*
[4]*Dep Ped Metab Dis, Cerrahpasa Med Fac, Istanbul, Turkey*

Glutaric aciduria type I (GA-I) is a cerebral organic acid disorder caused by an inherited glutaryl-CoA dehydrogenase deficiency. The accumulation of glutaric acid causes neurotoxicity in both the basal ganglia and fronto-temporal cortex, which can lead to progressive dystonia, hypotonia, permanently impaired speech and seizures. Approximately 75% of the patients with macrocephaly present during infancy. Here, we describe a 3-year-old boy who presented with macrocephaly, speaking and walking delays and significant hemihypertrophy of his right upper and lower extremities. The patient was born preterm (at 36 week of gestation) with a weight of 1450 g (<3 rd percentile) and a length of 40 cm (<3 rd percentile) and had a 34.5 cm (75–90th percentile) head circumference. An MRI and urinary organic acid chromatography confirmed glutaric aciduria type I at 7 months of age. A specialized low-protein diet with L-carnitine was initiated. At 12 months of age, hemihypertrophy was diagnosed. An ultrasound scan of the abdomen ruled out any intra-abdominal pathology. Hemihypertrophy is defined as a unilateral overgrowth of part or all of one side of the body that can either be isolated or part of a well-defined syndrome such as Beckwith-Wiedemann syndrome, Proteus syndrome, Russell-Silver syndrome, Neurofibromatosis type I, or Klippel-Trénaunay syndrome. Our patient had no other syndromic component. This is the first report of hemihypertrophy in a patient with GA-I.

119-P

MUTATION ANALYSIS AND METABOLOMICS CHARACTERIZE A NEW VARIANT OF ISOVALERIC ACIDEMIA

Dercksen M.[1], Duran M.[2], Koekemoer G.[1], Mienie J.L.[1], Wanders R.J.A.[2], Waterham H.R.[2], Reinecke C.J.[1]
[1]*Lab Metab Def, Cent Hum Metab, NW Univ, Potchefstroom, South Africa*
[2]*AMC, Dep Ped & Clin Chem, UvA, Amsterdam, Netherlands*

Background: Isovaleric acidemia (IVA) due to an autosomal recessive deficiency of isovaleryl-CoA dehydrogenase is characterized by phenotypic, biochemical, and genetic variation.
Objectives: We characterized the genetic defect in several IVA South African patients diagnosed by us and determined their metabolomics fingerprint through an untargeted GC-MS analysis of their urinary organic acids.
Materials and Methods: Mutation analysis was performed by direct sequencing of PCR-amplified products with an Applied Biosystems automated DNA sequencer. Controls (19 infants and 32 adults) and three IVA groups were used for the metabolomics analyses (10 homozygotes before and after treatment and 12 obligate heterozygotes). Untargeted metabolomics profiles were generated by GC-MS and AMDIS analysis. The metabolomics matrix was developed through data pre-treatment, processing, and evaluated through univariate and multivariate analyses.
Results: (1) A homozygous mutation: 358 G>A (G120R) was identified in all IVA patients and confirmed in their heterozygous relatives. (2) Principal component analyses (PCA) showed a complete separation between all four groups, related to major and minor metabolites associated with the leucine degradation pathway as well as other biochemical pathways.
Conclusion: We identified a new 358 G>A (G120R) variant of IVA for which only one compound heterozygous 358 G>A (G120R) / 932 C>T (AV311V) case had been described before. The metabolomics analysis of IVA revealed a metabolic fingerprint of the leucine metabolites as well as the perturbation of other systems such as the citric acid cycle. It may assist in heterozygote detection and the assessment of the efficacy therapeutic intervention.

120-P

PROGRESSION AND TREATMENT OF RENAL DISEASE IN METHYLMALONIC ACIDEMIA

Valayannopoulos V[1], Cosson M.A[1], Benoist J.F[2], Touati G[1], Arnoux J.B.[1], Dechaux M[3], Boddaert N[4], Barbier V[1], Rabier D[5], Desguerre I[6], Niaudet P[3], de Lonlay P[1]
[1]*Ref Center IEM, Necker Enfants-Malades H, Paris, France*
[2]*Biochem Lab, Robert-Debre Hosp, paris, France*
[3]*Ped Nephrol, Necker Enfants-Malades Hosp, Paris, France*
[4]*Ped Radiol, Necker-Enfants Malades Hosp, Paris, France*
[5]*Biochem Lab, Necker Enfants Malades Hosp, Paris, France*
[6]*Ped Neurol, Necker-Enfants Malades Hosp, Paris, France*

Background: Thirty patients with vitamin-B12-unresponsive methyl malonic aciduria (MMA) were managed following standardized guidelines and studied retrospectively with a median follow-up of 8.3 years (y).
Methods: All patients were investigated with glomerular function rate studies (GFR), biochemical and genetic studies.
Results: Fifteen patients had a neonatal onset. Chronic renal disease (CRD) occurred in 14 patients (47%) with a median age of onset of 6.5 y. Renal function further deteriorated in 4 patients within a median period of 5.8 y. Of 25 patients, 17 were classified mut°, 3 mut- and 5 cblA. Mortality, number of acute decompensations, median MMA urinary excretion and neurological impairment were higher in mut° patients compared to mut-/cblA patients. Concerning the CRD, no difference incidence was found although the onset of CRD occurred earlier in mut° patients and was more severe. Four patients underwent successful renal transplantation leading to a correction of the renal function and/or improvement of their metabolic status. One patient died from hepatoblastoma 2 years after transplantation; all other patients are alive and well.
Conclusions: Patients with mut° phenotype have a more severe phenotype and probably an earlier and more severe CRD than patients with mut-/cblA phenotype. Renal transplantation is an interesting treatment option for MMA patients with CRD.

121-P

TREATMENT OF A METHYLMALONIC ACIDURIA MOUSE MODEL WITH INTRA-LIVER TRANSPLANTATION OF A DEVELOPED NOVEL LIVER PROGENITOR CELL LINE

Buck N[1], Ahmad Hafad F[1], Pitt J[2], Yeoh G[3], Peters H[1]
[1]*Murdoch Childrens research Institute/ Ro, Melbourne, Australia*
[2]*VCGS pathology, MCRI, Melbourne, Australia*
[3]*School of Biomed, Biomol and Chem scienc, Perth, Australia*

Background: Methylmalonic aciduria (MMA) is an organic acidaemia resulting from a functional defect in methylmalonyl-CoA mutase. We have developed a mouse model which recapitulates the key aspects of MMA: elevated methylmalonic acid levels in urine, blood and various organs. Mice are smaller and thus there is a phenotype and biochemical markers for determining disease correction.
In this study we investigate the use of intra-liver transplantation of a developed liver progenitor cell (LPC) line for the treatment of MMA using a mouse model with an intermediate phenotype.
Methods: Pups were intra-liver injected with 0.2 million cells at 1 and 4 days old. Several groups were set up: untransplanted, sham and F-EGFP cell lines (an immature LPC line established from congenic embryonic day 14 fetal liver cells). Each group contained at least 3 litters of pups. Mice were monitored for 16 weeks before analysis.
Results: Three of twenty three mice in the study had detectable cell engraftment and repopulation. Increased body weight and decreased blood C3 carnitine, plasma MMA and urine MMA levels were observed in a proportion of cell-transplanted MMA mice indicating potential disease correction by the transplanted cells.
Conclusions: Intra-liver transplantation provides a promising therapy as biochemical and phenotypic improvement were observed in MMA cell-transplanted mice. Although the percentage of EGFP DNA was low, this indicated that the cells were dividing and functional in the liver. Disease correction demonstrated in this study suggests a great opportunity in the clinical field and further investigations into this possibility are warranted.

122-P

BACTERIAL EXPRESSION AND ELUCIDATION OF THE CATALYTIC MECHANISM OF GLYCINE N-ACYLTRANSFERASE

Badenhorst CPS[1], Snyders M[1], van Dijk AA[1]
[1]North-West University, Potchefstroom, South Africa

Conjugation of glycine to organic acids by glycine N-acyltransferase (E.C. 2.1.3.13, GLYAT) is an important phase II detoxification mechanism. Several toxic organic acids, including metabolites of aspirin and industrial solvents, benzoic acid and several endogenous organic acids, are detoxified by conjugation to glycine. There is significant inter-individual variation in glycine conjugation capacity, although the underlying basis of this variation is not understood. We suspect, based on a group of isovaleric acidemia patients that respond poorly to glycine supplementation, that variation in glycine conjugation capacity may contribute to the variability in clinical outcome of this disease. One factor that may influence the glycine conjugation capacity of an individual is sequence variation of the enzyme itself. In order to investigate this possibility, we established a system for the bacterial expression of enzymatically active bovine and human GLYAT enzymes. The partially purified recombinant bovine GLYAT was characterised and compared to GLYAT isolated from bovine liver. The KM values of the recombinant and bovine liver GLYAT enzymes compare well for a range of substrates, including isovaleryl-coenzyme A. This suggests that the recombinant GLYAT enzyme can be used to investigate the effect of sequence variations on enzyme activity and substrate specificity. To demonstrate this, we identified a putative catalytic glutamate residue using bioinformatics. Using site-directed mutagenesis we demonstrated that E226 of bovine GLYAT is a catalytically important residue, acting as a general base catalyst to remove a proton from glycine. Mutation of this residue to glutamine resulted in loss of enzyme activity.

123-O

D-GLYCERIC ACIDURIA CAUSED BY GENETIC DEFICIENCY OF D-GLYCERATE KINASE

Sass JO[1], Fischer K[1], Wang R[2], Christensen E[3], Scholl-Burgi S[4], Chang R[2], Kapelari K[4], Walter M[1]
[1]Lab Clin Biochem Metab, Univ Child Hosp, Freiburg, Germany
[2]Div Metab Dis, CHOC, Orange, CA, United States
[3]Dept Clin Genet, Rigshospitalet, Copenhagen, Denmark
[4]Ped IV, Univ Child Hosp, Innsbruck, Austria

D-glyceric aciduria (DGA) is a disorder of serine and fructose metabolism that has been described in about 20 patients since its discovery in 1974. Affected individuals have presented with severe metabolic acidosis from birth, intermittent metabolic decompensations, or mainly neurological abnormalities. Some have been considered asymptomatic. The molecular basis of DGA is largely unknown; possible causes of DGA that have been discussed are deficiencies of D-glycerate dehydrogenase, triokinase, and D-glycerate kinase. In 1989, van Schaftingen reported decreased D-glycerate kinase activity in the liver of a single patient with DGA. However, his assay has not been performed in other DGA patients, and the underlying defect has remained unknown on the gene level until now. We report three patients with DGA of Serbian, Mexican, and Turkish origin, including the patient initially reported in 1974. All had homozygous mutations in exon 5 of the GLYCTK gene encoding D-glycerate kinase. Two were frameshift mutations (p.Phe483SerfsX1 and p.Leu520CysfsX108) resulting in premature stop of translation or a massively altered, overlong product of protein synthesis (probably prone to degradation), while the third patient had a homozygous missense mutation (p.Phe493Cys). The three parents whose DNA could be studied were heterozygous for their child's mutation. No mutation was found in 210 control chromosomes. Overexpression of the variant GLYCTK genes in HEK293 cells clearly showed loss of enzyme activity when compared to the wild type enzyme. Our work has revealed mutations in the GLYCTK gene as the cause of D-glycerate kinase deficiency and DGA.

124-P

PERSPECTIVES OF THE ORGANIC ACIDEMIAS IN DEVELOPING COUNTRIES USING THE COLOMBIAN EXPERIENCE

Barrera L.A[1], Echeverri OY.[1], Guevara J.M.[1], Espinosa E[2], Pulido N.F.[3]
[1]I.E.I.M. Universidad Javeriana, Bogota, Colombia
[2]Serv. Neuropediatria. Hosp. Militar, Bogota, Colombia
[3]Hospital San Ignacio, Bogota, Colombia

Organic Acidurias (OA) are rare diseases with a broad spectrum of onset ages and clinical manifestations which in general are bizarre, making its diagnosis a difficult task for clinicians. The biochemical diagnosis of these entities is done by demonstrating abnormal profiles of organic acids excretion in urine by gas chromatography/mass spectrometry (GC/MS), a method that was established in developed countries since 1980. Due to the small number of experienced clinical biochemists and clinicians in this field and also the short availability of GC/MS in our country and in general in Latin-America, few reports exist from developing countries like Colombia. This is a summary of the Colombian experience in the diagnosis of OA during the last 10 years at the Institute for the Study of Inborn Errors of Metabolism in Bogota, and is related to cases diagnosed, performance, difficulties and challenges as a snapshot of diagnosis of OA in the Latin-American context. The most frequent OA diagnosed were Isovaleric, methylmalonic and MSUD, additionally it have been observed very rare entities with few cases reported worldwide such as methylglutaconic, ethylmalonic and 3-hydroxy-3-methylglutaric acidurias; metylcrotonylglycinuria; and deficiency of Succinyl-CoA transferase and Acetoacetyl-CoA thiolase. The amount of remissions has increase about 100% and among high risk population remitted for analysis, near 15% of total samples analyzed showed an abnormal profile, but just 10% of them were confirmed as OA, showing that although the diagnosis of OA has improved, there is lot of work to be done to get adequate coverage and diagnoses

125-P

SEVERE NEONATAL PRESENTATION IN A cblD NEW PATIENT

Furlan F[1], Rigoldi M[1], Corbetta C[2], Codazzi D[3], Barbanti C[3], Merinero B[4], Perez B[4], Ugarte M[4], Parini R[1]
[1]Dep Ped, San Gerardo Hosp, Monza, Italy
[2]Lab Metab Dis, Ist Clin Perfezionamento, Milano, Italy
[3]Ped Intens Care Unit, Ospedali Riuniti, Bergamo, Italy
[4]CBMSO- Universidad Autonoma de Madrid, Madrid, Spain

Background: Cbl D is a rare disorder of intracellular cobalamin metabolism with three clinical and biochemical variants: combined Homocistinuria (Homo)-Methylmalonic aciduria (MMA), isolated Homo and isolated MMA. We describe an isolated MMA case with a severe neonatal onset.

Case Report: male, born at term from related parents after normal pregnancy and delivery, at birth meconium stained amniotic fluid, Apgar 7–10. At 4 days hypotonia, hyporeactivity, polypnea and pathological weight loss with inflammatory indexes increase and metabolic acidosis: antibiotics were started. At 6 days severe respiratory distress, myoclonic jerks and coma with severe hyperammonemia (1348 μmol/l), hyperlactacidemia (6 mMol/L) and metabolic acidosis (pH 7.29, BE-15), ketonuria (+++). Haemodiafiltration, arginine, sodium benzoate, carnitine, hydroxycobalamin and glucolipidic iv calories were started. Plasma ammonia decreased from 1.348 to 53 μmol/l in 24 h. In the meanwhile the diagnosis of isolated MMA was made (MMA 5875 mM/M urinary creatinine; normal plasma homocysteine). In cultured fibroblasts, propionate incorporation was low and normalized after hydroxycobalamin incubation. MMA CoA mutase activity, CBl A and B complementation analysis were normal, Cbl D molecular analysis showed a homozygous mutation (c.57–64del8) of the MMADHC gene. Now, he is 5 years old, has normal growth and development, MMA excretion is between 250 and 1100 mM/M urinary creatinine, with an intake of 14 grams of natural proteins.

Conclusion: This patient is the first case of isolated MMA Cbl D who had a very severe neonatal onset. Prompt B12 treatment in neonates with metabolic acidosis and a suspect of organic aciduria is advised.

126-O

INSIGHTS INTO THE PATHOPHYSIOLOGY OF METHYLMALONIC ACIDEMIA (MMA) FROM TISSUE-SPECIFIC TRANSGENIC MOUSE MODELS

Manoli I[1], Sysol JR[1], Chandler RJ[1], Sloan J[1], Cusmano-Ozog K[2], Zerfas P[3], Hoffmann V[3], Abu-Asab M[4], Tsokos M[4], Enns GM[2], Venditti CP[1]
[1]*Gen Mol Biol Branch, NHGRI, NIH, Bethesda, MD, United States*
[2]*Div Med Gen, Stanford Univ, Stanford, CA, United States*
[3]*Div Veterin Res, ORS, NIH, Bethesda, MD, United States*
[4]*Ultrastr Path Sec, CCR, NIH, Bethesda, MD, United States*

Background: To elucidate the consequences of organ-specific correction in MMA and probe disease pathophysiology, we created transgenic mice that expressed the Mut gene under the control of liver [Mut-/-;Tg(INS-Alb-Mut)] or muscle [Mut-/-;Tg(INS-MCK-Mut)] tissue-specific promoters. Phenotypic, metabolic and pathological findings were correlated with pathologic findings in tissues from MMA patients undergoing liver and kidney transplantation (LKT).
Results: [Mut-/-;Tg(INS-Alb-Mut)] and [Mut-/-;Tg(INS-MCK-Mut)] animals were born in Mendelian proportions, survived beyond weaning and exhibited tissue restricted Mut expression in the liver [Mut-/-;Tg(INS-Alb-Mut); 8.2±1%] or muscle [Mut-/-;Tg(INS-MCK-Mut); 103±2%] vs heterozygote controls. 1–13C-propionate conversion (%) into 13CO2, an in vivo measure of whole animal propionate oxidative capacity, for the various genotypes were: 54.7±9.2—[Mut-/-;Tg(INS-Alb-Mut)]; 18.4±3.6—[Mut-/-;Tg(INS-MCK-Mut)], 76.5±4.5—Mut+/- and 10±2—Mut-/-. Megamitochondria formation in the proximal tubular epithelial cells was seen in both models and a decreased glomerular filtration rate in the [Mut-/-;Tg(INS-Alb-Mut)] mice could be induced with a high-protein diet. The ultrastructural changes in the liver and kidneys of the transgenic mice resembled the findings in the native tissues obtained from MMA patients undergoing LKT (N=4).
Conclusions: Selective hepatic or muscle expression of the Mut enzyme by transgenesis resulted in rescue of the neonatal lethal phenotype displayed by the Mut-/- mice. Similar to the patients, the transgenic animals manifest megamitochondria formation associated with a bioenergetic defect in the hepatocytes and/or a proximal tubulopathy that evolves into chronic renal insufficiency. These novel mouse models provide a platform for testing of organ-targeted cell, gene and pharmacological therapies for MMA.

127-P

METHYLMALONYL CoA EPIMERASE DEFICIENCY PRESENTING WITH ACUTE METABOLIC ACIDOSIS

Chronopoulou E[1], Chapman S[1], Turner C[1], Waterham H[2], Champion MP[1]
[1]*Evelina Children's Hosp, St Thomas Hosp, London, United Kingdom*
[2]*Academic Medical Centre, Amsterdam, Netherlands*

Methylmalonic aciduria (MMA), despite being frequently classified as a "classical" organic aciduria, comprises a heterogeneous group of inborn errors in propionate catabolism. Enzyme/protein deficiencies along this pathway, which breaks down four amino acids, odd-chain fatty acids, thymine and cholesterol to fuel the Krebs cycle with succinyl-CoA, lead to a wide spectrum of clinical presentations characterised by accumulation of MMA in body fluids to variable extent.
We describe the case of a 15 month old boy presenting with hypoglycaemia (glucose 2.6 mmol/l) and profound metabolic acidosis (pH 6.94, pCO2 2.5, bicarbonate 4.1, BE -26.9) following a 3 day vomiting illness. Rapid acylcarnitine screen revealed raised propionyl and methylmalonyl carnitine suggesting a diagnosis of methylmalonic aciduria confirmed with plasma MMA 130mcmol/l. Subsequent and urine organic acids confirmed elevated MMA and methylcitrate. He made a full recovery with fluid resuscitation, protein restriction and carnitine supplementation.
Further investigations revealed that methylmalonyl-CoA mutase activity was normal despite reduced propionate uptake in cultured fibroblasts. Cobalamin metabolism disorders were excluded. Mutation analysis of the methylmalonyl-CoA epimerase gene (MCEE) showed a homozygous nonsense mutation (c.139C>T). Previously there has been debate as to the physiological role of this enzyme and whether deficiency is disease causing in man. Screening of cases of methylmalonic aciduria with moderate MMA excretion for this gene has been suggested. The child now aged four years continues to thrive with near normal cognition off diet and medication. This case, however, shows that patients can present with life-threatening decompensations and therefore should not be considered so benign.

128-P

CLINICAL IMPROVEMENT IN A PATIENT WITH METHYLMALONIC ACIDAEMIA WHEN SUPPLEMENTED WITH AMINO ACID MIXTURE: CASE REPORT

Rahman Y[1]
[1]*Dep Inh Metab Dis, GSTFT, London, United Kingdom*

Introduction: Methylmalonic Acidaemia (MMA) is an inherited metabolic disorder caused by a deficiency of methyl-malonyl-CoA mutase, a vitamin B12–dependent enzyme. Severity varies depending on the degree of enzyme deficiency. Some MMA patients are responsive to co-factor vitamin B12 while unresponsive patients need to limit natural protein intake. More severe variants are at risk of developing chronic renal failure. Dietary management varies with regards to using synthetic amino acids which are free from the precursor amino acids.
Case Report: A 19 year old female with B12-unresponsive MMA, complicated with a metabolic stroke in childhood and deteriorating renal function. Frequent hospital admissions due to nausea and retching.
The patient was gastrostomy fed, providing 32 g protein (0.7 g protein/kg/day). In an effort to slow down renal deterioration, by decreasing MMA production, the feeding regimen was altered. Natural protein intake was decreased and supplemented with an amino acid supplement free from methionine, threonine, valine and isoleucine (XMTVI Maxamum).This regimen provided 20 g natural protein and 20 g protein equivalent (total protein 0.89 g/kg/day, 50% from synthetic amino acids).
Since implementing this regimen, the patient has had 2 hospital admissions over a 12 month period and family commented that the new regimen had "changed their lives". Renal function has remained stable and the MMA level has dropped from 1,942,030 nmol/L to 723,175 nmol/L. The patient reports that her quality of life has improved dramatically.
Conclusion: The clinical value of these amino acid supplements remains controversial. Further work is required to assess the use of these synthetic amino acid supplements in MMA.

129-P

COBALAMIN C DEFICIENT METHYLMALONIC ACIDURIA AND HOMOCYSTINURIA: A CASE REPORT

Aydogdu SD[1], Yarar C[2], Bor O[3], Yakut A[2], Coskun T[4]
[1]*Dept Ped Nutr Metab, ESOGU Med Fac, Eskisehir, Turkey*
[2]*Ped Neuro, ESOGU Med Fac, Eskisehir, Turkey*
[3]*Ped Hemato, ESOGU Med Fac, Eskisehir, Turkey*
[4]*Ped Nutr Metab, Hacettepe Uni Child Hosp, Ankara, Turkey*

Methylmalonic aciduria is an organic aciduria caused by a deficiency of methylmalonyl CoA mutase or a defect of cobalamin biosynthesis. Here, we presented a thirteen years old boy with cobalamin C deficient methylmalonic aciduria and homocystinuria. The patient has been suffered from recurrent metabolic acidosis attacks, lung infections, neuromotor retardation, seizures and megaloblastic anemia since six months of age. The parents are first degree relatives. Serum vitamin B12 level was normal. Plasma homocysteine level was 50 micromol/L (N: 5-12) and MMA was seven fold increased of normal level. Thyroid function tests, folic acid, orotic acid, uric acid and transcobalamin levels were normal. The fibroblast sample of the patient showed cobalamin C/D deficiency. We recently learned that he is homozygous for a mutation in exon 3 in the MMACHC gene, c.394C>T. Low protein diet, hydoxycobalamin, carnitine, betain and folic acid were given as treatment for three years. After treatment he became seizure free and no megaloblastic anemia. Plasma homocysteine level decreased to 29 micromol/L.

130-P

COMPARED BRAIN AND URINE MRS SPECTRUM IN 5 PATIENTS WITH 3-HYDROXY-3-METHYLGLUTARYL COENZYME A LYASE DEFICIENCY

Roland D[1], Jissendi P[2], Briand G[3], Dobbelaere D[4]
[1]Institut de Pathologie et de Génétique, Gosselies, Belgium
[2]Dept Neuroradio, Hôpital Roger Salengro, CHRU Lille, France
[3]Dept of Biochem and Mol Biol, CHRU Lille, France
[4]Ref Cent Inherit Met Dis, Univ Med Cen, Lille, France

3-Hydroxy-3-methylglutaryl coenzyme A (HMG CoA) lyase deficiency, an inborn error of leucine catabolism and ketogenesis is a rare condition which can be revealed after metabolic decompensation.
Rapid correction of nonketotic hypoglycemia and metabolic acidosis is mandatory.
In long-term follow-up, leucine and lipid restricted diet, carnitine supplement and long-time fasting avoidance are recommanded. With appropriate treatment, most children develop few neurologic symptoms.
The aim of our study was to evaluate brain MRI and proton MRS abnormalities in 5 patients treated for HMG CoA lyase deficiency and to identify the biochemical nature of the abnormal peaks observed on brain MRS comparing cerebral to urine MRS spectra.
Patients and methods: 5 patients (4 males, 1 female) aged from 25 days to 10 years with HMG CoA lyase deficiency were evaluated.
Urine samples, brain MRI and brain MRS were obtained the same day for each of the five patients.
Results: Brain MRI showed white matter lesions. Brain MRS spectra revealed abnormal peaks at 1,2 -1,4 ppm and 2,4 ppm.
Urine MRS analysis confirmed the presence of abnormal metabolites (3-hydroxyisovaleric, 3-methylglutaconic, 3-methylglutaric, 3-hydroxy-3-methylglutaric acids). Their chemical shift and ppm position was compared to brain MRS spectra.
Conclusions: All 5 patients diagnosed with HMG CoA lyase deficiency had abnormal brain MRI contrasting with their almost normal clinical development. Correlating brain and urine MRS spectrum allowed us to identify the biochemical nature of the abnormal peaks seen on brain MRS in the 1.2–1.4 ppm and 2.4 ppm region as 3-hydroxyisovaleric and 3-hydroxy-3-methylglutaric acid.

131-P

CASE STUDY OF METHYLMALONIC ACIDEMIA PRESENTING WITH ACUTE ENCEPHALOPATHY ASSOCIATED WITH BASAL NUCLEI LESIONS 20 MONTHS AFTER LIVER TRANSPLANTATION FROM A LIVING DONOR

Nakajima Y[1], Ito T[1], Ichiki S[1], Maeda Y[2], Kobayashi S[1], Ando N[1], Sugiyama N[3], Hashimoto T[4], Togari H[1]
[1]Dept Pediatr, Nagoya City Univ, Nagoya, Japan
[2]Dept Hosp Pharm, Nagoya City Univ, Nagoya, Japan
[3]Dept Pediatr, Aichi-Gakuin Univ, Nagoya, Japan
[4]Dept Pediatr Surg, Fujita Health Univ, Toyoake, Japan

Background: Recently, liver transplantation has been attempted for severe cases of methylmalonic acidemia (MMA) but long-term effectiveness for neurological complications has not been unvalued.
Case Report: A girl, 1376 g, was born as the second child of triplets after 34 weeks and 0 days gestation without severe complications. About at 2-month-old, lactation insufficiency, impaired liver function and metabolic acidosis were identified. Organic acid analysis and a subsequent gene analysis revealed MMA due to mutase deficiency. Despite protein restriction and L-carnitine treatment, metabolic decompensations were repeated. Given the parents' strong desire, a liver transplantation from the mother as a living donor was performed at 5-year-4-month-old. After transplantation, the patient showed an increased appetite, no longer required tube feeding and showed a marked reduction of metabolic decompensation. At 6-year-11-month-old, the patient was admitted to the hospital with a fever, loss of appetite and high inflammatory response. Fluid replacement and antibiotic treatment were started; the fever decreased, however, disturbance of consciousness, nystagmus, quadriplegia and involuntary movement appeared on the fifth day after admission. Head MRI, MRS and clinical findings indicated Leigh's encephalopathy, and vitamin B1 and coenzyme Q treatments were started. Disturbance of consciousness and neurological symptoms improved several days later. At present, the patient has improved her symptoms and of oral ingestion of food.
Discussion: Liver transplantation allowed to be weaned from tube feeding, stabilized metabolic dynamic and improved the quality of life, but neurological complications occurred. Fortunately the symptoms improved, making us realize the difficulty of post-liver transplantation management.

132-P

ELEVATED EXCRETION OF 3,6-EPOXYDICARBOXYLIC ACIDS AND 2-HYDROXYSEBACIC ACID IN URINE AS A MARKER OF PEROXISOMAL DISORDERS

Krouská L[1], Hrubá E[1], Bártl J[1], Ko-ich V[1]
[1]Inst Inher Metab Dis, Gen Fac Hosp, Prague, Czech Republic

Background: Increased levels of phytanic, pristanic, very-long-chain fatty acids and C27-bile acid intermediates together with deficiency of plasmalogens in erytrocytes are considered the major biochemical markers of peroxisomal disorders. Profiling of urinary organic acid may also indicate a peroxisomal disorder if elevated excretion of 3,6-epoxytetradecanedioic (E14DCA), 3,6-epoxydodecanedioic (E12DCA) and 2-hydroxysebacic acid (2HS) is observed.
Aim: To assess the specificity and sensitivity of elevated excretion of E14DCA, E12DCA and 2HS in diagnosis of peroxisomal disorders.
Method: Organic acids were analysed by GC/MS system (GCQ Finnigan MAT) after oximation, solvent extraction with ethylacetate and trimethylsilyl (TMS) derivatization in urines of patients with peroxisome biogenesis disorders (PBDs, n=7), X-adrenoleukodystrophy (X-ALD, n=6), cholestatic hepatopathy (n=10), ketonuria (n=20), infants receiving MCT (n=20) and of controls (n=50).
Results: The reference range of C14-DCA, C12-DCA and 2-HS was below 0.2, 0.2 and 0.3 mmol/mol creat., respectively. Excretion of E14DCA, E12DCA and 2HS was grossly increased in patients with PBDs (0.6–115, 0.1–33 and 8–82 mmol/mol creat., respectively) in contrast to normal excretion in patient with X-ALD. However, mild to moderate elevation of E14DCA, E12DCA and 2HS (i.e. 0.2–11, 0.2–2 and 0.3–16, respectively) was observed also in patients suffering from cholestatic hepatopathy, and mild elevation of only 2HS (up to 10 mmol/mol creat.) in patients with ketonuria and in patients receiving MCT.
Conclusion: Elevated excretion of E14DCA, E12DCA and 2HS may be a marker of peroxisomal biogenesis disorders with rather poor specificity. We propose that increased excretion of these compounds should prompt a diagnostic work-up for peroxisomal disorders.

133-P

METHYLMALONIC ACIDURIA IN RUSSIA

Shekhter OV[1], Baydakova GV[1], Zakharova EY[1]
[1]*Lab Inh Met Dis, Res Cen Med Gen, Moscow, Russian Federation*

Methylmalonic aciduria (MMA) is one of the most common hereditary diseases within the organic acidurias. The clinical phenotype of MMA is heterogeneous, with a varying period of illness. In 80% of cases the age of onset is the neonatal period or the 1st year of life. Observed patients displayed recurrent vomiting, dehydration, dyspnea, respiratory distress syndrome, lethargy, coma, ketoacidosis, hyperammonemia and hypoglycemia. During the period from 2004 to present, we identified 16 patients with suspected MMA using tandem mass spectrometry (increasing concentration of propionylcarnitine: 7,1–58 mM /L, normal range: 0–6,8 mM/L). The diagnosis was confirmed in 9 patients by quantitative determination of organic acids urine using gas chromatography-mass spectrometry. The concentration of methylmalonic acid ranged from 1108 to 20000 mM/Mcr, normal range: 0–2 mM/Mcr. 7 patients revealed isolated MMA and one patient combined with MMA and homocystinuria. 6 patients were analyzed gene MUT. The genotype is fully set in 4 patients. Diagnosis revealed five previously described mutations, as well as two novels: Leu358Pro and IVS 8–1 G> C.

134-P

A NOVEL MUTATION IN BETA KETOTHIOLASE DEFICIENCY

Unal O[1], Hismi B[1], Kilic M[1], Dursun A[1], Kalkanoglu-Sivri HS[1], Tokatli A[1], Coskun T[1], Sass O[2]
[1]*Metabolism Unit, Hacettepe University, Ankara, Turkey*
[2]*Zentrum Kinder Jugendmed, Univ Freiburg, Freiburg, Germany*

Beta-ketothiolase deficiency is a rare, autosomal recessive metabolic disorder in which the body cannot properly process the amino acid isoleucine or the products of lipid breakdown. In this study we present two cases with ß ketothiolase deficiency confirmed by genetic analysis. One of the patients carries a novel mutation homozygously. Case 1, an 8 –month-old boy, was referred to our hospital after a severe metabolic acidosis attack. Physical examination showed failure to thrive, developmental delay and axial hypotonia. Blood gas and urine analysis were compatible with massive ketoacidosis. Increase of C5-OH-3OH isovaleryl carnitine, C10:1, C10, C4DC, C16, C18:1, and C18 levels were detected on tandem mass spectrometry. Urine organic acid analysis showed increased lactic acid, fumaric acid, 3-OH butiric acid, 3-OH isovaleric acid, 2-methyl-OH butyric acid, acetoacetic acid and tiglylglycine in urine. Mutation analysis of the ACAT1 gene in the patient revelaed c.1040 T >C nucleotide change homozygously, resulting in substitution of isoleucine for threonine at position 347 T reported previously. Case 2, a 15 -month- old girl, was referred to our hospital after a severe and refractory metabolic acidosis and vomiting attack. Tandem MS analysis showed increased tiglyl carnitine, C5 isovaleryl carnitine and 3-OH isovaleryl carnitine, and urine organic analysis showed increased acetoacetic acid, lactic acid, tiglylglycine, 2-methyl 3-OH butyric acid, 3-OH butyric acid, suberic acid, 3-OH sebacic acid, adipic acid. Mutation analysis of the patient showed that the patient carries a frame shift mutation (c.416delG), resulting in a premature stop of protein synthesis (p.Ser139IlefsX9) not reported previously.

135-O

LIVER TRANSPLANTATION FOR PROPIONIC ACIDAEMIA IN CHILDREN

Vara R[1], Turner C[1], Mundy H[1], Heaton N[2], Rela M[2], Mieli-Vergani G[2], Hadzic N[2], Champion M P[1]
[1]*Dept of IMD, Evelina Children's Hosp, London, United Kingdom*
[2]*Paed Liver, GI & Nu, King's College Hosp, London, United Kingdom*

Background: Propionic academia (PA) is a rare inherited disorder of branched chain amino acid metabolism and despite improvements in conventional management, long-term outcome remains disappointing. Liver transplantation (LT) has been proposed to minmise the risk of future metabolic decompensation and improves quality of life.
Methods: Retrospective review of all children with PA referred for LT between 1987–2008.
Results: Five children were identified; median age 1.2 years (0.7–4.1y) at referral. Initial presentation was antenatal in one and prior to 3 weeks of age in 4. All presented with metabolic acidosis and median (range) lactate 1.1 mmol/L (1–10 and ammonia 268 μmol/L (47–1978. Two had seizures requiring intensive care, one required continuous veno-venous haemofiltration (CVVH). All were managed with protein restricted diet, carnitine supplementation, sodium benzoate and metronidazole (3). Due to frequent metabolic decompensations (2), sibling death (2) and medical advice (1); all were assessed for elective LT. After a median waiting time of 50 days (range 5–410 days), one received an auxiliary LT and 4 received cadaveric grafts. Median age at LT was 1.5 years (range 0.8–7). One required re-transplantation 3 months later due to hepatic artery thrombosis. One suffered a metabolic stroke 1 year after LT with minimal residual neurology. At last follow up; median 6 years (1–14 y) all children have protein-unrestricted diet, normal graft function and good quality of life with no further decompensations.
Conclusions: LT has a role in the management of PA, reducing the risk of decompensation and improving quality of life. The potential for neurological decompensation remains.

136-P

OXIDATIVE STRESS INDUCTION BY ACUTE ETHYLMALONIC ACID ADMINISTRATION IN RAT BRAIN AND SKELETAL MUSCLE

Varela AG[1], Simon KR[1], Felisberto F[1], Tonin AM[2], Viegas CM[2], Petronilho F[1], Ferreira GC[2], Dal Pizzol F[1], Wajner M[2], Streck EL[1], Schuck PF[2]
[1]*FISIOPAT, UNESC, Criciúma, Brazil*
[2]*Dept Biochem, UFRGS, Porto Alegre, Brazil*

Background: Ethylmalonic acid (EMA) accumulates in tissues and biological fluids of patients affected by short-chain acyl-CoA dehydrogenase deficiency (SCADD) and ethylmalonic encephalopathy, illnesses characterized by neurological and muscular symptoms. Considering that the mechanisms responsible for the brain and skeletal muscle damage in these diseases are poorly known, in the present work we investigated the effects of acute EMA administration on oxidative stress parameters in cerebral cortex and skeletal muscle from 30-day-old rats.
Methods: Animals received three subcutaneous injections of EMA (6 μmol/g; 90 min interval between injections) and were killed 1 h after the last injection. Control animals received saline in the same volumes.
Results: EMA administration significantly increased thiobarbituric acid-reactive substances levels and carbonyl content in cerebral cortex and skeletal muscle when compared to the saline group. EMA administration also significantly increased DCFH oxidation and superoxide production and decreased glutathione peroxidase activity in cerebral cortex, while glutathione levels were decreased in skeletal muscle.
Conclusion: The present results show that acute EMA administration elicits oxidative stress in rat brain and skeletal muscle, suggesting that oxidative damage may be involved in the pathophysiology of the brain and muscle symptoms found in patients affected by SCADD and ethylmalonic encephalopathy.

137-P

EVIDENCE THAT AMMONIA POTENTIATES THE TOXICITY OF MEDIUM-CHAIN FATTY ACIDS ACCUMULATING IN MCAD DEFICIENCY ON BIOENERGETICS IN CEREBRAL CORTEX AND LIVER OF RATS

Scaini G[1], Ferreira GC[2], Ferreira GK[1], Schuck PF[1], Streck EL[1]
[1]*FISIOPAT, UNESC, Criciúma, Brazil*, [2]*PPGCS, UNISUL, Tubarão, Brazil*

Background: Medium-chain acyl-CoA dehydrogenase deficiency (MCADD) patients suffer from acute episodes of encephalopathy, rhabdomyolysis, hepatic dysfunction, and some times hyperammonemia. However, the underlying mechanisms of tissues damage are unclear. We investigated the in vitro effect of octanoic (OA) and decanoic (DA) acids in the presence of ammonium acetate (AA) on the activity of mitochondrial respiratory chain complexes in cerebral cortex and liver of rats.
Methods: Thirty-day-old Wistar rats were used. OA and DA in the absence or presence of AA were added to the reaction medium and the activities of complexes I-III, II, II-III and IV were evaluated.
Results: Our results show that DA inhibited complexes II and II-III in cerebral cortex, while the combination of DA and AA inhibited complex I-III. Complex IV was inhibited by AA, OA and AA plus OA. In liver, DA and DA plus AA inhibited complexes I-III, II and II-III, whereas complex IV was impaired only by the combination of DA and AA; OA plus AA only inhibited complex II-III.
Conclusions: The present data suggest that the MCADD-accumulating metabolites impair energy metabolism, especially in association with AA. These findings may contribute to the pathophysiology of MCADD.

138-P

RIBOFLAVIN-RESPONSIVE MULTIPLE Acyl-CoA DEHYDROGENASE DEFICIENCY (RR-MADD); A SYNERGISTIC EFFECT OF RIBOFLAVIN AND TEMPERATURE

Cornelius N[1], Frerman F[2], Corydon TJ[3], Gregersen N[1], Olsen RKJ[1]
[1]*Res Unit for Mol Med, Aarhus Univ Hosp, Aarhus, Denmark*
[2]*Dept of pedia, Univ of Colorado-Denver, Aurora, United States*
[3]*Dept of Hum Gen, Aarhus Univ, Aarhus, Denmark*

Riboflavin-Responsive-Multiple-Acyl-CoA-Dehydrogenase-Deficiency (RR-MADD) is a rare neuromuscular disorder. Until recently it was thought to be caused by an inherited defect in the availability of riboflavin-derived flavin cofactors, thus causing dysfunction of cellular flavoproteins; many of which are acyl-CoA dehydrogenases. We and others have recently established that RR-MADD is caused mainly by mutations in the mitochondrial electron transfer protein, Electron-Transfer-Flavoprotein-Ubiquinone-Oxidoreductase (ETF-QO).
In our current study using transiently transfected HEK-cells we have tested the riboflavin sensitivity of variant ETF-QO proteins identified in riboflavin-responsive and classical MADD patients. We show that there is a general response to a decreased riboflavin concentration; both wild-type as well as variant ETF-QO proteins being impaired. Interestingly, expression of the riboflavin-responsive variations result in a significantly lower level of ETF-QO protein and enzyme activity compared to wild-type. Variant ETF-QO proteins associated with a RR-MADD phenotype have a much milder impact on protein amount and activity compared to variant ETF-QO proteins associated with a classical MADD phenotype. We also studied the thermal protein stability and showed that the riboflavin-responsive variant proteins are more unstable than the wild-type protein, even under high riboflavin concentration (530 nmol/L). In conclusion, the results show that there is a clear difference between variant ETF-QO proteins associated with a RR-MADD phenotype and those associated with a classical MADD phenotype. Low riboflavin and high temperature have a synergistic effect on ETF-QO amount/enzyme activity. We suggest that variations associated with a RR-MADD phenotype only give rise to significant disease when expressed under low flavin-content (riboflavin deficiency) and/or high temperature (fever).

139-O

LPIN1 GENE MUTATIONS: A MAJOR CAUSE OF SEVERE RHABDOMYOLYSIS IN EARLY CHILDHOOD

Michot C[1], Hubert L[1], Brivet M[2], De Meirleir L[3], Valayannopoulos V[1], Muller-Felber W[4], Venkateswaran R[5], Ogier H[6], Desguerre I[1], Altuzarra C[7], Thompson E[8], Smitka M[9], Huebner A[9], Husson M[10], Horvath R[11], Chinnery P[12], Vaz FM[13], Munnich A[1], Elpeleg O[14], Delahodde A[15], De Keyzer Y[1], De Lonlay P[1]
[1]*Ref Center Metab Dis, Necker Hosp, Paris, France*
[2]*Dep Biochem, Kremlin-Bicêtre Hosp, Kremlin-Bicêtre, France*
[3]*Pediatr Neurol-Metab Dis, UZ Brussel, Brussels, Belgium*
[4]*Dep Pediatrics, Ludwig-Maximilians Univ, Munich, Germany*
[5]*Dep Pediatrics, Newcastle General Hosp, Newcastle upon Tyne, United Kingdom*
[6]*Ref Center Metab Dis, Robert-Debré Hosp, Paris, France*
[7]*Dep Pediatrics, Besancon C.H.U., Besancon, France*
[8]*SA Clin Genet, Women's & Children's Hosp, North Adelaide, Australia*
[9]*Dep Neuropediatrics, Children's Hosp, Dresden, Germany*
[10]*Dep Pediatrics, Bordeaux C.H.U., Bordeaux, France*
[11]*Friedrich-Baur Inst, L-Maximilians Univ, Munich, Germany*
[12]*Inst for Aging & Health, Newcastle Univ, Newcastle upon Tyne, United Kingdom*
[13]*Dep Clin Chem, Academic Medical Center, Amsterdam, Netherlands*
[14]*Dep Genet-Metab Dis, Hadassah-Hebrew Univ, Jerusalem, Israel*
[15]*CNRS-UMR8621, Paris-Sud Univ, Orsay, France*

Background: Autosomal recessive LPIN1 (lipin 1) mutations have been recently described as a novel cause of rhabdomyolysis in a few families.
Objectives: The purpose of the study was to evaluate the prevalence of LPIN1 mutations in patients exhibiting severe episodes of rhabdomyolysis in infancy.
Patients and methods: After exclusion of primary fatty acid oxidation disorders, LPIN1 coding sequence was determined in genomic DNA and cDNA (complementary DNA).
Results: Among the 29 patients studied, 17 (59%) carried recessive nonsense or frameshift mutations, or a large scale intragenic deletion. In these 17 patients, episodes of rhabdomyolysis occurred at a mean age of 21 months. Secondary defect of mitochondrial fatty oxidation or respiratory chain was found in skeletal muscle of two patients. The intragenic deletion, c.2295–866_2410,-30del, was identified in 8/17 patients (47%), all Caucasians, and occurred on the background of a common haplotype, suggesting a founder effect. This deleted human LPIN1 form was unable to complement Δpah1 yeast for growth on glycerol, in contrast to normal LPIN1.
Discussion: Since more than 50% of our series harboured LPIN1 mutations, LPIN1 should be regarded as a major cause of severe myoglobinuria in early childhood. The high frequency of the intragenic LPIN1 deletion should provide a valuable criterion for fast diagnosis, prior to muscle biopsy.

140-P

NOVEL MUTATIONS IN TURKISH PATIENTS WITH PRIMARY CARNITINE DEFICIENCY

Kilic M[1], Ozgul RK[1], Yucel D[2], Karaca M[3], Dursun A[1], Sivri HS[1], Tokatli A[1], Sahin M[4], Karagoz T[4], Coskun T[1]
[1]Pediatr Metab Dis, Hacettepe University, Ankara, Turkey
[2]Dept of Biology, Hacettepe University, Ankara, Turkey
[3]Dept of Biology, Aksaray University, Aksaray, Turkey
[4]Pediatr Cardiol, Hacettepe University, Ankara, Turkey

Primary systemic carnitine deficiency (SCD) is an autosomal recessive disorder caused by defective cellular carnitine transport. Patients usually present with predominant metabolic or cardiac manifestations. SCD is caused by mutations in the organic cation/carnitine transporter OCTN2 (SLC22A5) gene. Mutation analysis of SLC22A5 gene was done in 8 Turkish patients from six families. Six patients presented with sypmtoms of heart failure, dilated cardiomyopathy by echocardiography and low plasma carnitine levels, five of them with concurrent anemia. A patient with dilated cardiomyopathy had also facial dysmorphia, microcephaly, and developmental delay. Tandem MS analyses in siblings of the patients revealed 2 more cases with low plasma carnitine levels. SCD diagnosis was confirmed in these 2 cases by mutational screening. These two cases were asymptomatic but echocardiography revealed left ventricular dilatation in one of them. Treatment was started before the systemic signs and symptoms developed in these patients. Mean value of serum carnitine levels of the patients was 2.63±1.92 μmol/L at the time of diagnosis. After one year of treatment, carnitine values increased to 16.62±5.11 ($p<0.001$) and all responded to carnitine supplementation clinically. Mutation screening of the OCTN2 gene study in the patients revealed two novel (p.G411V, p.G152R), and four previously identified mutations (p.R254X, p.R282X, p.R289X, p.T337P (fs)10X). Early recognition and carnitine supplementation can be lifesaving in this inborn error of fatty acid oxidation.

141-P

EFFECT OF HEAT STRESS AND BEZAFIBRATE ON MITOCHONDRIAL FATTY ACID OXIDATION (FAO) IN FAO DISORDERS: EVALUATION BY IN VITRO PROBE ACYLCARNITINE ASSAY

Yamaguchi S[1], Li H[2], Purevsuren J[1], Mushimoto Y[1], Kobayashi H[1], Hasegawa Y[1], Fukuda S[1]
[1]Dept Pediatr, Shimane Univ School of Med, Izumo, Japan
[2]Dept Pediatr, Ningxia Med Univ, Yinchuan, China

Background: Hyperpyrexia occasionally triggers serious febrile illnesses such as febrile convulsion, or acute encephalopathy in childhood. It was reported that hyperpyrexia might be responsible for impaired fatty acid oxidation (FAO). We investigated the effect of heat stress and bezafibrate, a hypolipidemic drug, on mitochondrial FAO in cultured fibroblasts with FAO disorders.
Methods: Fibroblasts from children with FAO disorders including MCAD deficiency, VLCAD deficiency, CPT2 deficiency, MTP deficiency, and glutaric acidemia type 2 (GA2) were examined for acylcarnitine (AC) profiles by in vitro probe assay using MS/MS. The AC profiles in the culture medium were compared at 37oC and 41oC, and in the presence and absence of bezafibrate.
Results and Discussion: 1) At 41oC, acetylcarnitine (C2), which is final product of FAO cycle, increased in all cells of FAO disorders and controls. It is suggested that heat stress generally activated FAO. In controls and MCAD deficiency, ACs also decreased at higher temperature. In long-chain FAO disorders including VLCAD-, CPT2-, and MTP-deficiencies, however, accumulation of long-chain ACs including C16, significantly enhanced. In GA2, the short- to medium-chain ACs (C4 to C10) decreased at 41oC, while the long-chain ACs (C12 to C16) significantly increased.
2) In the presence of bezafibrate, C2 increased in all cell lines, while the other ACs including short- to long-chain ACs, all decreased.
Conclusions: Heat stress inhibits long-chain FAO, and some acute encephalopathy in childhood may be related to temporarily or congenitally impair FAO. Bezafibrate is expected as a treatment option for FAO disorders.

142-O

LIVER DISEASE IN MITOCHONDRIAL FATTY ACID OXIDATION DEFECTS: A FRENCH RETROSPECTIVE STUDY FROM 158 PATIENTS

Baruteau J[1], Sachs P[2], Broué P[3], Brivet M[4], Vianey-Saban C[5], Ogier de Baulny H[1]
[1]Div Metab Dis, Robert Debré Hosp, Paris, France
[2]Ped Intens Care Unit, Robert Debré Hosp, Paris, France
[3]Ped Hepatol, Univ Child Hosp, Toulouse, France
[4]Metab Bioch, Bicêtre Hosp, Paris, France
[5]Metab Bioch, Univ Child Hosp, Lyon, France

Background: Mitochondrial fatty acid oxidation defects (FAOD) are a group of severe inherited metabolic diseases which threaten the vital prognosis whereas their treatment could be effective. Although identified since the 1980 s, their description remains still incomplete. We sought to specify hepatic semiology.
Methods: Through a French retrospective multicentric study, we analysed data from 158 children aged less than 6 years affected with a FAOD confirmed by enzymatic study and/or molecular analysis. The hepatic involvement was defined by various combinations of hepatomegaly, raised blood transaminases or GGT levels, hepatic insufficiency with a prothrombin time less than 30% or INR >2, hepatic steatosis (liver hyperechogenicity and/or histological steatosis), and Reye syndrome (coma with cerebral edema and one of the following criterion: steatosis and raised blood transminases or hyperammoniemia).
Results: Hepatic involvement was found in 89% of patients, whatever the underlying defect. Hepatomegaly (92%), increased blood ALAT levels (82%), and steatosis (88%) are the most frequently observed symptoms. Reye syndrome (49%), increased GGT (37%), and liver failure (27%) were other main signs. Extra hepatic symptoms (neurological, muscular, cardiac, hemodynamic involvements, hypoketotic hypoglycemia) are often in the foreground while hepatopathy can progressively worse in the following hours. Isolated hepatic manifestations are exceptional (n=2).
Conclusions: Our results underline the regular hepatic involvement in the course of various beta-oxidation defects. It could have multiple causes such as severe cardiac failure or the expression of an energy deprivation or of toxicity in liver as it has already described in other high energy-dependent organs.

143-P

FATTY ACID OXIDATION DEFECTS REVEALED BY EXTREME PHYSICAL ACTIVITY, CASE REPORT WITH IMPLICATIONS FOR EXPANDED NEWBORN SCREENING

Engvall M[1], Barbaro M[2], Wibom R[1], Nennesmo I[3], Bieneck Haglind C[4], von Dobeln U[1]
[1]Centr for inher met dis, Karolinska Hosp, Stockholm, Sweden
[2]Dept mol med and surg, Karolinska Inst., Stockholm, Sweden
[3]Dept of Pathology, Karolinska Hospital, Stockholm, Sweden
[4]Astrid Lindgrens Cild Hosp, Karolinska, Stockholm, Sweden

We present three young adults who developed rhabdomyolysis during long-distance sports events. The patients terminated their respective competitions due to muscular cramps and experienced myoglobinuria followed by anuria/oliguria. The history revealed several previous episodes of exercise induced muscle cramps and myoglobinuria. All three patients were hospitalised for treatment of renal failure secondary to rhabdomyolysis. Two patients needed hemodialysis, the third received forced diuresis and alkalinisation of urine. All three eventually recovered completely regarding muscle and kidney function.
Muscle biopsies showed accumulation of fatty droplets in the sarcoplasm but were otherwise normal regarding morphology. Mitochondrial biochemical analysis showed reduced ATP-production rate when palmitoyl-carnitine was used as substrate. In two cases acylcarnitine profiling showed increased C14:1-acylcarnitine typical of very long chain acyl CoA dehydogenase (VLCAD) deficiency. This was confirmed by DNA-analysis showing two mutations in the gene for VLCAD. In the third case increased levels of C16- and C18-carnitine suggested deficiency of carnitine palmitoyl transferase II (CPT II). Genetic investigation later confirmed this diagnosis by the finding of compound heterozygosity for two mutations previously reported in patients with CPT II deficiency of adult muscular type.
The patients with VLCAD-deficiency are now treated with a sport drink containing medium chain triglycerides, which allows them to continue participation in a soccer team without development of symptoms.
These cases illustrate that some patients diagnosed by expanded newborn screening may have a mild fatty acid oxidation disorder that requires no other treatment than recommendation to avoid prolonged exercise.

144-P

DISTRIBUTION OF PIVALOYLCARNITINE AFTER ADMINISTRATION OF PIVALATE CONTAINING ANTIBIOTICS IN RAT

Ichiki S[1], Nakajima Y[1], Ito T[1], Maeda Y[2], Kurono Y[2], Sugiyama N[3], Togari H[1]
[1]*Dept Pediatr, Nagoya City Univ, Med, Nagoya, Japan*
[2]*Hosp Pharmacy, Nagoya City Univ, Pharm, Nagoya, Japan*
[3]*Dept Pediatr, Aichi-Gakuin, Pharm, Nagoya, Japan*

Background: Pivalate has been used to generate prodrugs to increase oral bioavailability. Long-term administration of pivalate-containing drugs causes hypocarnitinemia because carnitine is excreted into the urine as pivaloylcarnitine. Our previous report showed side effects of long term administration include carnitine-associated encephalopathy but details of the effect of pivalate induced hypocarnitinemia are not clear.
Objectives: To elucidate the effect of pivalate administration, we analyzed pivaloylcarnitine concentration in several tissues in rats after administration of pivalate containing antibiotics.
Material and methods: Male, four weeks old Wister rats were randomized into two groups: cefditoren pivoxil (CDTR-PI) treated and controls. CDTR-PI (850 mg/kg/day) was given every day for 30 days. On the 31st day; the animals were sacrificed and liver, heart, skeletal muscle, brain, urine and serum were collected. The samples were frozen immediately and stored in -20°C until analysis. After homogenizing with internal standard, centrifugation and solid phase extraction, the samples were injected an HPLC MS/MS.
Results: Levels of pivaloylcarnitine in treated rats were 6.15±2.09 nmol/g (mean±SD) in the liver, 0.80±0.34 nmol/g in the skeletal muscle and not detected in the brain. Free carnitine (FC) levels in treated rats were significantly reduced, i.e. in the liver; 27.2±8.30 nmol/g (controls; 159.8±25.5) and skeletal muscle; 154.3±39.2 nmol/g (controls; 316.1±40.8) and not detected in the brain (controls; 0.75±0.24 nmol/g).
Discussion: Pivaloylcarnitine was produced mainly in liver and less part in skeletal muscle. Even in the treated group, pivaloylcarnitine was not detected in the brain. Free pivalate analysis should be considered for further study.

145-P

A PUZZLING CASE OF CARNITINE-ACYLCARNITINE TRANSLOCASE (CACT) DEFICIENCY, DIAGNOSED ON NEWBORN SCREENING

Al-Hertani W[1], Cordeiro D[1], Burr L[1], Olpin S[2], Raiman J[1]
[1]*Dept of Metab Genet, Hosp Sick Children, Toronto, Canada*
[2]*Dept of Clin Chem, Sheffield Child Hosp, Sheffield, United Kingdom*

Case Report: We report a 15-month-old female, screened positive on newborn screening [NBS]. C16 and C16OH were elevated, raising suspicion of CPTII or LCHAD deficiency. There was a previous family history of intrauterine death at 30 weeks gestation. Follow up NBS blood showed elevations of the C4, C14, C14:1, C14:1OH and C16:1 species, and a low free carnitine (7.1 μM) with normal urine organic acids. On assessment, she was asymptomatic and feeding and growing appropriately. Echocardiography was normal. Our patient is now stable on carnitine, fat restricted diet and MCT oil supplementation.
Results: Fatty acid oxidation flux studies showed: octanoate 86%, myristate 15%, palmitate 13%, oleate 11% of controls, and therefore ruling out GAII, LCHAD/MTP and VLCAD. CPTII enzyme activity was normal, while CACT enzyme activity was low (0.0103 +/- 0.003; controls: 2.2 +/- 0.2), consistent with a CACT deficiency.
Conclusion: Distinguishing CPTII, CACT, LCHAD and MTP can prove difficult. Interestingly, our patient's phenotype is consistent with a mild CACT deficiency but with a very low CACT activity. Pending mutation analysis may contribute to the genotype-phenotype correlation for this disease. No clear explanation has been found yet for the elevated C4, though has been seen in other cases by the authors [S.O].

146-P

LATE-ONSET CARNITINE PALMITOYLTRANSFERASE TYPE II (CPT II) DEFICIENCY: DO BIOCHEMICAL PARAMETERS CORRELATE WITH CLINICAL PICTURE?

Kostalova E[1], Stastna S[1], Chrastina P[1]
[1]*Inst Inher Metab Dis, Gen Fac Hosp, Prague, Czech Republic*

Background: CPT II deficiency (MIM 255110) is an autosomal recessive disorder of carnitine dependent transport of long-chain fatty acids into mitochondrial matrix. Patients with late-onset phenotype have recurrent episodes of myalgia, weakness, myoglobinuria.
Methods: We analysed clinical and biochemical data (blood acylcarnitine profiles and the (C16+C18:1)/C2 ratios, serum creatine phosphokinase and myoglobin levels) in two adult male patients with the muscular phenotype of CPT II deficiency.
Results: Patient 1 presented with myalgia/weakness in 5 of 21 examinations. Myalgia/weakness were accompanied by the elevation of the (C16+C18:1)/C2 ratios in 4/5, creatine phosphokinase (CPK) in 3/5 and myoglobin in 3/5. Asymtomatic patient 1 (16/21) had the elevation of the (C16+C18:1)/C2 ratios in 6/16, CPK in 7/14 and myoglobin in 2/14. Patient 2 was clinically symptomatic in 6/9 investigations. The elevation of the (C16+C18:1)/C2 ratios in 6/6, increased CPK in 0/5 and increased myoglobin in 1/5 were detected. Investigations of asymptomatic patient 2 (3/9) revealed the elevation of the (C16+C18:1)/C2 ratios (3/3), CPK (2/2) and myoglobin (1/2).
Conclusions: The elevation of the (C16+C18:1)/C2 ratios were detected not only in all except one symptomatic cases, but also occasionally between attacks. No correlation between the absolute values of the (C16+C18:1)/C2 ratios and clinical picture was found. Increased CPK and myoglobin did not always relate to clinical symptoms. Both patients had a number of subclinical elevations of CPK. The elevation of CPK and/or myoglobin was not always in association with the increased (C16+C18:1)/C2 ratio.
Supported by the grant MZ0VFN2005 of Ministry of Health of Czech Republic.

147-P

EVIDENCE AGAINST THE INVOLVEMENT OF CARNITINE PALMITOYLTRANSFERASE 1 TRANSMEMBRANE DOMAINS IN ACYLCARNITINE TRANSPORT ACROSS THE OUTER MITOCHONDRIAL MEMBRANE

Violante S[1], IJlst L[2], Denis S[2], Tavares de Almeida I[1], Houten SM[2], Ventura FV[1], Wanders RJ[2]
[1]*Met&Gen, iMed.UL, Fac Pharm, Univ Lisbon, Lisbon, Portugal*
[2]*Lab Gen Metab Dis, AMC, Univ Amsterdam, Amsterdam, Netherlands*

Carnitine palmitoyltransferase 1 (CPT1) is recognized as the enzyme catalyzing the rate limiting reaction in the β-oxidation of long-chain fatty acids, converting long-chain acyl-CoAs into the respective carnitine esters. It remains controversial how these acylcarnitine intermediates have access to the intermembrane space to be further transported by carnitine acylcarnitine translocase and reconverted back to long-chain acyl-CoAs by carnitine palmitoyltransferase 2. It has been previously shown that rat CPT1A has a hexameric quaternary structure arrangement, suggesting that this oligomeric complex could function as a pore channelling acylcarnitines through the outer mitochondrial membrane (OMM). We aimed to investigate if oligomerization of CPT1 to form such pore would indeed be the only pathway by which acylcarnitines enter into the intermembrane space. Oxidation of C16-carnitine was measured in control and CPT1 deficient intact human fibroblasts in order to disclose if this process is independent of CPT1 transferase activity but dependent on the hypothesized pore formation. We found that using C16-carnitine as substrate total β-oxidation in CPT1 deficient cell lines (carrying a nonsense mutation) is not affected. Thus, although both CPT1 function and structure are severely impaired in the deficient cell lines, C16-carnitine is still able to access the mitochondrial matrix to be further metabolized. In conclusion, the pore formed by oligomerization of CPT1 does not function as a channel for the produced acylcarnitines or this is not the exclusive route (and certainly not the major one) involved in acylcarnitines channelling through the OMM.

148-P

EARLY MCADD DEATH: 5 CASES

Patterson AL[1], Henderson MJ[1]
[1]Biochemical Genetics, St James's Hosp, Leeds, United Kingdom

MCADD is the most common disorder of fat oxidation affecting around I in 10,000 live births in the UK. Neonatal screening occurs between days 5–8 with referral to metabolic specialists typically around day 12. We report 5 cases in an 11 year period of children with confirmed MCADD deficiency that died between 2–4 days of life. The diagnosis was made after death in all cases by acyl carnitines analysis. In one child the cause of death had been erroneously attributed to an overwhelming infection. The clinical pictures prior to death showed minimal prodromal signs and the infants generally succumbed rapidly and without warning. This highlights the threat to babies at high risk of MCADD in the first 3 days of life especially if breast feed. We suggest these infant should remain in hospital until the biochemical investigations are complete. The Yorkshire region in this time period had prevalence for early MCADD death of 1:100,000. In the 4 year period 2004–2008 of the MCADD implementation pilot only 4 cases of early death in 2,400,000 births were reported to the UK Newborn screening programme centre. If the prevalence of MCADD death for Yorkshire was reflected nationally, the conclusion must be that early MCADD deaths are either under reported or under diagnosed.

149-P

ASSESSMENT OF CARNITINE PALMITOYLTRANSFERASE II ACTIVITY IN SMALL SKELETAL MUSCLE BIOPSIES

Hargreaves IP[1], Lynes GW[1], Land JM[1]
[1]Neurometabolic Unit, National Hospital, London, United Kingdom

Carnitine palmitoyltransferase II (CPT II) converts acylcarnitine esters to the corresponding acyl-CoA derivatives within the mitochondria prior to fatty acid β-oxidation. CPT II deficiency (McKUSICK 255110) may present early in life, with patients displaying hypoketonemia, hypoglycaemia, cardiomyopathy or multiorgan failure. A neonatal case of lethal myopathy has also been described (1). However, the most common presentation of CPT II deficiency is in adulthood invariably characterized by exercise induced rhabdomyolysis and myoglobinuria especially in the fasted state. In our laboratory we routinely measure mitochondrial respiratory chain enzyme (MRCE) activities in small skeletal muscle biopsies (50–100 mg). However, once the diagnosis of a MRCE deficiency has been excluded the ability to be able to measure CPT activity in the skeletal muscle biopsies of patients having suspected disorders of MRCE/fatty acid β-oxidation would improve the differential diagnosis. In order to assess skeletal muscle CPT activity we have developed a non-radioactive LC-MSMS method which measures the synthesis of palmitoyl-carnitine from palmitoyl-CoA and carnitine in the absence (CPT I + II) and presence (CPT II) of malonyl-CoA (50 uM). Using this method we have been able to determine both CPT I + II (4.53 ± 1.6 nmol/min/mg; mean ± SEM) and CPT II (2.66 ± 0.90 nmol/min/mg; mean ± SEM) activities in skeletal muscle samples. In conclusion, it is now possible to assay CPT activity in small skeletal muscle biopsies without the use of radioactive reagents.

(1). Land JM et al., 1995. Neuromusc Disord 5; 129–137.

150-P

SIMILAR HISTOPATHOLOGICAL CHANGES IN LIVER BIOPSY, BUT DIFFERENT OUTCOMES IN TWO PATIENTS WITH LCHAD DEFICIENCY

Sykut-Cegielska J[1], Pronicki M[2], Taybert J[1], Kalicinski P[3], Broniszczak D[3], Migdal M[4], Witulska K[4], Pohorecka M[1], Socha P[5], Pawlowska J[5]
[1]Dept Metab Dis, Endocr & Diabet, CMHI, Warsaw, Poland
[2]Dept Pathol, CMHI, Warsaw, Poland
[3]Dept Surgery, CMHI, Warsaw, Poland
[4]Dept Intensive Care, CMHI, Warsaw, Poland
[5]Dept Gastroent, Hepat & Immunol, CMHI, Warsaw, Poland

Liver steatosis is typical for LCHAD deficiency, which is regarded as serious, life-threatening disease with unpredictable outcome. We report on two patients (GS and NL) with LCHAD deficiency detected presymptomatically in the pilot newborn screening by MS/MS. Both are homozygotes of the common mutation 1528G>C and developed hepatopathy: the patient GS—mainly hepatomegaly with cholestasis and hyperbilirubinemia without coagulation disturbances at the age of 1 month and the patient NL—progressive liver failure at the 5th month of life. Histopathological examination of liver biopsy (done at the age of 5 and 6 months in GS and NL, respectively) revealed strikingly similar pattern of severe fatty degeneration with marked fibrosis. The patient GS recovered completely spontaneously; now after 5 months does not manifest any liver involvement. While the second girl NL was qualified for liver transplantation performed from cadaver donor one month after liver biopsy. Explanted liver showed end-stage organ failure with diffuse fibrosis, but very few hepatocytes, so almost no steatosis. The postsurgical period was complicated by kidney failure (requiring dialysis); now after three months since the transplantation, she is alive, still on the diet with MCT, with mild transaminases elevation, and cardiac interventricular septum hypertrophy. To our knowledge there is a first report about liver transplantation in LCHAD deficiency.

151-P

ADULT MYOPATHIC PRESENTATION OF FATTY ACID OXIDATION DEFECTS

Waldek S[1], Molana S[1], Sharma R[1]
[1]Salford Royal Hospital, Foundation Trust, Manchester, United Kingdom

Abstract: Presentation of fatty acid oxidation defect (FAO) in adults can vary from mild almost asymptomatic to severe rhabdomyolysis with renal failure. Here we present 4 cases with varied presentation and diagnosis.

Case 1 : 65 year old female started getting aches and pains and difficulty in walking when commenced on statin 3y ago. She was diagnosed as having CPT II deficiency following her sister's recent diagnosis. Gene mutation confirmed CPT II deficiency.

Case 2 : 23 year old female was referred with muscle ache and raised CK 16,588. Since age of 16, she was complaining of muscle aches and cramps post exercise which was occasionally associated with dark urine. Skin biopsy confirmed VLCAD (Very Long Chain Acyl-CoA Dehydrogenase Deficiency).

Case 3 : 63 y old man with CPT II deficiency; he had multiple episodes of rhabdomyolysis precipitated by fasting or infection from very young age, He remained undiagnosed till age of 38. Then this led to renal failure requiring dialysis and renal transplantation at the age of 61.

Case 4 : 29 year keen biker, male, had 3y history of getting muscle pains following biking. Presented with rhabdomyolysis and renal failure (CK 500,000) following 2 days of mountain biking. Skin biopsy confirmed the diagnosis of CPT II deficiency.

Conclusion: Presentation of FAO is varied. Occasionally there is another insult precipitating rhabdomyolysis like use of statin, intense exercise etc. High index of suspicion is the key to early diagnosis. Clinicians have to be aware of the possibility and consider it.

152-P

NEWBORN SCREENING FOR VERY-LONG-CHAIN ACYL CoA DEHYDROGENASE DEFICIENCY AND MOLECULAR EVALUATION

Vilarinho L[1], Nogueira C[1], Gaspar A[2], Leao-Teles E[3], Garcia P[4], Santos H[5], Rocha H[1], Sousa C[1], Fonseca H[1], Marcao A[1]
[1]*National Institute of Health RJ, Porto, Portugal*
[2]*Hospital S. Maria,, Lisboa, Portugal*
[3]*Hospital S. Joao, Porto, Portugal*
[4]*Hospital Pediatrico de Coimbra, Coimbra, Portugal*
[5]*Centro Hospitalar de Gaia, Gaia, Portugal*

Newborn screening (NBS) for disorders of fatty-acid oxidation (FAOD) was implemented in 2004 and combined incidence of all FAOD is approximately 1:6,493. Very-Long-Chain-Acyl-CoA Dehydrogenase deficiency (VLCADD) is an autosomal recessive disorder considered as one of the most common FAOD, possibly associated with neonatal cardiomyopathy, infantile hepatic coma, or adult-onset myopathy. The molecular basis of VLCADD is complex, with about one hundred mutations described in ACADVL gene.

The implementation of MS/MS into our NBS program allowed the screening of 500,000 newborns and the identification of seven cases of VLCADD (1:71,428). Mutations of null type, which result in complet loss of enzymatic function, are always associated to severe disease presentation. However, the majority of diagnosed patients and all asymptomatic newborn so far, harbour missense mutations in the VLCAD gene. We found ten different mutations: seven missenses, one frameshift, one deletion, and one nonsense, from which, seven are novel mutations. In two patients, only one mutation was identified.

Pat	C14:1uM	Aminoacid change	(Nucleotide change)
1	3,09	p.G476A/p.G222R	(c.1427G>A/c.665G>A)
2	1,2	p.G441A/p.G441A	(c.1322G>C/ c.1322G>C)
3	1,67	p.R615X/p.G327A	(c.1844C>T/c.980G>C)
4	5,4	p.E331Q/p. L648LfsX2	(c.991G>C/ c.1944delCT)
5	7,2	p.E130del/ p.A425T	(c.388_390delGAG/ c.1273G>A)
6	6,22	p.G222R/ ?	(c.665G>A/?)
7	2,48	p.R341T/?	(c.1022G>C/?)

Despite marked progress the characterization of this disease, VLCADD is still considered as a relatively insidious disease because of its variable phenotype, and because of the lack of clear phenotype-genotype correlations for the identified mutations. The elucidation of the molecular and cellular effects of these various mutations is important for a therapeutic approach.

153-O

PROTEOMIC STUDY ON SCAD KNOCK OUT MICE REVEALS BROADER EFFECTS ON MITOCHONDRIAL FUNCTION

Wang W[1], Wang W[2], Vockley J[3]
[1]*Dep Hum Gen, GSPH, Univ Pitt, Pittsburgh, United States*
[2]*Res Cen Inher Metab Dis, SD Women Hosp, Jinan, China*
[3]*Dep Pediat, Sch Med, Dep Hum Gen, GSPH, Pittsburgh, United States*

Objective: Multiple mutations have been identified in patients with short chain Acyl-CoA dehydrogenase (SCAD). A defect in this enzyme leads to the accumulation of butyrylcarnitine and ethylmalonic acid in blood and urine and has been reported in association with a wide spectrum of clinical symptoms. However, most individuals identified through newborn screen have remained asymptomatic, raising the question of the clinical relevance of SCAD deficiency. The aim of this study was to better understand the molecular changes induced by SCAD deficiency by elucidating proteomic expression profile changes.

Methods: We examined the quantitative changes of mitochondrial proteome in SCAD knock out mice compared to wild type mice using two-dimensional gel difference electrophoresis (2DIGE) followed by protein identification with MALDI-TOF/TOF and iTRAQ labeling followed by nano-LC/MALDI-TOF/TOF.

Results: Our results revealed that a variety of energy metabolism related proteins including H+ transport synthase, ATPase, hydroxyacyl-coA dehydrogenase and sterol carrier protein2, a lipid transfer protein, changed significantly in mice carrying SCAD null alleles. Interestingly, ornithine carbamoyltransferase, a mitochondrial enzyme that catalyzes the carbamoylation of ornithine to form citrulline, was found to be decreased in SCAD knocked out mice.

Conclusion: Proteins in multiple energy related pathways expressed either increasingly or decreasingly due to deficiency in SCAD suggests a broader affect on mitochondrial function. Further study of diversity of protein changes in variable pathways in SCAD mice will lead to a broader appreciation of the complexity of the mitochondrial metabolome and identify compensatory changes that lead to protection from symptoms in most individuals with SCAD deficiency.

154-P

SELECTIVE SCREENING OF MITOCHONDRIAL ß-OXIDATION DISORDERS, A RETROSPECTIVE STUDY OF 900 CASES ANALYZED IN 1998–2010

Hrubá E.[1], Krouská L.[1], Chrastina P.[1], Paříková Z.[2], Dvořáková L.[1], Zeman J.[2], Stránecký V.[1], Kmoch S.[1]
[1]*Inst Inh Metab Dis, Gen Facul Hosp, Prague, Czech Republic*
[2]*Dept Pediat, Charles Univ&1st Facul Med, Prague, Czech Republic*

The production of the tritiated water from (2,3-3H) butyrate and (9,10–3H) oleate, palmitate and myristate in lymphocytes allows rapid screening and partial dissection of mitochondrial ß-oxidation disorders (BOX) into subgroups.

Using this method, we investigated 900 patients referred to our Institute with clinical and/or biochemical suspicion for any of the BOX disorders and 400 controls. On the basis of decreased ß-oxidation activities of 3H labeled substrates and specific changes in the activities ratios (C18/C16, C18/C14, C18/C4, C16/C14, C16/C4, C14/C4) we identified 34 patients with profiles characteristic for SCAD deficiency, 15 patients with MCAD deficiency, 6 patients with LCHAD/MTP defect, 4 patients with profiles characteristic for MAD deficiency, 3 patients with carnitine transport defects and 2 patients with VLCAD deficiency.

We also identified 21 cases with clearly decreased ß-oxidation activities and unspecific activities ratio profiles. This group includes 3 patients with proven respiratory chain defects and also patients with a potentially new BOX disorder(s). So far unidentified—will be subject to further biochemical and molecular analysis.

155-P

LONG-CHAIN 3-h=HYDROXYACYL-COENZYME A DEHYDROGENASE DEFICIENCY WITH UNUSUAL PRESENTATION OF EXTREMELY LOW VITAMIN D LEVEL

Kreile M[1], Dzivite-Krisane I[1], Krumina Z[1], Daneberga Z[1], Piekuse L[1]
[1]*Children's Clinical University Hospital, Riga, Latvia*

Background: Patients with long chain 3-hydroxyacyl CoA dehydrogenase (LCHAD) deficiency often present with acute metabolic crises with hypoketotic hypoglycemia that may be accompanied by cardiomyopathy, hypotonia and hepatopathy.
Case Report: We describe a 1y 9 mo old boy with unusual presentation of LCHAD deficency. Child was born from the third pregnancy, third delivery of healthy parents (consanguinity cannot be excluded). First pregnancy foetus mortus, suggested due to AFLP, second—healthy boy, third child-proband, fourth child died at age of 23 hours. The proband presented with seizures and nonketotic hypoglycemia. Till now only complaints from parents were poor weight gain, irritability, speech and gait delay. Wide spectrum of biochemical analysis was performed -observed changes: an extremely low vitamin D level (almost below detectable rate), slight hypocalcaemia, with normal serum alkaline phosphatase levels, slight hypochromatic microcytic anemia and slight hypocoagulation. The organic and amino acid profiles were also performed. Organic acid profile in urine was suggestive for LCHADD. Afterwards DNA analysis confirmed the diagnosis of LCHADD; patient was homozygous for common mutation 1528G>C of HADHA gene.
Discussion: Although hypoglycemia is frequent finding in patients with LCHADD, but severe D vitamin hypovitaminosis is not characteristic and is not previously suggested as a symptoms of LCHADD. D vitamin deficiency may be coincidental finding or sign of pathology, that is even more reliable as the serum alkaline phosphatase level was normal. Probably detection of serum vitamin D levels must be included in routine lab analysis for patients with LCHADD.

156-P

CLINICAL AND GENETIC CHARACTERIZATION OF FIVE PATIENTS WITH GLUTARIC ACIDURIA TYPE II

Martins E[1], Bandeira A[1], Rocha H[2], Nogueira C[2], Vilarinho L[2]
[1]*Unit Metab Dis, Child Hosp Maria Pia, Porto, Portugal*
[2]*CGM Jacinto Magalhães - INSA, Porto, Portugal*

Background: Glutaric acidemia type II (GA II) OMMIM # 231680, is caused by defects in the eletron transfer flavoprotein (ETF) or its electron acceptor, electron transfer flavoprotein-ubiquinone oxiredutase (ETF-QO). The clinical features are highly variable including age of onset and severity.
Patients and Methods: Clinical and biochemical evaluation of patients with GA II. Identify the genotype of these individuals trying to correlate with clinical manifestations and responsiveness to riboflavine.
Results: A total of five GA II patients were identified, from which four were diagnosed during acute episodes and one detected through newborn screening. In the symptomatic patients, two presented severe, neonatal onset disease with congenital anomalies, hypotonia, hypoketotic hypoglycaemia and metabolic acidosis and died in the first week of life. The remaining two, presented late onset disease with recurrent vomiting and metabolic acidosis. The fifth patient, diagnosed through newborn screening, was asymptomatic and started treatment at the 21st day of life. At present, with four years of age, his growth, development and neurological examinations are normal. Molecular analysis in four patients revealed four novel mutations in the ETFDH gene, two of them (p.X618QextX14 and c.34+5G>C) in the severe forms, and the remaining two (p.R155G and p.P534L) in the mild forms. The other patient with mild form presented an already described mutation in ETFB gene (p.R191C).
Comments: Four patients present a new mutation in ETFDH gene, two of them with mild forms had muscle involvement but only one is riboflavine responsive. This one is homozygous for P534L mutation.

157-P

FATTY ACID OXIDATION DISORDERS: A NEW PICTURE AFTER EXPANDED NEWBORN SCREENING

Rocha H[1], Marcão A[1], Sousa C[1], Fonseca H[1], Vilarinho L[1]
[1]*NBS Unit, CGMJM - INSA, Porto, Portugal*

Fatty acid oxidation disorders (FAOD) are a group of inborn errors of metabolism that affect mitochondrial fatty acid β-oxidation. FAOD are characterized by a variable age of onset and clinical severity, with symptoms being often triggered by mild viral illness and can range from asymptomatic adults to neonatal sudden death. In the majority of the cases, the treatment based in avoiding fasting is effective.
The possibility of a poor outcome, the global frequency, and the improved clinical outcome, achieved by presymptomatic initiation of treatment, made this group of disorders a main target for newborn screening (NBS).
Before NBS, FAOD were considered rare in Portugal, with very few cases diagnosed, and mainly associated with severe clinical outcomes. NBS for FAOD started in Portugal in 2004 and until now about 500,000 newborns were screened for a group of eight FAOD (MCADD, LCHADD, VLCADD, MADD, CUD, CPT1D, CPT2D, and SCHADD), with 77 positive cases detected (1: 6,493). The adopted detection algorithms, allowed a high detection rate for FAOD as well as the identification of rare forms of FAOD as SCHADD, preserving a low false positive rate. Some unexpected data was revealed, namely the high frequency of MCADD (1: 8,808; considered very rare in Portugal before NBS) and the detection of affected mothers with CUD. This data stretches even more the clinical spectrum of this group of disorders. Implementation of NBS has significantly reduced morbidity and mortality of FAOD. However, the most severe forms are still associated with poor outcomes and death.

158-P

BODY MASS INDEX (BMI) IN CHILDREN WITH MEDIUM CHAIN ACYL CoA DEHYDROGENASE DEFICIENCY

McSweeney M[1], McKay M[2], Dixon M[2]
[1]*Dept Metab Med, Gt Ormond St Hosp, London, United Kingdom*
[2]*Dietetic Dept, Gt Ormond St Hosp, London, United Kingdom*

Background: Medium Chain acyl Co-A dehydrogenase deficiency (MCADD) has been part of the Newborn Screening (NBS) programme in England since 2004. The MCADD Dietary Management Guidelines (www.bimdg.org.uk) advise a normal diet, avoidance of prolonged fasts and regular feeding when children are well. MCADD children in our centre are reviewed in a clinical nurse specialist led clinic supported by a metabolic dietitian. Parental anxieties about feed/foods quantities and feeding frequency expressed in clinic suggest a risk of overfeeding.
Objectives: To assess the risk of obesity in MCADD children using BMI
Methods: Cross-sectional data from 54 MCADD children aged 2 to 14 years.
(NBS n= 38 sibling high-risk screen, n= 4 clinical presentation n=12) from 2009 was reviewed. Weight and height were measured in clinic; Body Mass Index (BMI) calculated (kg/m2) and plotted on UK 90 Growth and BMI Charts. Overweight and obesity were defined according to the International Obesity Task Force.
Results: Height and weight were within normal reference ranges. BMI's were normal in 70.3% children (n=20 boys, 18 girls); 3.7% underweight (n=2 girls); overweight 20.3% (n=5 boys, 6 girls), UK prevalence 14.3%; obese 5.5% (n=3 girls), UK prevalence 16%.
Conclusion: compared to the UK population as a whole, our MCADD cohort has a greater incidence of overweight and lower incidence of obesity. Parental anxiety may be contributing to overweight. It is important we reduce overweight in our MCADD population by regular growth monitoring, reassurance about feeding issues and 'healthy lifestyle' advice in clinic.

159-P

TREATMENT OF SCOT DEFICIENCY WITH MODIFIED CORN STARCH (GLYCOSADE)

Verloo P[1], Van Driessche M[1], Van Coster R[1]
[1]Div Child Neur & Metab, Univ Hosp Ghent, Ghent, Belgium

A seven-year-old boy was referred to the metabolics department. He was followed in a pediatric nephrology department because of presumed renal tubular acidosis. However, acidotic crises occured more frequenty and became severe. Further increase of sodium bicarbonate suppletion had no beneficial effect on the clinical evolution. Analysis of blood gas during decompensation showed normoglycemic ketoacidosis. Lactate, organic acids and acylcarnitine profile were completely normal. Moreover, ketonuria was never absent. The diagnosis of succinyl-CoA acetoacetate transferase (SCOT) deficiency was considered. Enzymatic analysis is currently ongoing. Therapy was started with sufficient carbohydrate intake and home monitoring of urinary ketones. However, this therapy scheme led to severe fluctuations of ketosis during the day. To limit these fluctuations, therapy with uncooked corn starch as carbohydrate source was started. Unfortunately, the carbohydrate need of the patient was so high that the large amounts of uncooked corn starch intake (up to 200 g/day) led to gastrointestinal problems. As a next step, a therapy with modified corn starch (Glycosade) was started. Glycosade has been used for patients with glycogen storage diseases as a new source of carbohydrates. In the patient, modified corn starch was well tolerated. When dosed 60 grams two times daily, daytime fluctuations in ketosis were not anymore observed. Moreover, good control of ketoacidosis led to improved attention span and school performance.

160-P

IMPROVEMENT IN SEVERE HMG CO-LYASE DEFICIENCY WITH FAT RESTRICTION AND 3-HYDROXYBUTYRATE THERAPY

Bhattacharya K[1], Ho G[2], Dalkeith T[1], Dennison B[1], Thompson S[1], Christodoulou J[2]
[1]Gen Metab Service, Child Hosp Westmead, Sydney, Australia
[2]Kids Research Unit, Child Hosp Westmead, Sydney, Australia

Background: HMG Co-A Lyase (HMGCL) deficiency is a disorder of leucine and fat oxidation resulting in hypoketotic hypoglycaemia. Untreated, approximately 30% of patients present in the newborn period with a rapidly progressive fatal encephalopathy. Many go onto have recurrent episodes with developmental problems.

Case Presentation: A one year old boy of consanguineous Christian Palestinian origin presented on day 2 with vomiting and a rapidly progressive high anion gap metabolic acidosis with hypoglycaemia (2.4 mmol/L) and hyperammonaemia (245 μmol/L). Urine demonstrated elevations of 3-hydroxy-3-methylglutaric, 3-methylglutaconic and 3-hydroxyisovaleric acid. He was successfully treated with intravenous dextrose. He was commenced on a combination of breast feeds and glucose polymer providing total energy intake of 35% from fat and 60% from carbohydrate. Subsequent to a brain MRI at 6 weeks showing typical deep white matter changes, he was commenced on 300 mg/kg /day of 3-hydroxybutyrate. At 6 months, fat restriction was maintained but he was allowed more natural protein. Over the first year of life, he has shown normal developmental progress without any episodes of metabolic decompensation despite inter-current illnesses.

Initial investigation of the HMGCL gene showed it was impossible to amplify exons 4 and 5 using genomic DNA. Further studies showed that the proband had a novel homozygous sequence deletion of 4519 bases, deleting exons 4 and 5 and intron 4, with both parents being heterozygous.

Conclusion: Severe HMGCL deficiency can be treated with a fat and protein restricted diet and 3-hydoxybutyrate may be a useful adjunct to this therapy.

161-P

IMPROVED SENSITIVITY FOR HMG CoA SYNTHASE DETECTION USING KEY MARKERS ON ORGANIC ACID SCREEN

Carpenter K[1], Bhattacharya K[1], Ellaway C[1], Zschocke J[2], Pitt JJ[3]
[1]NSW Biochem Genet, Child Hosp Westmead, Sydney, Australia
[2]Div Human Genetic, Med Univ Innsbruck, Innsbruck, Austria
[3]VCGS Path, Murdoch Child Research Inst, Melbourne, Australia

Background: Mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase (HMGCS2) is an intermediary of ketone body production. Patients with a severe deficiency typically present with hypoglycaemia and a non-specific dicarboxylic aciduria during inter-current illness. Recently Pitt et al have shown particular fatty acid metabolites that appear more specific for the disorder. We have found these metabolites in 2 further patients.

Case Presentation: Case 1—of non consanguineous Caucasian origin presented at 8 months, after 2 day history of diarrhoea and vomiting. He had severe high anion gap metabolic acidosis (pH 6.96, bicarbonate 4 mmol/L, anion gap 44), hypoglycaemia and no ketosis. Urine organic acids demonstrated generalised dicarboxylic aciduria with particular elevations in adipic and glutaric acids. There were no significant elevations in corresponding carnitine or glycine conjugates. He was managed with high carbohydrate intravenous therapy and made a full recovery. Subsequent re-examination of the organic acid pattern revealed elevations of 4-hydroxy-6-methyl-2-pyrone (4HMP) and crotonyl glycine. Sequencing of the HMGCS2 gene demonstrated a compound heterozygote (p.G232V & p.Q283X) for 2 likely disease causing mutations.

Case 2, of consanguineous Arabic origin has Sanjad Sakati syndrome (hypoparathyroidism, mental retardation, facial dysmorphic syndrome). At 14 months of age she developed fever, vomiting and a mild metabolic acidosis with hypoglycaemia (p.H 7.31, bicarbonate 16 mmol/L, anion gap 21).Urine organic acids showed dicarboxylic aciduria, particularly of adipic acid with modest ketosis. 4HMP peak was identified as were several 3-hydroxyhexenoic species. Mutation analysis is awaited.

Conclusion: The sensitivity for HMGCS2 ascertainment is enhanced by identification of several additional metabolites.

162-O

CONFORMATIONAL DESTABILIZATION OF THE VARIANT MCAD PROTEIN IS THE STRUCTURAL MECHANISM UNDERLYING MCADD IDENTIFIED IN NEWBORN SCREENING

Maier EM[1], Gersting SW[1], Kemter KF[1], Jank JM[1], Truger MS[1], Reindl M[1], Muntau AC[1]
[1]*Molec Pediatr, Hauner Child Hosp, LMU, Munich, Germany*

Newborn screening (NBS) for medium-chain acyl-CoA dehydrogenase deficiency (MCADD) revealed a higher birth prevalence and genotypic variability than previously estimated, including numerous novel missense mutations in the ACADM gene. On average, these mutations are associated with milder biochemical phenotypes raising the question about their pathogenic relevance. In this study, we analyzed the impact of 10 ACADM mutations identified in NBS (A27V, Y42H, Y133H, R181C, R223G, D241G, K304E, R309K, I331T, R388S) on thermodynamics, conformational stability, and enzyme kinetics of the corresponding proteins. Partial to total rescue of aggregation by co-overexpression of GroESL indicated protein misfolding. This was confirmed by altered ground state thermodynamics, accelerated thermal unfolding in all variants, as well as decreased proteolytic stability and accelerated thermal inactivation in most variants. Catalytic function varied from high residual activity to markedly decreased activity or substrate affinity. Mutations mapping to the ß-domain of the protein predisposed to severe destabilization. In silico structural analyses of the affected amino acid residues revealed involvement in functionally relevant networks. Taken together, our results substantiate the hypothesis of protein misfolding with loss-of-function being the common molecular basis in MCADD. Moreover, considerable structural alterations in all analyzed variants do not support the view that novel mutations found in NBS bear a lower risk of metabolic decompensation than mutations detected in clinically ascertained patients.

163-P

LONG-CHAIN 3-HYDROXYACYL-CoA DEHYDROGENASE DEFICIENCY—THE MOST FREQUENT FATTY ACID OXIDATION DISORDER IN SELECTIVE SCREENING IN RUSSIA

Baydakova GV[1], Zakharova EY[1]
[1]*Lab Inh Met Dis, Res Cen Med Gen, Moscow, Russian Federation*

Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency is an autosomal recessive disorder and represents one of the most severe forms of the class of fatty acid oxidation disorders. The clinical phenotype of LCHAD is quite heterogeneous. Symptoms include cardiomyopathy, lethargy, hypoglycemia and hypotension. The most common form of failure LCHAD, with age of manifestation in the first two years of life, proceeds as a Reye-like syndrome. The clinical picture in the form of acute attacks, accompanied by vomiting, refusal of food, muscular hypotonia, hepatomegaly, tachypnea, lethargy and coma, develops in response to starvation or viral infections.. LCHAD gene mapped on chromosome 2p23 and consists of α-and β-subunits. 60–86% of reported patients with isolated LCHAD deficiency have a prevalent E474Q mutation in exon 15 of the HADHA gene. Since 2004 in our laboratory is carried out selective screening by ESI-MS/MS. During this period found 17 patients with disorders of mitochondrial β-oxidation, 11 of them with LCHAD deficiency. Using PCR-SSCP analysis, the common mutation E474Q was revealed on 80% of mutated alleles. It was also held 4 prenatal diagnosis of this disease. In the Czech Republic and Poland, LCHAD deficiency is also prevalent among other disorders of mitochondrial β-oxidation. It is possible LCHAD deficiency is the most common defect of mitochondrial β-oxidation among the Slavic population.

164-P

INFLUENCE OF SODIUM VALPROATE ON THE HOMEOSTASIS OF HEPATIC FREE COENZYME A

Luis PBM[1], IJlst L[2], van Cruchten A[2], Duran M[2], Almeida IT[1], Wanders RJA[2], Silva MFB[1]
[1]*iMed.UL, Fac Pharmacy, Univ Lisbon, Lisbon, Portugal*
[2]*Lab Gen Metab Dis Dept of Clin Chem Ped, Amsterdam, Netherlands*

Background: Microvesicular steatosis induced by valproic acid (VPA) treatment has been associated with mitochondrial dysfunction and decreased fatty acid b-oxidation. This branched-chain acidic drug produces acyl-CoA esters especially resistant to hydrolysis, both in mitochondrial and cytosolic compartments.
Objectives: To clarify the potential decrease in hepatocellular free CoA induced by VPA.
Methods: Two groups of Wistar rats (n=2 W10) were treated with one single i.p. injection of sodium valproate (100, 500 mg/kg). A third group of rats (n=10) was daily treated for 2 weeks (100 mg/kg). Control animals (n=10,n=12) were treated similarly with 0.9% saline, respectively. Free and total CoA levels in liver tissues were determined by HPLC with fluorescence detection.
Results: A significant decrease of free CoA was found in livers of rats treated with a single dose of VPA as compared with controls. In contrast, a significant increase of total hepatic CoA was found in the same animals. There were no significant changes of free and total CoA in livers of rats submitted to subchronic treatment. Discussion: A CoA depletion is unequivocally induced at the onset of VPA treatment. With subsequent VPA administrations, levels of free CoA seem to recover. The initial CoA depletion is most probably compensated by de novo synthesis of CoA in liver. The decreased bioavailability of CoA, may limit all crucial CoA-dependent reactions, accounting for the adverse effects associated either with the start of VPA therapy or an overdose. Under chronic treatment with VPA, hepatic CoA levels seem to be rescued by in vivo adaptation mechanisms.

165-P

A NOVEL METHOD FOR THE SYNTHESIS OF THIOESTER DERIVATIVES OF COENZYME A

Badenhorst CPS[1], Jansen van Rensburg PJ[1], van Dijk AA[1]
[1]*North-West University, Potchefstroom, South Africa*

Coenzyme A and its thioester derivatives are used as substrates by about 4% of known enzymes, including several enzymes that participate in fundamental biochemical pathways. These include the enzymes of glycolysis, fatty acid biosynthesis and degradation, the Krebs cycle and several other enzymes involved in organic acid and amino acid metabolism. Therefore, thioester derivatives of coenzyme A are valuable as analytical reagents for enzyme assays and as probes of enzyme chemistry. Several strategies have been developed for the synthesis of these compounds. Thioesters of coenzyme A can easily be synthesised by direct acylation of free coenzyme A. A disadvantage of this approach, however, is that functional groups on the coenzyme A, other than the thiol group, may be modified. Strategies that involve the direct chemical synthesis or enzymatic synthesis of coenzyme A derivatives have thus been developed, the most efficient of which utilises recombinant biosynthetic enzymes from E. coli as catalysts. We developed a novel method for the synthesis of thioester derivatives of coenzyme A from pantethine and ATP. An N-hydroxysuccinimide ester is used to synthesise the desired acyl-pantetheine, which is then used as a substrate for the enzyme-catalysed synthesis of the corresponding acyl-coenzyme A. The elimination of intermediate purification steps in the synthesis minimises loss of starting material. Stochiometric conversion of pantetheine to the desired acyl-coenzyme A is achieved. The method reported could facilitate research on and industrial usage of enzymes that use coenzyme A substrates, by presenting a cost effective and convenient means of synthesising these compounds.

166-P

GALACTOSEMIA, PRADER-WILLI SYNDROME AND DIABETES MELLITUS TYPE I: TWO CASE REPORTS

Prochazkova D[1], Gaillyova R[1], Slamova I[1], Vilemova M[1], Magnova O[1], Konecna P[1], Pouchla S[1], Dolezel Z[1]
[1]*Med Fac Masaryk Univ, Univ Hosp, Brno, Czech Republic*

Background: Galactosemia (OMIM 230 400) is the inborn error of carbohydrates metabolism with an incidence of 1:47.000 live birth. It is caused by mutation in galactose-1-phosphate uridyltransferase gene (GALT; 230400.0001, 9q13).
Methods: The aim of this study is to describe the coincidence of galactosemia with Prader-Willi syndrome (PWS; OMIM 176270; 15q12, 15q11-q13) and diabetes mellitus, type I (IDDM; OMIM %222100).
Results: PWS is characterised by diminished fetal activity, obesity, muscular hypotonia, mental retardation, short stature, hypogonadotropic hypogonadism, and small hands and feet. It is caused by deletion or disruption of a gene or several genes on the proximal long arm of paternal chromosome 15 or maternal uniparental disomy 15, because the gene(s) on maternal chromosome(s) are virtually inactive through imprinting. The diagnosis was established in our 1st patient afflicted with galactosemia [genotype p.Q188R/5UTR-119delGTCA+N314D (D2)] by molecular-cytogenetic examination—FISH (Vysis LSI D15S10/SNRPN DNA Probe: microdeletion 15q13)—at four years of age. PWS was associated with growth hormone deficiency and the treatment (Genotropin.) was initiated at the age of five years. IDDM was noticed in our 2nd patient suffered from galactosemia (genotype p.Q188R/p.Q188R) accidentally during regular outpatient clinic examination at the age of twelve years. The therapy with human recombinant insulin was started and lactose free and galactose restricted diet with diabetic diet were combined.
Conclusions: Also the relatively stable patients are noticeable, because it is not possible to eliminate accompanied disorders requiring comprehensive diagnostic and therapeutic access.

167-P

HIGH PREVALENCE OF VITAMIN D INSUFFICIENCY IN GALACTOSEMIC ADULTS DESPITE COMPLIANCE WITH SUPPLEMENTATION

Sirrs SM[1], Bosdet T[1], Branov J[1], O'Riley M[1], Paquin W[1], Rosen A[1], Sharpe J[1], Selvage C[1]
[1]*Adult Metabolic Diseases Clinic, Vancouver, Canada*

Background: Galactosemic patients are at risk for osteoporosis. Calcium and Vitamin D supplementation are routinely recommended. We measured 25-hydroxyvitamin D levels (25OHD) in our galactosemic patients to assess the adequacy of supplementation.
Methods: All patients with galactosemia at our center had 25OHD levels measured with their annual bloodwork. Patient interviews were used to assess compliance with supplements. Compliance was rated poor (taking < 50% of supplements), moderate (taking 51–74% of supplements) and good (taking >75% supplements). Vitamin D sufficiency was defined according to 25OHD levels: Deficient: <40 nmol/L, Insufficient: 40–79 nmol/L, Sufficient: >80 nmol/L. The recommended vitamin D supplement varied with patient (based vitamin D intake from other sources including soy beverages) but a total daily intake of 400 IU or more was recommended as per Osteoporosis Society of Canada guidelines.
Results: 16 of 18 adults (age range 17–55; mean 26.6+/-9 years; 7 females and 9 males) complied with bloodwork. Of these, 9 had 25OHD levels in the insufficient range and 1 was in the deficient range. Of the 10 patients with 25OHD levels below the sufficient range, 5 were rated as moderate or good in terms of their compliance with recommended vitamin D supplements. Information about daily vitamin D intake was available in 8 of these 10 patients showing a mean (+/-SD) estimated vitamin D intake of 536 (+/-400) IU per day.
Conclusions: Vitamin D insufficiency is prevalent in galactosemic patients despite reasonable compliance with routine supplement recommendations.

168-P

THE RESULTS OF BIOCHEMICAL AND GENETICAL APPROACH TO EXCLUSIVE GALACTOSEMIA CASES IN TURKEY THROUGH SELECTIVE SCREENING

Tanyalcin T[1], Kopish G[2], Tanyalcin I[3], Baker M[4], Hoffman G[2], Laessig R[5], Brokopp C[6]
[1]*Tanyalcin Medical Laboratory, Izmir, Turkey*
[2]*Wisconsin State Laboratory of Hygiene, Newborn Screening Laboratory, Madison, United States*
[3]*Vrije Universiteit Brussel Faculteit, Geneeskunde en Farmacie, Brussels, Belgium*
[4]*Wisconsin State Laboratory of Hygiene, UW Pediatrics & Public Health Madison, United States*
[5]*Wisconsin State Laboratory of Hygiene, Population Health Sciences †Deceased, United States*
[6]*Wisconsin State Laboratory of Hygiene, Dept Population Health Sciences Madison, United States*

Galactosemia is an inborn error of metabolism consisting of three types of enzyme deficiencies; galactokinase, galactose-1-phosphate uridyltransferase (GALT) and epimerase. Classical galactosemia with complete GALT deficiency is the most common form. In this long term prospective study, dried blood spots of infants with clinical symptoms like prolonged jaundice, cataract, sepsis, hypoglycemia, liver dysfunction have been analyzed for total galactose, free galactose and GALT measurements. Glucose-6-phosphate dehydrogenase activity on dried blood spots was also measured as an exclusion cause for hyperbilirubinemia. The galactose substrates and the GALT activity measurements are based on NADH/NADPH productions coupled with electron acceptor to produce a colored formazan in the presence of a colorless tetrazolium salt. The samples with high substrate levels and/or low enzyme activity were referred for GALT mutation analysis at the Wisconsin State Laboratory of Hygiene using tetra-primer ARMS-PCR. This method detects the Q188R, L195P, S135L and N314D alleles. There were 7110 babies screened between the years 2006–2010 of which 141 were referred for mutation analysis. The genotypes detected were Q188R/Q188R (39); Q188R/Wild Type (7), N314D/N314D (20), N314D/Wild type (21); Q188R/N314D (4); S135L/L195P (1). No mutations were detected in 20 cases. There were 112 galactosemia cases in this cohort. Mutations showed a remarkable regional distribution. The percentage (1.57 %) of galactosemia in this cohort would support consideration by the task force for the potential inclusion of galactosemia screening in Turkey newborn screening program.

169-P

HYPERCHOLESTEROLEMIA IN A GSD III PATIENT WITH A NOVEL GENOTYPE

Keularts IMLW[1], Rubio-Gozalbo ME[2], Dorland L[1], Dalton A[3], Hurkx GAPT[4], van der Ploeg EMC[5], Spaapen LJM[3]
[1]*Lab Bioch Gen, Dept Clin Gen, Univ Hosp, Maastricht, Netherlands*
[2]*Dept Clin Gen & Pediatr, Univ Med Center, Maastricht, Netherlands*
[3]*Sheffield Child NHS Foundation Trust, Sheffield, United Kingdom*
[4]*Dept Pediatr, Elkerliek Hospital, Helmond, Netherlands*
[5]*Dept Dietetics, Maastr Univ Med Center, Maastricht, Netherlands*

Background: Glycogen storage disease III (GSD III) is a rare autosomal recessive metabolic disorder due to a deficiency of the glycogen debrancher enzyme. We report a patient presenting with a hypercholesterolemia, growth retardation, hepatomegaly and motor developmental delay.
Methods: Basic clinical chemisty as well as selective metabolic screening was performed. Glycogen debrancher enzyme activity was measured in erythrocytes and AGL gene sequencing was done.
Results: The patient presented with strongly increased plasma cholesterol (14.1 mmol/l) and slightly elevated triglycerides (3.9 mmol/l). Clinical presentation and finding of a tetraglucose band in oligosaccharide analysis suggested GSD which was confirmed by strongly decreased activity of glycogen debrancher enzyme. Molecular genetic testing is revealed compound heterozygosity of c.1222C>T mutation (exon 11) and c.2120_2121delAA mutation (exon 17) of the AGL gene. On dietary therapy, consisting of high carbohydrate intake (65-energy%) and moderate fat restriction (22-energy%), plasma cholesterol decreased to (low-)normal within two months. Catch-up growth and improvement in motor development occurred within 8 months.
Discussion: We report a young GSD III patient with a novel genotype who presented with hepatomegaly, growth retardation and high plasma cholesterol responding well to dietary therapy. Hypercholesterolemia might reflect temporal derangement in hypoglycemic conditions. Compound heterozygosity for a classic (c.1222C>T) and novel AGL gene mutation (c.2120_2121delAA) led to classical GSD III phenotype with hepatomegaly and childhood onset growth retardation. The novel mutation is speculated to cause a premature truncation of the protein and hence to be pathogenic.

170-P

MITOCHONDRIAL INVOLVEMENT AND ERYTHRONIC ACID AS A NOVEL BIOMARKER IN TRANSALDOLASE DEFICIENCY

Engelke U[1], Zijlstra F[1], Mochel F[2], Valayannopoulos V[3], Rabier D[3], Kluijtmans L[1], Perl A[4], Verhoeven-Duif N[5], de Lonlay P[6], Wamelink M[7], Jakobs C[7], Morava E[1], Wevers R[1]
[1]*Dep Lab Med, Radboud UMCN, Nijmegen, Netherlands*
[2]*Dep Genetics, Hôp de La Salpêtrière, Paris, France*
[3]*Dep Met Dis, Hôp Necker-Enfants Malade, Paris, France*
[4]*Dep Med, SUNY Upstate Med Univ, NY, United States*
[5]*Metab Endo Dis, UMC Utrecht, Utrecht, Netherlands*
[6]*Hôpital Necker, Paris, France*, [7]*Dep Clin Chem, VU UMC, Amsterdam, Netherlands*

Background: Sedoheptulose, arabitol, ribitol and erythritol have been identified as key diagnostic metabolites in transaldolase (TALDO) deficiency (OMIM 606003).
Objective: To investigate the potential use of NMR spectroscopy to diagnose TALDO deficiency.
Case report: In 6 TALDO deficient patients, the diagnosis was confirmed at the metabolite level and the molecular genetics level.
Methods: Urine from patients and TALDO deficient knock-out mice were analyzed using 1H-NMR spectroscopy and GC-mass-spectrometry.
Results: Our data confirm the known metabolic characteristics in TALDO deficient patients. The beta-furanose form was the major sedoheptulose anomer in TALDO deficient patients. Erythronic acid was identified as a major abnormal metabolite in all patients and in knock-out TALDO mice implicating an as yet unknown biochemical pathway in this disease. A putative sequence of enzymatic reactions leading to the formation of erythronic acid is presented. The urinary concentration of the citric acid cycle intermediates 2-oxoglutaric acid and fumaric acid was increased in the majority of TALDO deficient patients but not in the knock-out mice.
Conclusion: Erythronic acid is a novel and major hallmark in TALDO deficiency. The pathway leading to its production may play a role in healthy humans as well. In TALDO deficient patients there is an increased flux through this pathway. The finding of increased citric acid cycle intermediates hints towards a disturbed mitochondrial metabolism in TALDO deficiency.

171-P

DIRECT NON-RADIOACTIVE ASSAY OF GALACTOSE-1-PHOSPHATE: URIDYLTRANSFERASE ACTIVITY USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

Lindhout M[1], Rubio-Gozalbo ME[2], Bakker JA[1], Bierau J[1]
[1]*Dept Clin Gen, Lab Biochem Gen, MUMC, Maastricht, Netherlands*
[2]*Dept Paed, MUMC, Maastricht, Netherlands*

Background: Galactose-1-phosphate: uridyltransferase (GALT) catalyses the conversion of galactose-1-phosphate (Gal-1-P) and UDP-Glucose (UDP-Glc) into glucose-6-phosphate and UDP-Galactose (UDP-Gal). Complete, or near complete, deficiency of GALT causes classic galactosaemia. The diagnosis is confirmed by measuring GALT activity in erythrocytes. The most commonly used assays require radio labelled substrates or are indirect coupled assays, both having major specific disadvantages.
Methods: GALT activity was measured in erythrocyte lysates using optimal concentrations of the substrates galactose-1-phosphate and UDP-Glc. UDP-Gal and UDP-Glc were separated using reversed-phase high performance liquid chromatography with UV detection. Clinical validity was assessed using blood samples from galactosaemic patients.
Results: UDP-Gal and UDP-Glc were separated with HPLC. The assay was linear with incubation times up 80 minutes and between 0 and 42.5 nmol haemoglobin. Within day and between day imprecision at 50, 75 and 100% enzyme activity was <1.4% and <2.4%, respectively. Mean GALT activity in 33 individuals was 601 ± 79 nmol UDP-Gal/(μmol Hb / hr) (range 492–697) . Patients with classical galactosaemia were easily detected by their extremely low activity.
Conclusions: We have developed a reliable and convenient direct method to measure GALT activity in human erythrocytes using HPLC with UV-detection.

172-P

GALT ACTIVITY REGULATION FROM PRENATAL TO ADULT LIFE IN A SHEEP MODEL

Rubio-Gozalbo ME[1], Lindhout M[2], van Waes S[2], Achten J[2], Kramer BW[1], Bakker JA[2], Bierau J[2]
[1]*Dept Clin Gen, Lab Biochem Gen, MUMC, Maastricht, Netherlands*

Background: Galactose-1-phosphate-uridyltransferase (GALT) deficiency causes classical galactosaemia. In the neonatal period acute complications in liver and kidney occur. Despite a life-long galactose-free diet, long-term complications including cognitive problems and gonadal dysfunction will occur. The involvement of these specific organs and its relation to GALT activity remains to be determined.
Objective: To determine the GALT activity in relevant organs in adult and pre-natal sheep.
Methods: Tissues of 5 preterm lambs and 3 adult ewes were used. GALT activity was measured by HPLC with UV detection.
Results: The GALT activity in liver was highest in both preterm lamb and ewe (5.58 and 1.19 nmol/μg protein/hr). In the preterm lamb, organs involved in acute complications revealed the highest activity (liver> kidney>small intestine). Ewes had higher GALT activity in cerebrum and cerebellum (0.29 and 0.55 nmol/μg protein/hr) in comparison with the preterm lamb (0.17 and 0.42 nmol/μg protein/hour).
Conclusion: Organs affected in acute complications, liver, kidney and small intestine, have the highest prenatal GALT activity. Conversely, brain and ovary, organs affected in adult life have low GALT activity. Whilst acute complications seem to be directly related to the GALT activity level in the affected organs, this is very likely not the case for long-term complications.

173-O

MUTATIONS IN UBIQUITOUSLY EXPRESSED GLUCOSE-6-PHOSPHATASE CATALYTIC SUBUNIT (G6PC3) CAUSE DURSUN SYNDROME

Banka S.[1], Newman W.G.[1], Donnai D.[1], Crow Y.J.[1], Chervinsky E.[2], Shalev S.[2], Yeganeh S.[3], Ozgul R.K.[4], Dursun A.[4]
[1]*Dep Genetic Med, Uni of Manchester, Manchester, United Kingdom*
[2]*Inst for Genetics, Ha`Emek Med Center, Afula, Israel*
[3]*Unit of Hemat, Poria Hospital, Tiberias, Israel*
[4]*Nut Metab Unit, Hacettepe Uni Child Hosp, Ankara, Turkey*

Background: Dursun syndrome (MIM 613034) is a triad of familial primary pulmonary hypertension (PHN), leucopenia and atrial septal defect. Mutations in G6PC3 are known to cause the autosomal recessive disorder, severe congenital neutropenia type 4 (SCN4), the common features of which are—congenital neutropenia, congenital heart defects, urogenital malformations and prominent superficial venous pattern.
Objective: To identify the genetic basis of Dursun syndrome.
Patients and Methods: G6PC3 was screened by DNA sequence analysis in a family with four individuals affected with SCN4. One of these patients also had features which significantly overlapped with Dursun syndrome. We therefore proposed that G6PC3 mutations may also cause Dursun syndrome. G6PC3 was sequenced in a patient with Dursun syndrome.
Results: All four patients with SCN4 were homozygous for a previously described c.758G>A mutation in G6PC3. In the patient with Dursun syndrome, we identified a novel pathogenic homozygous c.346A>G (p.M116V) mutation in G6PC3. The mutation is predicted to alter a highly conserved residue and was not present in a panel of ethnically matched controls.
Conclusions: We have discovered the genetic basis of Dursun syndrome which further defines and expands the phenotypic effects of mutations in G6PC3. Our work demonstrates that PHN, poor growth and developmental delay can be seen in children with G6PC3 mutations. In adults, these mutations can lead to development of varicose veins, endocrine and metabolic dysfunction. We propose that Dursun syndrome should be considered as a subset of SCN4 with PHN as an important clinical feature.

174-P

HYPERCALCAEMIA IN GLYCOGEN STORAGE DISEASE TYPE I PATIENTS OF TURKISH ORIGIN

Kasapkara CS[1], Tumer L[1], Okur I[1], Eminoglu FT[2], Ezgu FS[1], Hasanoglu A[1]
[1]*Div Ped Metab Dis, Gazi University Hosp, Ankara, Turkey*
[2]*Div Metab Dis, Dr. Sami Ulus Child Hosp, Ankara, Turkey*

Background: Glycogen storage disease type Ia is an autosomal recessive disorder caused by a deficiency in the glucose-6-phosphatase. The main clinical characteristics involve fasting hypoglycemia, failure to trive, hyperuricemia, hyperlacticemia and hyperlipidaemia. Hypercalcemia aroused to be an unknown problem in GSD type I patients especially in those with insufficient metabolic control.
Objectives: The aim of the present study was to obtain the prevalence of hypercalcemia and draw attention to the metabolic complications of GSD I patients including hypercalcemia at poor metabolic control.
Material-Method: Hypercalcemia frequency and the affecting factors were studied cross-sectionally in 23 GSD I pediatric subjects. Clinical diagnosis of GSD I was confirmed in all patients either through documentation of deficient G6Pase enzyme activity levels on liver biopsy samples or through G6PC gene sequencing of DNA.
Results: Hypercalcemia was detected in 78.3% of patients with GSD I. Different from the previous report about hypercalcemia in a GSD Ia patient who had R83H and 341delG mutations, we could not identify any genotype–phenotype correlation in our patients.
Hyperlacticemia and hypertriglyceridemia correlated significantly with hypercalcaemia. Furthermore, no differences in serum calcium concentrations could be demonstrated between patients with optimal metabolic control.
Discussion: We have observed hypercalcemia in our series of GSD I patients during acute metabolic decompensation. Therefore we speculate that hypercalcemia should be thought as one of the problems of GSD I patients during acute attacks. It may be related with prolonged lactic acidosis or may be a pseudohypercalcemia due to hyperlipidemia that can be seen in GSD I patients with poor metabolic control.

175-P

GALACTOSEMIA IN A TURKISH POPULATION WITH A HIGH PREVALENCE OF Q188R MUTATION

Guzel A[1], Ozgul RK[2], Dundar H[2], Coskun T[2], Sivri HS[2], Tokatli A[2], Goksun E[2], Hismi B[2], Dursun A[2]
[1]*Dept of Biology, Hacettepe Univ, Ankara, Turkey*
[2]*Dept of Ped, Metab Uni, Hacettepe Univ, Ankara, Turkey*

Classical galactosemia is an inherited recessive disorder of galactose metabolism caused by deficiency of the enzyme galactose-1-uridyltransferase (GALT). In GALT mutation database, 239 disease causing mutations have been reported for the GALT gene for classical galactosemia. In this study, mutation screening was carried out in 27 unrelated Turkish patients with galactosemia by using custom designed resequencing microarray and direct sequencing method. Nine different types of mutations (eight missense, one nonsense) were detected in 46 mutant alleles as a result of mutation screening of GALT gene. Q188R is the most common mutation with a frequency of 47 % of all mutated alleles among them in Turkish patients with galactosemia. The other detected mutations (M142K, R231H, R258G, K285N, F294Y, A320T, R328H, E340X) are less frequent in a Turkish population. R285G was depicted as a novel mutation and two Duarte-1 type (Los Angeles) variant were characterized in our group of patients with classical galactosemia.
The study was supported by State Planning Organization of TURKEY (Project No: DPT 2006 K120640)

176-P

GLYCOGEN STORAGE DISEASE TYPE III (GSDIII) PRESENTING AS LIVER DISEASE WITH A BIOCHEMICAL PHENOTYPE OF THE PRIMARY BILE ACID DISORDER 3-OXO-DELTA(4)-STEROID-5BETA-REDUCTASE DEFICIENCY

Sharrard MJ[1], Connolly SA[1], Olpin SE[2], Scott CAB[2], Manning NJ[2]
[1]*Paediatric Medicine, Children's Hospital, Sheffield, United Kingdom*
[2]*Clinical Chemistry, Children's Hospital, Sheffield, United Kingdom*

GSD III is a well recognized cause of liver disease presenting in infancy, often associated with hypoglycaemia. There are many disorders with the differential diagnosis of hepatosplenomegaly in infancy and the diagnosis of GSD III may be difficult.

Case Report: A male infant born to healthy unrelated Caucasian parents presented with mild leg weakness and inability to weight bear at 8 months of age. Development was otherwise normal. He was found to have hepatosplenomgaly and poor growth. There was no history suggestive of hypoglycaemia. Hepatic transaminases, creatine kinase and plasma lipids were markedly elevated. Ultrasound showed a diffuse increase in echotexture but normal vessels. Urinary bile salts by MS/MS showed gross excretion of glyco- and tauro- 3-oxo dihydroxycholinates, indicative of the bile acid synthesis disorder 3-oxo-delta(4)-steroid-5beta-reductase deficiency. He was started on cholic acid 75 mg twice daily and vitamin supplements with improvement in his transaminases and lipids and the gross excretion of glyco 3-oxo dihydroxycholenoate substantially decreased.

Subsequently leucocyte glycogen debrancher activity was found to be undetectable, with massively elevated erythrocyte glycogen and normal leucocyte phosphorylase, confirming GSD III. The bile acid profile has been highly variable despite continuing bile acid therapy.

Conclusion: Bile acid metabolism may be severely disturbed in GSD III, suggesting a primary bile acid disorder and complicating the diagnosis. The unusual variability in bile acids should suggest an alternative diagnosis to a primary bile acid disorder. Pharmacological bile acid replacement should be considered to reduce plasma lipids in GSD III.

177-P

PHENOTYPIC VARIABILITY WITHIN A CONSANGUINEOUS FAMILY AFFECTED WITH FRUCTOSE-1,6-BISPHOSPHATASE DEFICIENCY

Sharrard MJ[1], Manning NJ[2], Olpin SE[2], Scott CAB[2], Kirk RJ[3]
[1]*Clinical Chemistry, Children's Hospital, Sheffield, United Kingdom*
[2]*Molecular Genetics, Children's Hospital, Sheffield, United Kingdom*

Fructose-1,6-bisphosphatase deficiency is a rare recessively inherited defect of gluoconeogenesis usually presenting with hypoglycaemia and lactic acidosis.

Case Report: A two year old girl of Pakistani origin presented with a reduced level of consciousness following 24 hours of vomiting with no hypoglycaemia but a severe lactic acidosis (pH 7.09, lactate 9.6 mmol/L) and hepatomegaly. EEG was consistent with encephalitis. She was treated with intravenous dextrose, antibiotics and acyclovir and rapidly improved with resolution of hepatomegaly. She has previously experienced a vomiting illness with hepatomegaly but no encephalopathy, and a febrile convulsion. Growth and development were normal. Her father had been diagnosed with fructose intolerance as a child following a fructose load and had avoided eating fruit. He is now fructose tolerant and has had no encephalopathy. His brother had died age 4 years following a vomiting illness.

Urinary organic acids from the girl showed 3-hyroxyisobutyrate and 3-hydroxyisovalerate. A follow up sample showed a significant excretion of glycerol. Leukocyte fructose-1,6-bisphosphatase activity was low at 11 mmol/h/mg.protein (101–463). Analysis of the FBP1 gene showed she was homozygous for the missense mutation p.Arg158Trp, previously reported in an affected individual. Her father was also homozygous for this mutation.

Conclusion: Fructose-1,6-bisphosphatase deficiency usually presents with hypoglycaemia and lactic acidosis. Fructose intolerance is described but is rarely a problem except during crises. In this family there are two distinct phenotypes despite identical mutations. Other genetic factors may interact with the disorder to modify the phenotype. Fructose-1,6-bisphosphatase deficiency should be considered in those presenting with fructose intolerance.

178-P

DYSTONIC FAMILIAL TREMOR CAUSED BY MUTATION IN THE GLUCOSE TRANSPORTER 1 (GLUT1) GENE

Roubergue A[1], Apartis E[2], Mesnage V[1], Doummar D[3], Roze E[4], Trocello JM[5], Taieb G[6], Billette De Villemeur T[3], Vuillaumier-Barrot S[7], Vidailhet M[1], Levy R[1]
[1]*Dep Neurol, St Antoine Hosp, APHP, Paris, France*
[2]*Dep Physiology, St Antoine Hosp, APHP, Paris, France*
[3]*Dep Pediatr Neurol, Trousseau Hosp, APHP, Paris, France*
[4]*Dep Neurol, Salpétrière Hosp, APHP, Paris, France*
[5]*Ref Nat C Wilson Dis, Lariboisière Hosp, Paris, France*
[6]*Dep Neurol, Guy de Chauliac Hosp, Montpellier, France*
[7]*Dep Biochemistry, Bichat Hosp, APHP, Paris, France*

Background: Heterozygous mutations in the GLUT1 gene causes Glut1-deficiency syndrome (GLUT1-DS). Clinical features encompass movement disorders, seizures and mental retardation. Tremor is probably under-estimated according to a recent series reporting its occurrence up to 70% of the cases. Yet, its pattern has not been precisely described.

Objective: To describe the clinical and electrophysiological patterns of tremor in GLUT1-DS.

Case Reports/Results: We report a family followed for tremor (four affected generation) associated with mild dysarthria, cognitive impairment and seizures. Patients (index patient and mother) had upper limbs (postural, intention and writing), lower limb (sitting position) and vocal tremor with mild akinesia and unstable tandem walk. Further inquiries revealed hands, feet and laryngeal area paroxysmal dystonia, triggered by writing, exposure to cold, exercise, sitting position or long talk. Recordings showed an irregular 6 to 8.5 Hz tremor. Patients harbored a new heterozygous Thr137Ala mutation.

Discussion: The tremor, occurring in body areas affected by dystonia, fulfilled clinical diagnostic criteria for dystonic tremor (Deuschl G, 1998). The pattern of tremor recordings was compatible with this diagnosis.

Conclusion: Tremor may be a referral symptom for GLUT1-DS. GLUT1 gene mutation is a possible etiology to dystonic tremor especially when the tremor is familial and/or associated to mental retardation, seizures and/or movement disorders.

179-P

THE SPECTRUM AND FREQUENCY OF ALDOLASE B GENE MUTATIONS IN TURKISH PATIENTS WITH HEREDITARY FRUCTOSE INTOLERANCE

Yucel D[1], Ozgul RK[2], Yilmaz A[1], Sivri HS[2], Coskun T[2], Unal O[2], Tokatli A[2], Dursun A[2]
[1]*Dept of Biology, Hacettepe Univ, Ankara, Turkey*
[2]*Dept of Ped, Metab Uni, Hacettepe Univ, Ankara, Turkey*

Hereditary fructose intolerance (HFI) is an autosomal recessive disorder caused by deficiency in aldolase B enzyme. Since fructose load and enzymatic assay method are invasive and do not allow carrier detection, molecular genetic analysis are essential and useful diagnostic tool for HFI. In this study, mutation screening of nine exons in ALDOB gene was performed in 32 unrelated Turkish patients with HFI. The mutation screening was performed by a 50 K custom resequencing microarray chip (TR_06_01r520489/Affymetrix) and sequencing analysis using ABI3130 genetic analyser. Five different types of pathogenic mutations were detected in 22 of 32 patients suspected with HFI. The most common mutated alleles in 22 HFI patients are p.A175D (37.5%), p.A150P (22.5%) and c.865delC (22.5%), and c.360_363delCAAA (10%), respectively. In addition, one novel mutation (IVS2-3TdelTAGG (7.5%) was detected in three patients as heterozygously.

The study was supported by State Planning Organization of TURKEY (Project No: DPT 2006 K120640)

180-P

MOLECULAR STUDY OF CYPRIOT PATIENTS WITH CLASSICAL GALACTOSAEMIA: IDENTIFICATION OF A NOVEL LARGE DELETION IN THE GALT GENE

Papachristoforou R[1], Sawyer H[2], Petrou P[1], Stylianidou G[3], Burton-Jones S[2], Greenslade M[2], Williams M[2], Drousiotou A[1]
[1]*Biochem Genet Dept, Cyprus Inst Neur Gen, Nicosia, Cyprus*
[2]*Bristol Genetics Lab, Southmead Hosp, Bristol, United Kingdom*
[3]*Pediatrics Dept, Arch Mak III Hosp, Nicosia, Cyprus*

Classical galactosaemia is an autosomal recessive inborn error of carbohydrate metabolism, caused by mutations in the human galactose-1-phosphate uridyl transferase gene (GALT), occurring with an incidence of 1:30.000–1:40.000 births in Caucasian populations. Eight children with classical galactosaemia were diagnosed in Cyprus in the last seventeen years (number of births during this period about 150.000). The aim of the study was to characterize the mutations responsible for galactosaemia in Cyprus. DNA samples from the eight galactoseamic patients and thirteen of their parents were analyzed. All patients were negative for the p.Gln188Arg substitution, the most common mutation in European populations, tested by a real-time PCR Taqman genotyping assay. Bidirectional sequence analysis of all eleven exons revealed the presence of the p.Lys285Asn mutation in five patients. In the remaining three patients none of the eleven GALT exons could be amplified, suggesting homozygosity for a large deletion. These samples were examined by means of a junction fragment PCR assay for a previously described complex deletion of 5.5Kb and again no amplification was obtained. MLPA analysis (MRC-Holland) confirmed the presence of a large deletion encompassing all exons of the GALT gene and at least the first exon of the adjacent IL11RA gene. In conclusion, three patients were found to be homozygous for a large novel deletion, three were compound heterozygous for the p.Lys285Asn mutation and the deletion, and two were homozygous for the p.Lys285Asn mutation (siblings). Testing of parental samples confirmed expected heterozygosity status for either the p.Lys285Asn mutation or the large GALT deletion.

181-P

CLASSICAL GALACTOSEMIA: FULL GENOTYPING OF A GROUP OF PORTUGUESE PATIENTS

Coelho AI[1], Silva MJ[1], Leite M[1], Tavares Almeida I[1], Rivera I[1]
[1]*Met&Gen Group-iMed, FacPharm, UnivLisbon, Lisbon, Portugal*

Classical Galactosemia (CG, OMIM 230400) is an autosomal recessive disorder due to deficient activity of Galactose-1-Phosphate Uridylyltransferase (GALT, EC 2.7.7.12) and is caused by mutations in the GALT gene, where more than 200 mutations were described. GALT deficiency presents within the first weeks after birth as a life-threatening disease. In Portugal, CG screening is done by galactose measurement in blood samples from the neonatal screening program cards, and thus an early confirmation of the diagnosis is imperative for the initiation of therapy. The gold standard of the diagnosis is measurement of GALT activity in erythrocytes, which however can lead to false positives and, additionally, cannot be performed on blood transfusioned children. Therefore, molecular genetics characterization becomes mandatory.

This study aimed to implement a feasible and reliable molecular diagnostic approach, on daily-based lab work.

Seven potential patients were tested. Genomic DNA was isolated from peripheral blood samples. Specific primers were designed for amplification of all individual exons and respective intron boundaries, and a single amplification program was attempted. Several mutations were screened for by PCR-RFLP using appropriate restriction endonucleases. All exons were directly sequenced, either to confirm or to identify the disease-causing mutations. Allele assignment was established by parental DNA analysis when available.

The results allowed the confirmation of the diagnosis and the full genotyping of five patients, in whom three different pathogenic mutations were identified (S135L, Q188R, R333G). The other two individuals proved to carry the Duarte-2 allele, displaying in cis the N314D mutation and the associated intronic polymorphisms.

182-P

UTILITY OF SERUM BIOTINIDASE ACTIVITY AS A BIOMARKER FOR EVALUATING AND MONITORING HEPATIC GLYCOGEN STORAGE DISORDERS

Tolun AA[1], El-Gharbawy AH[1], Boyette K[1], Austin S[1], Goldstein JL[1], Kishnani PS[1], Bali D[1]
[1]*Div Med Genet, Duke Univ Med Cen, Durham, United States*

Due to increasing number of possible therapeutic interventions for Glycogen Storage Disorders (GSDs), the need for biomarkers as disease monitoring tools is recognized. Specific elevation of biotinidase activity in hepatic GSD patients has been reported. In non-GSD patients low biotinidase activity is considered an indicator of advanced liver disease. These findings suggest a role for biotinidase as a biomarker of liver disease progression. The significance of biotinidase activity in hepatic GSD patients with advanced liver disease has not been previously evaluated. Moreover, there is no systematic longitudinal study in GSD patients to evaluate the utility of biotinidase activity as a disease monitoring tool. We determined biotinidase activity using a colorimetric assay in serum of 45 hepatic GSD patients and 68 controls. Biotinidase activity was elevated in most of our patient samples (GSD I = 84%, GSD III = 53% and GSD IX = 78%). We are currently collecting repeat samples from patients at different time-points to determine the correlation of biotinidase activity with disease status and/or metabolic control. The type of GSD and the extent of liver damage may influence the level of biotinidase activity. Thus, we propose that, in hepatic GSD patients with elevated levels, biotinidase activity can be used to monitor disease progression.

183-P

FRUSTE FORM OF GLYCOGEN STORAGE DISEASE: A CAUSE SHOULD BE CONSIDERED IN THE DIFFERENTIAL DIAGNOSIS OF IDIOPATHIC HYPERURICEMIA AND HYPERLIPIDEMIA IN YOUNG ADULTS

Niu D[1], Huang C[1], Chong K[1], Hsu J[1], Sun C[2], Chu H[3], Cheng K[1], Yu H[1], Chang C[4]
[1]*Dep Pedia, Taipei Veterans General Hosp, Taipei, Taiwan*
[2]*Dep Patho, Taipei Veterans General hosp, Taipei, Taiwan*
[3]*Dep Radiology, Veterans General Hosp, Taipei, Taiwan*
[4]*Dep Nuc Med, Veterans General Hosp, Taipei, Taiwan*

Background: The manifestations of glycogen storage disease type 1a (GSD 1a) are usually so prominent in childhood that it is usually readily diagnosed by pediatricians. However, a forme fruste of the disease may only become apparent in adolescents or adults. We observed a brother and sister with subtle manifestations of the disease, discovered after the brother's son was diagnosed with typical GSD 1a.

Methods: We performed mutation analyses of the G6Pase gene and describe the clinical courses of a 25-month boy with typical GSD 1a, and of his 32-year-old father and 34-year-old aunt, who both presented with forme fruste of the disease.

Results: The adult siblings never suffered from hypoglycemia, had normal fasting blood glucose and liver enzymes at the time of diagnosis, and were taller than average Chinese. Their only notable disease manifestations were recurrent gouty arthritis with hyperuricemia and hyperlipidemia in adolescence. When diagnosed, the brother had multiple benign and malignant hepatic tumors, and died of fulminant metastatic hepatocellular carcinoma, 6 months after liver transplantation. p.M121V/p.R83H and p.M121V/p.M121V genotypic constellations of the G6Pase gene were identified in this family. Both siblings were homozygous for the newly identified p.M121V mutation. The infant had compound p.R83H and p.M121V heterozygous mutations.

Conclusions: We recommend a meticulous physical examination or/and abdominal ultrasound in patients with unexplained hyperuricemia and hyperlipidemia, especially at a young age, even in presence of normal liver function. If hepatomegaly is present, GSD should be strongly considered, despite the presence of a normal stature and normal blood glucose.

184-P

THE SCANNING OF COMMONLY SEEN MUTATIONS OF GLUCOSE-6-PHOSPHATASE AND GLUCOSE-6-PHOSPHATASE TRANSLOCASE GENES IN GLYCOGEN STORAGE TYPE 1A AND TYPE 1B DISEASE PATIENTS BY THE MICROELECTRONIC ARRAY TECHNOLOGY

Eminoglu FT[1], Tumer L[2], Ezgu FS[2], Okur I[2], Biberoglu G[2], Hasanoglu A[2]
[1]*Div Metab Dis, Sami Ulus Children' Hosp, Ankara, Turkey*
[2]*Div Metab Dis, Gazi Univ Hosp, Ankara, Turkey*

Background: Glycogen storage disease type 1 (GSD 1) is a group of conditions with autosomal recessive inheritance resulting in disfunction of Glucose-6-Phosphatase (G6Pase) system. Up to the present, 76 distinct mutations were defined in G6Pase gene, and 73 different mutations were defined in G6PT gene. Particularly in some ethnic groups and geographic regions, allelic homogeneity was detected in GSD 1.
Methods: In the present study, the most prevalent mutations in the world were searched by microelectronic array technology, a new method, in 27 Turkish patients diagnosed for GSD 1a and 3 with GSD 1b.
Results: Mutations causing the disease were detected in 45 (83.3%) of 54 alleles screened in the cases with GSD 1a. Allelic frequency of mutations (R83C, G270V, G188R, W77R) looked for were found as 68.5%, 7.4%, 3.7%, and 3.7%, respectively. G188R mutation was detected for the first time in a patient of Turkish origin. The patient with homozygous W77R mutation seemed to present milder clinical and laboratory findings, compared to other patients. G339C mutation was detected only in a single allele of two of three patients with the diagnosis of GSD 1b. Allelic frequency of G339C mutation was determined as 33.3%.
Conclusions: We suggest that microarray technology, which allows rapid analysis of frequently detected mutations and has considerably lower costs than other methods, can be successfully used in diagnosis of GSD 1a in populations with allelic homogeneity, such as patients of Turkish origin, instead of screening the whole gene.

185-P

FRUCTOSE-1,6-BISPHOSPHATE DEFICIENCY: COURSE IN 25 PATIENTS

Santos F[1], Grunewald S[2], Chakrapani A[3], Murphy E[4], Wright K[5], Broomfield A[2], Morris A[6]
[1]*Genet Med, Hosp Univ La Paz, Madrid, Spain*
[2]*Dept Metab Med, Great Ormond St Hosp, London, United Kingdom*
[3]*Dept Inherit Metab Dis, Children´s Hosp, Birmingham, United Kingdom*
[4]*Metab Unit, Nat Hosp Neurol&Neursurgery, London, United Kingdom*
[5]*Dept Clin Biochem, Alder Hey Child Hosp, Liverpool, United Kingdom*
[6]*Willink Unit Genet Med, St Mary´s Hosp, Manchester, United Kingdom*

No data have been published regarding the age & frequency of episodes of decompensation, and long-term outcome for fructose-1,6-bisphosphatase deficiency (FBPD) . We reviewed the notes for 25 FBPD patients from five different metabolic centres in the UK. Diagnosis was confirmed by enzyme assay in leukocytes in 23 patients and hepatocytes in 2. Patients' ages ranged from 3.5 to 35 years.
Most patients presented within the first 30 months of life (9 between day 1 and 5 and 12 between day 5 and 30 months). One patient presented at 9 years of age and one was diagnosed presymptomatically (sibling affected). All the patients for whom data were available had lactic acidaemia at presentation and all except one had hypoglycaemia. Hepatomegaly was reported in 6 patients.
All centres recommended the use of an emergency regimen during illness. Policies varied regarding the maximum duration of fasting and the need for fructose restriction in healthy patients. Six patients took uncooked corn-starch before bed during mid-childhood but only 2 of these had previously experienced early morning hypoglycaemia. There were no reports of decompensation triggered by fructose ingestion. The frequency of decompensation decreased with age but occasional episodes continued; 7 of the 10 patients aged over 10 years suffered episodes during their second decade.
Of the 25 patients, 20 are healthy, with normal development. Two died during episodes of decompensation (aged 5 and 8 years). One patient has spastic diplegia (probably due to prematurity) and two have learning difficulties (one due to a chromosomal microdeletion).

186-O

BIOCHEMICAL AND GLYCOMIC EFFECTS OF DIET RELAXATION IN CLASSICAL GALACTOSAEMIA

Coss K[1], Coman D[2], Brown A[3], Hendroff U[4], Carolan C[5], Mayne P[5], Walsh O[4], Struwe W[6], Rudd P[6], Treacy E[4]
[1]*UCD, Clin Research Ctr, Mater Hosp, Dublin, Ireland*
[2]*Dept Metabolic Med, Royal Children's Hos, Brisbane, Australia*
[3]*Dept Clin Biochemistry, Southmead Hosp, Bristol, United Kingdom*
[4]*NCIMD, Children's University Hospital, Dublin, Ireland*
[5]*Dept Biochem, Children's University Hosp, Dublin, Ireland*
[6]*NIBRT Oxford-Dublin Gly Lab, Conway Inst, Dublin, Ireland*

Background: Strict dietary galactose restriction is life-saving in Neonatal Galactosaemia. However over-restriction of galactose could contribute to the ongoing pathophysiology (Hughes et al, 2009).
Objectives: Study effects of galactose liberalisation, using our developed methods of serum N-glycan analysis and T-lymphocyte gene expression (Coman et al, 2010) in comparison to standard biochemical markers in Galactosaemia.
Materials and Methods: 10 Gal adults (19–26 yrs, Q188R/Q188R) with normal IQs were studied. 5 liberalised galactose from 300 mg to 4,000 mg over 16 weeks and were compared to 5 matched Gal controls with clinical and biochemical monitoring, (RBC Gal-1-P and urine Galactitol levels). Serum N-glycans were analyzed by NP-HPLC with galactose quantitation of IgG.
Results: No clinical changes were observed in the diet relaxed group. There were no significant differences noted between mean RBC Gal-1-P and urine galactitol levels, in comparison to the control group or association noted with increments in galactose intake. The mean Gal-1P levels in controls on galactose intake <300 mg/day was 0.55 μmol/gHb, (r:=0.43–0.77, n=15) in comparison to 0.52 μmol/gHb, (r=0.33–0.81, n=19). The mean urine galactitol was 124.4, (r=69–216, n=15) vs. 120 μmol/mmol creat, (r= 65–209, n=19). Analysis of serum N-glycan profiles showed favourable profiles for all patients on 1,000 mg galactose with increased bi- and tri-antennary sialylated glycan structures. 3 patients showed favourable galactosylation at 4,000 mg whereas 2 had profiles suggesting intoxication (glycan assembly defect) at 2,000 mg gal/day.
Conclusion: We propose that this method of N-glycan profiling (with IgG analysis) is an improved, sensitive method of monitoring and optimizing individual galactose tolerance and control in Galactosaemia.

187-P

NOVEL HETEROZYGOUS MUTATIONS IN TALDO1 GENE CAUSING TRANSALDOLASE DEFICIENCY AND EARLY INFANTILE LIVER FAILURE

Wamelink MMC[1], Salomons GS[1], Balasubramaniam S[2], Ngu LH[2], Keng WT[2], Jakobs C[1]
[1]*Dept of Clinical Chemistry, VUMC, Amsterdam, Netherlands*
[2]*Dept Clinical Genetics,Kuala Lumpur Hosp, Kuala Lumpur, Malaysia*

Transaldolase (TALDO; EC 2.2.1.2) deficiency (OMIM 606003), a recently recognized inborn error of the pentose phosphate pathway (PPP) has been reported to date in 10 patients from six families presenting with liver disease and variable clinical course. The PPP is a series of interconversions of sugar phosphates that has two major functions, generation of NADPH for reductive synthesis and provision of ribose-5-phosphate for nucleic acid biosynthesis. TALDO catalyzes the reversible transfer of dihydroxyacetone phosphate from sedoheptulose 7-phosphate (S7P) to glyceraldehyde 3-phosphate (G3P), thus generating fructose 6-phosphate (F6P) and erythrose-4-phosphate (E4P). The leading symptoms in the neonatal period in all 10 reported patients were bleeding tendencies with thrombocytopenia and abnormal liver function tests, hepatosplenomegaly, hemolytic anemia and dysmorphic features (anti-mongoloid slant, low-set ears and cutis laxa). Interestingly, mental and motor developments were normal in the majority who were assessed. The biochemical profile of all affected patients is similar: elevated urine erythritol, arabitol, ribitol, sedoheptitol, persitol, sedoheptolose, mannoheptulose and sedoheptulose-7P. Elevations of polyols and seven-carbon chain carbohydrates are most prominent in the neonatal period, improving slightly with age. Measurement of TALDO enzyme in fibroblast, lymphoblasts or liver tissue and sequence analysis of the TALDO1 gene confirm the diagnosis.
We describe the clinical and biochemical features of an additional patient with TALDO deficiency of Chinese origin with two novel heterozygous missense mutations. He presented with neonatal hydrops, progressive liver failure, cirrhosis with portal hypertension and hemolytic anemia. He unfortunately succumbed to his illness at 4. 5 months of age.

188-P

THE DIFFERENCE BETWEEN RARE AND EXCEPTIONALLY RARE: MOLECULAR CHARACTERISATION OF RIBOSE 5-PHOSPHATE ISOMERASE DEFICIENCY

Wamelink MMC[1], Gruning NM[2], Jansen EEW[1], Bleumlein K[2], Lehrach H[2], Jakobs C[1], Ralser M[2]
[1]*Max Planck Inst for Molecular Genetics, Berlin, Germany*

Background: Ribose 5-phosphate isomerase (RPI) deficiency is an enzymopathy of the pentose phosphate pathway. It manifests with progressive leukoencephalopathy and peripheral neuropathy and belongs, with one sole diagnosed case, to the rarest human disorders. The single patient was found compound-heterozygous for a RPI frameshift and a missense (RPIAla61Val) allele.
Methods: RPI enzyme activity, protein concentration and mRNA expression was analysed in fibroblasts and lymphoblasts derived from the patient and controls. A transgenic yeast model was generated encoding for human RPI, human RPIAla61Val and human RPI expressed under a weak promoter.
Results: We detected in the two patient-derived cell lines differences in RPI enzyme activity, enzyme concentration, and mRNA expression, higher in lymphoblasts than in fibroblasts. Furthermore, the transgenic yeast model exhibits metabolite- and enzyme-activity changes that correspond to the human syndrome and show that the decrease in RPI activity in patient cells is not fully attributable to the residue exchange.
Discussion: These results demonstrate that RPI deficiency is caused by the combination of a RPI null allele with an allele that encodes for a partially active enzyme which has, in addition, cell type dependent expression deficits. We speculate that a low probability for comparable traits accounts for the rareness of RPI deficiency.

189-P

FANCONI-BICKEL SYNDROME: THREE UNRELATED CASES FROM NORTHERN AND EASTERN EUROPE WITH THE SAME NOVEL MUTATION OF THE SLC2A2 GENE

Cimbalistiene L.[1], Tumiene B.[1], Utkus A.[1], Kucinskas V.[1], Brackman H.[2], Santer R.[3]
[1]*Dept Hum Med Genet, Vilnius Univ, Vilnius, Lithuania*
[2]*Barnekliniken, Haukeland Sygehus, Bergen, Norway*
[3]*Dept Ped, Univ Med Cent Hamburg-Ependorf, Hamburg, Germany*

Background: Fanconi–Bickel syndrome (FBS) is an inborn error of carbohydrate transport caused by a deficiency of GLUT2, encoded by SLC2A2. The most characteristic symptoms are hepatomegaly, fasting hypoglycemia with postprandial hyperglycemia, and renal proximal tubulopathy with disproportionately high glucosuria.
Objective: To describe three cases of FBS from our area with a common previously not reported mutation in the SLC2A2 gene.
Case Reports: Two cases come from unrelated non-consanguinous Lithuanian families. The first one presented with growth retardation, rickets at 4 months; renal proximal tubular dysfunction, metabolic acidosis, hepatomegaly were obvious at 10 months. She suffered from multiple bone fractures. Disproportionately high glucosuria, fasting hypoglycemia with postprandial hyperglycemia suggested FBS. Homozygosity for 1-bp deletion in exon 11 (c.1469delA [p.K490SfsX24]) was found. Clinical improvement was observed at 2,5 years.
The second case presented at 6 months with growth retardation and hepatomegaly, hyperlipidemia, increased liver enzymes, hypoglycemia, lactic acidemia, renal proximal tubulopathy with massive glucosuria, signs of glycogen accumulation in a liver biopsy, and a characteristic phenotype. This suggested FBS, and homozygosity for the same a 1-bp deletion was found. This patient succumbed to gastrointestinal infection at 1,5 years of age.
In a third typical 2-year-old FBS patient from Norway, we found the same mutation in the heterozygous state (together with SLC2A2 c.1411G>A [p.G471R]).
Conclusion: This novel SLC2A2 mutation results in characteristic FBS. It could be that it is more common in Northern and Eastern Europe, particularly the Baltic States.

190-P

NAIL POLISH REMOVER AND THE DEVELOPING BRAIN: ANTENATAL ONSET PYRUVATE CARBOXYLASE DEFICIENCY

Davison JE[1], Wilson M[1], Vijay S[2], MacPherson L[2], Peet AC[2], Gissen P[1]
[1]*University of Birmingham, Birmingham, United Kingdom*
[2]*Birmingham Children's Hospital, Birmingham, United Kingdom*

Core energy metabolism disorders frequently generate a severe, fatal clinical course. We describe a child with pyruvate carboxylase deficiency (PCD) who presented in utero with foetal magnetic resonance imaging (MRI) evidence of antenatal brain injury. Post-natal MRI and magnetic resonance spectroscopy (MRS) demonstrated brain injury patterns consistent with PCD, and revealed abnormal brain metabolism providing insight into pathogenic mechanisms.
Foetal MRI acquired at 31 weeks gestation because of abnormal foetal ultrasound scan demonstrated septated, enlarged ventricles and simple gyration. After term delivery the child developed abnormal respiratory patterns, hypothermia and significant lactic acidosis. The child received standard neonatal medical care including antibiotic cover. The acidosis initially responded to dichloroacetate and sodium bicarbonate, but after further deterioration the child died at 2 months. Post-natal MRI demonstrated diffusely abnormal white matter, subependymal cysts, ventricular septations and delayed myelination. The diagnosis of PCD was subsequently confirmed by fibroblast enzyme assay and DNA mutation analysis which demonstrated a 2 base-pair deletion in exon 8 (c.1154_1155delGC (p.Arg384fs)).
MRS is an in vivo technique performed concurrently with MRI that provides information about brain metabolites in the 1–10 mM range. MRS on day 10 (1.5 T, PRESS technique) demonstrated significantly elevated brain lactate and decreased glutamine and glutamate in white matter and basal ganglia, and another metabolite peak (singlet at 2.22 ppm) consistent with accumulating acetone. Acetone was not seen in a cohort of over 200 other neurometabolic cases and may be a specific marker of deranged brain metabolism in PCD consistent with exaggerated ketoacidosis and increased flux via acetyl-CoA.

191-P

THREE CASES WITH FRUCTOSE 1,6-BISPHOSPHATASE DEFICIENCY: TWO NOVEL MUTATIONS

Yucel D[1], Ozgul RK[2], Tokatli A[2], Sivri HS[2], Guzel A[1], Coskun T[2], Dursun A[2]
[1]*Dept of Biology, Hacettepe Univ, Ankara, Turkey*
[2]*Dept of Ped, Metab Uni, Hacettepe Univ, Ankara, Turkey*

Fructose 1,6-bisphosphatase (FBPase) deficiency is an autosomal recessive inborn error of metabolism in the gluconeogenetic pathway. FBPase catalyzes the converting of fructose-1,6-bisphosphate into fructose 6-phosphate and inorganic phosphate. FBPase deficiency is caused by mutations in the fructose-1,6-bisphosphatase 1 (FBP1). Mutations in the FBP1 gene result in impaired gluconeogenesis, which is characterized by episodes of hypoglycemia, ketonuria, lactic and metabolic acidosis.
In this study, mutation screening of seven exons in FBP1 gene was carried out in three patients with FBPase deficiency by using direct sequence analysis. Mutation analysis revealed that, detected nucleotide changes result in homozygously with one known mutation c.960–961insG (p.V131Gfs..71X) in exon 7 , and two novel mutations, c. 864insA (p.M289Nfs..44X) in exon 7 and c.392delT (p.V131Gfs..71X) in exon 3 in FBP1 gene.
The study was supported by State Planning Organization of TURKEY (Project No: DPT 2006 K120640).

192-P

BONE MINERALIZATION IN TYPE I GLYCOGEN STORAGE DISEASE: A TWO YEARS FOLLOW UP STUDY

Riva E.[1], Giulini Neri I.[1], Gasparri M.[1], Scotti Gerber J.[1], Salvatici E.[1], Minghetti D.[1], Cagnoli G.[1], Paci S.[1]
[1]*Dept. of Ped, S.Paolo Hosp, Univ of Milan, Milan, Italy*

Background: Patients with Glycogen Storage Disease I (GSD I) have reduced bone mineral density (BMD) with higher risk of osteopenia/osteoporosis.
Objectives: We analyzed the prevalence of osteopenia/osteoporosis and relationship between metabolic control, markers of bone control, BMD and calcium intake.
Patients and methods: 11 patients (5 Ia/6 Ib, 6 M/5 F) were studied during 2 years. All follow an intensive dietary treatment and are supplemented with calcium (orally) since diagnosis. We analyzed parameters of metabolic control, markers of bone control (serum osteocalcina, carboxyterminal-telopeptide of type I collagen-βcrosslaps, calciuria and tubular resorption of calcium-TRP) and areal BMD measured by DXA every 4–6 months. Z-scores were calculated by comparing BMD with age or age-sex matched reference values according to the manufacturer's reference database. A 3-day dietary schedule was filled. Three groups were distingued on the bases of DXA Z-score: 4 osteoporotic ($z>2,5$), 3 osteopenic ($2,5<z<1,5$), 4 normal ($z>1,5$). The prevalence of osteopenia/osteoporosis was higher in the Ib-group, probably because of granulokine-therapy or inflammatory bowel disease.
Results: Dietary calcium intake was markedly reduced, but with pharmacological supplementation total intake resulted adequate. Osteocalcina and βcrosslaps were elevated in most patients, suggesting a strain of bone repair; descriptive analysis showed that osteoporotic patients had elevated blood and urinary lactate; two had dyslipidemia, one chronic acidosis.
Conclusion: There is a correlation between bone mineralization and metabolic control but further studies are needed to confirm it and clarify whether reduced BMD is a natural consequence of GSD or it can be avoided by a good metabolic control.

193-O

ULTRA FAST AND SENSITIVE LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (LC-MS/MS)-BASED ASSAY FOR NEWBORN SCREENING OF GALACTOSE-1-PHOSPHATE URIDYLTRANSFERASE AND GALACTOKINASE DEFICIENCIES

Li Y[1], Ptolemy AS[2], Kellogg M[2], Berry GT[1]
[1]*Div Gen, Chidrens, Harvard Med School, Boston, United States*
[2]*Lab Med, Childrens, Harvard Med School, Boston, United States*

The diagnosis of transferase- and galactokinase (GALK)- deficiency galactosemia usually involves the measurement of galactose-1-phosphate uridyltransferase (GALT) and GALK enzyme activity, respectively, in erythrocytes. The current gold standard assays for these enzymes are radioactive assays, which are laborious and/or incapable of measuring low enzyme activities. To further our knowledge of genotype-phenotype relationships, we developed an assay for GALT activity using LC-MS/MS (Clin. Chem., 2010; 56: 772–780). Now we turned our attention into the development of an ultra fast and sensitive LC-MS/MS GALT and GALK assays for universal newborn screening. Stable isotope labeled substrates were utilized in the assays, with the enzymatic products ([13C6]-uridine diphosphate galactose in GALT assay and [13C6]-galactose-1-phosphate in GALK assay) quantitated in a 3 minutes LC-MS/MS run. The assays were sensitive enough to allow for the quantification of enzyme activities as low as 0.2% and 0.4% of normal control values in the GALT and GALK assays, respectively. Twenty two samples from non-galactosemic patients were assayed to have GALT activity of 23.2 ± 4.2 (mean ± SD) and GALK activity of 1.8 ± 0.47 (mean ± SD) μmol/g Hgb/hr. Erythrocyte GALT activity in a cohort of 43 patients with galactosemia were measured: 4 patients had GALT activity less than 1% of normal control values and the remaining 39 had no detectable GALT activity. With a 3 minutes per sample run time, this sensitive LC-MS/MS-based GALT/GALK assay can be adapted for newborn screening for galactosemia.

194-O

GENERATION AND CHARACTERIZATION OF MOUSE MODEL OF POLYGLUCOSAN BODY DISEASE

Akman HO[1], DiMauro S[2], Craigen WJ[1]
[1]*Baylor College of Medicine, Houston, United States*
[2]*Columbia University, New York, United States*

Background: The synthesis of glycogen is catalyzed by the sequential actions of two enzymes: (i) glycogen synthetase, attaches glucosyl units to linear chains of glycogen; and (ii) the branching enzyme, which attaches a short branch of glucosyl units to a linear chain. Glycogen storage disease type IV (GSD IV) (OMIM 232500) is an autosomal recessive disorder caused by glycogen branching enzyme (GBE) deficiency and leading to the accumulation of an abnormal amylopectin-like polysaccharide (polyglucosan) in multiple tissues, including liver, heart, skeletal muscles, and the central nervous system. A late-onset clinical variant, known as adult polyglucosan body disease (APBD), causes a neurodegenerative disorder simulating amyotrophic lateral sclerosis (ALS). The two naturally occurring animal models of this disorder, American quarter horses and Norwegian forest cats, are not practical laboratory animals.
Method: We have developed a mouse model for APBD, by introducing Y329S mutation in to mouse GBE1 gene by standard molecular biology techniques.
Results: Y329S mutation does not cause polyglucosan accumulation in heterozygous animals, and they have normal life as we have observed up to 8 months, however homozygous animals have severe polyglucosan accumulation in muscle and liver; and to a lesser extend in peripheral nerves. GBE1 activity in muscle and liver has been decreased respectively to 6% and 30% compare to the wild type littermates.
Conclusion: Introduction of Y329S mutation in to mouse GBE1 gene we have generated a mouse model for polyglucosan body disease. This animal model is suitable to investigate the pathogenesis and treatment of polyglucosan accumulation.

195-P

ANAPLEROTIC THERAPY FOR ADULT POLYGLUCOSAN BODY DISEASE (APBD) WITH GLYCOGEN BRANCHER ENZYME DEFICIENCY

Mochel F[1], Haller R.G.[2], Wallace M.[3], Lossos A.[4], Akman H.O.[5], Schiffmann R.[3]
[1]*INSERM, UMR 679, Hôpital La Salpêtrière, Paris, France*
[2]*Neuromuscular Center Institute, Dallas, United States*
[3]*Inst of Metab Dis, Baylor Res Inst, Dallas, United States*
[4]*Depart of Neurol, Hadassah Med Ctr, Jerusalem, Israel*
[5]*Depart Neurol, Columbia Uni Med Ctr, New York, United States*

APBD is a degenerative neurogenetic disorder that is often caused by glycogen brancher enzyme deficiency. It is therefore the adult form of glycogen storage disease type IV (GSD IV). There is currently no effective therapy and the pathogenesis of the disease is poorly understood. We identified indicators of energy deficiency such as symptomatic hypoglycemia in an APBD patient on submaximal exercise. Symptomatic hypoglycemia is known to occur in an animal model of GSD IV and in other glycogen storage diseases. We therefore hypothesized that (i) decreased glycogen degradation leads to energy deficit in neurons and glial cells; (ii) anaplerotic therapy using triheptanoin, a 7-carbon triglyceride, will supply needed substrate to the citric acid cycle to correct the energy deficit and thus slow, halt or reverse the progression of the disease. Open label trial at a triheptanoin dose of 1–2 g/kg of body weight/24 hours showed a mean improvement of 10% in the 6-minute walk test over a mean follow up of 8.5 months (n=5, p=0.06). One patient had a 126 feet improvement (9.5%) at the 25 months time point. Physical Function score increased in 4/5 patients on the SF-36 health survey questionnaire. We consequently initiated a randomized controlled study in 18 subjects ingesting a diet supplemented with triheptanoin or a control long chain vegetable oil. Outcome measures include 6-minute walk test, motion capture gait analysis and quality of life. Should this therapeutic approach be successful, it may also be applied to other glycogen storage disorders.

196-P

RETROSPECTIVE EVALUATION OF CLINICAL PRACTICE: USING A MODIFIED STARCH IN THE MANAGEMENT OF GLYCOGEN STORAGE DISEASE

Mullally M[1], Mundy H[2], Champion M[2], Gick J[2], Eardley J[2], Emery P[1]
[1]*King's College London, London, United Kingdom*
[2]*Dept of IMD, Evelina Child Hosp GSTT, London, United Kingdom*

Background: Cornstarch therapy has been the mainstay of treatment for Glycogen storage disease for over two decades. Recent cross over trials of a modified cornstarch (Glycosade) have demonstrated improved fasting times and short term metabolic control. Longer term studies have not yet been reported.
Objectives: To compare the use of Glycosade as part of a standard dietary regimen in the longer term in 11 Glycogen storage disease patients (Type Ia, Ib and III) vs traditional uncooked cornstarch therapy / Evaluating fasting tolerance, height and weight, appetite and secondary metabolites
Methods: Glycogen storage disease patients under care of the Evelina Children's hospital (Type Ia, Ib and III) aged 3–18 yrs taking cornstarch as part of their dietary management were included in the service evaluation. Data from starch loading tests and clinic reviews were collated from electronic patient records, medical and dietetics notes
Conclusion: Overall median fasting tolerance was 6 hrs on UCCS and 8 hrs on Glycosade. Most of the effect was observed in Type Ia. There were no remarkable improvements in growth; however 3 patients reduced their weight. 5 out of 9 patients who changed to Glycosade reported improvements in appetite. The lactate profiles of Type I patients taking Glycosade revealed higher mean profiles $\geq$ 4 hours fasting compared to UCCS. However in Type III patients fasting free fatty acids were comparable using both starches.

197-P

IMPROVING GALACTOSEMIA SCREENING BY DECREASING THE FALSE POSITIVE RECALL RATE: THE SWEDISH EXPERIENCE

Ohlsson A[1], Guthenberg C[1], von Dobeln U[1]
[1]*Centre of Inherited Metabolic Diseases, Stockholm, Sweden*

Background: High rates of false positive cases of galactose-1-phosphate uridyltransferase deficiency (GALT-deficiency) are common in newborn screening programmes. This results in high costs for follow up and anxiety for parents. To minimize this we have developed a rapid, simple and cheap method to verify positive cases of classical galactosemia. All samples with less that 15% activity in the Beutler-test are tested for elevated galactose levels using the rapid GAL-DH-test. It is ready within an hour and the patients can thus still be recalled urgently.
Method: The rapid GAL-DH-test is a fluorometric assay where galactose can be approximated. Galactose dehydrogenase (GAL-DH) catalyses the oxidation of galactose and the reduction of NAD+ to NADH. The amount of NADH is proportional to the amount of galactose. The test is considered positive for galactose if NADH is produced, as demonstrated by fluorescence under ultraviolet light.
Results: In 1,522,291 screened newborns between the years 1992–2008, 22 newborns were recalled and 18 cases of GALT-deficiency were confirmed. No missed cases have been reported.
Conclusions: The rapid GAL-DH-test has been in use for almost 20 years at the Swedish newborn screening laboratory. Since the introduction of it in combination with a cut off value of less than 15% for the Beutler test we have had less than one false positive case for every true positive case of classical galactosemia. All confirmed cases have had an activity of less than 10% in the Beutler-test. This 2-tier approach gives a negligible false positive rate at a low cost.

198-P

TRANSALDOLASE DEFICIENCY: 4 NEW PATIENTS AND NEW PATHOPHYSIOLOGICAL INSIGHTS ON THE PENTOSE-PHOSPHATE PATHWAY

Valayannopoulos V[1], Rio M[2], Wamelink M[3], Rabier D[4], Ottolenghi C[4], Salomons G[3], Habes D[5], Jacqmain E[5], Bernard O[5], de Lonlay P[1], Jakobs C[3]
[1]*Ref Center IEM, Necker Enfants-Malades H, Paris, France*
[2]*Genetics Dep, Necker-Enfants Malades Hos, Paris, France*
[3]*Clin Chem Dep, VU Univ Med Center, Amsterdam, Netherlands*
[4]*Biochem Lab, Necker Enfants Malades Hosp, Paris, France*
[5]*Ped Hepatology, Bicetre Hosp, Le Kremlin-Bicêtre, France*

Background: Transaldolase deficiency, an inborn error of the pentose phosphate pathway has been diagnosed so far in 10 patients from 6 families. All presented as neonates, or even prenatally, with liver disease and the clinical courses have been diverse.
Patients: We present 4 new patients from 2 families of African origin. The 2 siblings from the first family presented with low birth weight, cutis laxa, anemia, thrombopenia, cholestasis, elevated transaminases and liver failure. The 2 siblings from the second family presented with hepatosplenomegaly, elevated transaminases and failure to thrive within the first months of life. All patients developed progressive liver failure and cirrhosis at various ages.
Methods and Results: Elevation of erythritol, arabitol and ribitol, sedoheptulose and sedoheptulose-7 phosphate were found in all patients' urine. Enzyme studies and molecular investigations confirmed the diagnosis. In one patient, respiratory chain complex I deficiency was found in liver and fibroblasts.
Conclusions: The new patients confirm that TALDO deficiency is panethnic and the constant hallmarks include liver impairment with cirrhosis associated to hematological abnormalities and abnormal polyols in urine. The respiratory chain findings in the liver of one of our patients may suggest novel mechanisms for liver damage in TALDO deficiency that has to be confirmed in other patients.

199-P

TRANSFERRIN ISOELECTRIC FOCUSING AND PLASMA LYSOSOMAL ENZYME ACTIVITIES IN THE DIAGNOSIS AND FOLLOW UP OF FRUCTOSEMIA

Michelakakis H[1], Dimitriou E.[1], Mavridou I.[1], Georgouli H.[2], Ploski R.[3], Pollak A.[4], Moraitou M.[1]
[1]*1 Dept. of Enzymol and Cellul Function, Athens, Greece*
[2]*2nd Dept. Ped. Med. School Univ. Athens, Athens, Greece*
[3]*Dept. Med. Gen., Warsaw Medical Univ., Warsaw, Poland*
[4]*Instit. Physiol.-Pathol. of Hearing, Warsaw, Poland*

Glycoprotein hypoglycosylation and increased plasma lysosomal enzyme activities are described in hereditary fructose intolerance (HFI). We present the TfIEF and plasma lysosomal enzyme activities on diagnosis and follow up of two HFI patients (A, B), confirmed through DNA analysis. In both, a TfIEF type I CDG pattern was found associated with increased aspartylglucosaminidase and β-hexosaminidase activities, whereas a-mannosidase and β-mannosidase activities were at the normal range. In patient A, 25 and 46 days of partial fructose restriction led to gradual improvement of TfIEF, normalization of aspartylglucosaminidase and β-hexosaminidase activities, clinical improvement, normalization of transaminases and reduction in proteinuria. TfIEF and proteinuria normalized and enzyme activities were further reduced 1 month after total fructose restriction. In patient B, complete normalization of all laboratory and clinical parameters was observed following 20 days of fructose restriction.
Thus, although both abnormal TfIEF pattern and plasma lysosomal enzyme activities are observed in HFI, they respond differently to fructose restriction. TfIEF appears to be a better indicator of compliance.
The study was supported by the European Program: Euroglycanet LSHM – CT – 2005 – 512131.

200-P

PROTEOMIC ANALYSIS REVEALS POTENTIAL ALTERNATIVE MARKER PROTEINS FOR THE DIAGNOSIS OF CONGENITAL DISORDERS OF GLYCOSYLATION TYPES I AND II

Heywood WE[1], Mills KM[1], Yuzugulen J[1], Worthington V[2], Wang D[1], Mills PB[1], Burke D[3], Clayton PT[1], Grunewald S[3]
[1]*Institute of Child Health, UCL, London, United Kingdom*
[2]*Institute of Neurology, UCL, London, United Kingdom*
[3]*Great Ormond Street Hospital, London, United Kingdom*

N-glycosylation disorders are diagnosed typically by isoelectric focusing (IEF) of the plasma protein transferrin. However, there are limitations to this screening test as certain CDG subtypes might not result in abnormal IEF pattern and CDG patients with confirmed enzyme deficiencies have been missed. Therefore we have attempted to address this problem using 2D Differential Gel Electrophoresis (DIGE) and label free quantitative proteomics to look for more informative biomarkers for the diagnosis of N-glycosylation and combined N- and O glycosylation disorders.
In order to see the less abundant plasma proteins, albumin and IgG were removed using a combination of affinity and chemical chromatography. Using 2D DiGE, confirmed patients previously grouped as CDG I and II, demonstrated specific patterns in alpha-1- antitrypsin which showed altered mass and charge isoforms in CDG I cases and only isoform shifts in CDG II cases. Other proteins that showed an altered shift and mass profile were alpha-1 anti-chymotrypsin and alpha 2 HS glycoprotein (fetuin). Further analysis at the lower pH end also revealed altered profiles for alpha 1 acid glycoprotein 1 and plasma protease C1 inhibitor. However the most overwhelming change was seen by ceruloplasmin which showed a profound shift in all CDG I cases and only a typical isoform shift in CDG II cases. 2D DiGE was also used to investigate some suspected CDG cases with normal looking transferrin patterns. Altered profiles not fitting typical CDG patterns were observed and are being investigated further using label free quantitative proteomics.

201-P

THE MOLECULAR LANDSCAPE OF PHOSPHOMANNOMUTASE DEFICIENCY IN IBERIAN PENINSULA: IDENTIFICATION OF FOURTEEN POPULATION SPECIFIC MUTATIONS

Pérez B[1], Briones P[2], Quelhas D[3], Artuch R[4], Matthijs G[5], Ecay MJ[1], Vega AI[1], Gort L[6], Ugarte M[1], Pérez-Cerdá C[1]
[1]*CEDEM. CBM-SO. UAM. CIBERER, Madrid, Spain*
[2]*IBC/CDB, Hosp Clinic. CSIC. CIBERER, Barcelona, Spain*
[3]*Medical Genetics Center, Porto, Portugal*
[4]*Hosp San Joan de Deu. CIBERER, Barcelona, Spain*
[5]*Center for Human Genetics, Leuven, Belgium*
[6]*IBC/CDB, Hosp Clinic. CIBERER, Barcelona, Spain*

PMM2-CDG is an autosomal recessive disorder and the most frequent form of congenital disorder of N-glycosylation, with more than one hundred mutations identified to date. Sixty-seven unrelated patients from 59 families were diagnosed as PMM2-CDG (CDG-Ia) based on clinical signs or because of a previous affected sibling. They all presented an altered type 1 serum transferrin isoform pattern and in most cases the disease was confirmed by determining PMM activity in fibroblasts and/or lymphocytes. Residual PMM activity in fibroblasts ranged from not detectable to 60% of the mean controls. DNA and RNA were isolated from fresh blood or fibroblasts from patients to perform molecular studies of the PMM2 gene, resulting in the identification of thirty different mutations, three of them newly reported here (p.Y102C, p.P184D and p.D209G). From these 30 mutations, 14 have only been identified among Iberian PMM2-CDG patients. As in other Caucasian populations, p.R141H was the most frequent mutation (24 alleles, prevalence 20%), but less than in other European series in which this mutation represents 35–43% of the disease alleles. The next frequent mutations were pD65Y (12 alleles, prevalence 10%) and p.T237M (9 alleles, prevalence 7.6%), while p.F119L and p.E139K, the most frequent changes in Scandinavian and French populations respectively, were not found in our patients. The most common genotype was p.R141H/p.T237M, and only three homozygous patients, for p.D65Y, p.P113L and p.T237M, were detected. The broad mutational spectrum and the diversity of phenotypes found in the Iberian populations hamper genotype-phenotype correlation.

202-P

ADVANCES IN THE DIAGNOSIS OF CONGENITAL DISORDERS OF GLYCOSYLATION

Porta F[1], Turgeon C[1], Tortorelli S[1], Gavrilov D[1], Oglesbee D[1], Rinaldo P[1], Matern D[1], Raymond K[1]
[1]*BGL, Mayo Clinic College of Medicine, Rochester, MN, United States*

Background: Congenital disorders of glycosylation (CDG) are caused by defects in the assembly of the dolichol-linked oligosaccharide precursor (CDG I) or in the processing of protein N-linked glycan (CDG II), distinguished by the analysis of serum sialotransferrin.
Disorders of the processing pathway can also affect the core-1-mucyn type O-glycosylation, as revealed by the isoelectric focusing of apolipoprotein C-III (Apo C-III) in CDG IIe (COG7 deficiency) and autosomal recessive cutis laxa type 2 (ARCL2).
Objectives: To develop an online immunoaffinity liquid chromatography-mass spectrometry (LC-MS) method for serum Apo C-III isoforms.
Material and Methods: Serum (100 microl) was diluted 1:1 with water before application to an immunoaffinity column that sequestered Apo C-III isoforms before being eluted, concentrated and introduced into the MS. Analysis of the Apo C-III isoforms was entirely automated and completed in less than 10 min per sample.
Results: Reference ranges for the relative ratios among the possible Apo C-III isoforms were determined by analysis of 104 samples (a-/di-oligosaccharide: median=0.14, 99%ile=0.44; mono-/di-oligosaccharide: median=1.48, 99% ile=2.68). The Apo C-III analyses in two patients with confirmed defects of N- and O-glycosylation were clearly abnormal (COG7 deficiency: a-/di-oligosaccharide=0.84; ARCL2: mono-/di-oligosaccharide=3.65).
Conclusion: The LC-MS method for Apo C-III represents a sensitive and rapid approach to the diagnosis of the core-1-mucyn type O-glycosylation disorders. Its combination with LC-MS analysis of serum sialotransferrin improves the diagnosis and differentiation of CDG.

203-P

MILD CLINICAL AND BIOCHEMICAL PHENOTYPES IN 2 PATIENTS WITH CDG-IA

Casado M[1], Gort L[1], Montero R[1], Quintana E[2], Coll MJ[2], Perez-Cerdà C[3], Pérez B[3], Pineda M[1], Briones P[1], Artuch R[1]
[1]*Hospital Sant Joan de Déu, Barcelona, Spain*
[2]*Institut de Bioquímica Clínica, Barcelona, Spain*
[3]*Centro Diagn Enf Metabólicas, Madrid, Spain*

Aim: to report on 2 pediatric patients with genetic diagnosis of CDG-Ia presenting with very mild clinical and biochemical phenotypes.
Patients and Methods: Case 1 is the first child of healthy non consanguineous parents. At 9 months of age she presented psychomotor developmental delay corresponding to a 4-month-old child. Tremor and problems in manipulation were observed. Physical examination showed hypotonia with normal reflexes and lipodystrophy on her buttocks. Somatometry was normal, including cranial circumference. In the ophthalmological exploration neither strabismus nor retinopathy were evidenced. Routine biochemical parameters were normal including blood count, clotting factors, proteins thyroid hormones, etc. The pattern of transferrin by capillary zone electrophoresis showed a very slightly increased disialotransferrin value. PMM activity was on the low-normal range but mutational study showed p.R123Q and p.C241S mutations in PMM2 gene. Case 2 is also the first child of healthy non consanguineous parents. At 8 months of age psychomotor delay was observed. Hypotonia, convergent bilateral strabismus, tremor and lypodystrophy on buttocks were evident. Somatometry was normal, including cranial circumference. At the age of 16 months the MRI evidenced vermix cerebellar hypoplasia. In the first study of transferrin isoelectrofocusing, the pattern was normal, but slightly impaired by capillary zone electrophoresis and HPLC. Genetic analysis of PMM2 revealed p.F157S and p.C241S mutations.
Conclusions: Although the clinical phenotype was mild in both cases, it was indicative for CDG screening. However, the biochemical results, especially transferrin isoelectrofocusing pattern might have missed the diagnosis. Tremor was a remarkable clinical feature in both cases.

204-O

CLINICAL CHARACTERIZATION OF A NOVEL CONGENITAL DISORDER OF GLYCOSYLATION (DPM2 MUTATION)

Barone R[1], Race V[2], Sturiale L[3], Bammens R[2], Vleugels W[2], Keldermans L[2], Garozzo D[3], Foulquier F[4], Jaeken J[5], Sorge G[1], Fiumara A[1], Matthijs G[2]
[1]*Dept Pediatrics Univ Catania, Catania, Italy*
[2]*Centre for Human Genetics, Leuven, Belgium*
[3]*ICTP, National Res Council, Catania, Italy*
[4]*Glycobiologie UMR/CNRS, Lille, France*
[5]*Dept Pediatrics Univ Leuven, Leuven, Belgium*

Background: The human dolichol-phosphate-mannose (DPM) synthase is a heterotrimeric complex composed of DPM1, DPM2 and DPM3. Only mutations in DPM1 (the catalytic subunit) and more recently in DPM3 have been described.
Case Report: The girl was born to unrelated parents at the 32th week of gestation complicated by polyhydramnios. At birth she had normal growth parameters, severe hypotonia and dysmorphism. At six months, microcephaly (OFC: 33.5 cm), keel thorax, widely spaced nipples and joint flexion contractures were seen. Repeated laboratory investigations showed elevation of serum transaminases and CPK levels and coagulopathy. At age 20 months there were severe hypotonia, no head control, reduced active limb movements with normal osteotendineous reflexes. She experienced pharmacoresistant generalized tonic seizures and myoclonic jerks. Funduscopy showed optic atrophy. Brain MRI documented loss of cerebral white matter, without cerebellar atrophy. She died at age 36 months from pneumonia. Serum Transferrin (Tf) IEF showed overt increase of disialo-Tf and a very faint asialo-Tf band (type I pattern). PMM and PMI enzymes were normal. MALDI-MS of serum Tf showed di-glycosylated Tf and an additional glycoform owing to mono-glycosylated Tf. Unlike CDG-Ia (PMM deficiency), no a-glycosylated Tf species were detectable. Lipid-linked oligosaccharides analyses showed an accumulation of dol-PP-GlcNAc2-Man5. No mutations in DPM1, ALG3 or MPDU1 were detected. The patient was compound heterozygote for a splice mutation (c.4–1G>C) and a missense mutation (c.68A>G, p.Y23C) in the DPM2 gene.
Conclusions: Prominent neurological involvement, dysmorphism and fatal outcome were hallmark clinical features in the first CDG patient with DPM2 mutation.

205-P

CLINICAL TESTING FOR CONGENITAL DISORDERS OF GLYCOSYLATION (CDG): THE MAYO CLINIC EXPERIENCE

Raymond K[1], Grycki E[1], Minich S[1], Oglesbee D[1], Tortorelli S[1], Gavrilov D[1], Rinaldo P[1], Matern D[1]
[1]*Mayo Clinic, Rochester, MN, United States*

Background: Since 2000, our laboratory replaced traditional transferrin analysis by isoelectric focusing (IEF) with an immunoaffinity liquid chromatography mass spectrometry (LC-MS) method. Transferrin isoforms are sequestered on immunoaffinity and concentration on C4 column, and then eluted directly into the electrospray ion source of mass spectrometer. The advantages of the LC-MS approach over IEF include minimal sample requirements (5 μL), preparation ('dilute-and-shoot'), and analytical time 9 min). This allows for high throughput and rapid turn-around-time.
Results: During 5 years, total of 20,479 samples were analyzed from patients younger than 21 years old. And 769 (3.8%) had abnormal transferrin profile. Clinical follow up was obtainable for a cohort of 77 patients during the past 18 months. CDG type Ia was most frequent. Four adults' cases, 2 patients with a combined Type I/Type II profile, and 12 cases with increased trisialotransferrin were identified.
Conclusion: CDG type Ia was most frequently found. Clinically enzymatic confirmation of CDGs is currently limited to types Ia and Ib, while confirmation of other CDGs remains challenging. Among those are cases with transferrin isoforms missing one sialic acid moiety. Collaboration between clinical, diagnostic, and research centers is essential to improve test interpretation and implementation of research assays in clinical laboratories.

206-P

IDENTIFICATION OF NINE NOVEL EXT1 AND EXT2 MUTATIONS IN PORTUGUESE PATIENTS WITH HEREDITARY MULTIPLE OSTEOCHONDROMAS

Nogueira C[1], Matos G[2], Carvalho Barros S[1], Santos M[3], Vilarinho L[1]
[1]*Centro de Genética Médica, INSA, Porto, Portugal*
[2]*Centro Hospitalar de Coimbra, Coimbra, Portugal*
[3]*Centro Hospitalar de Vila Nova de Gaia, Porto, Portugal*

Hereditary multiple osteochondromas (HMO), a congenital disorder of glycosylation (CDG) that affects the synthesis of xylosylglycan, is an autosomal dominant disorder characterized by growths of multiple osteochondromas, benign cartilage-capped bone tumors. Mutations in EXT1 and EXT2 genes are most frequently associated with HMO. Osteocondromas are rarely present at birth, but gradually arise and increase in size in the first decade of life ceasing to grow at skeletal maturation. The risk for malignant degeneration to osteochondrosarcoma increases with age, although the lifetime risk of malignant degeneration is low (~1%). The prevalence is estimated at 1:50,000.
In this study, we investigated the molecular defects in 14 Portuguese patients with HMO, seven male and seven female, by direct sequencing analysis of EXT1 and EXT2 genes. We found disease causing mutation in 12 patients (86%), eight of which (57%) have EXT1 mutations and four (29%) EXT2 mutations. Of the eleven identified mutations, two were already described and nine were novel: seven in EXT1 gene (six causing frameshift and one missense mutation) and two in EXT2 gene (one nonsense and one splicing mutation).
In summary, this is the first molecular HMO study in Portugal, describing the genetic background of the most prevalent type of CDG. No mutational hotspots were found highlighting the genetic heterogeneity of these patients. Our date corroborate the importance of a molecular testing to confirm HMO patients and allows genetic counseling to couples at high risk of having affected children.

207-O

CDG-II CAUSED BY MUTATIONS IN A NOVEL GENE PROBABLY ASSOCIATED WITH GOLGI FUNCTION

Zeevaert R[1], Foulquier F[2], Amyere M[3], Schollen E[4], Van Schaftingen E[5], Vikkula M[3], Matthijs G[4], Jaeken J[1]
[1]Dep Pediatrics, Univ Hosp, Leuven, Belgium
[2]Struct and Funct Glycobiol, Univ, Lille, France
[3]Hum Mol Genet, De Duve Inst, Brussels, Belgium
[4]Cent Hum Genet, Univ Hosp, Leuven, Belgium
[5]Physiol Chem, De Duve Inst, Brussels, Belgium

Congenital Disorders of Glycosylation (CDG) are a group of genetic, mostly multisystem diseases caused by defects in the biosynthesis of the glycan moiety of glycoconjugates. Apart from defects in the specific Golgi glycosylation machinery, CDG-II can be caused by an aberrant Golgi structure and/or function.
Combining data from homozygosity mapping and expression profiling in a Jewish family with two siblings with CDG-IIx, we identified a mutation in TPARL, a gene with unknown function. The mutation disrupts normal splicing and leads to an alternative transcript with a frameshift and premature stop codon.
In a group of unsolved CDG-II patients we identified an unrelated patient with the same mutation and a similar phenotype including dwarfism with epi- and metaphyseal bone dysplasia and osteoporosis, facial dysmorphia, psychomotor retardation, feeding problems, hypotonia, muscle weakness and hepatomegaly. Serum transaminases (GOT>GPT) and creatine kinase were moderately elevated. A fourth patient showed a different homozygous mutation and a much milder phenotype (no dwarfism, no hepatomegaly, less psychomotor retardation).
Preliminary studies suggest that gene product has a role in Golgi pH homeostasis.

208-P

CONGENITAL DEFECT OF O-GLYCOSYLATION IN MULTIPLE HEREDITARY EXOSTOSES: FIRST STUDY IN A COHORT OF LATIN AMERICAN PATIENTS

Delgado MA[1], Sarrión P[2], Azar N[1], Zecchini L[3], Bistué Millón MB[1], Chiesa M[4], Robledo H[4], Dodelson de Kremer R[1], Ballcels S[2], Grinberg D[2], Asteggiano CG[1]
[1]CEMECO, Hosp Niños ST, UNC, Córdoba, Argentina
[2]Dpto Genética, Fac Biol, Univ Barc, Barcelona, Spain
[3]Serv Traum, Hosp Niños ST, UNC, Córdoba, Argentina
[4]Serv Bioimag, Hosp Niños, UNC, Córdoba, Argentina

Multiple Hereditary Exostoses (MHE) is an autosomal dominant disorder characterized by the formation of cartilaginous bone tumors (osteochondromas) at multiple sites. The most severe complication is malignant transformation to chondrosarcoma. Defects result from mutations in EXT1(8q24) or EXT2 (11p11.2) genes, which encode glycosyltransferases necessary for the synthesis of heparan sulfate proteoglycans (HSPGs). This study represents the first investigation of the genotype-phenotype correlation in Latin American patients.
We have studied 25 MHE patients with ages ranging from 2 to 50 years old. PCR and direct sequencing of both genes were performed after the written consents. Clinical assessments and functional rating were analyzed (Musculoskeletal Tumor Society Scale). A total of 10 exonic changes were identified. Six were novel mutations in EXT1 (p.Leu251Stop/p.Leu283Stop/p.Arg346Thr/p.Lys126AsnfsX62/p.Lys306Stop/p.Leu264Pro) and two in EXT2 (p.Trp393Stop/p.Asp307ValVfsX45). Two mutations were previously described in EXT1 (p.Leu490ArgfsX9 and p.Val78ArgfsX111) (http://medgen.ua.ac.be/LOVD/home.ph). Missense novel mutations were analyzed by prediction programs.
Clinical study: 8 patients presented a severe phenotype ranging from IS to IVS and 2 a mild one. A novel mutation (p.Leu283Stop) was associated with a malignant transformation to chondrosarcoma. We could not find a correlation between EXT1 and EXT2 genes with the grade of severity.
Our results identified novel mutations in a first cohort of Latin American patients. Preliminary analysis indicated that mutations on the first EXT1 exons correlate with a more severe phenotype. This interdisciplinary study represents the first genotype-phenotype investigation in Argentina and its progression will provide a wider vision of this pathology in our country and Latin America. CONICET/FONCYT-PICT2350/UCC.

209-P

A NEW B4GALT1-CDG PATIENT IDENTIFIED BY SERUM N-GLYCAN PROFILING BY MASS SPECTROMETRY

van Scherpenzeel M[1], Guillard M[1], Morava M[1], Bodamer O[2], Wevers RA[1], Lefeber DJ[1]
[1]Radboud Univ. Nijmegen Medical Centre, Nijmegen, Netherlands
[2]Universitatsklinik Kind.- Jugenheilkunde, Vienna, Austria

Congenital Disorders of Glycosylation (CDG) type II refer to defects in the processing of protein-linked glycans, mainly in the Golgi. Known defects include deficiencies in nucleotide sugar transporters, glycosyltransferases and Golgi trafficking complexes such as the COG complex or the Golgi based V-ATPase. The large number of candidate genes and the presence of secondary causes such as serum sialidase, complicate the identification of genetic defects. Nevertheless, this is of great importance for genetic counseling and to understand pathomechanisms.
We have applied MALDI-Ion Trap analysis for automated measurement and MSn sequencing of serum N-glycans of patients with known and unknown CDG type II defect and patients with a secondary cause. The contribution of mass spectrometry for the identification of primary genetic defects was exemplified by the identification of a second B4GALT1-CDG (CDG-IId) patient. This patient presented with recurrent episodes of diarrhea, mild hepatomegaly, short stature and dysmorphic facial features, yet her psychomotor development was age appropriate. Mass spectrometry revealed a dramatic increase of undergalactosylated N-glycans in serum, in accordance with a galactosyltransferase deficiency, as seen in B4GALT1-CDG. Mutational analysis confirmed this diagnosis. Additional investigations, including galactosyltransferase activity measurement and mass spectrometry of cellular glycans are in progress.

210-O

FIRST REPORT OF A MITOCHONDRIAL ENCEPHALOPATHY ASSOCIATED TO SDHD MUTATIONS

Hahn D.[1], Schaller A.[2], Jackson C.B.[2], Häberli A.[1], Gallati S.[2], Vella S.[3], Nuoffer J.M.[1]
[1]Inst. of Clinical Chemistry, Inselspital, Bern, Switzerland
[2]Division of Human Genetics, Inselspital, Bern, Switzerland
[3]Lindenhofspital, Bern, Switzerland

Background: Defects of the mitochondrial respiratory complex II (succinate dehydrogenase, SDH) are extremely rare. Of the four nuclear encoded proteins composing complex II, only mutations in the 70 kDa Flavoprotein (SDHA) and the recently identified complex II assembly factor (SDHAF1) have been found to be causative of a mitochondrial disorder. Mutations in the other three subunits (SDHB, SDHC, SDHD) and the second assembly factor (SDHAF2) have so far only been associated with hereditary paragangliomas and phaeochromocytomas.
Methods and Results: Here we report the first ever described case of a mutated subunit other than SDHA and SDHAF1 causing a mitochondrial disorder with an isolated complex II deficiency. The patient showed a progressive retardation after the age of six months evolving in a severe encephalopathy with choreo-athetotic movements, optic atrophy and intractable myoclonic seizures at the age of eight years. MRI and MRS were normal at the age of ten months. Complex II had a residual activity of 10% in muscle. Analysis by comparative 2D BN-PAGE following MALDI-TOF MS demonstrated the absence of SDHA. Further western blot analysis confirmed reduced expression of SDHA and SDHB in skeletal muscle. Molecular genetic analysis of SDHA, SDHB, SDHC, SDHD, SDHAF1 and SDHAF2 revealed compound heterozygosity for two mutations located in a transmembrane domain of SDHD.
Conclusion: We conclude that the mutations of the SDHD-gene result in abolition of its protein to integrate into the membrane impairing all other subunits to assemble into a functional SDH-complex and are subjected to degradation.

211-P

CLINICAL AND LABORATORY DATA IN 75 CHILDREN WITH NEONATAL ONSET OF MITOCHONDRIAL DISORDERS: PROPOSAL OF DIAGNOSTIC ALGORITHM

Honzik T.[1], Tesarova M.[1], Hansikova H.[1], Jesina P.[1], Magner M.[1], Szentivanyi K.[1], Langer J.[1], Zeman J.[1]
[1]*Dep.Pediatrics, Charles University, Prague, Czech Republic*

Mitochondrial disorders (MD) can present at any age. In our group of 357 patients with MD confirmed on enzymatic and/or molecular level, the first clinical symptoms developed in 75 patients (21%) in neonatal period. The aim of our study was to analyze the clinical and laboratory data in these 75 children and to suggest algorithms for the diagnosis of MD with neonatal onset.

Results: prematurity (36% of children) and intrauterine growth retardation (40%) were common. Hypotonia was present in most of neonates (95%) and the ventilation support was necessary in 66%. Apathy, feeding refusal and failure to thrive was most common in non-ventilated neonates. Hypertrophic cardiomyopathy was observed in 50%, seizures in 24% and Leigh syndrome in 11% of neonates. The prognosis was not favorable, one third of children died in the first three months of life. The lactic acidosis was present in 93%, the increased creatine kinase activity in 28% and increased excretion of the Krebs cycle intermediates in 75% of neonates. Hyperammonemia was recognized in 22% of patients. All newborns with Tmem70 protein deficit exhibited increased urinary excretion of 3-methylglutaconate.

Conclusion: Mitochondrial disorders often manifest in newborns. The diagnostic algorithm for a critically ill newborn with suspicion of MD was suggested. This algorithm enables to indicate direct enzymatic or mutation analysis with no need of muscle biopsy in cases such as Alpers, Barth, NARP and Pearson syndromes and PDH, Sco1, Sco2 and Tmem70 deficiencies.

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212-P

SPINOCEREBELLAR ATAXIA WITH HYPOGONADISM: AN INTRIGUING GROUP OF GENETIC DISORDERS

Lourenco CM[1], Sobreira C[1], Barreira A[1], Marques Jr W[1]
[1]*Neurogenetics Unit, Univ of Sao Paulo, Ribeirao Preto, Brazil*

Background: The association between cerebellar ataxia and hypogonadism was first described in four sibs by Holmes, and has become known as Holmes type ataxia. Several syndromes with hypo/hypergonadotrophic hypogonadism and ataxia have been published; however there is a remarkable clinical heterogeneity among them.

Objectives: To present the clinical data and molecular/biochemical studies of fifteen Brazilian patients with cerebellar ataxia and hypogonadism.

Methods: All patients were evaluated by geneticists and neurologists. MRI, ophtalmological exam, EMG/NCV, biochemical tests, screening for CDGs, karyotype, muscle biopsy with chain respiratory enzyme assays and measurement of coenzyme Q10, molecular tests for Friedreich ataxia and for SCAs 1,2,3,6 and 7 were performed in the course of the investigation.

Results: All patients had cerebellar ataxia, but the age of the onset was variable; ten patients had early onset ataxia. Mental retardation was seen in two unrelated girls with hypergonadotropic hypogonadism. None of the patients had chromosomal anomalies. Molecular tests for Friedreich and SCAs were all negative. Optic atrophy and retinochoroidal degeneration were found in five patients; axonal neuropathy was present in four patients. In two patients, coenzyme Q10 deficiency was confirmed in muscle biopsy.

Conclusions: One family has features consistent with a rare neurological disorder, Boucher-Neuhauser syndrome; two other unrelated patients had coenzyme Q10 deficiency; CDG Ia was identified in one adult patient. The remaining patients had features that may fit in the Gordon-Holmes phenotype although we believe this entity should not be a homogenous disorder.

213-P

CLINICAL AND MOLECULAR FEATURES OF 3 FILIPINO PATIENTS WITH MITOCHONDRIAL RESPIRATORY CHAIN DISORDER

Chiong M.A.[1], Abacan M.A[2], Padilla C.D.[1]
[1]*Insitute of Human Genetics, NIH, UP Mla, Manila, Philippines*
[2]*Philippine General Hospital, UP Mla, Manila, Philippines*

Background: The mitochondrion, being the powerhouse of the cell is the organelle which supports aerobic respiration and provides energy for metabolic pathways. Mitochondrial disorders greatly involve high rate aerobic metabolism in most organs, specifically the brain, heart and skeletal muscles. Here we describe 3 Filipino children confirmed to have a mitochondrial respiratory chain disorder after presenting with non-specific neurologic symptoms.

Case report: The first patient, a 3 month old female, had Otahara syndrome and was later found to have complex I deficiency. The second was a 4 month old female who had a history of developmental delay and metabolic acidosis. She was found to have the m.8993 T>G mtDNA mutation consistent with either a Leigh or a NARP phenotype. The third patient was a 2 year old female who had developmental delay then presented with sudden onset of right sided hemiplegia and lactic acidosis. The diagnosis of MELAS was confirmed by mutation analysis on blood and hair which showed the typical mtDNA A3243G mutation.

Discussion/Conclusion: Mitochondrial disorders are a genetically, biochemically, and clinically heterogenous group of disorders and this characteristic was seen in our 3 patients. All of them presented with a neurologic disorder of different types and severity. The first patient had severe complex 1 deficiency leading to early death while patients 2 and 3 had made encouraging progress after decompensation although their clinical prognosis remains uncertain. Treatment is symptomatic and further investigations have to be done on the family members so that proper genetic counseling can be addressed.

214-P

OXIDATIVE STRESS AND ANTIOXIDANT DEFENCE ACCOMPANIED BY MITOCHONDRIAL COMPLEX I INHIBITION FOLLOWING SEIZURES INDUCED IN ANIMAL MODEL BY HOMOCYSTEIC ACID

Jesina P[1], Folbergrova J[2], Haugvicova R[2], Houstek J[2]
[1]*Dept.of Pediatrics, 1st Med Fac, Prague, Czech Republic*
[2]*Inst. of Physiol, Acad of Sciences, Prague, Czech Republic*

We have demonstrated recently mitochondrial complex I inhibition in cerebral cortex of immature rats during seizures induced by homocysteic acid (HCA) and its persistence during long periods of survival following these seizures. Inhibition of complex I activity was substantially reduced by selected free radical scavengers. Furthermore, it was accompanied by significant increases of mitochondrial markers of oxidative damage, 3-nitrotyrosine, 4-hydroxynonenal and protein carbonyls, indicating the presence of oxidative stress in HCA-treated animals. It was of interest to examine antioxidant defense mechanisms under these conditions.

Seizures were induced by bilateral icv infusion of DL-HCA in 12-day-old male Wistar rats. During acute phase of seizures and at desired times of survival after seizures (20 h, 6 days, 3 and 5 weeks, respectively), mitochondria were isolated from the cerebral cortex of animals for determination of complex I activity, MnSOD, Cu/ZnSOD and catalase content (by SDS-electrophoresis/WB analysis).

The results have revealed increased SOD activity (by ~ 20%) at 20 h and 6 days following these seizures. At the same time periods, there was also an upregulation of MnSOD content. Cu/ZnSOD and catalase levels did not significantly differ from the appropriate controls at none of the studied periods.

The present results suggest that the defence of mitochondria from immature HCA-treated animals against oxidative stress is apparently limited and can contribute to neuronal injury demonstrated in this model of seizures.

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215-P

TOWARDS THE GENETIC DEFECT IN MEGDEL-SYNDROME: FOUR NOVEL PATIENTS

Wortmann SB[1], Rodenburg RJ[2], de Brouwer APM[3], Kalkan Ucar S[4], Coker M[4], Baric I[5], Kluijtmans LAJ[2], Engelke UFH[2], Smeitink JAM[1], Wevers RA[2], Morava E[1]
[1]*IGMD, Dep of Ped, RUNMC, Nijmegen, Netherlands*
[2]*Dep of Lab Med, RUNMC, Nijmegen, Netherlands*
[3]*Dep of Hum Gen, RUNMC, Nijmegen, Netherlands*
[4]*Dep of Ped, Ege Univ, Izmir, Turkey*
[5]*Dep of Ped, Div Hum Gen, Univ Hosp, Zagreb, Croatia*

Recently we defined MEGDEL syndrome as 3-MEthylGlutaconic aciduria type IV with sensori-neural Deafness, Encephalopathy, and Leigh-like syndrome. In addition to the four original patients, we found four new patients with the same distinctive phenotype in Pakistan, Croatia, Turkey and the Netherlands. In all eight patients (four girls, four boys) 3-methylglutaconic aciduria type I, II, III, V and ATP-ase deficiency were excluded. The children presented with characteristic association of 3-methylglutaconic aciduria (25–196 mmol/mol creatinine), sensori-neural deafness and neuro-radiological evidence of Leigh disease. They also had neonatal hypotonia, recurrent lactic acidemia and hypoglycemia, severe recurrent infections, feeding difficulties, failure to thrive, developmental delay, and progressive spasticity with extrapyramidal symptoms. One showed liver involvement. Muscle biopsy revealed OXPHOS dysfunction in six patients. Homozygosity mapping was performed in three unrelated children from consanguineous families. The largest overlapping homozygous region was 4.6 Mb on chromosome 2 and contained promising candidate genes, which we are now analyzing. In another patient we found a complex chromosome aberration. Although MEGDEL may be genetically heterogeneous further patients from a consanguineous background may help to unravel its genetic basis.

216-P

ELEVATED CSF-LACTATE IS A RELIABLE MARKER OF MITOCHONDRIAL DISORDERS IN CHILDREN EVEN AFTER BRIEF SEIZURES

Szentivanyi K.[1], Magner M.[1], Svandova I.[2], Jesina P.[1], Tesarova M.[1], Langer J.[1], Honzik T.[1], Zeman J.[1]
[1]*Dep.Pediatrics, Charles University, Prague, Czech Republic*
[2]*Dep.Physiology, Faculty of Science, Prague, Czech Republic*

Increased lactate is an important biochemical marker in diagnostics of children with suspicion of mitochondrial disorders. A diagnostic dilemma may originate if analyses are performed after seizures, when the increased lactate levels may be considered to result from the seizures. To address this problem, we ascertained the diagnostic value of lactate and alanine in blood (B) and cerebrospinal fluid (CSF) in children with mitochondrial disorders (n=24), epilepsy (n=32), psychomotor retardation (n=23), meningitis (n=12) and meningism (n=16).

Methods: Lactate concentration was measured using a spectrophotometric method. Amino acids in serum and CSF were analyzed by ion exchange chromatography with ninhydrin detection.

Results: Average blood and CSF lactate levels were significantly higher in children with mitochondrial disorders (3.87±0.48 and 4.43±0.55 mmol/L) and meningitis (2.77±0.45 and 8.58±1.08 mmol/L) than in children with epilepsy (1.72±0.13 and 1.62±0.04 mmol/L), psychomotor retardation (1.79±1.40 and 1.68±0.06 mmol/L) or meningism (1.70±0.13 and 1.64±0.07 mmol/L). Blood and CSF alanine levels were also higher in children with mitochondrial disorders (558±44 and 51±8 μmol/L) than in children with epilepsy (327±23 and 27±3 μmol/L) or psychomotor retardation (323±27 and 26±3 μmol/L). The CSF lactate levels of children with epilepsy were similar whether the samples were obtained 3±0.6 hours after an attack of brief seizures or from children without history of recent seizures.

Conclusion: Even in children who have recently suffered from short-lasting seizures, elevated cerebrospinal fluid lactate level is a reliable marker pointing to the mitochondrial origin of disease.

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217-P

LIVER DISEASE WITH MITOCHONDRIAL RESPIRATORY CHAIN DISORDER IN JAPAN

Fujinami A[1], Murayama K[1], Ajima M[1], Sanayama Y[1], Tsuruoka T[1], Yamazaki T[2], Harashima H[2], Mori M[3], Takayanagi M[1], Ohtake A[2]
[1]*Dep Metab Dis, Chiba Child Hosp, Chiba, Japan*
[2]*Dep Ped, Saitama Medical Univ, Saitama, Japan*
[3]*Dep Ped, Jichi Medical Univ, Tochigi, Japan*

Aim: Mitochondrial respiratory chain disorder (MRCD) is the common group of inborn errors of metabolism, affecting at least 1 in 5,000 individuals. Liver and the gastrointestinal tract are the major target organs in MRCD. The aim of the present study is to show the contribution of mitochondrial respiratory chain enzymes in liver disease in children.

Material and Methods: 267 patients were investigated, all with at least a possible diagnosis using the criteria of Bernier et al. (Neurology 59: 1406–1411, 2002)

Enzyme activities of individual respiratory chain complexes were measured in organ homogenates and mitochondrial fractions from fibroblasts. Western blotting after BN-PAGE was carried out using monoclonal antibodies specific for Complex I to IV subunits. Mitochondrial DNA and nuclear DNA copy numbers within tissues were determined by quantitative polymerase chain reaction.

Result: With both in vitro enzyme assay and BN-PAGE, 110 patients were diagnosed to have MRCD. 25 out of 110 patients have liver disorders as a major symptom. Complex I deficiency and multiple complexes deficiencies are highly associated with MtDNA depletion syndrome (MDS), and are most common among diagnosed MRCD. Twelve patients in 10 families were diagnosed to have hepatic MDS. Out of 12 hepatic MDS, we discovered nuclear gene mutations of DGUOK, POLG and MPV17 in 6 patients from 4 families.

Conclusion: Mitochondrial hepatopathy is extremely important in metabolic liver disease and should be diagnosed by enzymatic analysis of liver specimens. Furthermore, gene analysis will be a priority topic to be resolved in future.

218-P

GOOD CLINICAL OUTCOME IN TWO PATIENTS WITH COMPLEX I DEFICIENCY AFTER RIBOFLAVIN TREATMENT

Tricomi G[1], Lamantea E[1], Invernizzi F[1], Bizzi A[1], Zeviani M[1], Moroni I[1], Uziel G[1]
[1]*IRCCS Istituto Neurologico C. Besta, Milano, Italy*

Background: White matter involvement has been described in several mitochondrial disorders being associated with either mtDNA or nuclear DNA mutations.

Patients: We report two children with isolated complex I deficiency in muscle and fibroblasts and deep white matter involvement responding to riboflavin therapy. MRI and H-MRS revealed in both patients abnormal signal intensity of the deep white matter with rarefaction appearance, cystic-like lesions and a lactate peak. The first child, presented with a progressive spastic tetraparesis, language regression and dysphagia at the age of 14 months. She, now 5 years old, completely recovered after riboflavin administration and MRI greatly improved after two years. Compound heterozygosis for 2083 T>C and 2084A>G mutations were identified on NDUFS1 gene. The second child developed ataxia, dysarthria and language regression at 33 months of age; his recovery was complete after one month of riboflavin treatment. NDUFS1and mtDNA sequence were both normal, analysis of nuclear genes related to complex I deficiency has not yet been completed.

Conclusions: This report emphasizes the importance of MRI-based early diagnosis and highlights the beneficial effect of riboflavin at least in a subgroup of patients with Complex I deficiency.

219-P

REVERSIBLE SUCCINATE DEHYDROGENASE DEFICIENCY AFTER RHABDOMYOLYSIS IN ISCU MYOPATHY

Kollberg G[1], Melberg A[2], Holme E[1], Oldfors A[1]
[1]*Inst Biomed, Sahlgrenska Univ Hosp, Goteborg, Sweden*
[2]*Uppsala Univ Hosp, Uppsala, Sweden*

Patients with iron-sulphur cluster deficiency myopathy suffer from muscle fatigability, dyspnea, cardiac palpitations and episodic myoglobinuria. Morphological hallmarks in skeletal muscle tissue include deficiency of succinate dehydrogenase (SDH) and accumulation of iron within mitochondria. Biochemical investigations of the respiratory chain show complex I deficiency, SDH deficiency, aconitase deficieny as well as deficiency of other iron-sulphur proteins. Most patients are homozygous for a deep intronic IVS5+382 G>C mutation in ISCU, the gene encoding the iron-sulphur cluster assembly protein (IscU). The mutation causes alternative splicing of the pre-mRNA resulting in premature truncation and severely reduced levels of the IscU protein in muscle tissue. The mutation is not completely inactivating and both normal and aberrantly spliced mRNA is always present. Analysis of muscle tissue in a patient homozygous for the intronic mutation, demonstrated all typical morphological hallmarks. However, another biopsy specimen from the same patient, shortly after an episode of rhabdomyolysis, revealed regenerating muscle without SDH deficiency and only minor pathological iron accumulation. RT-PCR and western blot revealed upregulation of ISCU and increased protein expression in regenerating muscle tissue. However, the ratio of normally/aberrantly spliced ISCU mRNA in the two muscle biopsy specimens was the same. Our study suggests that the phenotype with the typical hallmarks associated with the disease will only be manifest if the absolute amount of normally spliced mRNA decreases below a certain threshold level, and supports the concept that RNA modulating therapy with upregulation of normally spliced ISCU mRNA may be a therapeutic possibility for these patients.

220-P

BIOCHEMICAL STUDIES TO SELECT PATIENTS WITH CoQ10 DEFICIENCY

Bujan N[1], Montero R[2], Artuch R[2], Briones P[3]
[1]*Dep Bioch Mole Gen, Hosp Clinic, CIBERER, Barcelona, Spain*
[2]*Div Bioch Neuro, Hosp St Joan D, CIBERER, Barcelona, Spain*
[3]*Hosp Clinic, CSIC, CIBERER, Barcelona, Spain*

Background: 16 genes are known to be involved in CoQ10 metabolism, but few patients with mutations in these genes have been described. Nevertheless, it is very important to obtain a diagnosis because CoQ10 deficiency is a pharmacologically treatable disease. Our aim was to select CoQ10 deficient patients.
Methods: Respiratory-chain enzymes have been assayed by spectrophotometry, and CoQ10 concentration by HPLC.
Results: During about 15 months, we have determined mitochondrial respiratory-chain activities and CoQ10 concentration in muscle homogenates of 134 patients. 58 of them (43.2%) presented enzymatic activities within reference values, 8 patients showed deficient complex IV (one carrying the A3243G MELAS mutation), 11 showed deficient complex III, 31 patients had combined complex II+III deficiency and 22 had multiple deficiencies. In 4 patients the muscle biopsies were insufficient for a conclusive result. Only 4 patients of the total studied showed the typical pattern suggestive of CoQ10 defect (CI+III and CII+III deficiencies) and in two of them the deficiency was demonstrated by HPLC analysis. Moreover, 28 additional patients presented deficient CoQ10 concentration. These results must be confirmed in fibroblasts and afterwards with genetic studies; however the patients have started medication with CoQ10 and in some cases the effect is favorable. In the molecular studies, 3 patients showed heterozygous mutations in genes involved in CoQ10 biosynthesis (CABC1, COQ4 and PDSS1). The studies to find a second mutation that explains the deficiency are on course.
Conclusion: When investigating OXPHOS deficiencies, it is advisable to include CoQ10 determinations, because of the treatability of deficient patients.

221-P

QUANTITATIVE ANALYSIS OF mtDNA CONTENT IN FORMALIN-FIXED PARAFFIN EMBEDDED MUSCLE BIOPSIES

Font A[1], Navarro-Sastre A[1], Cusi V[2], Tort F[1], Briones P[1], Ribes A[1]
[1]*Dept Biochem Mol Gen, Hosp Clinic, Barcelona, Spain*
[2]*Dep Patho, Hosp Sant Joan de Deu, Barcelona, Spain*

Background: Mitochondrial DNA (mtDNA) depletion syndromes (MDS) are heterogeneous group of genetic disorders characterized by a decrease of mtDNA copy number. Quantification of mtDNA content in patients with mitochondrial disease has become an essencial tool for the diagnosis of MDS. Analysis of mtDNA from formalin-fixed paraffin-embedded (FFPE) tissue samples, which represent the largest source of archived morphologically defined biopsies, would facilitate diagnosis procedures and allow retrospective studies.
Objective: In the present study, we optimized the methodology to extract and analyze mtDNA by Real-Time PCR from FFPE samples and compared these results with those obtained from a portion of the same frozen tissue as a reference method.
Materials: We analyzed 18 individuals: 14 controls and 4 patients including genetically confirmed DGUOK and SUCLA2, in order to validate the sensitivity of the technique. Quantification of mtDNA copy number was performed by multiplex Real-Time PCR.
Results: We set up the methodology to analyze mtDNA copy number and we established a reference range for mtDNA content, which was similar in both frozen and FFPE tissue controls (0.57–2.90 versus 0.41–2.02 relative units, respectively). Interestingly, the 4 patients analyzed were below this range, from 0.04 to 0.26, showing a decrease in mt DNA content with either material.
Conclusion: Despite the difficulties due to degradation of DNA in FFPE material and the natural variability of mtDNA content in human tissues, in our experience, studies of mtDNA in FFPE by real-time PCR can be useful for molecular screening of patients suspected to have MDS when frozen biopsies are not available.

222-P

GENETIC ANALYSIS IN THE ATP SYNTHASE ASSEMBLY FACTORS TMEM70 AND ATP12 GENES IN PATIENTS WITH 3-METHYLGLUTACONIC ACIDURIA

Tort F[1], Lissens W[2], Montoya J[3], Fernandez-Burriel M[4], del Toro M[5], Arranz JA[5], Riudor E[5], Briones P[1], Ribes A[1]
[1]*Dept Biochem Clin Gen, Hosp Clin, Barcelona, Spain*
[2]*Cen Med Gen, Univ Hosp, Brussels, Belgium*
[3]*Dept Biochem Mol Cel Biol, Univ Zar, Zaragoza, Spain*
[4]*Lab Mol Biol, Hosp, Merida, Spain*
[5]*Dept Child Neu Clin Psich, Hosp VH, Barcelona, Spain*

ATP synthase defects are clinically characterized by cardiomyopathy, nervous system involvement, lactic acidosis and 3-methylglutaconic accumulation acid in urine. Genetic defects have been found in one patient carrying a mutation in the ATP12 gene. More recently mutations in the TMEM70 gene were found in affected individuals of gypsy origin.
In our study we have selected a series of five unrelated patients of Caucasian origin with 3-methylglutaconic aciduria that were screened for the presence of pathogenic mutations in the ATP12 and TMEM70 genes. These patients had a clinical presentation with central nervous system involvement in early childhood and a marked increase of 3-methylglutaconic and 3-methylglutaric acids. Three of these patients presented cardiomyopathy and one of them also had a marked alteration of ATP synthase complex demonstrated by blue native studies. Molecular markers and activity of respiratory chain complexes I-IV were normal in all studied cases, and no mutations were found in the ATP12 gene. On the other hand, our results showed the previously described TMEM70 mutation (c.317–2A>G) in homozygosity in one of the patients and a newly identified heterozygous missense mutation (c.580G>A; p.V194M) in the paternal allele of another patient with complex V alteration. Current work is in progress in order to find out the genetic alteration in the maternal allele.
In conclusion TMEM70 mutations are involved in the pathogenesis of 3-methylglutaconic aciduria in populations from different ethnic origins and might be considered as a useful genetic marker for this disease, especially in cases with encephalocardiomyopathic presentation.

223-P

HETEROLOGOUS EXPRESSION OF R224G E1α MUTANT FORM OF PYRUVATE DEHYDROGENASE COMPLEX

Florindo C[1], Mendes M[1], Pinheiro A[1], Tavares de Almeida I[1], Silva MJ[1], Leandro P[1], Rivera I[1]
[1]*Met&Gen, iMed UL, Fac Pharm, Univ Lisbon, Lisbon, Portugal*

Pyruvate dehydrogenase (E1; EC 1.2.4.1) is one of the three enzymatic components of the intramitochondrial pyruvate dehydrogenase complex (PDHc), which catalyses the oxidative decarboxylation of pyruvate to acetyl-CoA. Genetic defects in PDHc lead to lactic acidemia and neurological abnormalities. E1 is a heterotetrameric enzyme composed of two α and two β subunits, encoded by PDHA1 and PDHB genes, respectively. Recently, our group identified a novel and "de novo" c.757A>G mutation in PDHA1 (p.R224G in the mature E1α subunit) in a Portuguese boy presenting mild neurological involvement and low PDHc activity with absence of E1α on immunoblotting analysis.

Here we describe the characterization of the mutant R224G E1a protein produced in a prokaryotic expression system.

Wild-type E1α and E1β cDNAs (provided by Prof. MS Patel) were cloned into the bicistronic expression vector pETDuet (Clontech) which contains two multiple cloning sites (MCS1 and MCS2). The presence, N-terminally to MCS1, of a sequence encoding a 6xHis tag allows further purification by IMAC. The referred mutation was introduced by site directed mutagenesis. Recombinant proteins were produced in E. coli BL21 (DE3), after IPTG induction, in LB supplemented with thiamine. After purification, the enzymatic activity (DCPIP method) and the oligomeric profile were evaluated (SEC analysis).

The developed expression system allowed us to obtain high yields of functional E1wild-type protein mainly as tetramers. When compared to the wild-type form, the mutant p.R224G E1α presented lower expression levels. We postulate that a loss-of-function, due to protein misfolding, should be the main pathogenic mechanism of this disease-causing mutation.

224-P

TWO LARGE GENE DELETIONS AND ONE POINT MUTATION IN THE TAZ GENE OF PATIENTS WITH BARTH SYNDROME

Ferri L[1], Vaz FM[2], Bertini E[3], Houtkooper RH[2], Malvagia S[4], Catarzi S[1], Funghini S[4], Gasperini S[4], Pérez-Cerdà C[5], Guerrini R[4], Donati MA[4], Morrone A[1]
[1]*Dept for Woman and Child's Health, Florence, Italy*
[2]*Dept of Clinical Chemistry and Paediat, Amsterdam, Netherlands*
[3]*Dept Lab Med, Bambino Gesu' Child' Hosp, Rome, Italy*
[4]*Metab and Musc Unit, Meyer Child Hosp, Florence, Italy*
[5]*Universidad Autónoma de Madrid, Madrid, Spain*

Background: The X-linked Barth Syndrome (BS) is a cardioskeletal myopathy that presents in infancy with cardiomyopathy, hypotonia, growth delays, and cyclic neutropenia generally associated with 3-methylglutaconic aciduria. It is caused by mutations in the tafazzin gene encoding tafazzin proteins involved in the metabolism of cardiolipin, a mitochondrial specific phospholipid that plays a role in mitochondrial energy production and apoptosis.

Objectives: Molecular analysis of the TAZ gene in suspected patients with cardiomyopathy and/or left ventricular non compaction.

Materials and methods: PCR amplification and direct sequencing of exons and intron-exon boundaries of the TAZ gene in a cohort of 12 suspected Barth patients.

Results: Molecular analysis led to the identification of genetic lesions in 5 BS patients. A new c.641A>G p.[His214Arg] and a known c.367C>T (p. Arg123X) nucleotide substitutions in two male patients. Moreover two large deletions were also identified: from exons 1 to 5 in one Hispanic male patient and from exons 6 to11 in two unrelated Italian male patients. Deletions were confirmed by multiplex PCR assay. PCR amplification and primer walking of deleted regions led to the identification of breakpoints. Quantitative TAZ RNA analysis revealed its complete absence in deleted patients.

Conclusion: The new genetic variant identified modifies a conserved tafazzin amino acid residue. Two new large deletions leading to a complete absence of RNA/proteins were reported and the detection of the breakpoint allows the identification of heterozygous females.

225-P

DIHYDROLIPOAMIDE DEHYDROGENASE (DLD) DEFICIENCY IN A SPANISH PATIENT WITH MYOPATHIC PRESENTATION DUE TO A NEW MUTATION IN THE INTERFACE DOMAIN

Font A[1], Quintana E[1], Vilaseca MA[2], Tort F[3], Ribes A[3], Pineda M[4], Briones P[5]
[1]*Dept Biochem Mol Gen, Hosp Clinic, Barcelona, Spain*
[2]*Dept Biochem, Hosp Sant Joan de Deu, Barcelona, Spain*
[3]*Dept Biochem Mol Gen, H Clinic, CIBERER, Barcelona, Spain*
[4]*Dept Neuro, Hosp Sant Joan de Deu, Barcelona, Spain*
[5]*Dept Biochem, H Clinic, CIBERER, CSIC, Barcelona, Spain*

We present a 32 year-old patient that started at 7 months with photophobia and left-eye ptosis progressively increasing to muscular weakness. Visited at 7, she showed normal psychomotor development, bilateral ptosis and normal strength that decreased after exercise, accompanied by severe acidosis. Basal blood and urine lactate were normal, increasing dramatically post effort. Muscle and skin biopsies analysis evidenced PDHc deficiency. No mutations in PDHA1 were detected. Unlike cetogenic diet, thiamine gave good response though bilateral ptosis persists, with great fatigability and acidosis after an effort.

Recently, analysis of DLD detected the homozygous change c.1440 A>G (p.I480M), in the interface domain. Both parents are carriers and DLD activity in patient's fibroblasts was undetectable.

The five patients reported with DLD-interface mutations suffered fatal deteriorations. In our patient the disease is milder, only myopatic, similar to that due to mutation p.G229C in the NAD-binding domain.

Two of those five patients present mutations (p.D479V and p.R482G) very close to our patient's (p.I480M). All three, despite different clinical severity, showed mild clues to DLD deficiency, with sporadic small increases of alpha-ketoglutarate and branched-chain-amino-acids. Curiously, those two cases presented hypertrophic cardiomyopathy, attributed to moonlighting proteolytic activity of DLD when monomeric, removing mitochondrial proteins, such as frataxin. In our patient, without cardiomyopathy, the p.I480M mutation probably does not so severely affect the ability of DLD to dimerize as p.D479V or p.R482G.

Our patient, with a new mutation in the DLD interface and mild clinical symptoms reinforces that moonlighting activities widen the clinical spectrum in this deficiency.

226-P

MILD MCAD MAY ALSO BE DIAGNOSED BY MEASURING WHOLE BLOOD ACYLCARNITINE PRODUCTION RATES GENERATED FROM DEUTERATED PALMITATE

Dessein AF[1], Fontaine M[1], Andresen BS[2], Gregersen N[3], Brivet M[4], Rabier D[5], Napuri-Gouel S[6], Dobbelaere D[7], Mention-Mulliez K[7], Martin-Ponthieu A[1], Briand G[8], Millington DS[9], Vianey-Saban C[10], Wanders RJA[11], Vamecq J[12]
[1]*Clin Biol, Univ Hosp, Lille, France*
[2]*Clin Med, Aarhus Univ, Aarhus, Denmark*
[3]*Biochem Mol Biol, Univ South. Denmark, Odense, Denmark*
[4]*Univ Hosp Bicêtre, Le Kremlin-Bicêtre, France*
[5]*Clin Biol, Hôpital Necker-Enfants Malade, Paris, France*
[6]*Pediatr Neurol, Univ Hosp, Rennes, France*
[7]*Refer Center Metabol, Univ Hosp, Lille, France*
[8]*MS Applic Lab, Univ Lille2, Lille, France*
[9]*Pediatrics, Duke Univ Med Center, Durham, United States*
[10]*Pediatr Biochem, Debrousse Univ Hosp, Lyon, France*
[11]*Lab Gen Metab Dis, Acad Med Center, Univ, Amsterdam, Netherlands*
[12]*Inserm, Lille, France*

Background: Whole blood samples were previously validated for rapid diagnosis of mitochondrial fatty acid oxidation disorders including common MCAD (homozygoty for the 985A>G mutation), VLCAD and SCAD deficiencies (Dessein et al., CCA 406, 23–26, 2009).
Objective: Acylcarnitine production rates in a patient with mild MCAD deficiency (MCADD) (compound heterozygote for common 985A>G and a new 145C>G mutations with 15% residual fibroblast 1–14C-octanoate along with normal 1–14C-palmitate and -butyrate oxidation rates) are compared with rates in controls, patients with common (985A>G mutation) MCADD and in an adult carrier.
Methods: Whole blood samples were incubated with 1 μmolL-carnitine and 200 nmol [16-2H3, 15-2H2]-palmitate at 37°C for 6 hours. Labeled acylcarnitines were quantified by electrospray-ionization tandem mass spectrometry after thawing, extraction and derivatization to their butyl esters.
Results: The various chain-lengthened acyl-carnitines generated by whole blood from deuterated palmitate were produced in the patient with mild MCADD at rates differing from controls, being increased and decreased for C8 and C4 acylcarnitines, respectively. These results were similar to those in patients with common MCADD but distinct from adult carrier data which were normal.
Conclusion: Whole blood samples were successful in identifying the patient with mild MCADD. This result is very encouraging because, like common MCADD, mild MCADD (high residual enzyme activity and undetectable glycine conjugates), similar to some of the mild MCADDs detected by MS/MS newborn screening, is at risk for disease presentation and metabolic decompensation.

227-O

ESTABLISHMENT OF A NEURONAL CELL MODEL OF COENZYME Q10 DEFICIENCY: IMPLICATIONS FOR PATHOGENESIS AND TREATMENT OF DISORDERS OF COENZYME Q10 BIOSYNTHESIS

Duberley KEC[1], Heales SJR[2], Rahman S[3], Allen G[1], Hargreaves IP[4]
[1]*Institute of Neurology, UCL, London, United Kingdom*
[2]*Enzyme Laboratory, GOSH, London, United Kingdom*
[3]*Institute of Child Health, UCL, London, United Kingdom*
[4]*Neurometabolic Unit, National Hospital, London, United Kingdom*

Background: Coenzyme Q10 (CoQ10) deficiency is a rare but often treatable autosomal recessive 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 the neuroblastoma cell line (SHSY-5Y) using para-aminobenzoic acid (PABA) treatment. PABA is a benzoic acid derivative that is able to competitively inhibit the CoQ10 biosynthetic pathway enzyme, 4-hydroxybenzoate poly prenyl transferase.
Methods: Treatment of SHSY-5Y cells with 1 mM PABA induced a significant ($p < 0.05$) decrease of 46.21% in the CoQ10 status compared to control levels (Control: 57.18 pmol/mg ±2.47; 1 mM: 30.76 pmol/mg ±2.52).
Results: The effect of this deficit was further investigated on mitochondrial electron transport (ETC) function by measurement of complex II/III (CII/III) activity, an enzyme that is dependent upon endogenous CoQ10. CII-III activity expressed as a ratio to citrate synthase activity, a mitochondrial marker enzyme showed a decrease in activity of 99.66% compared to control (Control: 0.059 ±0.87; 1 mM: 0.00020 ±0.011), ATP production also decreased by 84.61% in comparison to control (Control: 0.82 μmol/mg; 1 mM: 0.13 μmol/mg).
Conclusion: In conclusion, this neuronal cell model will enable the effect of a deficit in CoQ10 status to be investigated upon mitochondrial oxidative metabolism as well as assessing the therapeutic efficacy of exogenous quinones to restore mitochondrial function. This project is funded by Ataxia UK (www.ataxia.org.uk).

228-P

NITRIC OXIDE PRODUCTION IN SUBJECTS WITH MELAS SYNDROME AND THE EFFECT OF ARGININE AND CITRULLINE SUPPLEMENTATION: INTERIM RESULTS

El-Hattab A[1], Craigen W[2], Jahoor F[2], Wong LJ[1], Scaglia F[1]
[1]*Mol & Hum Genet, Baylor College of Med, Houston, United States*
[2]*Dept Pediatrics, Baylor College of Med, Houston, United States*

Background: The mitochondrial, encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome is one of the most frequent maternally inherited mitochondrial disorders. It is believed that epithelial dysfunction leads to nitric oxide (NO) deficiency and ischemic events. Both arginine and citrulline act as NO precursors.
Methods: To assess whether NO production is lower in patients with MELAS and the effect of arginine and citrulline supplementation, we designed a study to measure NO production rate via stable isotope infusion technique and the concentrations of nitric oxide metabolites (NOx), arginine, and citrulline in 20 control subjects and 20 subjects with MELAS before and after arginine or citrulline supplementation.
Results: Five patients and 3 controls have completed the study. No significant differences between patients and controls were found in plasma arginine and citrulline concentrations. Patients have lower NOx concentration than controls (16.56±1.40 versus 20.01±2.44 μMol/L, p=0.12). Citrulline supplementation resulted in more significant increase in plasma arginine and citrulline concentrations than arginine supplementation. Plasma NOx concentration increased after arginine supplementation (17.04±1.71→18.54±0.99 μMol/L, p=0.18); however, the increment was higher after citrulline (16.08±1.78→19.50±2.84 μMol/L, p=0.06). NO production rate increased after arginine supplementation (0.058±0.016→0.282±0.153 μMol/L, p=0.12); however, the increment was higher after citrulline (0.056±0.018→0.352±0.144 μMol/L, p=0.045).
Conclusion: The interim analysis showed that in comparison to arginine, citrulline supplementation to subjects with MELAS has led to more significant increase in NOx, arginine, and citrulline concentrations and NO production rate. Ultimately, the completion of this pilot study could aid in the design of a better therapeutic strategy for MELAS syndrome.

229-P

A NOVEL MUTATION IN DGUOK GENE IN A TURKISH NEWBORN

Kilic M[1], Dursun A[1], Sivri HS[1], Tokatli A[1], Akcoren Z[2], Yigit S[3], Vezir E[1], Seneca S[4], Demerlier L[5], Coskun T[1]
[1]Pediatr Metab Dis, Hacettepe University, Ankara, Turkey
[2]Pediatr Pathology, Hacettepe University, Ankara, Turkey
[3]Neonatology Unit, Hacettepe University, Ankara, Turkey
[4]Centrum Mediche Genetics, Brussel, Belgium
[5]Pediatr Neurol, CMG,, Brussel, Belgium

Mitochondrial DNA (mtDNA) depletion syndromes (MIM 251880) are genetically and clinically a heterogeneous group. mtDNA depletion syndromes, presenting mainly with liver failure, have been commonly associated with bi-allelic mutations in the nuclear genes; POLG, DGUOK, MPV17, and C10orf2 (TWINKLE). A patient was admitted to hospital for respiratory difficulty and poor suction developed in the first hours after birth. Physical examination showed mild hepatomegaly, jaundice, abdominal distension, and ascites. Laboratory study in blood showed pH 6.9, HCO3 13 mmol/L, glucose 40 mg/dl, ALT 53 IU/L, AST 203 IU/L, GGT 122 IU/L, ALP 310 IU/L, Tbl 7 mg/dl, Dbl 4 mg/dl, INR 5, aPTT 62 s, lactate 200 mg/dl, tyrosine 19.2 mg/dl, ferritin 2522 mg/dl, NH3 138 μg/dl, AFP 9983. Urine organic acid analysis showed elevated levels of lactic acid, pyruvic acid, and para-hydroxy compounds. Echocardiography revealed patent ductus arteriosis and pulmonary hypertension. He developed progressive hepatic dysfunction and died at the age of 42 days. Molecular analysis of DGUOK gene detected a novel mutation, c.34C>T (p.Arg12X) in the patient. Considering the genotype-phenotype relation it might be concluded that this novel mutation is one of the very severe pathogenic nucleotide changes in DGUOK gene.

230-P

MALE SIBLINGS WITH SUCCINYL-CoA LIGASE B SUBUNIT DEFICIENCY WITH IDENTICAL MUTATIONS IN SUCLA2 GENE AND DIFFERENT CLINICAL PRESENTATIONS

Anselm IA[1], Wong LJ[2], Harris D[3], Levy H[3], Berry GT[3]
[1]Dep Neuro, Child Hosp, Boston, United States
[2]Dep Mol and Hum Genet, Baylor Coll Med, Houston, United States
[3]Div Genet, Child Hosp, Boston, United States

Succinyl -CoA ligase is the enzyme responsible for catalyzing the conversion of succinyl-CoA to succinate, a key Kreb's cycle intermediate. Deficiency of this enzyme leads to reduced Kreb's cycle TCA function, hypermethylmalonic acidemia and mitochondrial depletion with lactic acidosis, Leigh-like syndrome, and shortened life span.

We present a case of two affected siblings with identical mutations (A307V/M329V) in the SUCLA2 gene, but different phenotypes. The sibling with the milder phenotype was treated with DCA. The sibling with a severe phenotype has unique clinical characteristics, such as severe immunodeficiency.

Patient 1 presented with developmental delay, dystonia, lactic acidosis and abnormal brain MRI. He was treated with DCA since the age 2 years and tolerated it well, without developing peripheral neuropathy over 5 years. At age 7 he has poor head control, is unable to sit or stand. He has severe cyclical vomiting resistant to drug therapy.

Patient 2 presented with lactic acidosis in the first year of life, severe developmental delay and hypotonia, and MRI findings consistent with Leigh disease. He was not treated with DCA, but he developed peripheral neuropathy at age 2 years. He has severe GI dysmotility, intermittent anemia, bone demineralization and immunodeficiency, requiring therapy with IVIG.

Conclusions: The sibling with more severe phenotype was not treated with DCA; however, DCA treatment alone cannot explain the starkly different phenotypes in these two brothers with the same A307V/M329V SUCLA2 genotype. This is yet another example of discordancy in clinical phenotype in patients with biochemically and genetically identical metabolic disease.

231-P

MITOCHONDRIAL NEUROGASTROINTESTINAL ENCEPHALOMYOPATHY (MNGIE) WITH EARLY ONSET AND A RAPIDLY FATAL COURSE

Libernini L[1], Lupis C[1], Mastrangelo M[1], Santorelli FM[2], Ferrara M[1], Donati MA[3], Inghilleri M[4], Leuzzi V[1]
[1]Dept Child Neurol and Psych, La Sapienza, Roma, Italy
[2]D Molecular Medicine, Bambino Gesu Ch. H., Roma, Italy
[3]Meyer Children Hospital, Firenze, Italy
[4]Dept Neurology, La Sapienza, Roma, Italy

Background: Mitochondrial Neurogastrointestinal Encephalomyopathy (MNGIE) is a rare autosomal recessive disorder characterized by gastrointestinal dysmotility, cachexia, ptosis or ophtalmoparesis, peripheral neuropathy, and leukoencephalopathy. We report two affected Italian brothers carrying a novel TYMP/ECGF1genotype.

Case Report: The index case presented at the age of 15 hyporexia, weight loss, and recurring vomiting. Gastrointestinal endoscopy showed duodenoileal hypotonia and diverticulosis. Neurologic examination evidenced severe muscular hypotrophy, osteotendineous hyporeflexia, claw feet, and normal mental development with mood disorder. Brain MRI and electrophysiologic studies revealed a bilateral periventricular leukoencephalopathy and a demyelinating peripheral neuropathy. Urinary thymidine (4.04 mmol/mol creatinine; r.v. 0.04–0.05) and deoxiuridine (13.51 mmol/mol creatinine; r.v. 0.02–0.04) were increased. Direct sequencing of the TYMP/ECGF1 gene identified two novel pathogenic mutations (c.215–14del13ins4 / c.1159+2 T>A). The mutations segregated in healthy parents and were not found in 100 control chromosomes. At the age of 17, the patient died of acute pulmonary edema after a dramatic worsening of his general condition.

The 13-year-old younger brother presented a neurosensorial hypoacusia and failure-to-thrive. Brain MRI evidenced a bilateral periventricular leukoencephalopathy. Analysis of TYMP/ECGF1 detected the same genotype of the elder brother.

Discussion: The two brothers herein described harbored severe mutations of the gene encoding for thymidine phosphorylase (TP), a crucial enzyme for intramitochondrial nucleotide homeostasis. Although displaying the same genotype, the two brothers expressed different phenotype and clinical course. Adding to the list of variants in TYMP/ECGF1, our report represents additional evidence that attempts to establish genotype-phenotype correlations in MNGIE is generally disappointing. Other factors might influence intrafamilial variability.

232-P

INTESTINAL MANIFESTATIONS IN PATIENTS WITH THE MELAS MUTATION m.3243A>G — A CLINICAL AND HISTOLOGICAL CASE STUDY

Fazeli W[1], Tsiakas K[1], Herberhold T[1], Rolinski B[2], Hagel C[3], Santer R[1]
[1]Univ Child Hosp, Hamburg Eppendorf, Germany
[2]Inst f Clin Chem, Hosp, Muenchen Schwabing, Germany
[3]Dep of Neuropath, Univ Hosp, Hamburg Eppendorf, Germany

Background: Apart from MNGIE syndrome (mitochondrial neuro-, gastrointestinal-, encephalomyopathy caused by mutations of the thymidine phosphorylase gene) gastrointestinal symptoms have rarely been reported in mitochondrial disorders. We describe two patients with typical MELAS syndrome (mitochondrial encephalo,myopathy, lactic acidosis, stroke-like episodes) who presented with severely impaired gastrointestinal motility. While constipation and intestinal pseudo-obstruction have been mentioned in few such cases, histological und therapeutic aspects have barely been discussed in the literature.

Patients: Two previously diagnosed MELAS patients were admitted with paralytic ileus at ages 16 and 20 years, respectively. The clinical course was protracted with considerable weight loss in both. On endoscopy, no remarkable macroscopic changes were seen. Intestinal biopsies showed an increased number and marked hyperplasia of mitochondria both in enterocytes and the Lamina muscularis mucosae. Mitochondria showed an abnormal structure, e.g., intramitochondrial vacuoles were observed. In both patients the m.3243A>G mutation was found in several tissues including the gastrointestinal tract with a degree of heteroplasmy of >80% . Pro-kinetic treatment with erythromycin considerably enhanced gut motility and thus reproducibly improved intestinal symptoms in both patients.

Conclusion: Our cases demonstrate that characteristic histological signs can also be found in intestinal tissue in patients with a mitochondrial disorder. They show that the spectrum of clinical signs in MELAS cases has to be expanded and that the underlying cause of cases presenting as MNGIE syndrome is not restricted to mutations of the thymidine phosphorylase gene. A therapeutic trial with erythromycin is warranted in mitochondrial intestinal motility disorders.

233-P

MUTATION SPECTRUM IN A COHORT OF 65 PATIENTS WITH PYRUVATE DEHYDROGENASE COMPLEX DEFICIENCY

Imbard A[1], Boutron A[1], Zater M[1], Saudubray JM[2], Ogier de Baulny H[3], Desguerres I[4], Rio M[5], Delonlay P[2], Desportes V[6], Lamireau D[7], Sedel F[8], Mignot C[9], Goldenberg A[10], Tardieu M[11], Rivier F[12], Chabrol B[13], Thauvin C[14], Mine M[15], Benelli C[16], Marsac C[2], Brivet M[1]
[1]*Bioch. Gen., Hôpital de Bicêtre, APHP, Bicêtre, France*
[2]*Metabolic Unit, Hôpital Necker, APHP, Paris, France*
[3]*Metabolic Unit, Hôpital R.Debre, APHP, Paris, France*
[4]*Neuroped, Hôpital Necker, APHP, Paris, France*
[5]*Clin. Gen., Hôpital Necker, APHP, Paris, France*
[6]*Neuroped., GH Est, Lyon, France*
[7]*Neonat. Intens. Care unit, CHU Bordeaux, Bordeaux, France*
[8]*Neurology, GH Pitié Salpêtrière, APHP, Paris, France*
[9]*Neuroped., Hôpital Trousseau, APHP, Paris, France*
[10]*Clin. Gen., Hôpital Charles Nicolle, Rouen, France*
[11]*Neuroped., Hôpital de Bicêtre, APHP, Bicêtre, France*
[12]*Neuroped., Hôpital Gui de Chauliac, Montpellier, France*
[13]*Metabolic Unit, Hôpital de la Timone, Marseille, France*
[14]*Gen. Dep., Hôpital d'Enfants, Dijon, France*
[15]*Gen. Dep., Hopital Lariboisière, APHP, Paris, France*
[16]*INSERM UMR S747, Univ Paris Descartes, Paris, France*

Background: Defects in pyruvate dehydrogenase complex (PDHc) are an important cause of primary lactic acidosis. Majority of PDHc deficiencies result from mutations in the X-linked PDHA1 gene. Mutations can also occur in PDHB, PDHX, DLAT or DLD genes.
Methods: Our cohort consisted of 65 unrelated French patients with PDHc deficiency, diagnosed in the period 1990–2010, by PDHc assay in lymphocytes or fibroblasts. Mutation screening was performed by direct gDNA sequencing from a long range PCR template, cDNA analysis and gene copy number determination by qPCR and CGH array.
Results: PDHA1 mutations were found in 22 girls and 26 boys, PDHB mutations in 7 patients, PDHX mutations in 9 patients and DLD mutations in 1 patient; 27 different novel mutations in PDHA1 have been identified: (V8G) in the mitochondrial targeting sequence, 13 missense mutations [E75A, R88S, R119W, G144D, A169V, P217R, R235G, Y243C, Y243S, R245G, P250L, G278R, G298E], 3 splicing mutations [Y161Y, I166I, IVS7+26G>A], 3 nonsense mutations [R263X in a mosaic male, R304X, W383X in two girls], 5 small ins or del mutations (249dup, 937_942dup, 960_1008+5dup, 1050_1133dup, 1153_1158del) and 2 large rearrangements [a 4.2 kb intragenic deletion and a contiguous gene deletion syndrome involving PDHA1 and 18 other genes (3.4 Mb deletion)]. Mutations in PDHX were mostly severe mutations leading to null alleles. A previously described mutation (M101V) in PDHB was found in 5 patients of North-African origins, suggesting a founder effect.
Conclusion: This is to date the largest cohort of molecular resolved PDHc deficient patients.

234-P

POLYNEUROPATHY AS THE MAIN PRESENTING SYMPTOM IN PDH DEFICIENCY

Unal O[1], Hismi B[1], Kilic M[1], Dursun A[1], Kalkanoglu-Sivri HS[1], Tokatli A[1], Coskun T[1], Zeviani M[2]
[1]*Metabolism Unit, Hacettepe University, Ankara, Turkey*
[2]*Istituto Neurologico Carlo Besta, Milano, Italy*

Peripheral neuropathy is a rare presenting symptom of PDH deficiency. We present a patient with PDH deficiency presented with polyneuropathy. A 2.5 -year- old boy patient followed up with developmental delay, hypotonia from six months old and epileptic seizures that started at 2 years old was admitted to our hospital. After polyneuropathy had been diagnosed at 6 months of age and confirmed by EMG investigation and he had been treated with intravenous immunglobulin and oral steroid therapy. Physical examination revealed growth retardation, hypoactive deep tendon reflexes, and distal atrophy in both lower limbs. Elevated serum lactic and pyruvic acid in serum and elevated urinary 2-ketoglutaric acid on organic acid analysis were detected. Cranial MRI and MRS showed high signal activity in the globus pallidus and elevated lactate peak in globus pallidus and the other parts of brain. EMG showed mild axonal and severe sensorineural polyneuropathy. Activity of PDH complex in cultured skin fibroblasts kindly performed by Zeviani from Istituto Neurologico Carlo Besta, showed low basal activity, 1.2 (N 1.5–3.7), low activated activity of enzyme, 3.1 (N 4.3–7), and low citrate synthase activity, 160 nmol/minmg (N 90–170). We want to emphasise that polyneuropathy can be the main presenting symptom in PDH deficiency and should be taken into the consideration in the differential diagnosis of polyneuropathy.

235-P

ADULT MITOCHONDRIAL RESPIRATORY CHAIN DISORDERS IN NEUROMETABOLIC ADULT CLINIC

Macario C[1], Grazina M[2], Rebelo O[1], Garcia P[3], Diogo L[3], Oliveira C[2], Cunha L[1]
[1]*Neurology Coimbra University Hospital, Coimbra, Portugal*
[2]*Institute Biochem Faculty Medicine Un, Coimbra, Portugal*
[3]*Metabolic Unit Pediatric Hospital, Coimbra, Portugal*

Mitochondrial respiratory chain disorders (MRC), defined as primary diseases of the oxidative phosphorylation system are clinically, neuroradiologically, histologically, enzymatic and genetically heterogeneous. Then they are difficult to diagnose and classify.
This study intends to evaluate the main clinical features, phenotype classification, and biochemical, anatomopatologic and genetic abnormalities characterization of patients with definite primary mitochondrial disorders.
We classify 200 patients studied for possible MRC, based on Walker et al original criteria. We analyze the clinical symptoms, enzymatic activity of mitochondrial respiratory chain complexes I-V, genetic mtDNA, some nuclear evaluation (SURF1, POLG1), standard muscular pathologic evaluation and abnormalities on MRI.
A total of 56 patients were assigned with definite primary mitochondrial disorders, 11 of them presenting in the firsts 10 years of life. The classical mitochondrial syndrome was identified: 19 had Progressive External Oftalmoplegia(PEO), 9 Kearn-Sayre S, 5 Leigh S, 3 MELAS (mitochondrial encephalopathy lactic acidosis stroke like episodes), 4 LHON (Leber hereditary optic neuropathy), 1 MERRF (mioclonic epilepsy, ragged red fibers), 1 DMDF (diabetes mellitus deafness), 9 patients had Generalized Miopathy, 2 severe cardiomiopathy plus PEO and 9 encephalopathy (delay or regression with various types of neurologic signs). All patients with muscular involvement had pathologic abnormalities. 30 patients had low activities of chain complexes, most of them IV. 29 patients had multiple deletions on mtDNA, 4 single large deletion, 8 punctual mutations, 2 SURF1 mutations and 1 POLG1 mutation.
Adult Mitochondrial respiratory chain disorders are the most frequents hereditary errors of metabolism. Most patients have muscular involvement and mitDNA abnormalities.

236-P

EXPANDED CLINICAL SPECTRUM AND NEONATAL HYPERAMMONEMIA ASSOCIATED WITH MUTATIONS IN TMEM70

Shchelochkov O[1], Li F[2], Wang J[2], Towbin JA[3], Jefferies JL[4], Wong L-JC[2], Scaglia F[2]
[1]Div Genetics, Department Ped, Univ Iowa, Iowa City, United States
[2]Dep Mol Human Genet, Baylor Coll Med, Houston, United States
[3]Dep Ped, Hum Genet, Univ of Cincinnati, Cincinnati, United States
[4]Dep Ped, Div Card, Baylor Coll Med, Houston, United States

Background: Mitochondrial disorders are a genetically heterogeneous group of disorders posing a significant diagnostic challenge. The majority of patients likely harbor mutations in nuclear genes, most of which are still poorly characterized, and therefore not amenable to efficient screening using currently available molecular methods.
Methods: Here we present a patient, followed since birth after presenting with neonatal hyperammonemia, Reye-like syndrome episodes, and ventricular tachyarrhythmia. Initial biochemical work-up revealed mild orotic aciduria and significant amounts of 3-methylglutaconic and 3-methylglutaric acids in the urine. Muscle biopsy demonstrated ragged-red fibers and non-specific structural abnormalities of mitochondria. The activities of respiratory chain enzymes (complexes I-IV) were normal. Mutational analysis of the entire mitochondrial genome did not reveal deleterious point mutations or large deletions. Long-term follow-up revealed a later-onset hypertrophic cardiomyopathy, muscle weakness, and exercise intolerance. Although she had frequent episodes of Reye-like episodes in infancy and early childhood, triggered by illnesses, these symptoms improved significantly with the onset of puberty.
Results: In light of recent reports linking cases of type IV 3-methylglutaconic aciduria and hypertrophic cardiomyopathy to mutations in TMEM70, sequencing of this gene was conducted. We identified one previously reported splice site mutation, c.317–2A>G and a novel, unclassified missense variant, c.494G>A (p.G165D) in an evolutionarily conserved region predicted to be deleterious.
Conclusions: In comparison to previously reported cases, our patient had normal growth parameters and cognitive development, absence of structural heart defects, no dysmorphic features, improvement of symptoms with age, and persistence of hypertrophic cardiomyopathy, expanding the clinical phenotype of this mitochondrial syndrome.

237-P

MITOCHONDRIAL RESPIRATORY CHAIN DISORDERS IN NEONATE IN JAPAN

Murayama K[1], Ito A[2], Ajima M[1], Tsuruoka T[2], Aizawa M[2], Harashima H[3], Mori M[4], Ohtake A[3], Takayanagi M[1]
[1]Dep of Metab, Chiba Child Hosp, Chiba, Japan
[2]Dep of Neonatol, Chiba Child Hosp, Chiba, Japan
[3]Dep of Pediatr, Saitama Med Univ, Moroyama, Japan
[4]Dep of Pediatr, Jichi Med Univ, Tochigi, Japan

Background & Objectives: Congenital disorders of the mitochondrial respiratory chain enzymes are the most common group of inborn errors of metabolism, affecting at least 1 in 5000 individuals. But there are only a few reports about neonatal-onset mitochondrial respiratory chain disorders (MRCD) so far. The present study was aimed to know the clinical manifestation of MRCD in naonate.
Material & Methods: 273 patients were investigated, all with at least a possible diagnosis using the criteria of Bernier et al. (Neurology 59:2002). Combination of enzyme activities and BN-PAGE analysis of individual respiratory chain complexes were performed to diagnose the MRCD using organ homogenates and mitochondrial fractions from fibroblasts.
Result: Forty four patients out of 107 were neonatal-onset. Eighteen neonates out of 50 were indicated some perinatal disorder including IUGR, brain anormaly, and cardiac hypertrophy. Twelve naonates were preterm infants and 23 neonates were born weighing less than 2,500 gram. Thirteen neonates were presented as neonatal asphyxia. Major initial symptom was respiratory distress and feeding difficulty. Of 50 neonates, 31 died. Complex I deficiency was identified for 21 neonates and multiple complexes deficiency for 20 neonates, complex III deficiency for 3 neonates, and complex IV deficiency for 5 neonates. Five neonates had the mitochondrial DNA mutation.
Conclusion: The prognosis of MRCD with neonatal-onset was mortal compared with child-onset. Initial symptom was not peculiar including asphyxia, respiratory distress, and feeding difficulty. Most of MRCD with neonatal-onset are seems to be nuclear DNA mutation, so it is important for diagnosis to perform the enzymatic study.

238-O

N-ACETYLCYSTEINE A NEW AND EFFECTIVE TREATMENT IN ETHYLMALONIC ENCEPHALOPATHY

Burlina A[1], Viscomi C[2], Dweikat I[3], Savoiardo M[4], Bordugo A[1], Del Rizzo M[1], Tiranti V[2], Zeviani M[2]
[1]Div Metab Dis, Univ Child Hosp, Padova, Italy
[2]Mol Neurogen Unit, Besta Institute, Milan, Italy
[3]Div Metab Dis, Makassed Hospital,, Jerusalem, Israel
[4]Neurorad Unit, Besta Institute, Milan, Italy

Ethylmalonic Encephalopathy (EE) is an autosomal recessive, (OMIM#602473), characterized by progressive encephalopathy, chronic diarrhoea, petechiae and severe orthostatic acrocyanosis and early death (Burlina, 1994). EE is a peculiar entity, since it combines severe deficiency of cytochrome c oxidase (COX) with blood and urinary accumulation of ethyl-malonic acid (EMA), a di-carboxylic derivative of butyrate which is produced in short-chain acyl CoA dehydrogenase (SCAD) deficiency. EE is caused by failure to detoxify sulfide, a mitochondrial poison for both COX and SCAD, produced by intestinal anaerobes and, in trace amount, by tissues. Absence of a mitochondrial matrix sulfur dioxygenase, encoded by the ETHE1 gene, is responsible for EE. We treated four EE children for a period ranging from 3 to 9 months with acetylcisteine (Fluimucil. 100 mg/kg/d) and metronidazole (20 mg/kg/d). All patients showed including disappearance of diarrhoea and skin lesions, and improvement of neurological symptoms, with hardly any collateral effect. Since neurological symptoms in EE do not appear before 2–4 months after birth, and more slowly progressive cases, albeit however severe, early diagnosis by either prenatal analysis of the ETHE1 gene in at-risk fetuses, or MS/MS spectrometry screening of EMA aciduria in neonates could prompt to pre-symptomatic treatment with the aim of preventing irreversible brain damage. The results presented here warrant the development of future investigation,including a larger clinical trial with the same or similar compounds, and alternative experimental therapies that will first be attempted in the mouse model, such as gene or bone marrow cell replacement.

239-P

A DELETION IN THE POLG1 GENE CAUSING ALPERS SYNDROME

Naess K[1], Barbaro M[2], Bruhn H[1], Wibom R[1], Nennesmo I[1], von Dobeln U[1], Larsson N-G[3], Nemeth A[4], Lesko N[1]
[1]Div Lab Med, Metab Dis, Karolinska Inst, Stockholm, Sweden
[2]Div Molec Med, Surg, karolinska Inst, Stockholm, Sweden
[3]Max Planck Inst Biol Ageing, Cologne, Germany
[4]Div Clin Sci, Pediat, Karolinska Inst, Stockholm, Sweden

Mutation in the gene encoding the catalytic subunit of mitochondrial DNA polymerase γ (POLG1) is a major cause of human mitochondrial disease. More than 150 different point mutations in the gene have been reported. Here we report the first identified large deletion of the POLG1 gene, comprising both the entire POLG1 gene and an adjacent gene; FANCI. The deletion was found in a boy with Alpers syndrome, presenting at 18 months of age with slightly retarded motor development, balance problems and seizures. Treatment with valproic acid was started from 28 months of age with the patient responding well to treatment; there were no further seizures. Three months later he rapidly deteriorated, due to a valproic acid-induced acute liver failure. MRI of the brain showed bilateral, symmetrical, high signaling abnormalities and oedema of thalamus and the basal ganglia. Signs of frontal, temporal and cerebellar atrophy were also seen.
Sequence analysis of the POLG1 gene identified the mutation W748S in cis with the polymorphism E1143G on one allele, whereas Multiplex Ligation-dependent Probe Amplification analysis revealed a deletion of the entire gene on the other allele.
The patient deteriorated further and died at the age of 32 months, due to hepatic failure.
This case illustrates the devastating hepatic consequences of valproic acid when given to a patient with mitochondrial disease due to POLG mutations. It further emphasises the importance of considering the possibility of POLG disease and liberally performing POLG DNA testing in pediatric seizure disorders of unknown aetiology.

240-P

COENZYME Q10 LEVEL IN FETAL MUSCLE AND LIVER TISSUES

Hansikova H[1], Pejznochova M[1], Havlickova V[1], Magner M[1], Hulkova H[2], Langer J[1], Zeman J[1]
[1]*Dept of Pediatrics, Charles University, Prague, Czech Republic*
[2]*Inst of Inher Metab Dis, Charles Univer, Prague, Czech Republic*

Coenzyme Q10 (CoQ10) is an electron carrier in mitochondrial inner membrane. Although CoQ10 is important for fetal development, only scare data exist about the levels of CoQ10 and activities of respiratory chain complexes (RCC) during fetal development. In our study we analyzed the levels of total CoQ10 and the COQ2 mRNA in fetal muscle and liver tissues with respect to activities of RCC and citrate synthase (CS). Material: 11 liver and 13 muscle tissue samples were obtained from fetuses aborted spontaneously or after genetic indication between 13 and 28 week of gestation. Control liver tissue was obtained at autopsy in 10 children at the age between 1 month and 8 years. Control muscle tissue was obtained from 20 "disease free controls" at the age 0,5–2 years.
Methods: CoQ10 content was determined by HPLC with UV detection. Activities of RCC I, II, I+III, II+III and IV and CS were measured spectrophotometrically. mRNA level for COQ2 was analyzed by qRT-PCR.
Results: CoQ10 content in fetal muscle and liver was significantly lower in comparison with controls ($p<0,001$ and $p<0,05$, respectively). Significant correlation between gestation and CoQ10 content normalized to CS was found both in liver ($p<0,05$) and muscle ($p<0,001$). mRNA level for COQ2 in muscle significantly increased during gestation ($p<0,05$). Significant correlation was also found between CoQ10 content and complex II activity in liver ($p<0.001$).
Conclusion: Our results are indicative for tissue specific developmental changes in maturation of mitochondria in fetal muscle and liver tissue during 13–28 week of gestation.
Supported by MSM0021620806.

241-P

ANALYSIS OF COENZYME Q10 IN LYMPHOCYTES BY HPLC–MS/MS

Arias A[1], Buján N[1], Pajares S[1], García-Villoria J[1], Briones P[1], Ribes A[1]
[1]*Dep Bioch Mol Gen, Hosp Clinic, CIBERER, Barcelona, Spain*

Background: Coenzyme Q10 (CoQ10) is an essential carrier for the electron transfer in the mitochondrial respiratory chain for ATP production. CoQ10 is not only present in all the cells and membranes of the eukaryotic, but it is also synthesized and broken down inside the cell. Its deficiency has been reported in patients presenting heterogeneous clinical symptoms. Plasma is not the appropriate biological fluid to analyze CoQ10 due to dietary influences on its concentration. Our objective was to set up a quick, reliable and non invasive analytical method for the evaluation of CoQ10 deficiency in lymphocytes, as well as to establish the reference values in healthy population.
Patients and Methods: CoQ10 concentration was analyzed by HPLC–MS/MS in 30 controls; in addition, and with the aim to know the influence of dietary CQ10 in lymphocytes, 9 healthy volunteers from our laboratory were given 100 mg CoQ10 daily for 6 days. CoQ10 was analyzed both in plasma and lymphocytes. Peripheral blood mononuclear cells were isolated from 8 ml heparinised blood by isopycnic centrifugation. CQ9 was used as internal standard.
Result: The reference interval for lymphocytes CoQ10 in the control population (n=30) was 1,41–3,20 nmol/U CS with a mean of 2,26 + 0,496. After 6 days of CoQ10 administration the concentration in all healthy volunteers was found significantly increased in plasma ($p=0,001$), but not in lymphocytes ($p=0,082$).
Conclusion: Therefore, lymphocytes seem to be a reliable material for the diagnosis of primary CoQ10 deficiencies.

242-P

ISOLATED ATP SYNTHASE DEFICIENCY DUE TO TMEM70 MUTATIONS: TWO NEW CASES WITH DIFFERENT PHENOTYPES

Behulova D[1], Fabriciova K[2], Frankova E[2], Zeman J[3], Tesarova M[3], Skodova J[1], Sebova C[1], Saligova J[4], Madarova J[5]
[1]*Dept Lab Med, Univ Child Hosp, Bratislava, Slovakia*
[2]*1st Dept Pediatr, Univ Child Hosp, Bratislava, Slovakia*
[3]*Dept Pediatr, 1st Fac Med, Charles Univ, Prague, Czech Republic*
[4]*Univ Child Hosp, Kosice, Slovakia*
[5]*Stara Lubovna Hospital, Stara Lubovna, Slovakia*

Background: Recently disease-causing mutations in nuclear gene TMEM70 were discovered in more than 30 individuals with severe loss of ATP synthase activity. Two new cases with homozygous mutation 317–2A>G detected in Slovakia are reported.
Case 1: A hypotrophic boy was born preterm to Romany (Gypsy) parents. At age 13 hours extreme metabolic acidosis, marked hyperlactacidemia, ketonuria and moderate hyperammonemia were revealed. The baby presented with coma requiring arteficial ventilation, hypotony, hypertrophic cardiomyopathy, mild hepatopathy, epispadia. Clinical state and laboratory findings improved, detected 3-methylglutaconic aciduria persisted. The boy died at age 26 days.
Case 2: A hypotrophic girl was born preterm to Romany parents. She presented with congenital anomaly of intestine (operation on the 2nd day of life), bilateral syndactyly of the 2nd and 3 rd toes, hypertelorism. At age 3 years during acute gastroenteritis the patient was unconscious showing metabolic acidosis, ketonuria, marked hyperammonemia and mild orotic aciduria. At age 7 years girl presents with hypotony, hypotrophy, microcephaly, mental retardation, hypertrophic cardiomyopathy, mild hepatomegaly, fluctuation of plasma lactate and persistent 3-methylglutaconic aciduria.
Conclusion: Affected individuals have been reported mainly in Romanies so far. The incidence could be high in Slovakia where this ethnic group represents approximately 8% of population.

243-O

MITOCHONDRIAL ARGINYL-tRNA SYNTHETASE DEFICIENCY: ACUTE NEONATAL PRESENTATION WITH LACTIC ACIDOSIS

Brown RM[1], Glamuzina E[2], Grunewald S[2], Chong WK[2], Rahman S[2]
[1]*Dept Biochem, Univ Oxford, Oxford, United Kingdom*
[2]*Gt Ormond St Children's Hospital, London, United Kingdom*

Pontocerebellar hypoplasia, type 6, a fatal infantile encephalopathy with variable mitochondrial respiratory chain abnormalities, is caused by mutation in the RARS2 gene which encodes the mitochondrial arginyl-tRNA synthetase. With only four patients from two families reported to date, the extent of clinical and biochemical variability in this condition remains to be defined. We now describe a fifth patient who developed symptoms immediately after birth.
The female patient presented on the first day of life with seizures associated with a profound lactic acidosis (blood lactate 14 mmol/L). A brain MRI on day 6 showed a small cerebellum and pons, increased signal intensity of cerebellar white matter and peripheral cysts in the inferior cerebellar hemispheres. A repeat MRI at 2 months showed progressive atrophy of the cerebral hemispheres, cerebellum and pons. Muscle histology was unremarkable and, apart from a mild reduction in cytochrome oxidase, activity of the respiratory chain complexes was normal. Currently, at age 9 months, she has progressive neurological deterioration with severe seizures and a dystonic movment disorder.
Analysis of the RARS2 gene revealed compound heterozygosity for a missense mutation (c.1211 T>A, M404K) and a three base deletion after position c.471 resulting in deletion of lysine 158. Both of these mutations affect highly conserved residues. Each parent is heterozygous for one of these mutations.
Although mitochondrial dysfunction is highly variable in this condition, the presence of lactic acidosis, with or without respiratory chain abnormalities, in patients with characteristic MRI changes is a clear indication for RARS2 gene analysis.

244-P

DEMETHYLATION OF THE CODING REGION IS PIVOTAL FOR TRANSCRIPTION OF THE HUMAN TESTIS-SPECIFIC PDHA2 GENE

Pinheiro A[1], Faustino I[1], Silva MJ[1], Silva J[2], Sá R[3], Sousa M[3], Barros A[4], Almeida IT[1], Rivera I[1]
[1]*Met&Gen - iMed, Faculty Pharmacy UL, Lisbon, Portugal*
[2]*Centre Reprod Genetics Alberto Barros, Porto, Portugal*
[3]*Lab Cell Biol - ICBAS, Univ Porto, Porto, Portugal*
[4]*Dep Med Genetics, Faculty Medicine UP, Porto, Portugal*

DNA methylation is a crucial mechanism regulating the expression of tissue-specific genes in animal cells, and several studies have already reported a strong correlation between genomic methylation status and transcriptional activity in testis-specific genes.

The E1alpha subunit of Pyruvate Dehydrogenase Complex, an essential and rate-limiting enzyme system in energy metabolism, exists under two different isoforms encoded by distinct genes: PDHA1 located on chromosome X and expressed in somatic tissues, and PDHA2 located on chromosome 4 and exclusively expressed in post-meiotic spermatogenic cells.

The objective of this work was to elucidate the role of DNA methylation on PDHA2 gene expression in human tissues.

Human somatic and testicular tissues, including isolated fractions of haploid and diploid germ cells, were analysed at genomic and transcriptional levels. After primer design with appropriate software, sodium bisulfite PCR sequencing of the PDHA2 core promoter and coding regions was performed. The presence of PDHA2 mRNA was evaluated by RT-PCR followed by agarose gel electrophoresis.

The analysis of PDHA2 genomic sequence revealed the presence of two CpG islands: one in the core promoter which is methylated in all tissue types, and the other in the coding region which is methylated in somatic tissues but fully demethylated in spermatogenic cells. Furthermore, this methylation status displayed a perfect correlation with transcription activity, once PDHA2 transcript was only detected in haploid and diploid germ cells. These results led us to hypothesize that PDHA2 tissue-specific expression must be controlled by DNA methylation and chromatin remodelling.

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245-P

MUTATIONS IN TMEM70 CAUSES SEVERE ENCEPHALOCARDIOMYOPATHY AS WELL AS MILD ENCEPHALOPATHY

Van Coster R[1], Smet J[1], Lissens W[2], De Paepe B[1], Seneca S[2], De Meirleir L[2], Spilioti M[3], Fitsioris X[4], Evangeliou A[5]
[1]*Div Ped Neurol and Metabol, Univ Hospit, Ghent, Belgium*
[2]*Div Ped Neur - Cent Med Gen, UZ Brussel, Brussels, Belgium*
[3]*1st Dep Neurol, Aristotle Univ, Thessaloniki, Greece*
[4]*Dep Neurol, Papegeorgiou Hosp, Thessaloniki, Greece*
[5]*4th Ped Clinic, Aristotle Univ, Thessaloniki, Greece*

Background: Recently, TMEM70 was identified as a novel ancillary factor essential in biogenesis of mammalian complex V. We report clinical, biochemical and genetic data of two unrelated patients with pathogenic mutations in TMEM70. The two patients presented with remarkable difference in age of onset and severity of clinical symptoms.

Material and Methods: Oxidative phosphorylation (OXPHOS) enzyme activities were measured using spectrophotometrical analysis. Functional integrity of the five complexes was evaluated using BN-PAGE followed by in-gel activity staining. Western blotting was performed to document amounts of residual protein in the OXPHOS complexes. All coding exons and part of flanking introns of TMEM70 were PCR amplified and sequenced.

Results: In one patient, who presented with severe neonatal phenotype, sequencing of TMEM70 showed a homozygous splice site mutation (c.317–2A>G). The adolescent patient with milder symptoms was found to be compound heterozygote for the c.317–2A>G mutation and c.251delC deletion. In the adolescent patient, 3-methylglutaconic acid was detected in urine.

Almost complete absence of functional holocomplex V was demonstrated in several tissues from both patients.

Conclusions: Complex V deficiency caused by mutations in TMEM70 is apparently not extremely rare. Patients can present with a severe neonatal form, as well as with milder non progressive encephalopathy.

246-O

MITOCHONDRIAL HEPATOENCEPHALOPATHIES CAUSED BY MUTATIONS IN DGUOK, MPV17, POLG AND C10orf2 GENES

Yamazaki T[1], Compton A[2], Sugiana C[2], Laskowski A[2], Sceneay J[2], Kirby D[2], Pantaleo S[3], Sart D[3], Boneh A[3], Murayama K[4], Higasayama A[1], Ohtake A[1], Thorburn D[2]
[1]*Pediatrics, Saitama Medical University, Saitama, Japan*
[2]*Mitochondrial lab. MCRI, Melbourne, Australia*
[3]*VCGS pathology, RCH, Melbourne, Australia*
[4]*Metabolism, Chiba Children's Hospital, Chiba, Japan*

Background: Mitochondrial DNA (mtDNA) depletion syndrome (MDS) involves a tissue-specific reduction in mtDNA copy number. There are three main clinical presentations: hepatocerebral, myopathic and encephalomyopathic forms. Most patients with hepatocerebral MDS have neonatal or infantile onset liver failure and neurological symptoms, with a severe lactic acidosis. Hepatocerebral MDS has been associated with defects in four genes, however, the presenting clinical features of hepatocerebral MDS caused by the different genetic disorders have not been well studied.

Objectives: To determine the actual incidence of pathogenic DGUOK, MPV17, POLG and C10orf2 mutations in Australian (27) and Japanese (11) hepatocerebral MDS patients, and perform genotype-phenotype correlations by combining these patients with a larger cohort of previously published patients (62 DGUOK patients, 23 MPV17 patients and 7 recessive C10orf2 patients) and 34 patients we previously diagnosed POLG mutations.

Results: Mutations in DGUOK, MPV17, POLG and C10orf2 were identified in 10 Australian and 6 Japanese hepatocerebral MDS patients. All defects led to similar levels of hepatic mtDNA depletion. DGUOK patients had an earlier median age of onset while POLG patients had the latest median onset. POLG patients also had longer median survival but there isconsiderable overlap in onset and survival between each group. Patients with nonsense, frameshift and splicing mutations tended to have a more severe phenotype than those with missense mutations.

Conclusions: Our results provide new insights into hepatocerebral MDS mutation genetics, suggesting that patients may have different gene dependent presentation within this disease spectrum and that patients with missense mutations have a better prognosis.

247-P

A CASE OF DEOXYGUANOSINE KINASE (DGUOK) DEFICIENCY PRESENTING AS NEONATAL HEMOCHROMATOSIS

Hanchard N[1], Shchelochkov OA[2], Brundage E[1], Schmitt E[1], Li F[1], Wong L-JC[1], Scaglia F[1]
[1]*Dep Mol Human Genet, Baylor Coll Med, Houston, United States*
[2]*Div Genetics, Department Ped, Univ Iowa, Iowa City, United States*

Background: Mutations in the nuclear gene deoxyguanosine kinase (DGUOK) result in mitochondrial DNA (mtDNA) depletion, which may present as neonatal liver failure. The association between DGUOK deficiency and severe hepatic failure of neonatal hemochromatosis (NH), however, remains under-recognized.
Methods: We report a female neonate born to non-consanguineous African American parents, who failed her newborn screening for tyrosinemia. By two weeks of age she was hospitalized with progressive liver failure. Succinylacetone was absent in urine. Plasma amino acids were consistent with hepatic failure. An elevated ferritin and an abdominal MRI suggestive of hepatic iron deposition led to a presumptive diagnosis of NH. Her hepatic dysfunction and coagulopathy worsened, leading to death. Subsequent autopsy demonstrated hepatic siderosis and iron deposition in the pancreas, thyroid and heart. The abnormal newborn screening, severe liver dysfunction, and elevated plasma lactate suggested a hepatic mtDNA depletion syndrome.
Results: DGUOK sequencing revealed an apparent homozygous variant c.572A>G (p.Y191C) predicted to disrupt a critical tyrosine-glutamate interaction in the enzyme and previously reported in a neonate with the hepatocerebral form of DGUOK deficiency. Oligonucleotide arrayCGH did not detect intragenic deletions, but revealed mtDNA depletion. Parental samples were unavailable for analysis. A high resolution SNP-array demonstrated a region of absent heterozygosity encompassing the DGUOK gene, confirming homozygosity of DGUOK.
Conclusion: This report underscores the importance of considering DGUOK deficiency as a cause of NH, highlights mtDNA depletion-mediated hepatic failure in the differential diagnosis of neonatal tyrosinemia, and broadens its ethnic phenotype to include subjects of African American heritage.

248-P

DELETERIOUS MUTATIONS IN RRM2B RESULT IN SEVERE REDUCTION OF MTDNA CONTENT IN SKELETAL MUSCLE

Van Coster R[1], Smet J[1], De Paepe B[1], Lissens W[2], De Meirleir L[2], Seneca S[2]
[1]*Div Ped Neurol and Metabol, Univ Hospit, Ghent, Belgium*
[2]*Div Ped Neur - Cent Med Gen, UZ Brussel, Brussels, Belgium*

Introduction: Mutations in POLG1, DGUOK, TK2, SUCLG1, SUCLA2, PEO1, MPV17 and TYMP can cause mtDNA depletion. Recently, deleterious mutations in RRM2B encoding the p53 inducible ribonucleotide reductase subunit (p53R2) were shown to be associated with severe mtDNA depletion. We report on a patient with severe hypotonia, deafness, blindness, hyperammonemia and increased lactate. The metabolic disorder progressed rapidly and patient died at the age of two months.
Material and Methods: Oxidative phosphorylation (OXPHOS) enzyme activities were measured using spectrophotometrical analysis. Functional integrity of the five complexes was evaluated using BN-PAGE followed by in-gel activity staining. Western blotting was performed to investigate residual protein amounts in the OXPHOS complexes. Amount of mtDNA was evaluated using qPCR. Direct PCR nucleotide sequencing analysis screening of all coding exons and part of flanking introns of RRM2B was used.
Results: Severe reduction of activities from partially mtDNA encoded OXPHOS complexes (I, III, IV and V) was demonstrated in skeletal muscle using spectrophotometrical analysis and BN-PAGE. Western blotting using antibodies against selective OXPHOS subunits showed that the nuclearly encoded complex II was preserved. The amount of mtDNA in muscle was 3.1 %. Sequencing of RRM2B showed that the patient harboured a homozygous 3-bp in-frame deletion (nt 253–255delGAG, p.Glu85del) mutation in exon 3.
Conclusions: Apart from mutations in enzymes involved in salvage pathway of dNTPs, also deficient enzymes involved in de novo synthesis of deoxyribonucleotides can cause mtDNA depletion. In patients with myopathy, primary lactic acidosis and severe mtDNA depletion, screening of RRM2B gene is highly recommended.

249-P

RARE MTDNA MUTATIONS IN EARLY ONSET CASES OF MITOCHONDRIAL DISEASE

Itkis YS[1], Tsygankova PG[1], Zakharova EY[1], Rudenskaya GE[1], Dadali EL[1], Mikhailova SV[2]
[1]*Dep Inher Metab Dis, Res Cen Med Gen, Moscow, Russian Federation*
[2]*Russian Children's Hospital, Moscow, Russian Federation*

Mitochondrial diseases are associated with mitochondrial respiratory chain deficiency in muscles and in a wide variety of tissues. The clinical manifestation of these diseases is extremely heterogeneous because the different components involved in this process are encoded by nuclear and mitochondrial genome. One type of such disorders is Leigh syndrome—subacute necrotising encephalomyopathy, which mostly has onset at first years of life. Detection of causative mutations is usually limited to frequent mutations in nuclear genes (e.g. SURF1) and common mutations in the mitochondrial DNA (mtDNA). However, mutations in other mtDNA regions can be an important cause of oxidative phosphorylation disease as well. The etiology of a mutation and a proportion of mutant mtDNA in a cell and organs define the severity of a disease.
In our laboratory we performed mtDNA sequence analysis in blood cells of a small group of patients with Leigh-like phenotype and with excluded mutations in SURF1 gene. We identified a total four different substitutions in five patients. Among these, two unknown mutations (14441 T>C in ND6 region and 8839G>C in ATP6) were found. In two different families it was the same substitution (13094 T>C, ND5) but in patients with unlike manifestation. In the last patient we have found a mutation 13513G>A (ND5), which is reported as a frequent cause of Leigh-like syndrome.
Thus, it's recommended to deploy an analysis of mtDNA as a routine screening for mitochondrial disorders with Leigh (Leigh-like) phenotype, because very often there is no evidence of causative mutation, if frequent mutations are excluded.

250-O

DIAGNOSES AND MOLECULAR BASES OF MITOCHONDRIAL RESPIRATORY CHAIN DISORDERS IN JAPAN

Ohtake A[1], Murayama K[2], Yamazaki T[1], Honda M[1], Harashima H[1], Itoh A[2], Fujinami A[2], Tsuruoka T[2], Ajima M[2], Baba K[2], Takayanagi M[2], Compton A[3], Yamamoto S[4], Iwasa H[5], Okazaki Y[5], Mori M[6], Ryan MT[7], Thorburn DR[3]

[1]*Dept of Pediatr, Saitama Medical Uni, Moroyama, Saitama, Japan*
[2]*Dept of Metab, Chiba Children's Hosp, Chiba, Japan*
[3]*Mitochondrial Research Lab, MCRI, Melbourne, Australia*
[4]*Dept of Pediatr, Shimoshizu Natl Hosp, Yotsukaido, Chiba, Japan*
[5]*Transl Res Cent, Saitama Medical Uni, Hidaka, Saitama, Japan*
[6]*Dept of Pediatr, Jichi Medical Uni, Shimotsuke, Tochigi, Japan*
[7]*Dept of Biochem, La Trobe Uni, Melbourne, Australia*

Background: Congenital and primary lactic acidosis is one of the most frequent inborn errors of metabolism, of whom only 30% have had its precise cause identified. Our aim is to make a prompt and correct diagnosis of mitochondrial respiratory chain disorders (MRCD).
Methods: Activities of the individual respiratory chain complexes and citrate synthase were measured in tissue homogenates and mitochondrial fractions isolated from fibroblasts. Samples were solubilised and subjected to Blue Native Polyacrylamide Gel Electrophoresis (BN-PAGE) and western blotting using monoclonal antibodies. Mitochondrial DNA and nuclear DNA copy numbers within tissues were determined by quantitative PCR.
Results: One hundred and ten patients were diagnosed to have MRCD out of 267 candidate patients. Most frequent was complex I deficiency, of whom many patients had tissue-specific type deficiency. Twenty patients out of 110 had mitochondrial DNA pathogenic mutations, which meant the majority of childhood-onset MRCD was nuclear origin. Patients with mtDNA mutations had milder symptoms than those suspected to have nuclear mutation. MtDNA depletion syndrome (MDS) was a prevalent cause of multiple MRCD. Twelve patients in 10 families were diagnosed to have hepatic MDS, and 5 patients were diagnosed to have myopathic MDS. Out of 12 hepatic MDS, compound heterozygous MPV17 and POLG mutations were detected in one family, each, and homozygous DGUOK deletion (c.143–308_169del335) in the other. This DGUOK deletion was also detected in the other patient, and might be a common mutation in Japanese.
Conclusion: We must have a suspicion that almost every disease may be a MRCD.

251-P

STROKE IN PATIENTS WITH POLYMERASE GAMMA 1 (POLG1) MUTATIONS

Gavrilova RH[1], Zabel CA[1], Swanson JW[2]

[1]*Mayo Clinic, Dept Med Genetics, Rochester, United States*
[2]*Mayo Clinic, Dept Neurology, Rochester, United States*

Background: Mitochondrial Encephalopathy Lactic Acidosis Strokes (MELAS) is prototypically associated with cerebral infarcts possibly due to impairment of arterial vasodilatation related to abundance of intracellular reactive oxygen species that inactivate nitric oxide (NO). Administration of arginine to MELAS patients demonstrated decrease in severity and frequency of stroke episodes.
Polymerase gamma mutations lead to mitochondrial DNA depletion/deletions. Several phenotypes exist including sensory ataxic neuropathy (SANDO), Alpers, and myopathy. Some patients have strokes which mechanism is unknown. Genotype/phenotype correlation with stroke is not clearly established.
Methods/Results: We describe two patients with SANDO phenotype. Interestingly both had occipital strokes and were homozygous for the same mutation (c.1399G>A, pA467T) of POLG1 gene encoding linker domain. 8 additional patients with POLG1 gene mutations and strokes were identified. 3 were with SANDO and 5 with Alpers phenotypes. 4/8 were compound heterozygotes for linker/linker and 4/8 for linker/polymerase domain mutations. 5/8 shared A467T linker mutation. 2/8 had occipital infarcts, 4/8 hemiparesis.
Conclusions: Strokes in POLG1 occur in association with SANDO and Alpers phenotypes. Strokes are more frequently located in the occipital region or affect motor function. All stroke patients had mutations in the linker domain but none in the exonuclease domain.
Molecular and biochemical characterization might be helpful in determining the specific variations leading to stroke in POLG. If deficiency of NO is responsible for strokes in some POLG1 patients, arginine therapy might be applicable in order to prevent stroke frequency and associated disability.

252-O

LACTIC ACID AND BEYOND: QUANTITATIVE IN VIVO METABOLOMIC ANALYSIS OF MITOCHONDRIAL DISEASE USING MAGNETIC RESONANCE SPECTROSCOPY

Davison JE[1], Sun Y[1], Wilson M[1], Chakrapani A[2], Hendriksz CJ[2], Vijay S[2], Wassmer E[2], Davies N[1], Peet AC[2], Gissen P[1]

[1]*University of Birmingham, Birmingham, United Kingdom*
[2]*Birmingham Children's Hospital, Birmingham, United Kingdom*

Background: Diagnosis in mitochondrial disorders (MD) is challenging due to heterogeneous clinical presentation. Magnetic resonance spectroscopy (MRS) performed concurrently with MRI quantifies brain metabolites (range 1–10 mM). MRS lactate measurement is used as a marker of mitochondrial disease. Detection of other brain metabolites may provide additional information.
Aim: To evaluate clinical utility of MRS in diagnosing mitochondrial disorders.
Patients/Methods: MRS was performed at 1.5 T in a cohort of children being evaluated for various neurodevelopmental or metabolic disorders. Quantitative concentrations of twenty-four metabolites from basal ganglia (BG) and parieto-occipital white matter (WM) were acquired using LCModel software. Individual spectra were evaluated for metabolite peaks not fitted by the standard metabolite basis-set. Mean spectra and metabolite concentrations in the MD cohort (n=11, proven mutations and/or enzyme deficiency) were compared with a cohort of children with normal imaging (n=63). Diagnostic performance of MRS-detected lactate was assessed against a heterogeneous (neuro/metabolic disease) cohort of children (n=173).
Results: Lactate was reliably detected above 0.6 mM and was significantly elevated in BG and WM in MD compared to the normal-imaging cohort ($p<0.01$), with 50% sensitivity, 80% specificity and ROC-area 0.71 in BG compared to the heterogeneous cohort. N-acetylaspartate, creatine and glutamate were decreased in BG ($p<0.01$), but there was no difference in choline or myo-inositol. Analysis of MRS peaks unfitted by standard metabolites revealed one case with a single (2.4 ppm) in WM but not BG consistent with succinate suggesting complex II deficiency with regionalised pathogenic effects.
Conclusion: Quantitative MRS can aid diagnosis and understanding of mitochondrial disease processes.

253-P

IN VIVO PROTON MR SPECTROSCOPY FINDINGS SPECIFIC FOR ADENYLOSUCCINATE LYASE DEFICIENCY

Dreha-Kulaczewski S[1], Henneke M[1], Brockmann K[1], van der Graaf M[2], Willemsen M[3], Engelke U[4], Dechent P[5], Heerschap A[6], Helms G[5], Wevers RA[7], Gärtner J[1]
[1]*Dept Ped and Ped Neurology, UMG, Gottingen, Germany*
[2]*Clin Phys Lab, Dept Ped, Radboud Univ, Nijmegen, Netherlands*
[3]*Dept Ped Neurology, Radboud Univ, Nijmegen, Netherlands*
[4]*Dept Neurol, Lab Ped Neurol, Radboud Uni, Nijmegen, Netherlands*
[5]*MR-Research, UMG, Gottingen, Germany*
[6]*Dept Radiology, Radboud Univ, Nijmegen, Netherlands*
[7]*Dept Lab Med, Radboud Univ, Nijmegen, Netherlands*

Background: Adenylosuccinate lyase (ADSL) deficiency is an inborn neurometabolic disorder of purin metabolism with inconsistent clinical symptoms and unspecific leukodystrophy pattern on brain MR-imaging (MRI). Biochemically, it is characterized by the accumulation of succinylaminoimidazolecarboxamide riboside (SAICAr) and succinyladenosine (S-Ado) in body fluids which can be determined by high resolution in vitro MR-spectroscopy (MRS).
Patients and Methods: In three boys (age 4–9 yrs) in vitro MRS from cerebrospinal fluid (CSF) revealed ADSL deficiency that was further confirmed genetically. Clinical symptoms included psychomotor delay and behavioral abnormalities. In vivo MRS studies were carried out at 3 and 1.5 T MRI systems (Siemens). For localization STEAM (repetition time (TR)/echo time (TE)/middle interval (TM) 6000/20/10 or 30 msec, volume of interest (VOI) 4 ml or 8 ml) or PRESS (TR/TE 6000/30 msec, or 2000/136 msec, VOI 8 ml) were applied. VOI were placed in white and gray matter and spectra analysed by LCModel.
Results: In vitro MRS of CSF in ADSL deficiency showed at pH 7.4 singlets at 8.27 and 8.29 ppm and a doublet at 6.08 ppm corresponding S-Ado as well as a singlet at 7.48 ppm and a doublet at 5.66 ppm corresponding to SAICAr. In vivo MRS resonance revealed a singlet at 8.3 ppm in all patients in all brain regions. Given the lower spectral resolution of in vivo MRS, the 8.3 ppm peak represents the merged singlets at 8.27 and 8.29 ppm of S-Ado.
Conclusion: In vivo proton MRS provides a conclusive finding in ADSL deficiency and represents a reliable noninvasive diagnostic tool for this neurometabolic disorder.

254-P

SUCCESSFUL USE OF ALBUTEROL IN A PATIENT WITH CENTRAL CORE 5 DISEASE AND MITOCHONDRIAL DYSFUNCTION

Schreuder LTW[1], Nijhuis-Van der Sanden MWG[1], de Hair A[2], Peters G[1], Wortmann S[1], Bok LA[2], Morava E[1]
[1]*UMC St Radboud, Nijmegen, Netherlands*
[2]*Máxima Medical Centre Veldhoven, Veldhoven, Netherlands*

Background: Albuterol, a selective beta-adrenergic agonist, has been used experimentally in combination with exercise therapy in a few inherited neuromuscular disorders to increase muscle strength and muscle volume.
Methods: We report on a 9-year-old boy with central core disease and mitochondrial dysfunction due to compound heterozygous RYR1 mutations receiving albuterol treatment for 1 year. Throughout the period of albuterol administration, the patient underwent an aerobic exercise regime of training sessions three times a week that lasted 20 min each.
Results: No side effects of albuterol use were seen. Significant clinical progress, including self care, sitting up, raising arms above the shoulders, independent feeding, and better speech and writing were observed compared with minimal development of these abilities in the previous year's physiotherapy. Improved forced expiratory volume in 1 s (FEV1) score was detected and increased muscle strength was noted: progress was measured using various functional tests and assessment scales. The only complication observed was a mild progression of the joint contractures, possibly due to an unbalance between the flexor and extensor muscula- ture.
Conclusions: In general, in this pilot study in a complex case of metabolic myopathy our patient has shown promising results following albuterol treatment and aerobic exercise therapy.

255-O

SEVERE X-LINKED MITOCHONDRIAL ENCEPHALOMYOPATHY ASSOCIATED WITH A MUTATION IN APOPTOSIS-INDUCING FACTOR

Uziel G[1], Ghezzi D[1], Sevrioukova I[2], Invernizzi F[1], Moroni I[1], Lamperti C[1], Mora M[3], Zeviani M[1]
[1]*IRCCS Istituto Neurologico C. Besta, Milano, Italy*
[2]*Dep Mol Biol Biochem Univ of California, Irvine, United States*
[3]*IRCCS Istituto Neurologico C. Besta, Milano, Italy*

The majority of mitochondrial disorders are not due to mutations in genes encoding structural components of the respiratory chain, but in gene products involved in respiratory chain assembly, turnover and activity control. We investigated two male infants affected by a severe and progressive encephalomyopathy with OXPHOS failure due to a deleterious mutation in a master gene product involved in mitochondrial-dependent programmed cell death.
The patients were born from monozygotic twin sisters and unrelated fathers, suggesting an X-linked trait. Fibroblasts from both showed reduction of respiratory chain cIII and cIV. In muscle, defective cI-cIII-cIV was associated with low mtDNA copy number (30% of the normal). We found a disease-segregating deletion of arginine 201 in the Apoptosis-Inducing Factor (AIF), encoded by the X-linked AIFM1 gene. Under normal conditions, mature AIF is a FAD-dependent NADH oxidase of unknown function that binds to the inner mitochondrial membrane projecting into the intermembrane space. Upon apoptogenic stimuli, a soluble form (AIFsol) is released by proteolytic cleavage and migrates to the nucleus, where it induces a caspase-independent fragmentation of chromosomal DNA termed pathanatos. In vitro, the AIFR201-del mutation decreases the stability of both AIFmit and AIFsol and increases the AIFsol DNA binding affinity, a prerequisite for nuclear fragmentation. In AIFR201-del fibroblasts, staurosporine-induced parthanatos was markedly increased, whereas re-expression of AIFwt induced recovery of RC activities. We conclude that AIF has a role in RC integrity and mtDNA maintenance and that AIFR201 del is an unstable mutant variant associated with increased parthanatos-linked cell death.

256-P

MUTATIONAL SPECTRUM OF POLG IN PORTUGUESE PATIENTS

Almeida LS[1], Ferreira M[1], Evangelista T[2], Macário MC[3], Martins J[4], Martins E[5], Santorelli FM[6], Vilarinho L[1]
[1]*Centro de Genética Médica, INSA, Porto, Portugal*
[2]*Hospital Sta. Maria, Neurologia, Lisboa, Portugal*
[3]*HUC, Neurologia, Coimbra, Portugal*
[4]*Hospital Egas Moniz, Neuromusculares, Lisboa, Portugal*
[5]*Un. Doencas Metabólicas, CHP, Porto, Portugal*
[6]*IRCCS Fondazione Stella Maris, Pisa, Italy*

Mitochondrial diseases are devastating disorders with no cure and no proven treatment. Mitochondrial DNA is replicated by complex machinery including, among others, DNA polymerase γ (POLG). POLG is a heterotrimer, consisting of a catalytic subunit (POLGα, encoded by POLG) and an accessory subunit dimer (POLGβ, encoded by POLG2). In 2001 the first disease mutations were identified in POLG.
POLG-related disorders comprise a continuum of broad and overlapping phenotypes, being characterized by mtDNA deletions/depletion. Genotype-phenotype correlations are not possible because all combinations of mutation type and location have been associated with the entire phenotypic spectrum and both autosomal recessive/dominant inheritance. To date more than 150 disease mutations have been identified placing POLG as a major locus of mitochondrial disease.
In this study we investigated a cohort of Portuguese patients suspicious of POLG disorder having a wide variety of clinical manifestations such as external ophthalmoplegia, ptosis, peripheral neuropathy, Parkinsonism.
In 11 patients (29% of the cases) we detected 12 different mutations in the POLG gene from which two of them have not been described (p. Arg1081Gln and p.Trp585X). A number of already described single nucleotide polymorphisms were also found.
Neurodegenerative phenotypes are gaining importance as showed by the identification of a new mutation in a patient with Parkinsonism and peripheral neuropathy. Overall, in our cohort of patients we expand the array of mutations in POLG gene and we also confirm that the associated phenotypic range is ample.

257-P

DIFFICULTY IN THE DIAGNOSIS OF A GIRL WITH PYRUVATE DEHYDROGENASE DEFICIENCY AND A LARGE X-CHROMOSOMAL DELETION

Sperl W[1], Koch J[1], Rauscher C[1], Zimmermann F[1], Zschocke J[2], Fauth C[2], Mayr JA[1]
[1]*Dept. Pediatrics, Paracelsus Med. Univ., Salzburg, Austria*
[2]*Dept. Human Genetics, Medical University, Innsbruck, Austria*

Mutations in the X-linked E1a subunit of the pyruvate dehydrogenase complex are the most frequent causes of PDHC deficiency. The clinical picture is heterogeneous depending on residual enzyme activity and X-inactivation.

We report on a girl with low birth weight who presented at an age of 3 weeks with muscular hypotonia, vomiting, hyperlactatemia, microcephaly with enlarged ventricles, partial agenesis of corpus callosum and seizures.

At the age of 10 months normal respiratory chain enzyme activities including PDHC were measured in muscle. Substrate oxidation rates revealed a moderately diminished pyruvate oxidation. PDHA1 sequencing was normal however quantitative analysis revealed a hemizygosity of the whole PDHA1 gene. Homozygosity mapping and determination of the breakpoint revealed a 1.1 million base pair deletion on the X-chromosome including the CDKL5 and PDHA1 gene.

In this patient the difficulty in the diagnosis of PDHC deficiency is evident: 1. Enzyme activity can be normal depending on the X-inactivation. 2. Large deletions can be missed by routine genetic analysis. 3. Only quantification of the PDHA1 gene content revealed the genetic defect in our patient. Therefore we recommend revisiting patients who are clinically suspicious for a mitochondrial disorder especially for a hidden PDHA1 mutation.

258-O

ATP SYNTHASE DEFICIENCY DUE TO A MUTATION OF SUBUNIT E THE FIRST OF A NUCLEAR ENCODED SUBUNIT

Mayr JA[1], Havlíčková V[2], Zimmermann F[1], Magler I[1], Kaplanová V[2], Jeina P[2], Pecinová A[2], Nůsková H[2], Koch J[1], Houtěk J[2], Sperl W[1]
[1]*Dept. Bioenergetics, Acad. Sciences, Prague, Czech Republic*

Mitochondrial diseases due to isolated disorders of the F1Fo-ATP synthase are so far known to be caused by mutations in mtDNA genes for the subunits ATP6 and ATP8 or in nuclear genes encoding the biogenesis factors TMEM70 and ATPAF2. Here we describe a patient with a homozygous p.Tyr12Cys mutation in the ε subunit encoded by the nuclear gene ATP5E. The 22 year old woman presented with neonatal onset, lactic acidosis, 3-methylglutaconic aciduria, mild mental retardation and developed peripheral neuropathy. Patient fibroblasts showed 60–70% decrease in both oligomycin-sensitive ATPase activity and mitochondrial ATP synthesis. The mitochondrial content of the ATP synthase complex was equally reduced, but its size was normal and it contained the mutated ε subunit. A similar reduction was found in all investigated F1 and Fo subunits with the exception of Fo subunit c, which was found to accumulate in a detergent-insoluble form. This is the first case of mitochondrial disease due to a mutation in a nuclear encoded structural subunit of ATP synthase. Our results indicate an essential role of the ε subunit in the biosynthesis and assembly of the F1 part of the ATP synthase.

259-O

THE UTILITY OF TARGETED ARRAY CGH IN THE DIAGNOSIS OF MITOCHONDRIAL RELATED DISORDERS

Zhan HL[1], Li FY[1], Brundage E[1], Pursley A[1], Chinault C[1], Wang J[1], Wong L[1]
[1]*Dept Mol & Hum Genet., Baylor Col Med, Houston, TX, United States*

Background: Mitochondrial disorders constitute a group of clinically and genetically heterogeneous diseases. Current molecular diagnosis is based on direct DNA sequencing. However, sequencing does not detect large deletions. We report the utility of a custom targeted oligonucleotide array comparative genomic hybridization (oligo aCGH) for the detection of large deletions.

Methods: A custom oligo aCGH with high-density coverage of the mitochondrial genome and a set of nuclear genes involved in mitochondrial related disorders was used to evaluate large deletions in nuclear and mitochondrial genomes in a total of 1000 DNA samples. Deletions detected were confirmed by PCR and sequencing to determine the deletion breakpoints.

Results: Oligo aCGH identified a total of 61 (6.1%) cases with 21 large deletions in urea cycle genes (16 OTC, 4CPS1, and 1 ASS1), 5 in genes causing mitochondrial DNA depletion (2 DGUOK, 1 each in POLG1, TK2, and MPV17), 9 deletions in metabolic genes (SLC25A13, ABCB11, CACT, OCTN2, ALDOB, STS, MCCC1, MECP2, and GK), and 9 with large deletions in mtDNA. The breakpoints were confirmed and the percentage of mtDNA deletion heteroplasmy was estimated. In addition, 9 and 8 cases, respectively, of large copy number loss and gain involving multiple genes believed to be pathogenic were identified.

Conclusions: These examples illustrate the successful utilization of targeted custom oligonucleotide arrays to detect large deletions in both nuclear and mitochondrial genomes. This technology is particularly useful as a complementary diagnostic test in the context of a recessive disease when only one mutant allele is found by sequencing.

260-O

MITOCHONDRIAL VDAC1 IS CENTRAL TO GLUCOSE HOMEOSTASIS BY INTERACTING WITH HEXOKINASE 2 AND ENHANCING AKT SIGNALING

Raghavan A[1], Xin H[1], Cai Z[1], Murphy E J[2], Craigen WJ[1]
[1]*Dept of Molecular and Human Genetics, BCM, Houston, United States*
[2]*Department of PPT, Univ. of North Dakota, Grand Forks, United States*

Background: Voltage dependant anion channels (VDACs) are 30–35-kilodalton (kDa) pore-forming proteins present within the mitochondrial outer membrane (MOM). VDACs form complexes with other proteins that localize to MOM, and serve as the main pathway for transporting metabolites between the cytoplasm and mitochondria. There are three isoforms in humans and mice; VDACs 1, 2 and 3.

Methods and Results: We have generated knockout mice for each isoform by gene targeting. VDAC1 knockout mice exhibit impaired glucose tolerance and altered hepatic lipid metabolism, although the causal mechanism has yet to be elucidated. Using a hyperinsulinemic, euglycemic clamp protocol, VDAC1 deficient mice demonstrate a significant reduction in glucose tolerance, glucose infusion rate (GIR), glucose uptake in muscle tissues, and glucose disposal rate (GDR), all of which are characteristic features of Type-2 diabetes.

Conclusions: The localization to the MOM of various proteins involved in glucose metabolism suggests that an interaction between these proteins could explain the VDAC1-/- mouse phenotype. In fact, the hexokinase isoforms HKI and HKII that are central to glycolysis have been shown to interact specifically with VDAC1, in the MOM. At a cellular level, VDAC1 knockout mice have reduced mitochondrial bound hexokinase, and this is associated with reduced AKT activation. We hypothesize that VDAC1 plays an important role in facilitating ATP access for HKs in the MOM and that absence of VDAC1 could lead to impaired glycolysis, insulin insensitivity, and diabetes.

261-P

CORRELATION BETWEEN PARIETAL WHITE MATTER, BASAL GANGLIA, AND CEREBELLAR MRI/MRS LACTATE LEVELS TO BLOOD OR CSF LACTATE LEVELS IN 742 CHILDREN

Renaud DL[1], Aboian MA[1], Port JD[1]
[1]Mayo Clinic Foundation, Rochester, MN, United States

Purpose: To identify and correlate brain MR spectroscopy (MRS) lactate measurements with blood and CSF lactate values in children under evaluation for developmental delay at Mayo Clinic Rochester from 2003 to present.
Methods: MRS data was collected from voxels in right basal ganglia (BG), parietal white matter (PWM), and cerebellum on a 1.5 T MRI. Brain lactate peaks were fitted with LCModel. Bivariate correlation was performed to calculate Pearson's correlation coefficients using SPSS software.
Results: 742 patients were included. Elevated blood lactate was found in 23 (3.1%) patients. Elevated CSF lactate was found in 12 (1.5%) patients. Elevated lactate on MRS was found in 38 (5.1%) patients within BG, in 41 (5.5%) patients within PWM, and in 27 (2.6%) patients within the cerebellum. Elevated blood alanine value was found in 13 (1.8%) patients. Elevated PWM and BG Lac/Cr values correlated with elevated CSF lactate values with R=0.928 ($p<0.0001$) and R=0.924 ($p<0.001$), respectively. Elevated cerebellar Lac/Cr values did not correlate with either the CSF or blood lactate values. Elevated blood lactate values loosely correlated with elevated CSF lactate values and elevated blood alanine values. There was a positive correlation trend between elevated blood lactate value and the BG Lac/Cr elevation.
Conclusion: MRS lactate values in the PWM and BG have a statistically significant correlation with elevated CSF lactate values and a positive correlation with elevated blood lactate values. As such, these non-invasive brain MRS lactate measurements may be used in lieu of CSF-lactate measurements to evaluate mitochondrial abnormalities in children.

262-P

PEDIATRIC ONSET OF MYOPATHY AND CARDIOMYOPATHY DUE TO THE MITOCHONDRIAL DNA 3302A>G MUTATION IN THE tRNA (LEU (UUR)) GENE: A CASE REPORT

Costa C[1], Garcia P[1], Santos I[2], Rodrigues F[1], Grazina M[3], Vilarinho L[4], Diogo L[5]
[1]Met Dis Unit, Child Dev Center, Ped Hosp, Coimbra, Portugal
[2]Cardiology Department, Ped Hosp,, Coimbra, Portugal
[3]Bioch Inst, Facult Med, Cent Neuroc, Univ, Coimbra, Portugal
[4]Med Gen Cent, Nation Health Instit, INSA, Porto, Portugal
[5]Met Dis Unit, Child Dev Center, Ped Hosp, Coimbra, Portugal

Background: The main clinical features of the mitochondrial DNA 3302A>G mutation is an adult onset of progressive mitochondrial proximal myopathy.
Case Report: This girl is the first child of a Caucasian nonconsanguineous couple who came to medical attention in her second year of life because of with frequent falls, easy fatigability and language delay. Family history was irrelevant except for ptosis and ophthalmoplegia in the maternal grand-mother. On clinical exam at 5 years of age, a slight right ptosis with normal ocular movements, axial hypotonia and hyperreflexia were noticed. The investigation revealed persistent hiperlactacidemia with increased lactate/ pyruvate ratio in plasma and cerebral spinal fluid, a paradoxical ketonemia and elevated beta-hydroxibutirate/acetoacetate ratio. Brain MRI and heart evaluation were normal. Muscle biopsy showed ragged red fibbers. Mitochondrial respiratory chain complexes function (I to V) was normal in muscle and mtDNA screening was negative for common mutations and deletions.
Progressive fatigability, especially during infections, exercise intolerance and proximal muscle weakness ensued. At twelve years of age hyper-throphic myocardiopathy with abnormal function was diagnosed. Her IQ was 62. Finally, a 3302 A>G mtDNA heteroplasmic (85–88%) mutation was found in the patient's tissues and also in her mother's lymphocytes (64%). She died with sudden cardiac arrest at the age of 18.
Conclusion: In this patient, a mitochondrial disorder was strongly suspected due to the evidence of ragged red fibers at an early age and progressive multisystem involvement. To our knowledge, this is the first reported paediatric presentation of the mtDNA 3302A>G.

263-P

LEIGH SYNDROME: MOLECULAR DIAGNOSTICS

Tsygankova PG[1], Zakharova EYu[1], Mikhailova SV[2], Pichkur NA[3], Rudenskaya GE[1], Dadali EL[1], Il'ina ES[2], Nikolaeva EA[4]
[1]Research centre for medical genetics, Moscow, Russian Federation
[2]Rus Nat Child Hospital, Moscow, Russian Federation
[3]Ukr Children's Spec Hosp OXMATDET, Kiev, Ukraine
[4]Ins Pediatr child Surgery, Moscow, Russian Federation

Leigh syndrome is the most frequent OXPHOS disorder of in children. Besides, Leigh syndrome is mitochondrial disorder with the largest genetic heterogeneity. Pathological mutations have been found either in the mitochondrial or in the nuclear genome.
Aim: Develop a reliable protocol for mutation searching in Leigh syndrome patients.
Results: We conducted molecular genetics research in a series of 54 patients with Leigh and Leigh-like phenotypes. We performed sequence analysis of nuclear genes (NDUFV1, NDUFS3, NDUFS4, NDUFS7, NDUFS8 and SURF1). Later we continued with sequence of MTND3-ND6, MTATP6 regions of mtDNA. We revealed mutations in SURF1 gene to be the major cause of Leigh syndrome in our patients (70% of cases). We found 7 novel mutations in the SURF1 gene (IVS7-1G>C, c.554_555insA, c.703A>G, c.65delG, p.L76term, p.G17term, [IVS7-45_749delAG_818]dup), including complex rearrangement. The most frequent mutation is c.845delCT. Mutations in mtDNA were found in 8 patients, 5 of them have Leigh-like phenotypes. Three patients have 8993 T>G mutation. One patient has 8339A>G transition which wasn't described earlier. Four patients have mutations in ND5 and ND6 regions (two with 13094 T>C, one with 13513G>A and one with novel 14441 T>C). We managed to reveal molecular defect in 85 % of Leigh syndrome cases.
Conclusion: We analyzed clinical, biochemical and genetic features of Leigh syndrome and made the algorithm for molecular diagnostic of Leigh syndrome that could be used in the genetics laboratories, especially in Eastern Europe countries. The algorithm include SURF1 gene screening for frequent mutations, 8993 T>G, 13513G>A screening test, SURF1 whole gene sequencing, ND3-ND6 sequencing.

264-P

INTERLABORATORY COMPARISON OF RESPIRATORY CHAIN ACTIVITY MEASUREMENT

Rolinski B[1], Mayr H[2], Ahting U[3], Gempel K[4], Makowski C[5], Freisinger P[6], Sperl W[2]
[1]Dep Lab Pathol Elblab Elblandklinikum, Riesa, Germany
[2]Univ Child Hosp SALK, Salzburg, Austria
[3]Dep Clin Chem Munich Municipal Hospital, Munich, Germany
[4]Labor Becker Olgemoller, Bamberg, Germany
[5]Univ Child Hosp TUM, Munich, Germany
[6]Kinderklinik Kreiskliniken Reutlingen, Reutlingen, Germany

Background: Proficiency testing is one of the most indispensable features of quality assurance for laboratory testing. However, for orphan assays proficiency testing is often not available. In order to establish a surrogate system for proficiency testing respiratory chain activities in muscle biopsies were measured in two different laboratories and results compared.
Methods: In an eight year period a total of 130 muscle biopsies were measured in parallel in the laboratories for mitochondrial disorders in Munich and in Salzburg. Patient's median age was 2 years with 65 males and 65 females. All biopsies were performed as open biopsies in the University Children's Hospital in Munich. Material was split in equal parts in the operation theatre and sent to the laboratories.
Results: As expected we observed a large interlaboratory variability when comparing the respiratory chain activities related to protein concentration. Only little improvement in the degree of variability was achieved by relating the values to the mitochondrial reference enzyme citrate synthase. However, when measured values were calculated as percent of the mean of the reference range variability substantially decreased. With regard to the diagnostic accuracy we analyzed the number of samples where both laboratories gave positive or negative or discordant results with the number of discordant results reached up to 30%. However when comparing the final diagnosis discordant results were only found in 3 % of samples.
Conclusion: Respiratory chain activities obtained from different laboratories can vary considerably. With respect to diagnostic accuracy, however, agreement was high.

265-P

NOVEL MITOCHONDRIAL DNA DELETION IN PATIENT WITH DISTINCT PRESENTATION OF PEARSON SYNDROME

Kecman B[1], Mayr J[2], Djordjevic M[1], Sarajlija A[1], Stajic N[3]
[1]*Metab Div, Moth and Child Health Inst, Belgrade, Serbia and Montenegro*
[2]*Dept of Pediatrics, Paracelsus Med Univ, Salzburg, Austria*
[3]*Nephr Div, Moth and Child Health Inst, Belgrade, Serbia and Montenegro*

Introduction: Pearson syndrome (PS) represents a multisystemic mitochondrial disorder typically presenting with early hematologic abnormalities and exocrine pancreas insufficiency. Involvement of numerous other organ systems has been described in PS, with renal tubular dysfunction verified in more than 20% of patients. Further insights to the clinical and genetic spectrum of disease showed marked heterogenicity of manifestations previously considered as typical.
Objective: To present a patient with novel deletion of mitochondrial DNA (mtDNA) and distinct clinical presentation.
Case Report: A boy born to a pair of healthy non-consanguineous parents (Roma and Korean descent) presented at two months of age with pancytopenia. He was treated for suspected myelodysplastic syndrome. Pancytopenia subsided during the following months, but in the second year failure to thrive became overt, and lactic acidosis was noted during an episode of enterocolitis. Diagnostic evaluation revealed persistent polyuria, aminoaciduria, glycosuria, phosphaturia and hypoparathyroidism. Exocrine pancreatic insufficiency was not proven since steatorrhea was absent with normal levels of fecal elastase. Ophthalmologic manifestations included corneal clouding and retinal degeneration. Amplification of the whole mtDNA revealed a single large deletion 8283_13792del5510 that has not been previously described. At 5 years of age, the boy has Toni-Debré-Fanconi syndrome with chronic renal insufficiency and signs of cerebellar ataxia, areflexia and moderate mental retardation.
Conclusion: Large deletions of mtDNA can be associated with distinct clinical phenotypes of Pearson syndrome.

266-P

mtDNA DEPLETION INVESTIGATION IN PORTUGUESE PATIENTS

Santos MJ[1], Truong CK[2], Pratas J[1], Diogo L[3], Garcia P[3], Scaglia F[2], Oliveira CR[4], Wong LJ[2], Grazina M[4]
[1]*Lab Biochem Genet - CNC/UC, Coimbra, Portugal*
[2]*Baylor College of Medicine, Houston, Texas, United States*
[3]*Metabolic Unit, Pediatric Hospital, Coimbra, Portugal*
[4]*Inst Biochem, Fac Medicine, Un Coimbra, Coimbra, Portugal*

Background: Reduction of mtDNA content has been implicated as a major cause of mitochondrial disease in children, particularly with liver and brain dysfunction.
Methods: We have analyzed the mtDNA copy number in 60 samples (14 liver, 23 muscle and 23 blood) from 41 paediatric patients with possible mitochondrial OXPHOS disease. Total DNA was extracted by standard methods. The copy numbers of both mtDNA and nDNA were determined by real-time PCR, using SYBR green and primers specific for mitochondrial and nuclear genes. The intensity of fluorescent signals was analyzed by SDS v2.2.2 software. The relative contents of mtDNA was calculated by ΔCt (ΔCt is the difference of Ct value between nuclear and mtDNA genes.
Results: We have found mtDNA depletion (<30% compared with age matched controls) in about 15% of cases: 7 samples (4 liver, 1 muscle and 2 blood) samples, from 6 patients. In three subjects (50%), the depletion could be related with mutations found in DGUOK gene.
Conclusions: As expected, other nuclear genes are also involved in mtDNA depletion. To our knowledge, this is the first report of an mtDNA depletion investigation in Portuguese patients, illustrating the importance of copy number impairment as a cause of OXPHOS disease in children.

268-P

MITOCHONDRIAL rRNA GENES VARIATIONS IN FROTOTEMPORAL DEMENTIA PATIENTS

Santos MJ[1], Silva F[2], Santana I[3], Santiago B[3], Pires P[3], Oliveira CR[2], Grazina M[2]
[1]*Lab Biochem Genet - CNC/UC, Coimbra, Portugal*
[2]*Inst Biochem, Fac Medicine, Un Coimbra, Coimbra, Portugal*
[3]*University Hospitals of Coimbra, Coimbra, Portugal*

Background: Frontotemporal dementia (FTD) is the second most common type of primary degenerative dementia, only preceded by Alzheimer's disease (AD). It usually presents a neuropathological and clinical overlapping with AD that suggests some common pathways in physiopathology mechanisms in both phenotypes, specially the mitochondrial DNA (mtDNA) alterations. We have found several variations in MTRNA16S gene sequence in FTD patients. Accordingly, we considered that it would be relevant to investigate also sequence variations in MTRNA12S gene.
Methods: We studied 7 patients recruited at the Neurology Unit of HUC with probable diagnosis of FTD. Total DNA was extracted from peripheral blood by standard methods and the analysis of MTRNA12S sequence was performed using the Kit mitoSEQr Resequencing System.
Results: We have found 3 mtDNA polymorphic sequence variations, in 2 patients (29%), while the other 5 patients (71%) do not present any alteration in this gene. The 2 patients that have variations in MTRNA12S have also variations in the MTRNA16S gene. We have also studied the rRNA structure modifications for the alterations found and there is one alteration that could influence the mitochondrial protein synthesis, leading to unknown problems.
Conclusions: This is an original report that suggests the contribution of mtDNA encoded rRNAs variations to FTD pathology. However, a larger sample needs to be studied for drawing major conclusions.

269-P

BIGENOMIC INVESTIGATION IN PAEDIATRIC PATIENTS WITH MULTIORGAN IMPAIRMENT

Ribeiro C[1], Silva F[1], Santos MJ[2], Truong CK[3], Diogo L[4], Pratas J[2], Garcia P[4], Oliveira CR[1], Wong LJ[3], Grazina M[1]
[1]*Lab Biochem Genet - CNC/UC, Coimbra, Portugal*
[2]*Baylor College of Medicine, Houston, Texas, United States*
[3]*Metabolic Unit, Pediatric Hospital, Coimbra, Portugal*

Background: Mitochondrial cytopathies are a heterogeneous group of diseases that include a broad spectrum of clinical presentations. They may appear at any age, be transmitted by any type of heredity and are characterized by functional changes in mitochondria, leading to mitochondrial respiratory chain (MRC) dysfunction. Polymerase gamma (POLG) is encoded by 2 nuclear genes, POLG1 and POLG2 and is responsible for constant and exact replication of mitochondrial DNA (mtDNA), maintaining the adequate copy number and preserving MRC structure and functions. The association between human disease and POLG genes' mutations was described in 2001. Since then, more than 100 mutations have been reported. Currently, it is estimated that 25% of mitochondrial diseases are related to POLG mutations.
Methods: The aim of this study is to investigate the association of mitochondrial disorders with hepatic involvement with POLG1,2 mutations and mtDNA abnormalities. We have studied 6 pediatric patients with liver dysfunction. The methods used included PCR, sequencing and real-time PCR.
Results: We have found 19 sequence variations, 18 located in non-coding regions and one described as pathogenic (Q1236H) in Alpers syndrome. This patient did not present mtDNA depletion. Polymorphic mtDNA variations were found in 2 cases. Reduction of mtDNA copy number was found in 3 cases, 20% in 2 and one with a 87% mtDNA copy number reduction, without POLG mutations.
Conclusions: The results suggest that Q1236H mutation does not affect liver mtDNA copy number in this case. Additionally, other genes than POLG1,2 are involved in copy number reduction.

270-P

MITOCHONDRIAL ENERGY IMPAIRMENT IN KJER-TYPE OPTIC ATROPHY

Mendes C[1], Simues M[1], Silva E[2], Reis A[3], Oliveira CR[4], Castelo-Branco M[3], Grazina M[4]
[1]University Hospitals of Coimbra, Coimbra, Portugal
[2]IBILI, Fac Medicine, Un Coimbra, Coimbra, Portugal
[3]Inst Biochem, Fac Medicine, Un Coimbra, Coimbra, Portugal

Background: Kjer-type optic atrophy (OAK) is a form of slowly progressive disease and is considered the most common form of autosomal inherited optic neuropathy. Enzymatic deficiencies of mitochondrial respiratory chain (MRC), with impaired energy metabolism in nervous system, are a common denominator in several neurodegenerative disorders. The pathogenic characteristics of OAK share some similarities with Leber's hereditary optic neuropathy (LHON), a known mitochondrial disease, due to mtDNA mutations. OPA1 gene codes for a mitochondrial protein and harbors mutations leading to OAK phenotypes, and possibly leading to insufficient energy supply in the highly energy-demanding neurons of the optic nerve.
Methods: Aiming to investigate the possible involvement of MRC dysfunction in OAK, we have evaluated MRC activity (complexes I to V) in circulating lymphocytes and ATP content in plasma of 25 patients with OAK phenotype.
ATP was measured using luciferin-luciferase cheluminescent method and MRC complexes were analyzed by double wavelength spectrophotometry. Citrate Synthase activity was used as a mitochondrial reference enzyme.
Results: The MRC evaluation revealed 60% of cases with deficiency: 32% in CIII, 8% in CII, 24% in CI and 36% in CV. The plasma ATP levels were noticeably decreased (65%) compared to controls.
Conclusions: Further studies are necessary, including the analysis of a larger sample. Additionally, the analysis of results concerning age and/or variability of clinical expression reflected by the extent of optic atrophy is useful to clarify the MRC involvement. However, our data are relevant in showing evidence that MRC impairment with ATP reduction may contribute to OAK phenotype.

271-P

BIOCHEMICAL AND GENETIC ANALYSIS ON A LARGE COHORT OF PATIENTS WITH SUSPECTED MITOCHONDRIOPATHY

Ahting U[1], Freisinger P[2], Prokisch H[3], Gempel K[4], Hofmann W[1], Rolinski B[5]
[1]Dep Clin Chem Munich Municipal Hospital, Munich, Germany
[2]Kinderklinik Kreiskliniken Reutlingen, Reutlingen, Germany
[3]Inst Hum Gen Helmholtz Center, Munich, Germany
[4]Labor Becker Olgemoller, Bamberg, Germany
[5]Dep Lab Pathol Elblab Elblandklinikum, Riesa, Germany

The "metabolic disease centre" at the Department of Clinical Chemistry, Munich Municipal Hospital is a center for the diagnosis of mitochondrial diseases and glycogenoses since now more than twenty years. We analyzed the incoming samples from different institutions from 2005 to 2008. This samples include muscle biopsies (1181) and/or material for molecular genetics diagnostics (798).
We measured the respiratory chain enzyme activities (complex I-IV) in 761 biopsies. In 246 of them (32.3 %) we found a defect in one or more complexes of the respiratory chain. Up to now molecular genetics diagnostic could reveal a diagnosis in 45 cases (18.3 %) of these 246 patients. The mayor found diagnoses are mtDNA depletion or deletions in the mtDNA.
Interestingly in addition genetic diagnosis was found in 15 patients with measured respiratory chain activities, but without any measurable defect in the respiratory chain. Most of these patients showed deletions in the mtDNA. In contrast MELAS- and MERRF-Mutations were found only in patients with measurable respiratory chain defect. We found 13 pathogenic mutations in the mtDNA and 13 in nuclear genes.
From 2005 to 2008 we analyzed 798 patients molecular genetically. In 172 cases we analyzed for mtDNA depletion and found in 23 patients clear cut depletion (13.3 %), in 183 cases we checked for deletions in the mtDNA, which we found to be true in 27 patients (14.8 %).
The analysis shows for diagnosis of mitochondriopathies both enzymatic measurements and molecular genetic diagnostics should be applied.

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MITOCHONDRIAL A3243G MUTATION LOAD IN DIFFERENT SAMPLES IN A FAMILY AFFECTED OF MELAS

O'Callaghan M[1], Pineda M[1], Montero R[2], Artuch R[2], Vilaseca MA[2], Ruiz - Pesini E[3], Montoya J[3]
[1]Depart of Neurology, Sant joan Deu Hosp, Barcelona, Spain
[2]Depart Bioch, Sant Joan Deu Hosp, Barcelona, Spain
[3]Bioch and Mol Biol, Univ Zaragoza, Zaragoza, Spain

Background: In more than 80% of the cases, Mitochondrial Encephalo-myopathy with Lactic Acidosis and Strokelike episodes (MELAS) syndrome is caused by the transition 3243A>G in the tRNA Leu(UUR). Due to the phenomenon of mtDNA heteroplasmia, the A3243G mutation load varies between different tissues. The ratio of A3243G mutation is higher in the mtDNA isolated from muscle than from blood. However, muscle biopsy is an invasive procedure impossible to perform routinely in clinical practice and it is extended to look for the A3243G mutation in blood.
Objectives: To describe the clinical features in a MELAS's family and to find out an accessible sample with highest mutation load.
Material and Methods: From an index case of MELAS we reported the clinical features in 21 relatives of the same family and the A3243G mutation load in four accessible tissues (blood, urine, saliva and oral mucosa) in 13 members.
Results: Three women died (MELAS detected). The most common symptoms in the rest of patients have been deafness, fatigue and migraines. In 9 of the 13 cases, the urine expressed the highest percentage of the A3243G mutation whereas in 5 cases the blood expressed the lowest percentage of the A3243G mutation.
Conclusions: Since urine is an accessible sample and expresses a percentage of the A3243G mutation higher than other tissues, this should be sample of choice for the diagnosis of MELAS disease instead of the invasive muscle biopsy or the blood sample.

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INCREASING MUTATION LOAD IN MUSCLE AFTER 4 YEARS OF KETOGENIC DIET IN A GIRL WITH MELAS MUTATION

Freisinger PJK[1], Rolinski B[2], Baumeister FAM[3], Ahting U[2], Sperl W[4], Mayr J[4]
[1]Childrens Hospital, Metab Dep Techn Univ, Munchen, Germany
[2]Clin Chem, Klinikum Schwabing, Munchen, Germany
[3]Childrens Hospital, Neuropediatrics, Rosenheim, Germany
[4]Childrens Hospital, Metab Dep. Univ., Salzburg, Austria

There are still few therapeutic options in mitochondrial disorders. One approach is ketogenic diet in PDH-deficiency but there are also some reports of ketogenic diet in patients with respiratory chain (RC) complex I deficiency. Santra et al. (Ann Neurol. 2004, 56:662–9) analyzed cell lines with mtDNA –mutations after feeding with keton bodies and reported an increase of wild type mtDNA and a decrease of mutated mtDNA . ("heteroplasmic shifting") suggesting a positive effect of ketogenic diet in cells with mtDNA-mutations.
In a 4-year old girl with mental retardation, failure to thrive and lactic acidosis, biochemical analysis of RC activity in muscle (m.quadriceps) showed a clear reduction of complex I activity (0,10 U/U CS normal: 0,17–0,56). A mutation of the mtDNA was identified at position 3243 (MELAS). The degree of heteroplasmy was appr. 60 %. Considering two different possibly beneficial mechanisms we started ketogenic diet and observed a clear decrease in lactic acid and a stabilization of the clinical picture over years. A second muscle biopsy (quadriceps) was analysed after 4 years. The activity of complex I was still reduced (0,010 U/U CS), the degree of heteroplasmy increased to 70 %.
In this patient no "heteroplasmic shifting" with increase of wild type mtDNA could be found—on the contrary—there was a slight increase of mutant mtDNA. Nevertheless a clinical benefit can be observed. This might be the effect activating the citric cycle and bypassing RC complex I.

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T8993G MUTATION IN ATP-SYNTHASE SUBUNIT 6 ASSOCIATED WITH SEVERE HYPERTROPHIC CARDIOMYOPATHY IN 2 PATIENTS

Freisinger PJK[1], Mayr J[2], Ahting U[3], Kolker S[4], Gharavi B[5], Sperl W[2], Rolinski B[3]
[1]*Childrens Hospital, Metab Dep. Univ., Salzburg, Austria*
[2]*Clin Chem, Klinikum Schwabing, Munchen, Germany*
[3]*Childrens Hospital Metab Dep Univ, Heidelberg, Germany*
[4]*Marien-Hospital, Witten, Germany*

The thymine to guanine transversion in the ATP synthase subunit 6 gene (T8993G) is one of the most common pathogenic point mutations of mtDNA leading to reduced activity of the respiratory chain (RC) complex V. This mutation typically causes a neurological picture like NARP-syndrome (neurogenic weakness with ataxia and retinitis pigmentosa) and/or Leigh-Syndrome (Morava et al. 2005, Am J Med Genet 140A8, 863–868), other organs are rarely involved. The severity of the clinical presentation is rather variable depending on the mutation load.

We report 2 patients with the T8993G mutation and severe hypertrophic cardiomyopathy as a leading symptom. Patient 1, a male newborn, presented 4 days after birth with lactic acidosis, muscular hypotonia and bilateral lesions of the basal ganglia. Patient 2, a baby girl, presented at 3 months with failure to thrive, lactic acidosis, seizures and severe hypertrophic cardiomyopathy. She died at the age of 4 months. In both patients RC activity in fresh muscle revealed ATP- synthase deficiency. Mutation screening of ATP synthase subunit 6 showed homoplasmic T8993G mutation in both.

We conclude: 1) in newborns/young infants with signs of mitochondrial disorders and hypertrophic cardiomyopathy the "NARP-mutation" T8993G should be excluded before muscle biopsy is performed. 2) The spectrum of severity in patients with T8993G-mutations is large and the clinical course difficult to predict. However if hypertrophic cardiomyopathy is associated the outcome is probably very poor.

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VALPROATE INHIBITS THE ACTIVITY OF ATP- AND GTP-DEPENDENT SUCCINYL-CoA SYNTHETASE

Luis PBM[1], Ruiter J[1], IJlst L[1], Duran M[1], Almeida IT[2], Wanders RJA[1], Silva MFB[2]
[1]*Lab Gen Metab Dis Dept of Clin Chem Ped, Amsterdam, Netherlands*
[2]*iMed.UL, Fac Pharmacy, Univ Lisbon, Lisbon, Portugal*

Background: The impairment of mitochondrial function may be an important effect of valproic acid (VPA) treatment with potential adverse consequences.

Objectives: To investigate the influence of VPA on the activity of GTP and ATP-dependent succinyl CoA synthetases (SCS).

Methods: The GTP and ATP-dependent SCS activities were measured in human fibroblasts in the reverse direction, at different concentrations of succinate. Incubations were performed in the presence of VPA, valproyl-CoA, octanoyl-CoA and zinc chloride (as a positive control). Activities were measured using an optimized HPLC procedure.

Results: ATP- and GTP-SCS activities were inhibited by valproyl-CoA (1 mM), 50–60% and 30–50%, respectively. Octanoyl-CoA (at 1 mM) inhibited both enzymes 20 to 50%. VPA (1 mM) had no influence on the two enzymatic activities. In contrast, zinc chloride inhibited completely the activity of both forms of SCS. Discussion: Valproyl-CoA appears to interfere with the activity of SCS, especially with the ATP-dependent enzyme. This inhibition is not specific for the tested drug metabolite since octanoyl CoA also inhibits the activity of SCS but to a lower extent. Considering the involvement of SCS in the Krebs cycle, interference with its activity might impair the cellular energy status. Moreover, ATP-SCS is bound to nucleoside diphosphate kinase (NDPK), which is crucial for the equilibrium between di- and triphosphate nucleotides in the cell. An inhibition of ATP-SCS might influence the activity of NDPK inducing an imbalance of nucleotides in the mitochondria. The VPA induced impairment of SCS activity may account for the mitochondrial dysfunction associated with the drug.

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LIVER TRANSPLANTATION FOR MITOCHONDRIAL CYTOPATHIES IN CHILDREN: A SINGLE CENTER EXPERIENCE

Vara R[1], Fratter C[2], Poulton J[2], Portmann B[3], Mieli-Vergani G[3], Heaton N[3], Raiman J[1], Champion MP[1], Hadzic N[3]
[1]*Dept of IMD, Evelina Children's Hosp, London, United Kingdom*
[2]*Oxford Medical Genetics Laboratory, Oxford, United Kingdom*
[3]*Paed Liver, GI & Nu, King's College Hosp, London, United Kingdom*

Aim: To review the outcome liver transplantation (LT) in children diagnosed with mitochondrial cytopathy (MC).

Methods: Retrospective study of all patients transplanted with a diagnosis of mitochondrial cytopathy.

Results: 29 children with MC presented between 1987–2009. 3 were considered for transplantation but not listed due to neurological involvement and identification of common POLG mutations in 1. One was listed, with normal muscle enzymology and brain imaging, but died prior to LT; mitochondrial depletion syndrome was found in post-mortem liver.

6 patients (21%) were transplanted; median age 1.25 years (0.24–2.17), 1 had living-related LT. 4 presented with acute liver failure (ALF); 2 with deranged liver function. 3 presenting with ALF, died following LT (3, 6 and 18 months respectively) secondary to neurological deterioration prior to diagnosis.

3 surviving patients, 1 presented with ALF; CNS imaging could not be obtained prior to emergency LT (CIV deficiency). In 1, CIV deficiency was found only in liver, muscle and CNS imaging normal; he is well 6 years post LT. The other was transplanted due to progressive liver disease; diagnosis made following later presentation with seizures, she has well controlled epilepsy aged 22y.

Conclusion: MC's with liver involvement are rare and heterogeneous. LT is often required as a life saving treatment and diagnosis made retrospectively. 50% have survived with a good quality of life. Therefore MC should not be considered an absolute contraindication to LT and clinical assessment made on an individual basis. Prompt clinical screening tests are urgently needed.

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DEMETHYLATION OF PDHA2 GENE LEADS TO ITS EXPRESSION IN SOMATIC TISSUES

Pinheiro A[1], Milagre I[2], Nunes MJ[2], Rodrigues E[2], Silva MJ[1], Almeida IT[1], Rivera I[1]
[1]*Met&Gen - iMed, Faculty Pharmacy UL, Lisbon, Portugal*
[2]*Mol&CellBiol -iMed, Faculty Pharmacy UL, Lisbon, Portugal*

Pyruvate Dehydrogenase Complex (PDHc) deficiency is a severe inborn error of metabolism and most cases result from mutations in PDHA1 gene, encoding the alpha subunit of the rate-limiting enzyme of PDHc (E1). The existence of a testis-specific isoform of E1alpha, encoded by PDHA2 gene, led to hypothesize that PDHA2 activation in somatic tissues could constitute an ideal therapy for this type of disorder. Our previous studies revealed the presence of two CpG islands in PDHA2, one in the core promoter and the other in the coding region. Furthermore, we showed a correlation between gene expression and methylation status of the exonic CpG island, which is demethylated in spermatogenic cells and methylated in somatic ones.

This work aimed to disclose the role of epigenetic mechanisms, namely DNA methylation and histone acetylation, in PDHA2 gene expression.

Somatic cell lines (SH-SY5Y) were treated with 5′-aza-2′-deoxycytidine (DAC—inhibitor of DNA methyltransferase) and trichostatin A (TSA—histone deacetylases inhibitor). Genomic DNA was isolated to evaluate the methylation status of the two CpG islands by MS-PCR, and PDHA2 mRNA levels were quantified by qRT-PCR.

MS-PCR analysis showed a considerable demethylation of the exonic CpG island, while the core promoter remained fully methylated. Transcriptional analysis revealed that pre-treatment with the DNA demethylating agent caused marked synergistic activation of PDHA2 gene by TSA.

These results demonstrate the possibility to activate PDHA2 expression in somatic cells and show that PDHA2 tissue-specific expression is strongly controlled by DNA methylation and chromatin remodeling.

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HIGH SERUM LACTIC ACID LEVELS AS A NEW BIOLOGICAL MARKER IN PATIENTS WITH MONOCARBOXYLATE TRANSPORTER 8 DEFICIENCIES

Itoh M[1], Sato H[1], Yamamoto A[1], Kakinuma H[2], Saikawa Y[1]
[1]*Dept Pediatr, Kanazawa Medical Univ, Uchinada, Kahoku-gun, Ishikawa, Japan*
[2]*Chiba City Sakuragien, Chiba, Chiba, Japan*

Objective: Monocarboxylate transporter 8 (MCT8) deficiency is an X-linked disorder characterized by a combination of thyroid and neurological abnormalities. Mutations in the MCT8 gene have been accumulating in this disorder; however, the functional consequence of the mutations remains unclear. We aimed to clarify the mutations that account for the reduction of MCT8 activities, and biological markers as an early manifestation of this deficiency.
Materials and Methods: The MTC8 genes with each of 3 novel and one known mutations (p.L304P, p.N193LfsX47, p.D498N and p.235dupV) were transiently expressed in CHO-K1 cells. The cells were analyzed for I-131 labeled T3 uptake. Profiling of thyroid function tests and measurement of serum lactate and pyruvate were performed at first examination of the patients (5–8 months of age).
Results: The CHO-K1 cells carrying each of 4 MCT8 gene mutations showed significantly lower T3 uptake compared to the cells with wild type of MCT8. The levels of serum lactic acid were elevated (26.3±6.2 mg/dl, normal range 3–17 mg/dl) in all the cases, in which the lactate/pyruvate ratios revealed above 20.
Conclusions: The mutations examined in this study were responsible for the reduction of MCT8 activities. Precise mechanisms of molecular involvement need to be investigated. Profiling of the biological tests demonstrated the elevation of serum lactic acid in the patients specifically in an early infancy. High serum lactate accompanied by an abnormal thyroid function test can provide a diagnostic biological marker of MCT8 deficiency in infants with undefined severe psychomotor delay.

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THE SPECTRUM OF PEROXISOMAL DISORDERS IN SINGAPORE

Hart C E[1], Tan E-S[2], Sharp P[3], Fietz M[3]
[1]*Biochemical Genetics Lab, KK Hospital, Singapore, Singapore*
[2]*Department of Paeds Med, KK Hospital, Singapore, Singapore*
[3]*National Ref Lab, SA Pathology (at WCH), Adelaide, Australia*

Introduction: Testing for peroxisomal disorders was not previously available in Singapore, samples were sent to Australia which was costly and inconvenient thereby acting as a barrier to testing, raising the question as to whether peroxisomal disorders were under diagnosed. Therefore a study was started to set up very long chain fatty acid (VLCFA), phytanate and pristanate testing and to target patients with relevant clinical features to ascertain the spectrum of peroxisomal disorders in Singapore.
Methods: The analytical method is a stable isotope dilution GC-MS method covering C22, C24, C26, phytanate and pristanate. Patients with infantile hypotonia or boys with neurological regression were recruited over 2 years. 26 patients were recruited. Samples from the first 10 patients were tested in duplicate, in Singapore and Adelaide.
Results: There was good comparison of results between labs despite differences in methodology. Although data is limited, reference ranges for the local population do not appear different to elsewhere. Two cases of x-linked adrenoleuokodystrophy (ALD) were identified. Case 1 was confirmed by mutation analysis (p.L79P, c.236 T>C), Case 2 DNA analysis is pending. Mother of Case 1 was confirmed as a carrier by DNA and VLCFA analysis. Relatives of Case 2 are under investigation but mother and one sister have VLCFA results consistent with carrier status.
Conclusions: X-linked ALD is present in the local population, probably with an incidence similar to the rest of the world. No other peroxisomal disorders were identified but, given their incidences and the size of the population covered, this was not unexpected.

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PHYTANIC ACID INDUCES OXIDATIVE STRESS IN CEREBELLUM AND CEREBRAL CORTEX OF YOUNG RATS

Leipnitz G[1], Amaral AU[1], Zanatta A[1], Seminotti B[1], Fernandes CG[1], Knebel LA[1], Eichler P[1], Vargas CR[2], Wajner M[3]
[1]*Departamento de Bioquímica, ICBS, UFRGS, Porto Alegre RS, Brazil*
[2]*Departamento de Análises Clínicas, UFRGS, Porto Alegre RS, Brazil*
[3]*Servico de Genética Médica, HCPA, Porto Alegre, RS, Brazil*

Background: Phytanic acid (Phyt) is a branched-chain fatty acid that accumulates in Refsum disease and other peroxisomal disorders characterized by neurological symptoms and abnormalities whose pathogenesis is virtually unknown.
Objectives: In the present study we investigated the in vitro effects of Phyt on parameters of oxidative stress in cerebellum and cerebral cortex of 30-day-old rats.
Methods: The following parameters of oxidative stress were determined: thiobarbituric acid-reactive substances (TBA-RS; lipid peroxidation), carbonyl formation and sulfhydryl oxidation (protein oxidative damage) and the concentrations of reduced glutathione (GSH).
Results: Phyt significantly increased TBA-RS levels in both cerebral structures. This effect was prevented by the antioxidants α-tocopherol and melatonin, suggesting the involvement of free radicals. Phyt also provoked protein oxidative damage in both cerebellum and cerebral cortex, as determined by increased carbonyl formation and sulfhydryl oxidation. Furthermore, Phyt significantly diminished GSH, while melatonin and α-tocopherol treatment totally blocked this effect. We also verified that the decrease of GSH levels provoked by Phyt in brain structures was not due to a direct oxidative effect of this fatty acid, since exposition of a commercial GSH solution to Phyt in a free cell medium did not decrease GSH content.
Conclusions: Our data indicate that oxidative damage and decrease of brain antioxidant defenses are elicited by Phyt, a mechanism that may contribute at least in part to the pathophysiology of Refsum disease and other peroxisomal disorders where Phyt is accumulated.
Financial support: Research grants from CNPq, FAPERGS, FINEP Rede Instituto Brasileiro de Neurociência (IBN-Net) # 01.06.0842-00 and INCT-EN.

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IN VITRO EVIDENCE THAT PHYTANIC ACID COMPROMISES Na+,K+-ATPase ACTIVITY AND THE ELECTRON FLOW THROUGH THE RESPIRATORY CHAIN IN BRAIN CORTEX FROM YOUNG RATS

Busanello EN[1], Viegas CM[1], Moura AP[1], Tonin AM[1], Schuck PF[2], Ferreira GC[1], Wannmacher CMD[1], Wajner M[3]
[1]*Lab Fisiopatologia Experimental Unesc, Criciúma SC, Brazil*
[2]*Servico de Genética Médica, HCPA, Porto Alegre, RS, Brazil*

Background: Phytanic acid (Phyt) tissue concentrations are increased in Refsum disease and other peroxisomal disorders characterized by neurologic damage and brain abnormalities.
Objectives: The present work investigated the in vitro effects of Phyt, at concentrations found in these peroxisomal disorders, on important parameters of energy metabolism in brain cortex of young rats.
Material and Methods: The parameters analyzed were CO2 production from labeled acetate and glucose, the activities of the citric acid cycle enzymes citrate synthase, aconitase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinate dehydrogenase, fumarase and malate dehydrogenase, as well as the respiratory chain complexes I-IV, creatine kinase and Na+,K±ATPase.
Results: Our results show that Phyt did not alter citric acid cycle enzyme activities and CO2 production from acetate, reflecting no impairment of the functionality of the citric acid cycle. In contrast, respiratory chain activities were reduced at complexes I, II, I-III, II-III and IV. Membrane synaptical Na+,K±ATPase activity was also reduced by Phyt, with no alteration of creatine kinase activity.
Conclusion: Considering the importance of the electron flow through the respiratory chain for brain energy metabolism (oxidative phosphorylation) and of Na+,K±ATPase activity for maintaining membrane potential necessary for neurotransmission, the data indicate that Phyt impairs brain bioenergetics at the level of energy formation, as well as neurotransmission. It is presumed that Phyt-induced impairment of these important systems may be involved at least in part in the neurological damage found in patients affected by disorders in which brain Phyt concentrations are increased.
Financial Support: CNPq, PRONEX, FINEP rede IBN-Net #01.06.0842-00, INCT-EM.

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X-LINKED ADRENOLEUKODYSTROPHY IN SOUTHERN CONE: IDENTIFICATION OF 23 MUTATIONS IN THE ABCD1 GENE IN 24 INDEX CASES AND 83 RELATIVES

Pereira FS[1], Giugliani R[2], Blank D[2], Castilhos RM[1], Habekost CT[2], Vargas CR[2], Matte US[3], Jardim LB[2]
[1]*PPGCM, UFRGS, Porto Alegre, Brazil*
[2]*Med Genet Serv, Hosp Clin, Porto Alegre, Brazil*
[3]*CenGeneTherapy, Hosp Clin, Porto Alegre, Brazil*

Background: X-linked adrenoleukodystrophy is caused by a defect in the gene for the adenosine triphosphate (ATP)-binding cassette ABCD1. This gene codes for ALD protein (ALDP), a peroxisomal membrane protein that belongs to the ATP-binding cassette superfamily of membrane transport proteins.
Objective: In this study, we analyzed the ABCD1 gene in X-ALD patients and relatives from 35 unrelated families.
Methods: male ALD patients originated from Brazil, Uruguay and Argentina, have been previously diagnosed by VLCFA analysis. Families were then invited to molecular studies to improve genetic counseling. After consent, blood was collected and DNA was extracted. All samples were screened by SSCP analysis of PCR fragments, followed by DNA sequencing to establish the specific mutation in each family.
Results: We identified twenty-three different mutations, of which 12 were novel and 11 have been previously described. This population had an important allelic heterogeneity: 23 mutations were exclusive to a single family (96%). Only p.Arg518Gln mutation (exon 8) was found in two families. Intra-familiar phenotype variability was observed in all pedigrees. Twenty four families were sufficiently studied in order to define mother status: among them, we found two de novo mutations (2/24, or 8%). Sixty women older than 18 years old were analyzed in these families: 31 were heterozygous (51%). Two women with previous high levels of VLCFA had actually normal genotyping.
Conclusions: This study extends the spectrum of mutations in X-ALD, confirmed the usual rate of de novo mutations and facilitated the identification of heterozygous females.

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CONTRIBUTION OF PEX GENE SCREEN FOR THE DIAGNOSIS OF PEROXISOME BIOGENESIS DISORDERS

Vianey-Saban C[1], Luangkhot E[1], Doray B[2], Couderc F[3], Chamouine A[4], Amsalem D[4], Lyonnet S[5], Pennerath A[6], Ogier H[7], Chabrol B[8], Tranchant C[9], Levade T[10], Bieth E[11], Calvas P[11], Guffon N[1], Cheillan D[1]
[1]*CR Metab Dis - Hospices Civils de Lyon, Bron, France*
[2]*Génétique médicale, Hop Hautepierre, Strasbourg, France*
[3]*Pédiatrie, CH d'Aix en Provence, Aix en Provence, France*
[4]*Réanimation infantile, Hop St Jacques, Besancon, France*
[5]*Génétique médicale, Hôpital Necker, Paris, France*
[6]*Pédiatrie, CH de Colmar, Colmar, France*
[7]*CR Metab Dis - Hôpital Robert Debré, Paris, France*
[8]*CR Metab Dis - Hôpital de la Timone, Marseille, France*
[9]*Neurologie, Hôpital Hautepierre, Strasbourg, France*
[10]*Laboratoire de biochimie, Hôpital Purpan, Toulouse, France*
[11]*Génétique médicale, Hôpital Purpan, Toulouse, France*

Background: Peroxisome biogenesis defects (PBD) in the Zellweger syndrome spectrum (ZSS) are caused by alterations in one of the 13 PEX genes required for normal peroxisome assembly. Their diagnosis is assessed on clinical and biochemical arguments; the confirmation could only be done by molecular analysis of one of the PEX genes after complex complementation studies.
Methods: Our objective was to develop a screening protocol of the most commonly defective PEX genes in PBD, without preliminary step of complementation. Sixteen exons, distributed on 6 different PEX genes (PEX1, PEX2, PEX6, PEX10, PEX12 and PEX26), were selected covering more than 80% of the already described pathogenic alleles.
Results: Fifteen ZSS patients were investigated using this protocol. We identified 2 pathogenic alleles for 6 patients, 2 of them presenting a third pathogenic allele on another PEX gene. One pathogenic allele was identified for 3 patients. However, for the 6 remaining patients, the screening was negative. Among the 8 mutations detected, 3 have not been described before but are probably deleterious because they affect a highly conserved protein domain: p.Cys796Arg (PEX6 - Exon 13, c.2392 T>C), p.Cys296Phe (PEX10 – Exon 5, c.887G>T) and p.Trp250Arg (PEX2 – Exon 4, c.748 T>C).
Conclusions: Finally, our protocol allowed, in an easy and fast way, the determination of the involved PEX gene in 60% of the patients, confirming the diagnosis of PBD, and allowing a reliable genetic counseling. With these results, we propose a strategy for the investigation of patients suspected of PBD.

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INFANTILE REFSUM DISEASE IN A TURKISH PATIENT: CASE REPORT

Kilic M[1], Karli OK[2], Coskun T[1], Haliloglu G[3], Wanders RJA[4], Dursun A[1], Sivri HS[1], Tokatli A[1], Topcu M[3]
[1]Pediatr Metab Dis, Hacettepe University, Ankara, Turkey
[2]Dept of Radiology, Hacettepe University, Ankara, Turkey
[3]Pediatr Neurol, Hacettepe University, Ankara, Turkey
[4]Genet Metab Dis, Univ of Amsterdam, Amsterdam, Netherlands

Infantile Refsum disease (IRD) is an autosomal recessive peroxisomopathy comprising retinitis pigmentosa, sensorineural hearing loss, psychomotor and mental developmental delays, hypotonia, and cerebellar ataxia. A 3-year 4-month old girl presented with visual and hearing impairment and developmental delay with onset in the first six months of life. Physical examination showed generalized hypotonia, bilateral sensorineural hearing loss, decreased deep tendon reflexes, developmental delay, retinal pigmentation anomalies, and decreased vision. She was unable to sit without support and was not able to recognize her parents. She was under levothyroxine treatment due to congenital hypothyroidism. Biochemical analysis showed C26:0 2.52 μmol/L (N 0.6–1.3), phytanic acid 47.63 μmol/L (N 0.42–3.77), pristanic acid 14.28 μmol/L (N 0–1.5), and C26/22 to 0.11 μmol/L (N 0.011–0.026), consistent with the infantile Refsum disease. Phytanic acid oxidation in cultured cells was deficient. MR imaging revealed symmetrical signal changes in the peritrigonal white matter, centrum semiovale, corticospinal tracts, corpus callosum splenium and cerebellar pedincles. 1H MRS displayed elevated choline and myoinositol with decreased NAA; presence of lactate and lipid peaks in affected areas. All findings were interpreted as suggestive of peroxisomal disorder. Although rare, this diagnosis should be considered in the appropriate clinical, biochemical and radiological settings.

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DIFFERENTIAL DIAGNOSIS OF AUTOSOMAL RECESSIVE RHIZOMELIC AND X LINKED RECESSIVE CHONDRODYSPLASIA PUNCTATA

Payas A[1], Dikme G[1], Topal N[1], Tuysuz B[1]
[1]Dep Ped Genet, Ist Uni Cerrahpasa Med, Istanbul, Turkey

Rhizomelic chondrodysplasia punctata type 1 (RCDP1) is a peroxisome biogenesis disorder and characterized by proximal shortening of the humerus and to a lesser degree of the femur, punctate calcifications in cartilage, cataract and elevated phytanic acid. X-linked recessive chondrodysplasia punctata (CDPX1) caused by enzyme arylsulfatase E deficiency and characterized by chondrodysplasia punctata, brachytelephalangy and nasomaxillary hypoplasia. First patient was a 7 months-old girl from first cousin marriage. She had severe growth and motor retardation, microcephaly, flat face, low nasal bridge, rhizomelic shortness and cataract. Radiography showed proximal shortness of humerus and punctate calcifications around large cartilages. Cranial MR imaging revealed cortical atrophy. Plasma concentration of phytanic acid was elevated. She was diagnosed as RCDP1. Second patient was a 2,5 months-old boy. He was the first child of nonconsanguineous parents. He had hypertelorism, low nasal bridge and small nose. Radiography revealed punctate calcifications in cartilage mostly around vertebral bodies. At age of 3 months he had a septicemia after respiratory infection and died. The clinical findings were compatible with CDPX1. CDP have been classified as RCDP type 1, 2 and 3, CDPX1, X-linked dominant CDP and autosomal dominant CDP tibia-metacarpal type. Maternal systemic lupus erythematosis, warfarin embryopathy and other vitamin K deficiencies also result in similar phenotype. CDPX1 and RCDP1 are very rare disorders. Punctate calcifications were seen around large cartilage in RCDP1. However, the calcification showed vertical involvement in CDPX1. Distribution of the punctate calcification, rhizomelic shortness and pattern of inheritance are important in the differential diagnosis.

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HACETTEPE EXPERIENCE WITH PEROXISOMAL DISORDERS UNDER FOUR YEARS OF AGE

Kilic M[1], Tokatli A[1], Sivri HS[1], Dursun A[1], Topaloglu H[2], Wanders RJA[3], Coskun T[1]
[1]Pediatr Metab Dis, Hacettepe University, Ankara, Turkey
[2]Pediatr Neurol, Hacettepe University, Ankara, Turkey
[3]Genet Metab Dis, Univ of Amsterdam, Amsterdam, Netherlands

Peroxisomal diseases are genetically determined disorders caused either by failure to form or maintain the peroxisome or by a defect in the function of a single or multiple enzyme that is normally located in this organelle. The combined incidence of the peroxisomal disorders (except X-ALD) is estimated to be 1/50000. We had seen 7 patients aged between 7- days-neonate and 3,5-years-child during the last three years. Studies in cultured fibroblasts revealed 3 D-Bifunctional Protein (D-BP) deficiency, 1 Zellweger syndrome (ZS), 1 Zellweger spectrum disorder (ZSD), 1 Infantile Refsum Disease (IRD), and 1 CADDS (contiguous ABCD1 DXS1357E deletion syndrome). All except IRD, had the common features of peroxisomal disorders like severe hypotonia, low reflex, dysmorphic appearance with high forehead, high-arched palate, enlarged fontanel, long filtrum, epicantal folds, hypertelorism, retrognathia, low-set ears, sensory neural deafness, decreased vision, severe developmental and growth retardation. There were early encephalopathy, and resistant epilepsy except IRD. Retinitis pigmentosa was seen only in IRD patient. Cranial MRIs demonstrated widespread polymicrogyria and possible heterotopia in D-BP deficiency patients whereas symmetrical signal changes in the peritrigonal white matter, centrum semiovale, corticospinal tracts, corpus callosum splenium and cerebellar pedincles in IRD patient. VLCFA were elevated in all. Our cases expired soon after the diagnosis without gaining any developmental milestones before 1 years of age except IRD and ZSP. Bleeding diathesis and lipid soluble vitamin deficiency was seen in ZSD patient. D-BP deficiency is the most common peroxisomal disorder in Turkey.

287-O

NEW INSIGHTS INTO QUARTERNARY STRUCTURE OF PEX26 POINT TO POTENTIAL ALTERNATIVE FUNCTIONS OF THIS PEROXIN

Lotz AS[1], Guder P[1], Woidy M[1], Messing DD[1], Danecka MK[1], Schatz UA[1], Gersting SW[1], Muntau AC[1]
[1]Molec Pediatr, Hauner Child Hosp, LMU, Munich, Germany

Background: Mutations in the PEX26 gene cause peroxisomal biogenesis disorders of complementation group 8. PEX26 encodes a tail-anchored peroxisomal membrane protein targeted to the peroxisome in a PEX19-dependent manner. PEX26 has been shown to bind PEX6, to recruit the PEX1-PEX6 complex to the peroxisome, and to be involved in PTS1/PTS2 import. Despite its endoplasmatic and cytosolic localization the splice variant PEX26-Δex5 has been described to complement PEX26 deficient cells suggesting that PEX26 does not require peroxisomal localization to exert its function.

Results: In the present work, we identified homomeric interaction of PEX26 in living cells using bioluminescence-resonance energy transfer (BRET) and confirmed this finding by coimmunoprecipitation. The binding domains were characterized by BRET using PEX26 truncation constructs. Western blot analyses under reducing and non-reducing conditions as well as native PAGE indicated formation of dimers with the cysteine residue of the transmembrane domain involved in the interaction. Using protein structure predictions (I-Tasser) and docking analyses we propose a 3D model of PEX26 dimers supporting the position of the binding domains identified experimentally. Intriguingly, we also observed homomeric interaction of PEX26-Δex5 variants lacking the transmembrane domain. Moreover, PEX26-Δex5 was shown to interact with PEX26 despite its distinct subcellular distribution.

Conclusion: In conclusion, we describe homo- and heterooligomerization of PEX26 and its splice variant PEX26-Δex5. Considering the interaction of PEX26 and PEX26-Δex5, our data further support the notion that PEX26 does not require peroxisomal localization and we hypothesize that PEX26 may have different functions inside and outside the peroxisome.

288-P

MOLECULAR AND COMPUTATIONAL ANALYSES IN ARGENTINEAN PATIENTS WITH X-LINKED ADRENOLEUKODYSTROPHY (X-ALD)

Amorosi CA[1], Dvoráková L[2], Myskova H[2], Cismondi A[1], Bender S[1], Guelbert N[1], Coll MJ[3], Dodelson de Kremer R[1], Oller de Ramírez A[1]
[1]*CEMECO, Med School, Univ Child Hosp, Cordoba, Argentina*
[2]*Instit InheR Metab Disorders, Prague, Czech Republic*
[3]*Institut de Bioquímica Clínica, Barcelona, Spain*

Background: X-ALD is a neurodegenerative disorder characterized by an increase of very long-chain fatty acids.
Patients and Methods: We analyzed genomic variations in the ABCD1 gene in 10 unrelated probands. PCR/sequencing of all exons was accomplished. Each mutation was verified by two methods. The validation of new missense and intronic changes are accomplishing through a combination of methods:
a) Bioinformatics tools
(PolyPhen (http://genetics.bwh.harvard.edu/pph), SIFT (http://blocks.fhcrc.org/sift/SIFT.html), ME (http://gener.mit.edu/bungelab/maxent/Xmaxentscan_scoreseg_acc.html) and NN (http://www.fruitfly.org/seq_tools/splice.html))
b) Functional analysis.
Results: We identified 9 novel and 1 known mutations that allowed us the molecular characterization of 10/10 patients. New mutations identified: 3 frameshift, an insertion (p.Ser284fs), a deletion (p.Thr254ArgfsX82), both in exon 1 and a duplication in exon 3 (p.Glu380ArgfsX21); a deletion in exon 7 (p.Ser572_Asp575del), a splicing (c.1081+5G>C) and 4 missense changes: in exon 1 (p.Ala19Ser); in exon 2 (p.Ala341Asp) in exon 4 (p.His420Pro) and in exon 7 (p.Tyr547Cys) and a known missense mutation (p.His669Arg) in exon 10.
ME: For normal allele 9.22, for splicing change (c.1081+5G>C) 5.49. NN can produce lost of splice normal site. SIFT: p.His669Arg, p.Ala19Ser and p.His420Pro are predicted to be tolerated; p.Ala341Asp and p.Tyr547Cys are predicted to be affected. PolyPhen: p.His669Arg and p.Ala19Ser are predicted to be bening; p.His420Pro, p.Ala341Asp and p.Tyr547Cys are predicted to be probably damaging. The intronic and the last 2 changes according these programs could be mutations, while the others 3 could be polymorphisms. The functional analyses are in progress.
Discussion: The precise definition of genotype in X-ALD patients is essential for diagnostic reasons and evaluation of future therapeutic interventions.

289-P

EVALUATION OF VERY LONG CHAIN FATTY ACIDS BY UPLC-MS/MS IN A NORMAL PEDIATRIC POPULATION

Liu A[1], Bunker A[1], Roberts W[2], Longo N[3], Pasquali M[2]
[1]*ARUP Laboratories, Salt Lake City, United States*
[2]*Dept of Pathology, University of Utah, Salt Lake City, United States*
[3]*Dept of Pediatrics, University of Utah, Salt Lake City, United States*

Background: Peroxisomal disorders are a group of diseases caused by defective activity of one (single enzyme deficiencies) or more peroxisomal enzymes (peroxisome biogenesis disorders). These diseases are biochemically characterized by accumulation in tissues and body fluids of very long chain fatty acids (VLCFAs), hexacosanoic (C26:0), tetracosanoic (C24:0), and docosanoic (C22:0) acids, and branched chain fatty acids, pristanic (C19:0-br) and phytanic (C20:0-br) acids. Analysis of VLCFAs in plasma is used in the evaluation of patients with peroxisomal disorders.
Methods: In this study we have evaluated VLCFAs in 250 normal controls, ranging in age from 6 months to 18 years, using Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS). Plasma samples were collected as part of the CHILDx pediatric reference range study. VLCFAs were converted to trimethylaminoethyl iodide esters prior to chromatographic separation and MS/MS analysis.
Results: The concentration of straight chain VLCFAs (C26:0, C24:0, and C22:0) did not change significantly with age. By contrast, the concentration of pristanic and phytanic acids (BCFA) peaked around 1–2 years of age, then decreased with age and remained constant after 7 years of age. This may reflect dietary changes with age (phytanic acid is present in products derived from ruminant animals). There was, however, a clear separation in the concentration of VLCFAs and/or their ratios between normal controls and patients with peroxisomal disorders.

290-P

CLINICAL, BIOCHEMICAL AND GENETIC FEATURES IN TUNISIAN ZELLWEGER SYNDROME

Nasrallah F[1], Hammami MB[1], Hadj Taieb S[1], Sethoum MM[1], Azzouz H[2], Ben Turkia H[2], Ben Dridi MM[2], Feki M[1], Kaabachi N[1]
[1]*Laboratory of Biochemistry, Rabta Hosp, Tunis, Tunisia*
[2]*Paediatric department, Rabta Hosp, Tunis, Tunisia*

Background: Zellweger syndrome (ZS) is a clinically and genetically heterogeneous disease entity. Patients potentially suffering of ZS are screened by elevated levels of very long chain fatty acids (VLCFA). Confirmation of ZS is made by molecular diagnostic. The aim of this work is to report the clinical features and the biochemical and genetic data in two Zellweger patients.
Materials and methods: Plasma VLCFA was analyzed with gas chromatography allowing the assessment of plasma hexacosanoic acid (C26:0), tetracosanoic acid (C24:0) and docosanoic acid (C22:0). Study of peroxisome and enzymes activities of peroxisome metabolism were corroborated by analyzing cultured fibroblasts. Confirmation of the diagnosis has been made by molecular analysis.
Results: Two children, aged 4 months and 15 days, respectively were issued from a consanguineous marriage. The clinical features include hypotonia and failure to thrive for the first patient and hypotonia, seizures, hepatomegaly, respiratory distress, craniofacial dismorphism and growth retardation for the second. The C24/C22 ratio was 2.24 and 3.76 (NV: 0.5–0.98) and the C26/C22 ratio was 0.26 and 0.76 (NV: 0.002–0.018) for the first and the second patients, respectively. For the first patient, peroxisomal enzymatic activities were drastically reduced and peroxisomes were absent in fibroblasts. Molecular analysis highlights a homozygous W105X mutation of the PEX 26 gene.
Conclusion: The clinical spectrum of ZS is very heterogeneous. The diagnosis was confirmed by enzymatic activity study and molecular analysis but VLCFA chromatography remains a reliable tool for the diagnosis.

291-P

MAGNETIC RESONANCE IMAGING DEFINES REGIONS IN THE BRAIN OF GUANIDINOACETATE N-METHYLTRANSFERASE (GAMT) DEFICIENT MICE THAT DIFFER FROM WILD-TYPE LITTERMATES

von Both I[1], Laliberté C[2], van Eede M[2], Henkelman M[2], Schulze A[3]
[1]*Progr Genet & Genom Biol, SickKids Hosp, Toronto, Ontario, Canada*
[2]*Mouse Imaging Centre, SickKids Hosp, Toronto, Ontario, Canada*
[3]*Div Clin & Metab Genet, SickKids Hosp, Toronto, Ontario, Canada*

Background: In creatine deficiency syndromes, symptoms are mainly cerebral in nature and patients with GAMT deficiency are most affected. Aim of our study was to use a knockout mouse model to investigate the cerebral morphology of GAMT mice using high-resolution magnetic resonance imaging.
Methods: We use a series of image registration methods to bring 24 brains (wild-type, heterozygous, mutant, 8 each; age at perfusion-fixation 10–12 weeks) into alignment, and then investigate where the brains had to grow or shrink in order to be like the others using a Jacobian metric [NeuroImage 50: 409–415, 2010]. The changes in volume of morphological structures were also evaluated.
Results: When analyzing morphological structures using the mouse brain atlas, we found the corpus callosum and the internal capsule to be significantly different and the globus pallidus to be almost significantly different in size (absolute volume) between mutant and heterozygous/wild-type mice. Significant peaks (local areas of difference in relative size) were found in the cerebellar cortex, the striatum, the nucleus accumbens, the hypothalamus, the olfactory bulbs, the medulla, the frontal lobe of the cerebral cortex, the hippocampus and the pons.
Conclusion: Our studies on fixed mouse brains found significant changes in regions that have been identified as pathological in humans, which makes our mouse model ideal to further study the cerebral effects of creatine deficiency syndromes.

292-O

NEWBORN SCREENING FOR GAMT DEFICIENCY: EXPERIENCE WITH GUANIDINOACETATE BY FIA-MS/MS AND UPLC-MS/MS SECOND TIER TESTING

Sweetman L[1], Ashcraft P[1]
[1]*Inst Metab Dis, Baylor Res. Inst, Dallas, United States*

Background: The creatine synthetic defect, guanidinoacetate methyltransferase (GAMT) deficiency can be detected by the elevation of guanidinoacetate (GAA) in dried blood spots. Treatment with creatine can improve the clinical outcome and early detection would be desirable.
Objective: The objective was to evaluate the addition of GAA with [13C2] GAA internal standard into routine derivatized MS/MS newborn screening and the use of a second tier UPLC-MS/MS test for false positives.
Results: Approximately 12,000 specimens have been screened for GAA elevations with about half from Mexico. The initial cut off of 2.94 μM resulted in a positive rate of 0.5 %. The second tier test showed that these were all false positives. We requested repeat specimens when there was insufficient specimen for the second tier test. GAA was below the cutoff for all repeat cards received. These results and the second tier data were used to raise the cutoff to 4.0 μM and the positive rate decreased to 0.1%.
Conclusion: Adding GAA to the routine derivatized MS/MS newborn screen is simple and adds little to the cost.

293-P

AAV2 AND AAV5 VIRUSES TO TRANSDUCE RNAi-INDUCED KNOCK-DOWN OF GAMT AND SLC6A8 IN 3D ORGANOTYPIC BRAIN CELL CULTURES IN AGGREGATES

Béard E[1], Braissant O[1]
[1]*Clinical Chemistry, University Hospital, Lausanne, Switzerland*

Creatine deficiency syndromes, due to AGAT, GAMT or SLC6A8 defects, are among the most frequent inborn errors of metabolism and principally affect CNS development. To investigate the effects of creatine deficiencies on developing brain cells, we developed new experimental models by gene knock-down of GAMT and SLC6A8 in 3D organotypic rat brain cell cultures in aggregates.
Specific siRNA sequences for rat GAMT and SLC6A8 were selected, and tested first for their RNAi effect in ROC cells (hybridoma between C6 astroglioma and oligodendrocytes), which express GAMT and SLC6A8. The best siRNA sequences caused a knock-down effect of 80% (GAMT) and 86% (SLC6A8) in these ROC cells cultured in monolayer.
The best selected interfering sequences were then transduced in brain cell aggregates by two different adeno-associated virus serotypes, AAV2 and AAV5, as shRNAs expressed under control of the CMV promoter. The selected AAV2 and AAV5 vectors also transduced Green Fluorescent Protein (GFP) to allow the follow-up of AAV-infected cells. Following GFP expression from day 5 to day 13 post-infection, we show that AAV2 and AAV5 serotypes are able to transduce GFP as well as GAMT and SLC6A8 shRNA interfering sequences, which appear to affect brain cells in our experimental model of creatine deficiencies.
AAV2 or AAV5 viruses appear as a powerful tool for knocking down GAMT and SLC6A8 expression by RNAi in developing brain cells, allowing the analysis of specific alterations of CNS development in creatine deficiency syndromes.
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294-P

A CREATINE METABOLISM DISORDER IN A CHILD SUFFERING FROM DUCHENNE MUSCULAR DYSTROPHY

Joncquel M[1], Cuisset JM[2], Mention K[3], Briand G[1]
[1]*Laboratoire de biochimie, CHRU Lille, Lille, France*
[2]*Serv Neuropédiatrie, CHRU Lille, Lille, France*
[3]*Serv Pédiatrie, CHRU Lille, Lille, France*

We report a case of an 11 years old boy with familiar history of Duchenne muscular dystrophy (DMD) also affected by a creatine deficiency syndrome.
When he was 18 months, he was referred to neuropaediatricians because of motor delay: neurological examination showed no other abnormality. He started walking at 2 years and a half with a waddling gait and frequent falls. The diagnosis of DMD was confirmed on muscle biopsy at the age of 4 years. At the same time, he presented hyperkinesia. Ambulation was lost when he was 8 years old. Additionally, he presented an attention deficit disorder and a treatment by methylphenidate was started. At 11, because of mild mental retardation with attention disorder, metabolic investigations were on principle performed. Analysis showed very high urinay creatine level (11700 μmol/L) (controls: 36–4964 μmol/L) and very high ratio creatine/creatinine (3039 mmol/mol) (controls: 17–720 mmol/mol) with a normal guanidinoacetate level (850 μmol/L) (controls: 9–1142 μmol/L) and a normal ratio guanidoacetate/creatinine(221 mmol/mol) (controls: 4–220 mmol/mol). Results are consistent with creatine transporter (CT1) deficiency
Since CT1 deficiency and DMD are two X-linked disorders, this report would suggest further genetic analysis (high resolution karyotype, CGH micro-array).

295-P

AN ATYPICAL FORM OF CREATINE DEFICIENCY SYNDROME

Nasrallah F[1], Hammami MB[1], Hadj Taieb S[1], Khemir S[1], Feki M[1], Briand G[2], Kaabachi N[1]
[1]*Laboratory of Biochemistry, Rabta Hosp, Tunis, Tunisia*
[2]*bLaboratory of Biochemistry, CHRU, Lille, France*

Introduction: Creatine deficiency syndromes (CDS) are a group of disorders caused by defects in biosynthesis or transport of creatine (Cr). Levels of Cr and its precursor guanidinoacetate (GAA) in body fluids are informative for biochemical diagnosis of CDS. Diagnosis must be confirmed by enzymatic activity determination and molecular analysis. We report the observation of two patients addressed for a high suspicion of CDS.
Materiel and Methods: Two patients aged 1 year (P1) and 18 years (P2), issued from a first degree consanguineous marriage were admitted with symptoms suggestive of CDS. Amino acids and organic acids abnormalities and hypomethylation syndrome were excluded. Urinary Cr and GAA was analyzed by GC/MS. Guanidinoacetate methyltransferase (GAMT) activity was measured in lymphoblasts using tandem mass spectrometry.
Results: Both patients exhibited mental retardation, epilepsy, autistic behavior, severe mental language delay and axial hypotonia. Urinary analysis showed low levels of Cr associated with relatively high GAA concentration. The Cr / GAA ratio was of 0.25 and 0.20 for P1 and P2, respectively (Normal value: Cr / GAA >1). GAMT activity was clearly detectable and similar to the controls in both patients.
Conclusion: Association of low Cr/GAA ratio with these clinical features is suggestive of GAMT deficiency, but normal GAMT activity disagrees with this diagnosis. Two hypotheses may be advanced to explain this discrepancy. The low Cr/GAA ratio may be related to high endogen consumption of Cr. We also may speculate the diagnosis of an atypical form of CDS with non ubiquitous GAMT deficiency.

296-P

ASSESSMENT OF CREATINE AND GUANIDINOACETIC ACID IN BRAIN, LIVER AND KIDNEY OF AGAT AND GAMT DEFICIENT MICE BY MEANS OF CATION-EXCHANGE HPLC WITH POST COLUMN DERIVATIZATION

Sinha A[1], Von Both I[1], Schulze A[1]
[1]*Res Inst, Hosp for Sick Children, Toronto, Canada*

Creatine (Cr) with phosphocreatine acts as an energy buffer for brain and muscle tissue, while guanidinoacetic acid (GAA) is neuro-toxic intermediate of Cr production. Additionally, AGAT deficiency causes decrease in Cr and GAA, where as GAMT deficiency causes decrease in Cr and accumulation of GAA. The focus was to observe levels of Cr and GAA in brain, kidney and liver of AGAT and GAMT wild-type, heterozygous and mutant mice models. The method used was cation exchange HPLC with post column derivatization using ninhydrin [1].
Comparative analysis shows that Cr levels in brains of AGAT and GAMT mutant mice were much higher than expected and previously described for GAMT [2]. GAA was below detection limit in all of the three organs of AGAT mutant mice while respective levels in GAMT mice varied according to tissue; highest level was detected in liver and lowest in kidney. In the kidney of AGAT mutant mice, GAA was below the detection limit; yet Cr was present in all three tissues. This could be due to coprophagia, as mice were caged together based on littermates and not according to genotype.
Our study provides information on tissue specific distribution of Cr and GAA in AGAT and GAMT mouse models. Coprophagia needs to be considered when planning animal experiments because unexpected findings such as detectable Cr levels observed in GAMT and AGAT mice may occur.
References:
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297-O

TREATMENT OF INTRACTABLE EPILEPSY IN A FEMALE WITH X-LINKED CEREBRAL CREATINE TRANSPORTER (SLC6A8) DEFICIENCY

Mercimek-Mahmutoglu S[1], Connolly M[2], Poskitt K[3], Lowry N[4], Salomons GS[5], Casey B[6], Sinclair G[6], Jakobs C[5], Stockler-Ipsiroglu S[1]
[1]*Div Biochemical Dis, Dep Ped, Univ BC, Vancouver, Canada*
[2]*Div Neurol, Dep Ped, Univ BC,, Vancouver, Canada*
[3]*Dep Radiol, Univ BC, Vancouver, Canada*
[4]*Dep Ped, Royal Univ Hos, SASKATOON, Canada*
[5]*Dep Clin Chem, Metab Unit, VU Univ Med C, Amsterdam, Netherlands*
[6]*Dep Path, Univ BC, Vancouver, Canada*

Background: X-linked cerebral creatine transporter (SLC6A8) deficiency is characterized by mild to severe global developmental delay, behavioral problems and epilepsy in males. Females are asymptomatic or have mild intellectual disability according to skewed X-inactivation.
Case report and results: This 10-year-old female presented with intractable epilepsy and global developmental delay at age 3 years. She failed eight anti-epileptic medications and ketogenic diet for 3 years. Her investigations revealed partial creatine deficiency in cranial MR-spectroscopy at age 6 years. Subsequent investigations confirmed the diagnosis of SLC6A8 deficiency by a novel missense mutation (c.1067G>T; p.Gly356Val) in the SLC6A8 gene. The creatine uptake study in fibroblasts was partly impaired. There was no skewed X-inactivation in the peripheral blood. However unfavorable X-inactivation in the brain might explain her severe neurological manifestations. To enhance intracerebral creatine synthesis she was started on l-arginine, l-glycine and creatine monohydrate supplementation therapy. She became seizure free at 10 months of this combination therapy and has been seizure free for 20 months for the first time. Her caregivers and teachers described improvements in her behavior and speech language. Her EEG showed improvements in epileptiform activity and in background after one year of therapy. Her cranial MR-spectroscopy revealed mild increase in intracerebral creatine.
Conclusion: We present first female with severe clinical phenotype of SLC6A8 deficiency. Her intractable epilepsy was treated with l-arginine, l-glycine and creatine monohydrate supplementation therapy. SLC6A8 deficiency is a potentially treatable condition and should be considered in females with intractable epilepsy, global developmental delay and intellectual disability.

298-P

CREATINE METABOLISM AND METABOLIC STRESS: UNDERSTANDING THE POSSIBLE CROSS-TALK

Alcaide P[1], Merinero B[1], Ruiz-Sala P[1], Sanchez A[1], Ribes A[2], Artuch R[3], Campistol J[3], Rodríguez-Pombo P[1], Ugarte M[1]
[1]*CEDEM, CBMSO, UAM-CSIC, CIBERER, Madrid, Spain*
[2]*Inst. Bioquimica Clinica, CIBERER, Barcelona, Spain*
[3]*Hosp.Sant. Joan de Déu, CIBERER, Barcelona, Spain*

Since creatine deficiency syndromes (CDS) could result in energy depletion, our aim was to investigate parameters related to oxidative stress in CDS cell lines with biochemical and genetic diagnosis of CRTR (creatine transport) and GAMT defects.

We previously confirmed an increase of intracellular ROS (reactive oxygen species) content and percentage of apoptotic cells in some CDS cell lines. Increased ROS levels in cells were found to induce many types of DNA damage including cell cycle arrest, or the entry in an irreversible sub-G1 state when damage is severe. Herein, we present new data about the impact of Cr defects on the normal growth and cell cycle distribution in nine CDS fibroblasts cell lines and the possible linkage between ROS levels/DNA damage and intracellular Cr levels. Analysis of cell cycle was accomplished by flow-cytometry analysis. Intracellular Cr was quantified by HPLC/MS/MS.

Most of CDS cell lines investigated displayed different patterns of cell cycle arrest, including a significant percentage (>14%) of quiescent cells measured as sub-G1 population in those lines who's also displayed higher ROS levels (2 CRTR). These data are in discordance with the measured intracellular creatine levels, as a drastic decreased content was detected in all CRTR cell lines (5% of control values), whereas GAMT lines showed Cr levels similar to control. Together these data pointed out that Cr homeostasis in fibroblast is mainly kept by extracellular uptake and that Cr depletion is not the only cause to explain the apparent metabolic stress found in specific CDS cell lines

299-P

CLINICAL AND MR SPECTROSCOPY FOLLOW-UP OF CT1 DEFICIENT ITALIAN PATIENTS TREATED BY ORAL ARGININE

Battini R.[1], Casarano M.[1], Tosetti M.[1], Chilosi AM.[1], Mancardi MM.[2], Leuzzi V.[3], Cioni G.[4], -Cr GISMET[5]
[1]*Dpt Dev Neurosc, IRCCS Stella Maris, Pisa, Italy*
[2]*Dpt Ped Child Neuropsy IRCCS G.Gaslini, Genova, Ital*
[3]*Dpt Child Neurol Psych, Univ La Sapienza, Roma, Italy*
[4]*Div Child Neurol Psych Univ Pisa, Pisa, Italy*
[5]*GISMET-creatina Italian Group, Pisa, Genova, Roma, Italy*

Creatine transporter deficit (CT1) is an inherited metabolic disease that causes mental retardation, epilepsy, language and behavioral disorders. Results of different therapeutical attempts didn't show an agreement in this condition. In our experience the supplementation with Arginine, a Cr precursor capable to go along the blood brain barrier, appears a feasible therapeutic option. We report on the results of a longitudinal study of the five Italian CT1 patients, who were treated with oral supplementation of Arginine (300 mg/Kg/die) and underwent a comprehensive follow-up, including clinical and neuropsychological assessment as well as and serial 1H- and 31P-MRS brain examinations. The patients presented a similar clinical picture (mental retardation with language impairment, clumsiness, hyperactivity and epilepsy in 3 of them) and started oral supplementation with arginine at age 5.5 yrs, 5.6 yrs, 8.5 yrs, 8.6 yrs, 17 yrs respectively; three of them have completed three years of treatment. They did not complain any side effects.

Brain Cr and PCr fluctuations under treatment were monitored respectively by MRS: total Cr signal was analyzed with LCModel and brain PCr was computed as PCr/PDE ratio using PDE as internal reference. During treatment tCr showed a mild increase; ATP concentration increased too; PCr remains unchanged; baseline brain pH progressively normalized.

A significant improvement, mainly involving adaptive functions and behavior skills, was reported in all children. IQ score did not show significant changes. Clinical results seem remarkable when considering the degree of intellectual disability and the protracted phase of developmental stagnation before the beginning of the treatment.

300-P

TREATMENT OPTIONS FOR GAMT DEFICIENCY SYNDROME: RESULTS FROM BRAIN 31P MAGNETIC RESONANCE SPECTROSCOPY PILOT STUDY IN A MURINE KNOCK-OUT MODEL

Schmitt B[1], von Both I[2], Bachert P[1], Schulze A[2]
[1]*Med Phys in Rad, German Cancer Res Ctr, Heidelberg, Germany*
[2]*Res Inst, Hosp for Sick Children, Toronto, Canada*

Background: Inborn errors of creatine (Cr) metabolism such as the guanidinoacetate methyltransferase (GAMT) deficiency are genetic disorders which lead to a severe impairment of development at onset. Treatment focuses on normalization of cerebral Cr, but also on decreasing the intracerebral accumulation of guanidinoacetate product of the AGAT reaction. To investigate whether the latter can be achieved by ornithine (Orn) supplementation, this study used 31P magnetic resonance spectroscopy (MRS) to trace metabolic changes in the brain of a GAMT knock-out mouse-model undergoing Orn treatment.

Methods: Brain MR spectra were recorded from homozygous knock-out (GAMT-/-) and heterozygous (GAMT+/-) mice at baseline and after 5 days supplementation with Orn through drinking water. For comparison, wild-type (GAMT+/+) C57Bl/6 animals were also examined at baseline. Concentrations of 31P metabolites were calculated relatively to the concentration β-ATP.

Results: The baseline measurements showed phosphoguanidinoacetate (PGua) levels in GAMT knock-out animals which were distinctly higher than the phosphocreatine (PCr) concentration. In wild-type or heterozygous animals, respectively, no significant PGua levels compared to PCr were detected. After Orn supplementation, PGua levels decreased in 3 out of 4 GAMT-/- animals while PCr concentrations remained at low concentrations. In heterozygous animals, no apparent effect could be observed.

Conclusion: The results indicate that Orn supplementation can reduce intracerebral accumulation of PGua in GAMT deficiency. However, in the mouse model employed, the baseline PCr concentration seems to be higher compared to human GAMT patients. Further studies with higher number of animals and varying Orn concentration will be conducted to gain additional insight.

301-P

SIX NEW PATIENTS WITH CREATINE DEFICIENCY SYNDROMES IDENTIFIED BY SELECTIVE SCREENING IN BRITISH COLUMBIA

Mercimek-Mahmutoglu S[1], Roland E[2], Huh L[2], Steinraths M[3], Connolly M[2], Salomons GS[4], Sinclair G[5], Jakobs C[4], Stockler-Ipsiroglu S[1]
[1]Div Biochemical Dis, Dep Ped, Univ BC, Vancouver, Canada
[2]Div Neurol, Dep Ped, Univ BC,, Vancouver, Canada
[3]Medical Genetics, Victoria Gen Hos, Victoria, Canada
[4]Dep Clin Chem, Metab Unit, VU Univ Med C, Amsterdam, Netherlands
[5]Dep Path, Univ BC, Vancouver, Canada

Background: Creatine biosynthesis (guanidinoacetate methyltransferase (GAMT) and L-arginine:glycine amidinotransferase (AGAT) deficiencies) and transport (SLC6A8 deficiency) defects results in creatine deficiency syndromes. Global developmental delay, intellectual disability and seizures are common clinical features.
Methods: Guanidinoacetate and creatine/creatinine ratio were measured in urine by tandem-mass-spectrometer. Intracerebral creatine was measured by cranial-MR-spectroscopy. Mutation analysis of GAMT and SLC6A8 genes, GAMT enzyme activity and creatine uptake study were performed.
Results: Two female patients with GAMT deficiency and 4 patients with SLC6A8 deficiency (2 female siblings and 2 males) were identified within 5 years in British Columbia. All patients presented with global developmental delay. One female patient with SLC6A8 presented with intractable epilepsy. Five of the patients showed total or partial intracerebral creatine deficiency in cranial-MR-spectroscopy. Two patients with GAMT deficiency had elevated levels of guanidinoacetate in urine. Three previously reported mutations (c.327G>A;splice mutation) and a novel insertion (c.58insT) were identified. Both had none-detectable GAMT activity. Both were treated with creatine (400–550 mg/kg/d), ornithine (400–500 mg/kg/d) and arginine restricted-diet (300 mg/d). Two males had elevated and two females had normal urine creatine/creatinine ratio with SLC6A8 deficiency. Three (2 females and one male) patients had a novel missense mutation and one patient had a known 3 bp deletion in the SLC6A8 gene. All patients with SLC6A8 deficiency are on creatine, l-arginine and l-glycine supplementation therapy.
Conclusion: Creatine deficiency syndromes are treatable cause of intellectual disability and epilepsy. Patients with global developmental delay and epilepsy should be investigated for these disorders to identify treatable causes.

302-P

GUANIDINOACETATE METHYLTRANSFERASE (GAMT) DEFICIENCY IN TWO ADULT FEMALE SIBLINGS

Chronopoulou E[1], Hinnell C[2], Alkufri F[2], Samuel M[2], Turner C[1], Dalton N[1], Nashef L[2], Rahman Y[1]
[1]Evelina Children's Hosp, St Thomas Hosp, London, United Kingdom
[2]Dept of Neurology, King's College Hosp, London, United Kingdom

Creatine Deficiency syndromes, a group of inborn errors of creatine synthesis and transport, cause cerebral creatine deficiency, manifest as progressive central nervous dysfunction, are diagnosed by simple biochemistry of body fluids, are potentially treatable and may account for a considerable fraction of undiagnosed developmental delay. Guanidinoacetate methyltransferase (GAMT) deficiency has the most severe clinical phenotype presenting with intellectual disability associated with expressive speech delay, drug-resistant epilepsy, progressive extrapyramidal movement disorder and autism. We present 2 cases of female siblings, aged 29 and 31, born to consanguineous Turkish-Cypriot parents. Patient 1 had delayed motor milestones, developed focal dystonia at the age of 3 years and now independently mobilises with unilateral weakness. Speech is limited to short sentences and has autistic behaviour and partial seizures. Patient 2 had severe speech delay in childhood with near normal motor development up to age 21, and then developed severe global dystonia. Behavioural problems were reported; she does not suffer from epilepsy. Unremarkable investigations include copper/caeruloplasmin, autoantibodies, white cell enzymes, plasma aminoacids, VLCFA, urine oligosaccharides and organic acids, Fragile X, Rett's syndrome and mitochondrial mutations, nerve conduction studies, skin and muscle studies and brain imaging. Both patients had low plasma creatinine (35, 30 μmol/L), low plasma creatine (0.3, 3.4 μmol/L) and high plasma guanidinoacetic acid (21.6, 26.2 μmol/L). We report two very different clinical presentations of GAMT deficiency in two siblings diagnosed in adulthood. The cases highlight the importance of considering a potentially treatable metabolic disorder in patients presenting with developmental delay, seizures, autism and movement disorders.

303-O

LONG-TERM FOLLOW-UP OF TETRAHYDROBIOPTERIN (BH4) THERAPY IN PATIENTS WITH BH4 DEFICIENCY IN JAPAN

Shintaku P[1], Ohwada P[2], Kitagawa D[3]
[1]Dept Ped, Osaka City Univ Grad Sch Med, Osaka, Japan
[2]Dept Ped Nutr, Grad Sch Kagawa Nutr Univ, Sakato, Japan
[3]Tokyo Health Service Association, Tokyo, Japan

Background: Tetrahydrobiopterin(BH4) deficiency is a rare disorder developing not only hyperphenylalaninemia but also neurological disorders. The combination administration of BH4, L-Dopa and 5-hydroxy tryptophan (5-HTP) is a common therapeutic approach in these patients. About 1 million nenates are tested every year in newborn mass-screening in Japan. Among these, about 1 in 2 million neonates are diagnosed as having BH4 deficiency. In this post-marketing surveillance, we followed 19 patients with BH4 deficiency in which BH4 supplementation with Biopten. was initiated before age 4 years for an observation period of ≤28 years.
Methods: Patients who were screened positive for BH4 deficiency were treated with supplemental BH4 plus L-dopa and 5-HTP. Data on the patients' clinical course were collected once yearly at 10 medical centers in Japan.
Results: A total of 17 patients were diagnosed as having PTPS deficiency and 2 patients DHPR deficiency at an average age of 3.6 months; their mean age at end of follow-up was 14.6 years. Average duration of treatment with BH4 (mean, 5 mg/kg/day) was 13.2 years. Serum phenylalanine was reduced from >10 mg/dL at the start of drug administration to <2 mg/dL at end of follow-up. No abnormalities of height and weight were observed in all patients except one female patient with familial obesity. No unwarranted side effects were reported throughout the long-term course of treatment.
Conclusion: Biopten. therapy in patients with BH4 deficiency is highly efficacious at maintaining serum phenylalanine levels within normal control range and provides excellent safety throughout life with no unwarranted side effects.

304-P

PLASMA PHOSPHOLIPIDS LONG-CHAIN POLYUNSATURATED FATTY ACIDS PROFILE IN HYPERPHENYLALANINEMIC CHILDREN ON UNRESTRICTED DIET

Giovannini M[1], Verduci E[1], Radaelli G[1], Lammardo AM[1], Minghetti D[1], Cagnoli G[1], Salvatici E[1], Riva E.[1]
[1]*Dept. of Ped, S.Paolo Hosp, Univ of Milan, Milan, Italy*

Background: The phenylketonuric (PKU) diet determines a low intake of long chain polyunsaturated fatty acids (LCPUFA). The aim of the present study was to examine whether hyperphenylalaninemic children on unrestricted diet (MHP) may exhibit a different plasma phospholipids LCPUFA profile from PKU or healthy children.
Patients and Methods: Forty-five MHP children (age 9–14 years) were age and sex matched with 45 PKU and 45 healthy control children. Plasma phospholipds fatty acids were determined and expressed as % of total fatty acids.
Results: MHP children showed phospholipds docosahexaenoic acid levels higher than PKU children (mean difference, 0.2%; 95% confidence interval, 0.02% to 0.38%), although difference was not significant after correction for multiple comparisons (P=0.117), and lower than healthy children (0.8%; -1.01% to -0.59%).
Conclusions: The results suggest that MHP children may exhibit a plasma phospholipds LCPUFA profile not differing appreciably from PKU children whereas show lower DHA levels than healthy children.

305-A

RELATIONSHIP BETWEEN GROWTH AND INTAKE OF NOURISHING IN HYPERPHENILALANINEMIC CHILDREN: DIET (PKU) VS NON DIET SUBJECTS (MHPA) AND HEALTHY CONTROL (HC) GROUP

Riva E[1], Lammardo AM[1], Salvatici E[1], Damele CAL[1], Paci S[1], Zuvadelli J[1], Cagnoli C[1], Minghetti D[1], Giovannini M.[1]
[5]*Dept Pediatr, San Paolo Hosp Univ, Milan, Italy*

Background: PKU children have a restricted diet in natural protein and supplemented with a phenylalanine (Phe)-free mixture. Growth delay has been observed in PKU patients on a diet.
Objective: To evaluate the relation between the intake of natural protein and growth in PKU vs MHPA and HC children. Patients and methods: 67 children: 24 PKU with a good blood Phe control (mean age 106 ± 26 mths), 19 MHPA (mean age 104.0 ± 27 mths), 24 HC (mean age 106.3 ± 29 mths), matched for sex. 3-days-diet recalls were collected for three groups. Statistical analysis: Z-scores for Height (H/A), median ± SD.
Results: Median daily intake of protein expressed as g/100Kcal is for PKU 2.6±0.6 with a natural protein intake of 0.69 ± 0.31; for MHPA 3.7 ± 0.5 and for HC group 3.7 ± 0.5. H/A median is –1.13 ± 0.75 for PKU, -0.09 ± 0.99 for MHPA and 0.31 ± 0.61 for HC. Sharing PKU, MHPA and HC group in three sub-groups for age (5–7y; 7–10y and 10–12y) it can be observed that H/A median in the 2nd sub-group is –1.08 ± 0.61 in PKU patients vs 0.18 ± 0.58 in HC group. This result for PKU patients can be related to the decreasing of milk and yoghurt (animal protein admitted in PKU diet) registered by 3-days-diet recall.
Conclusion: From this preliminary study, it may be hypothesized that the nature of the protein rather than total protein would be in relation to growth of PKU children.

306-P

PREDICTION OF LONGTERM RESPONSIVENESS TO TETRAHYDROBIOPTERIN IN PHENYLKETONURIA

Hennermann JB[1], Roloff S[1], Weinhold N[1], Gebauer C[1], Klein J[1]
[1]*Dep of Ped, Charité Univ Med Center, Berlin, Germany*

BH4 responsive phenylketonuria (PKU) has been described more than 10 years ago. Though, criteria for the identification of PKU patients, who benefit from long-term treatment with BH4, have not yet been established. In our center, 20 patients with mild or classic PKU were treated over a medium period of 30 months (3–77 months) with BH4 in a medium dose of 17 mg/kg/day (10–20 mg/kg/day). Criteria for treatment with BH4 were defined by i. positive BH4 loading test, ii. identification of at least one milder BH4 responsive PAH mutation and/or iii. mild clinical phenotype. Three criteria were positive in 7 patients, two in 11 patients, and one in 2 patients.
15/20 patients showed long-term response to BH4 resulting in an increase of medium phenylalanine tolerance to 1013 mg/day (350–1840 mg/day), corresponding to 31 mg/kg/day (6–96 mg/kg/day). 9/15 patients still needed a phenylalanine-free amino acid mixture. In 5 patients long-term BH4 treatment was stopped due to missing response; furthermore, one of them complained of recurrent headache. All patients with three positive criteria showed long-term response to BH4. 7 of 11 patients with two positive criteria responded to long-term treatment with BH4, but none of those patients with only one positive criterion.
Thus, at least two of the designated criteria have to be positive to attain BH4 long-term responsiveness. No single criterion was specific enough to prognosticate long-term BH4 responsiveness.

307-P

BRAIN MRI IN THE OFFSPRING OF A PKU FEMALE ON NON-RESTRICTED DIET

Maertens P[1], Eyal F[1]
[1]*University of South Alabama, Mobile, United States*

Background: The maternal phenylketonuria (PKU) syndrome is caused by high blood phenylalanine (Phe) levels during pregnancy, leading to a host of birth defects, especially facial dysmorphism, microcephaly, development delay, learning difficulties and congenital heart disease.
Objective: We aimed to analyze brain anatomy in the offspring of women with PKU on non-restricted diet during pregnancy.
Case Report: A 9 year-old male is the product of a 23 year-old G6P1M5 PKU female who was on a non-restricted diet before or during pregnancy. He had congenital microcephaly and bilateral cataract at birth. Development was slow as he only sat up at age 2, said first word and walked at age 3. He is severely retarded with narrow forehead and small ears. He suffers from self-abusive and aggressive behavior towards other. He has poor fine motor skills. He never had seizures and his electroencephalogram is normal.
Result: MRI of brain shows a large face with small cerebrum and short corpus callosum. The right temporal lobe and hippocampus are dysplasic. There is relative sparing of the cerebellum.
Conclusion: Mental retardation in children born to PKU female on non-restricted diet is due to cerebral hypoplasia most prominent over temporal regions. Phe-restricted diet should start before conception and should be maintained throughout pregnancy.

308-P

OXIDATIVE STRESS IN PKU PATIENTS: EFFECT OF SUPPLEMENTATION WITH L-CARNITINE AND SELENIUM

Sitta A[1], Vanzin CS[1], Biancini GB[1], Manfredini V[2], Wayhs CAY[3], Ribas GOS[3], Giugliani L[4], Schwartz IVD[4], Souza CFM[4], Wajner M[1], Vargas CR[3]
[1]PPG Bioq, Univ Fed Rio Grande do Sul, Porto Alegre, Brazil
[2]Univ Reg Int Alto Uruguai e Missoes, Erechim, Brazil
[3]PPG Pharmacy, Univ Fed Rio Grande do Sul, Porto Alegre, Brazil
[4]Med Genet Serv, Clin Hosp Porto Alegre, Porto Alegre, Brazil

Background: In recent years it has been demonstrated the involvement of reactive species in pathophysiology of neurological damage in phenylketonuria (PKU). In a previous study of our group it was verified that PKU patients treated with a protein-restricted diet supplemented with an amino acid mixture (not containing L-carnitine and selenium) presented highest lipid and protein oxidative damage as well as a reduction of antioxidant defenses when compared to the healthy individuals. Our goal in the present study was to evaluate the effect of supplementation with L-carnitine and selenium 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 treatment with L-carnitine and selenium was capable to revert the lipid peroxidation, measured by thiobarbituric-acid reactive species, and the protein oxidative damage, measured by sulfhydryl oxidation, to the levels of controls. Additionally, the activity of glutathione peroxidase was normalized by the antioxidant supplementation, probably by selenium, the cofactor of the enzyme.
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 in PKU, which probably may be due to accumulation of toxic metabolites that lead to excessive production of free radicals, as well as to the alteration on antioxidant status when a restricted diet is applied.
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309-P

HIGH PHENYLALANINE LEVELS DIRECTLY AFFECT MOOD AND SUSTAINED ATTENTION IN ADULTS WITH PHENYLKETONURIA: A RANDOMISED, DOUBLE-BLIND, PLACEBO-CONTROLLED, CROSSOVER TRIAL

ten Hoedt AE[1], de Sonneville LMJ[2], Francois B[3], ter Horst NM[1], Janssen MCH[4], Rubio-Gozalbo ME[5], Wijburg FA[1], Hollak CEM[6], Bosch AM[1]
[1]Dept Pediatr, Ac Med Centre Amsterdam, Amsterdam, Netherlands
[2]Dept Clin Child & Adol Stud, Leiden Univ, Leiden, Netherlands
[3]Dept Metab Dis, Centrum Pinocchio, Diepenbeek, Belgium
[4]Dept Int Med, Radboud Univ Med Centre, Nijmegen, Netherlands
[5]Dept Pediatr, Maastricht Univ Med Centre, Maastricht, Netherlands
[6]Dept Int Med, Ac Med Centre Amsterdam, Amsterdam, Netherlands

Background: The main debate in the treatment of Phenylketonuria (PKU) is whether adult patients need the strict phenylalanine (Phe) restricted diet. Physicians and patients lack evidence based guidelines to help them make well-informed choices.
Objectives: To assess the effects of short term elevation of Phe levels on neuropsychological functions and mood of adults with PKU.
Patients and Methods: Nine continuously treated adults with PKU underwent two 4-week supplementation periods: one with Phe, mimicking normal dietary intake, and one with placebo in a double-blind crossover design. A set of neuropsychological tests of the Amsterdam Neuropsychological Tasks Program was administered at the end of each study period. In addition, patients or a friend or relative of each patient completed weekly questionnaires evaluating the patients' mood (Profile of Mood States). Phe levels were measured twice-weekly.
Results: Mean plasma Phe levels were significantly higher during Phe supplementation compared with placebo. Neuropsychological tests demonstrated impairment in sustained attention during Phe supplementation. Both patients and their friend or relative reported lower scores on the POMS questionnaires during Phe supplementation.
Conclusion: High plasma Phe levels have a direct negative effect on both sustained attention and on mood in adult patients with PKU. A Phe restricted "diet for life" may be an advisable option for many.

310-P

MATERNAL PHENYLKETONURIA (PKU) PRACTICAL MANAGEMENT IN UK METABOLIC CENTERS

Robertson LV[1], Macdonald A[2], Ripley S[3], Adams S[4], Chan H[5], Ellerton C[5], Maritz C[5], Mcstravick N[6], Micciche A[7], Terry A[8], Weetch E[9], Wildgoose J[10]
[1]Univ Hosp Birmingham NHS Trust, Birmingham, United Kingdom
[2]Birmingham Childrens Hosp, Birmingham, United Kingdom
[3]Salford Royal NHS Trust, Manchester, United Kingdom
[4]Royal Hosp for Sick Children, Glasgow, United Kingdom
[5]Univ College London Hosps NHS Trust, London, United Kingdom
[6]Belfast Health and Social Care Trust, Belfast, United Kingdom
[7]Guy's and St Thomas' NHS Trust, London, United Kingdom
[8]Alder Hey Children's Hosp NHS Trust, Liverpool, United Kingdom
[9]Sheffield Teaching Hosp NHS Trust, Sheffield, United Kingdom
[10]Bradford Teaching Hosp NHS Trust, Bradford, United Kingdom

Background: Pregnant women with PKU must adhere to strict dietary management. Dietary practice is mainly based on experience and expert opinion rather than scientific findings. There are no agreed UK best practice guidelines for the management of maternal PKU.
Methods: An online questionnaire was sent to specialist dietitians at adult metabolic centers (n=7) and to specialist paediatric dietitians who are responsible for adult patients in the UK (n=2).
Results: During preconception and pregnancy, all 9 centers recommended that blood phenylalanine concentrations were maintained within tight ranges. The time blood phenylalanine concentrations were expected to be within target range before recommending discontinuation of contraception varied from centre to centre. Eight of the 9 centers gave tyrosine supplementation, but started at different times during the pregnancy.
Folic acid supplements were routinely given by 2 centers; four gave no extra folic acid and 3 centers only supplemented if the folic acid intake from protein substitute did not meet the UK government recommendations for pregnancy. DHA supplements were only offered by 4 centers if not already included in the protein substitute.
Conclusions: In the UK, there are differences in management practices between centers.
There is a need for best practice guidelines to be produced.

311-O

MOLECULAR PATHOLOGY OF MUTATIONS IN PAH EXON 11, IMPACT ON MRNA PROCESSING, AND POTENTIAL IMPACT ON THERAPY WITH 6R-TETRAHYDROBIOPTERIN (BH4)

Heintz C[1], Dobrowolski SF[2], Blau N[1], Demirkol M[3], Andersen HS[4], Andresen BS[4]
[1]Div Cli Chem and Biochem, Univ Child Hosp, Zurich, Switzerland
[2]Dept Path, Sch Med, Univ Utah, Salt Lake City UT, United States
[3]Child Hosp Ist Fac Med, Ist Univ, Istanbul, Turkey
[4]Dept Biochem and Mol Biol, SDU, Odense, Denmark

Phenylalanine levels may be controlled in ~30% of PKU patients by BH4 therapy. The PAH genotype has utility to predict BH4-response, since this is dependent on production of functional PAH enzyme. Therefore correct assessment of the effect of mutations is important. Splicing of each exon in a gene is determined by a finely balanced interplay between splice-site strength and regulatory sequences like ESEs/ESSs. Mutations that disturb this balance may cause mis-splicing and disease, irrespective of the predicted amino acid change. We investigated two missense mutations, c.1139C>T and c.1144 T>C in PAH exon 11 and a 3′-splice site mutation (c.1066–3C>T) located outside the invariant AG-dinucleotide for their potential to cause mis-splicing.
We used PAH minigenes to study the molecular defect mechanism in detail and to exclude the effect of linked mutations and patient lymphoblasts to confirm the findings. This showed that c.1144 T>C and c.1066–3C>T causes exon 11 skipping, while c.1139C>T is neutral. We used RNA affinity purification to identify splicing regulatory proteins responsible for exon 11 splicing. We suggest that PAH exon 11 is a vulnerable exon, where splicing can be easily disrupted by mutations. This is due to the weak 3′-splice site, which requires maintenance of a fine balance between ESEs/ESSs in order for it to be recognized. Exonic mutations, like c.1144 T>C, that disrupt splicing are unlikely to facilitate response to BH4 and recognizing such mutations enhances our ability to predict BH4-response. Furthermore, the mechanism by which c.1066–3C>T manifests BH4-response is most probably by producing low levels of wild-type mRNA.

312-O

GENOTYPE-BASED PREDICTION OF TETRAHYDROBIOPTERIN (BH4)-RESPONSIVENESS IN PHENYLKETONURIA

Heintz C[1], Thony B[1], Blau N[1]
[1]Div Cli Chem and Biochem, Univ Child Hosp, Zurich, Switzerland

More than 70 specific mutations in the phenylalanine hydroxylase (PAH) gene, presenting with a substantial residual activity, were identified in BH4-responsive PKU patients. However, not a single mutation, but rather the full genotype is determining the BH4-responsive phenotype. The aim of our study was to provide more information on predictive value of BH4-responsive genotypes in PAH-deficient patients. For this purpose we extended the existing BIOPKU database (www.biopku.org) to 737 genotypes from PKU patients challenged with BH4 to investigate response upon blood-Phe testing. Database information is stratified according to the amount of BH4 administered, duration of the test, severity of the hyperphenylalaninemia, and BH4-responsiveness. The most frequent genotypes found in the BH4-responder type PKU patients are p.R261Q/ p.R261Q (6.0%), p.L48S/ p.L48S (4.7%), p.A300S/IVS10–11G>A (2.6%), and p.E390G/ p.R408W (2.6%). The predicted PAH activity from in vitro expression analysis of the mutant and normal PAH cDNA was also calculated and tabulated in the BIOPKU database. We found a positive correlation between the BH4-responsiveness and predicted PAH activity. Patients with predicted PAH activity of less than 15% can be considered as non-responder, while patients with predicted PAH activity of more than 39% are all BH4-responder. Particularly classical PKU patients with two null-mutations are certainly not candidates for the BH4 test. A number of common genotypes (e.g. p.L48S/ p.L48S, p.R261Q/ p.R261Q or a combination of both mutations) show inconsistent correlation between the BH4 testing and the phenotype.

313-P

HIGH PREVALENCE OF G352fsdelG MUTATION AND DETECTION OF NOVEL MUTATION p.K85X in PKU PATIENTS FROM MOROCCO

Dahri S[1], Desviat LR[2], Pérez B[2], Leal F[2], Ugarte M[2], Chabraoui L[1]
[1]Faculty of Medicine and Univ Child Hosp, Rabat, Morocco
[2]Univ Autónoma Centro de Invest Biomédica, Madrid, Spain

Objective: The knowledge of the molecular basis of the Phenylketonuria (PKU, MIM# 261600) in different countries provides relevant information for undertaking specific and rational mutation detection strategies in each population and for the implementation of adequate dietary and cofactor treatment. There is no data available in Moroccan population.
Design and methods: In this work we describe the genetic analysis by mutation scanning using denaturing gradient gel electrophoresis (DGGE) and subsequent direct sequencing of 20 different PKU families from Morocco. We have also included the study of 7 Moroccan PKU patients living in Spain detected by the Spanish newborn screening program.
Results: The mutational spectrum in the first sample included eight different changes, one of them, p.K85X was novel. The most common mutation was the frameshift change p.G352fsdelG identified in 62.5% of the mutant chromosomes studied. Other changes (p.R176X, IVS10nt-11 g>a, p.W120X, p.A165T, p.R243X and p.R243Q) were identified respectively in 2 or 3 mutant alleles. All detected mutations were severe according to the classical phenotype of the patients. In the 7 Moroccan patients living in Spain we have detected 4 severe mutations (p.G352fs, p. R176X, Y198fs and Exon3del) and also milder changes such as p.A403V, p.S196T, p.D145V and p.R408Q detected in 3 mild hyperphenylalaninemia (MHP) patients and a novel p.L258P found in a mild PKU patient.
Conclusion: The results provide important information on the distribution of PKU mutations in this Mediterranean area gaining insight into the genetic epidemiology of the disease.

314-P

TETRAHYDROBIOPTERIN RESPONSIVENESS IN BRAZILIAN PATIENTS WITH PHENYLALANINE HYDROXYLASE DEFICIENCY

Giugliani L[1], Sitta A[2], Vargas CR[3], Santana da Silva LC[4], Nalin T[5], Pereira LM[6], Schwartz IVD[7], Giugliani R[7]
[1]PPG SCA, UFRGS, Porto Alegre, Brazil
[2]PPGB, UFRGS, Porto Alegre, Brazil
[3]Faculty of Pharmacy, UFRGS, Porto Alegre, Brazil
[4]Federal University of Para, Para, Brazil
[5]PPGCM, UFRGS, Porto Alegre, Brazil
[6]Depart Biochem, UFRGS, Porto Alegre, Brazil
[7]Medical Genetics Service, HCPA, Porto Alegre, Brazil

Introduction: Recent studies showed that patients with hyperphenylalaninemia by phenylalanine-hydroxylase deficiency (HPA-PAH) can have serum levels reduced when receiving oral tetrahydrobiopterin(BH4).
Objective: to identify in a sample of Brazilian HPA-PAH patients the ones responsive to the oral administration of BH4.
Methods: the following inclusion criteria were used: diagnosis of HPA-PAH, age ≥7 years, on dietary treatment and Phe levels ≥6 mg/dL in all tests performed one year prior inclusion. Blood samples were also obtained at time points 0 h, 4 h, 8 h (Day 2) and 24 h (Day 3) after BH4 intake. Phe levels were determined by TMS. Criteria used to define responsiveness to BH4 were: Criterion 1: Phe reduction≥30% 8 h after BH4 administration; Criterion 2: Phe reduction ≥30% 24 hours after BH4 administration.
Results: From 18 patients studied, with a mean age of 14ys, 66.7% were male. Eleven presented the classical form of the disease and 3 the atypical form. Three patients(classical form: 1, atypical form: 2)and 5(classical form:2; atypical form:2; undefined form:1)were considered responsive to BH4 according to criteria 1 and 2, respectively. Phe serum levels on the Day 1 did not show any change on the established time point schedule (p=0.523). However, when comparing levels of Phe between Days 1 and 2, significant variation was found (p=0.006). The phenotype–genotype association analysis of patients with available data (n=6) showed that the association is multifactorial.
Conclusion: In accordance with the literature, our findings show that a significant proportion of Brazilian patients with HPA-PAH can benefit from the oral administration of BH4.

315-P

PHENYLKETONURIA—THE EFFECTS ON QUALITY OF LIFE AND PLASMA CONCENTRATIONS OF PHENYLALANINE AND TYROSINE OF TWO DIFFERENT AMINO-ACID-SUPLEMENTATIONS IN DIFFERENT CONCENTRATIONS

Ahring KK[1], Mxller LB[1], Nielsen JB[1], Andersen JR[2]
[1]The Kennedy Centre, Glostrup, Denmark
[2]University of Copenhagen, Copenhagen, Denmark

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. The aim of this study was to determine the effects of 2 different products (LN1 and LN2), containing LNAA in different combinations on plasma Phe levels and other metabolites in early treated adults with PKU, and to investigate the relationship between these metabolites and well being. Material (Patients) and methods: This was a prospective, double blind, cross over study consisting of four consecutive three-week phases. Twelve subjects (6 males, 6 females) with PKU were recruited, 11 completed the study. Each phase consisted of either LN1 or LN2, either in low or high dosage. Subjects were instructed to follow their usual SF diet, maintain energy intake, and complete a 3-day food record and a SF36 scheme during each phase and to take blood samples every day for the week of each period. At the end of each phase, plasma amino acid profile was quantified and other metabolites were measured.
Results: There was no correlation between plasma Phe level and LNAA dosage or type of LNAA supplement. However, 2 patients stated that they felt better when taking LN 2 in high dosage.
Conclusions: LN1 & 2 in higher dosage than usual do not lower Phe level. However, LNAA supplementation has been used for PKU patients > 18 years for 25 years in Denmark and proved to be a useful alternative for adults with PKU.

316-P

PHENYLKETONURIA IN ADULTS: DIETARY HABITS, DIETARY DEFICITS, BODY COMPOSITION AND MRI OF THE BRAIN

Das AM[1], Goedecke K[1], Meyer U[1], Kanzelmeyer N[1], Illsinger S[1], Lucke T[1], Janzen N[1], Ding XQ[2]
[1]*Dept, Paed. , Hannover Medical School, Hannover, Germany*
[2]*Inst. Diag. and Interv. Neuroradiology, Hannover, Germany*

Background: Diet for life is recommended in classical phenylketonuria (PKU). Adults do not strictly stick to the recommended diet some patients omit amino acid mixture (AM) although nutrition is often not normal. Deficits in micro- and macronutrients may therefore be possible.
Objective: The aim of this study was to assess dietary habits and nutritional status in adults/adolescents with PKU.
Patients and Methods: Dietary protocols from 51 patients with early-treated PKU (age 17–44 years, 31 female, 20 male) from our clinic were included in this study. Dietary protocols, micro- and macronutrients in blood, vitamin B12, body composition (via BIA) and cerebral MRI were analyzed.
Results: 41% of PKU-patients followed recommended protein restriction supplemented with AM. 14% said they follow a less restricted 'vegan' diet supplemented with AM. 45% claimed to have normal eating habits without AM.
Phenylalanine levels were within target levels in all patients as was body composition. Fat and energy intake was insufficient in many patients. Urea concentrations in serum were lower than normal in all patients indicating reduced protein intake even in those with presumably 'normal' nutrition. Micronutrient intake was reduced in 64% of patients from the latter group (partly reflected in blood values), only 5% in the group with PKU-diet and AM had deficits.
MRI findings did not correlate with nutrition. A quality of life – questionnaire revealed less problems in those following a relaxed diet.
Conclusion: 45% of adult PKU-patients claimed to have normal nutrition without AM, however dietary assessment showed deficits in protein and micronutrient intake.

317-O

QUANTITATIVE PROTON/T2-MAPPING AND DTI DISCLOSES MICROSTRUCTURAL CHANGES IN NORMAL APPEARING BRAIN TISSUE IN TREATED PKU-PATIENTS

Das AM[1], Goedecke K[1], Kanzelmeyer N[1], Illsinger S[1], Hartmann H[1], Raab P[2], Berndt M[2], Lanfermann H[2], Ding XQ[2]
[1]*Inst.. Diag. and Interv. Neuroradiology, Hannover, Germany*

Introduction: In early-treated patients with phenylketonuria (PKU) conventional MRI shows typical lesions. There is no correlation of lesions with neuropsychological/cognitive deficits. A possible explanation is the presence of microstructural brain abnormalities causing such deficits. Only subtle signal variances caused by microstructural changes or tissue alterations may not be evident in conventional MRI. Therefore, it is challenging to apply standardized quantitative MRI measurements.
Methods: Parameter mappings of relative proton density (PD, in ratio to an aqueous phantom), T2 relaxation time, as well as the fractional anisotropy (by DTI) maps were carried out from the raw MRI data of 12 PKU patients treated soon after birth with well-documented diet, and 12 healthy controls. Numeric values of the parameters in gray and white matter were measured on each subject with the method of region of interest. Morphological findings were evaluated using modified Zimmerman-scale. All studies were performed on a 1.5 T MR system with a standard quadrature head coil.
Results: Normal brain MRI was found in 4 patients, different grade of white matter (WM) abnormalities in 8 patients, without correlation to dietary compliance. Correspondingly, elevated T2 values were found in these abnormal WM. Interestingly, lower T2 values were mainly found in WM of patients with normal MRI. In addition, all patients revealed elevated proton density in white and gray matter. As fractional anisotropy was normal in PKU- patients preferred orientation of nerve fibers is normal in treated PKU-patients.
Conclusion: Quantitative proton/T2-mapping and DTI discloses microstructural changes in normal appearing brain tissue in treated PKU-patients.

318-P

BORN AT 27 WEEKS OF GESTATION WITH CLASSICAL PKU: CHALLENGES OF DIETETIC MANAGEMENT IN A VERY PRETERM INFANT

Ballhausen D[1], Egli D[2], Bickle-Graz M[3], Bianchi N[2], Bonafé L[1]
[1]*Div Mol Ped, CHUV, Lausanne, Switzerland*
[2]*Clin Nutr Unit, CHUV, Lausanne, Switzerland*
[3]*Dev Unit, CHUV, Lausanne, Switzerland*

Only few cases of classical phenylketonuria (PKU) in premature infants have been reported. Treatment of these patients is challenging due to the lack of a phenylalanine-free amino acid solution for parenteral infusion.
The boy was born at 27 weeks of gestation with a weight of 1000 g (P10). He received parenteral nutrition with a protein intake of 3 g/kg/day. On day 7 he was diagnosed with classical PKU (genotype IVS10-11G>A/IVS12+1G>A) due to highly elevated phenylalanine (Phe) level in newborn screening (2800 micromol/L). His maximum plasma Phe level reached 3696 micromol/L. Phe intake was stopped for 4 days. During this time the boy received intravenous glucose and lipids as well as little amounts of Phe-free formula by a nasogastric tube. Due to a deficit of essential amino acids and insufficient growth, a parenteral nutrition rich in branched-chain amino-acids and relatively poor in Phe was added, in order to promote protein synthesis without overloading in Phe. Under this regimen, Phe plasma levels normalized on day 19 when intake of natural protein was started. The boy has now a corrected age of 2 years. He shows normal growth parameters and psychomotor development.
Despite a long period of highly elevated Phe levels in the postnatal period our patient shows good psychomotor development. The management of premature infants with PKU depends on the child's tolerance to enteral nutrition. It demands an intensive follow-up by an experienced team and dedicated dietician. Appropriate Phe-free parenteral nutrition would be necessary especially in case of gastro-intestinal complications of prematurity.

319-P

WORKING WITH DIET AND SAPROPTERIN IN PHENYLKETONURIA (PKU): WHAT FACTORS SHOULD BE CONSIDERED?

Ahring K.K.[1], Bélanger-Quintana A[2], Dokoupil K.[3], Gokmen-Ozel H.[4], Lammardo AM.[5], MacDonald A.[6], Motzfeldt K.[7], Robert M.[8], Rocha J.C.[9], van Rijn M.[10]
[1]*The Kennedy Centre, Glostrup, Denmark*
[2]*Pediatria Hosp Ramon y Cajal, Madrid, Spain*
[3]*Dr von Hauner Child Hosp, Univ Munich, Munich, Germany*
[4]*Dept Nutr Dietetics, Hacettepe Univ, Ankara, Turkey*
[5]*Dept Pediatr, San Paolo Hosp Univ, Milan, Italy*
[6]*The Children's Hospital, Birmingham, United Kingdom*
[7]*Oslo Univ Hosp Rikshospitalet, Oslo, Norway*
[8]*Hôp Univ des Enfants Reine Fabiola, Brussels, Belgium*
[9]*Centro de Genética Médica, INSA, Porto, Portugal*
[10]*Univ Med Centre, Groningen, Netherlands*

Background: The recent introduction of sapropterin dihydrochloride (BH4, Kuvan.) into the management of PKU has implications for the management of phenylalanine-restricted diets in responders to sapropterin.
Objectives: Protocols for optimal adjustment of phenylalanine-restricted diets have not been determined in sapropterin-treated patients. We present initial recommendations and raise important areas for future research, and issues for discussion.
Discussion: Patients/carers should understand potential benefits/limitations of sapropterin. It is also important that any individual identified outcome from treatment with sapropterin (e.g. reduction in blood phenylalanine or relaxation in diet) must be understood by patients and carers from the outset. An accurate initial evaluation of pre-sapropterin phenylalanine tolerance is essential. Some responders to sapropterin can relax the special diet, while a minority can discontinue it. Phenylalanine introduction protocols should be individually determined, as the response to the quantity and the rate of phenylalanine introduction is variable but this requires further research. Care is necessary when altering the diet (e.g. preservation of safe total daily protein intake). Continuing education and support will be required thereafter, with further adjustment of diet and sapropterin dosage as a young patient grows.
Conclusions: New clinical protocols are needed for managing this change in diet, while maintaining control of blood phenylalanine, ensuring adequate nutrition, and preventing overweight or obesity.

320-O

THE EFFECTS OF PHENYLALANINE LEVELS ON THE ADULT BRAIN: THE USE OF A PORTABLE SACCADOMETER TO MEASURE REACTION TIME IN THE OUTPATIENT SETTING

Dawson C[1], Carpenter R[2], Ellerton C[1], Maritz C[1], Chan H[1], Murphy E[1], Lachmann RH[1]
[1]*Metab Unit, Nat Hosp for Neur & Neurosur, London, United Kingdom*
[2]*Dept Physiol, Univ Cambridge, Cambridge, United Kingdom*

Background: There is no evidence that high phenylalanine levels have irreversible effects on the adult brain. Many PKU adults no longer follow a protein-restricted diet. Neuropsychological studies show that reaction time in PKU adults is slower than controls. There is no data showing this is directly related to phenylalanine levels. Another way to assess reaction time is to measure saccadic latency. We have used a portable, head-mounted saccadometer to measure latency in outpatients who were off-diet, on a standard or maternal diet and controls.
Methods: Adult PKU patients were split into three groups: off diet (Phe>1200 μmol/L), on diet (Phe<800 μmol/L) and maternal diet (Phe 100–400 μmol/L). Reciprocal median latency (RML) was compared between groups.
Results: RML was significantly slower in patients who were off diet (5.56 ms-1) than in patients on diet (6.00 ms-1, p=0.043), on a maternal diet (6.03 ms-1, p=0.010), or in normal controls (5.92 ms-1, p=0.021). Reaction times in both diet-treated groups were not significantly different from normal controls. In 16 women planning pregnancy we obtained values before (5.86 msms-1) and after (6.25 ms-1) they commenced the maternal diet. Stricter control of Phe levels resulted in a significant improvement in reaction time (p=0.041).
Conclusions: Saccadometry is useful in monitoring PKU patients. Adult patients with PKU not on a protein-restricted diet have significantly slower reaction times than controls. In addition, off-diet patients have significantly slower reaction times than on-diet. Paired data show that effects of phenylalanine levels on reaction time are reversible.

321-P

INTERNATIONAL DEVELOPMENT OF DISEASE-SPECIFIC QUESTIONNAIRES TO ASSESS THE IMPACT OF PHENYLKETONURIA AND ITS TREATMENT ON DAILY LIFE: QUALITATIVE STEPS

Bettiol E[1], Marant C[2], Burlina A[3], Cunningham A[4], Gasteyger C[1], Benmedjahed K[2], Abetz L[5], Champigneulle A[1]
[1]*Merck Serono SA, Geneva, Switzerland*
[2]*Mapi Values, Lyon, France*
[3]*Div Metab Dis, Dpt Pediatrics, Univ Hosp, Padova, Italy*
[4]*Hayward Genetics Center, Tulane Univ Med, New Orleans, United States*
[5]*Mapi Values, Bollington, United Kingdom*

Background: Published studies evaluating impact of phenylketonuria (PKU) on the lives of patients and parents report inconsistent results. Our objective was to develop self-reported questionnaires with PKU-specific measures to evaluate impact of PKU on quality of life for children, adolescents, adults, and parents.
Methods: Semi-structured interviews with European PKU patients (N=29), parents of children with PKU (N=19), and clinicians (N=30) were conducted to explore the impact of living with PKU and its treatment. Thematic analysis yielded four conceptual models, which were finalized with an international PKU expert committee, and provided a foundation for development of child, adolescent, adult and parent versions of a PKU-specific impact questionnaire.
Results: Participants reported that the stringent low-phenylalanine diet and protein supplements often led to frustration, inconvenience, and non-adherence. Data from patients, parents, clinicians and expert opinion suggested these relevant domains: physical status, cognitive and emotional symptoms associated with diet/supplement non-adherence; emotional impact of the disease; impact of diet/ supplement on daily emotional, social, and family life; diet/supplement adherence levels; satisfaction with medication.
Based on this data, items measuring these domains were developed simultaneously in 6 languages for children (44 items), adolescents (52 items), and adults (55 items) with PKU, and their parents (45 items).
Conclusion: Qualitative research identified quality of life domains potentially impacted by PKU, which led to development of PKU-specific impact measures for which the face/content validity is currently being tested.

322-P

BODY MASS INDEX IN ADULT PATIENTS WITH DIET TREATED PHENYLKETONURIA

McStravick N[1], Robertson LV[2], Ripley S[3], Weetch E[4], Donald S[5], Adams S[6], Micciche A[7]
[1]Royal Group Hosp, Belfast, United Kingdom
[2]Univ Hosp Birmingham, Birmingham, United Kingdom
[3]Salford Royal NHS Foundation Trust, Manchester, United Kingdom
[4]Northern General Hosp, Sheffield, United Kingdom
[5]Cambridge Univ Hosp, Cambridge, United Kingdom
[6]Royal Hosp for Sick Children, Glasgow, United Kingdom
[7]Guys and St Thomas' NHS Trust, London, United Kingdom

Background: The number of patients with treated metabolic conditions reaching adulthood is increasing. This study aimed to identify Body Mass Index (BMI) and metabolic control in adult patients with diet treated Phenylketonuria (PKU).
Methods: Adult patient's, aged 16 years and over, with diet treated PKU was included. Those planning pregnancy or pregnant were excluded. Information collated included; average phenylalanine levels within the last twelve months; Body Mass Index (BMI); and gender. Seven adult metabolic centers in the UK participated.
Results: Two hundred and fifty two patients were included. Average phenylalanine concentration was 801 μmol/l; 44.5% achieved the recommended UK phenylalanine level (< 700 μmol/l). Average BMI was 26.1 kg/m2; 55% had BMI > 25 kg/m2; 25.8% had BMI > 30 kg/m2. Results indicated that males were more likely to be overweight compared to females (41.6% and 23% respectively), and females more likely to be obese (32.1% compared to 17.9%). The female PKU cohort had higher obesity rates compared to non-PKU peers (32.1% and 24% respectively). Results also indicated a direct correlation between average BMI and average phenylalanine concentrations.
Conclusion: The BMI patterns of diet treated PKU patients are similar to the general population with 55% having a BMI > 25 kg/m2. Of particular concern is the increased obesity risk in the female PKU cohort. Increased BMI, and the development of associated co-morbidities, will make dietary management more complex.

323-P

EFFECTS OF INTRACEREBROVENTRICULAR INJECTION OF PHENYLALANINE METABOLITES ON OXIDATIVE STRESS PARAMETERS IN RAT

Moraes TB[1], Moresco M[2], Rodrigues MV[2], Mazzola PN[1], Dutra AM[2], Wajner M[1], Dutra-Filho CS[1]
[1]Department of Biochemistry-UFRGS, Porto Alegre, Brazil
[2]Dep Basic Health Sciences-UFCSPA, Porto Alegre, Brazil

Background: Phenylketonuria (PKU) is an inherited metabolic disease caused by deficiency of the enzyme phenylalanine hydroxylase, leading to accumulation of phenylalanine and its metabolites phenylpyruvate (PP), phenylacetate (PA) and phenylactate (PL). Clinical features of PKU patients include severe mental retardation, microcephaly, and seizures, but the mechanisms of brain damage in this disease remain not completely understood.
Objectives: The aim of this study was to verify oxidative stress parameters in the brain after intracerebroventricular (icv) injection of the metabolites of phenylalanine.
Methods: PP, PA and PL were injected into lateral ventricle to 30-day-old rats. After 15 minutes, animals were killed, cerebral cortex was isolated and homogenated for measuring thiobarbituric acid reactive substances (TBA-RS), DNA damage and the activities of catalase (CAT) and glucose-6-phosphate dehydrogenase (G6PD).
Results: The icv administration of PL and PA reduced CAT activity and increased TBA-RS and G6PD activity, whereas PP did not alter any of these parameters. However, DNA damage was stimulated only by PP icv injection.
Conclusions: We and other investigators have been demonstrated that increased levels of phenylalanine can enhance oxidative stress in the brain. The present results suggest that the phenylalanine metabolites PP, PL, and PA may also be involved in this pathophysiological mechanism. (CNPq, CAPES, IBNnet, FAPERGS, and PROPESQ/UFRGS)

324-P

REDUCED BRAIN LARGE NEUTRAL AMINO ACID CONCENTRATIONS IN C57BL/6 PKU MICE

de Groot MJ[1], van der Zee EA[2], Struik D[2], Thony B[3], Reijngoud D-J[1], van Spronsen FJ[1]
[1]Beatrix Child Hosp, Univ Med Cent Gron, Groningen, Netherlands
[2]Dept Mol Neurobiol, Univ of Groningen, Groningen, Netherlands
[3]Dept of Pediatrics, Univ of Zurich, Zurich, Switzerland

Background: In phenylketonuria (PKU), elevated plasma phenylalanine (Phe) concentrations are considered to disrupt transport of non-Phe large neutral amino acids (LNAA) across the blood-brain-barrier, leading to elevated brain Phe concentrations and reduced brain non-Phe LNAA concentrations. The latter may impair cerebral protein and neurotransmitter synthesis, possibly contributing to the cognitive dysfunction observed in PKU. Aim to investigate whether elevated plasma Phe concentrations are associated with reduced brain non-Phe LNAA concentrations.
Materials and Methods: Untreated C57Bl/6 PKU mice were compared to healthy littermates. Mice were sacrificed after overnight food deprivation. Brains were freeze-clamped in liquid nitrogen. Amino acids were measured in plasma and brain homogenates by HPLC.
Results: Plasma Phe concentrations were significantly elevated in PKU mice (PKU 1573±126 μmol/L, n=7; controls 91±18 μmol/L, n=7; $p < 0.001$). Brain Phe concentrations were significantly elevated in PKU mice (PKU 567±54 nmol/g, controls 104±30 nmol/g; $p < 0.001$). Brain non-Phe LNAA concentrations were reduced by 15–50% (varying per LNAA) in PKU mice compared to controls. These reductions were statistically significant for threonine, valine, leucine, and tyrosine ($p < 0.05$). Unexpectedly, plasma Phe concentrations did not correlate to brain non-Phe LNAA concentrations.
Conclusion: Elevated plasma Phe concentrations were associated with (although not correlated to) reduced brain non-Phe LNAA concentrations in untreated C57Bl/6 PKU mice. Reduced brain non-Phe LNAA concentrations may reduce cerebral protein and neurotransmitter synthesis. Future studies should investigate the consequences of reduced brain non-Phe LNAA concentrations on cognitive function in PKU.

325-P

NEOPTERIN EXCRETION IN URINE AS POSSIBLE PERIPHERAL MARKER OF SEGAWA DISEASE

Leuzzi V[1], Carducci Cl[2], Giovanniello T[2], D'Agostino Costa C[1], D'Agnano D[1], Kolamunnage T[2], Antonozzi I[2], Carducci Ca[2]
[1]Dept Child Neurol and Psych, La Sapienza, Roma, Italy
[2]Dept Experimental Medicine, La Sapienza, Roma, Italy

Background: The diagnosis of autosomal dominant Dopa-responsive-dystonia (DYT-5) relies on GCH1 gene analysis, which detects alterations in about 75% of patients, and the dosage of Neopterin in CSF. The urine excretion of pterins is usually considered not affected by the disease. We report on a new DYT-5 family, in which the diagnosis could be addressed by the Neopterin reduction in the urine.
Case Report: This 12 year-old girl presented at the age of 9 with gait fatigability and incertitude emerging in the evening. The mother and the 8-year-old brother were asymptomatic. Her 35-year-old father had been complaining since the adolescence of lower limbs rigidity and gait difficulty worsening in the evening. On examination, at the age of 11, the girl showed generalized choreoathetosis and her father a very mild foot dystonia. Neopterin (normal range 0.3–4.0 mmol/mol creatinine) was low in urine from the proband (0.27), the father (0.23), and the brother (0.22). The biogenic amines were normal in the girl's CSF, while Neopterin was reduced (1.96 μg/L; v.n. 2.3–5.1). GCH1 gene analysis detected a frameshift mutation in exon 6 (c.631–632 del AT, p. H211fs) in the proband and her father; surprisingly, the mother and the brother turned out to be carrier of K224R (c.671 A>G) mutation on exon 6, which had already been associated with the disease.
Conclusions: The decrease of Neopterin in urine can be a marker of AD GTP-CH deficiency; 5-HIAA and HVA in CSF do not always reflect the severity of the clinical involvement in this condition.

326-P

RENAL AGENESIS IN ASSOCIATION WITH MATERNAL PKU SYNDROME

Kilic M[1], Sivri HS[1], Tokatli A[1], Dursun A[1], Coskun T[1]
[1]*Pediatr Metab Dis, Hacettepe University, Ankara, Turkey*

Maternal Phenylketonuria (PKU) Syndrome results in multiple congenital anomalies in the offspring, usually consisting of microcephaly, mental retardation, congenital heart disease, intrauterine growth retardation and dysmorphic facial features. A-10 month-old boy was admitted to the hospital with respiratory difficulty and cough. He was the second pregnancy of nonconsanguineous parents, born at 32 weeks of gestation with a birth weight of 2080 grams at home. The first pregnancy resulted in miscarriage at 12 weeks of gestation. Delivery was normal but respiratory difficulty was seen in the first day with cyanosis. Microcephaly and congenital heart disease were diagnosed and ventilatory support and antibiotics were given in neonatal care unit. The mother's phenylalanine level was found as 1920 μmol/l (N < 120 μmol/l). It was learned that she had PKU diagnosis and treated with low phenylalanine diet up to 10 years of age. She did not continue her diet and lost follow up. Due to Maternal PKU syndrome of her son; failure to thrive, developmental delay, microcephaly, right inguinal hernia, recurrent pulmonary infection, congenital heart disease (VSD, PDA, aort coarctation, severe pulmonary hypertension, aortic valve insufficiency, mild arcus aorta hypoplasia, mitral valve anomaly, and stenosis) were detected while renal ultrasonography showed the absence of left kidney. To the best of our knowledge this is the first case of maternal PKU syndrome presented with renal agenesis. This suggests that high phenylalanine levels of mother cause severe defects during organogenesis and needs careful examination of affected cases.

327-P

EXON DELETIONS IN PAH GENE IN ITALIAN HYPERPHENYLALANINEMIC PATIENTS

Carducci C[1], Cali' F[2], Pozzessere S[1], Artiola C[1], Chiavetta V[2], Ruggeri G[2], Ragalmuto A[2], Vinci M[2], Leuzzi V[3], Meli C[4], Antonozzi I[1], Romano V[2]
[1]*Dip Med Sper. Univ La Sapienza, Roma, Italy*
[2]*Lab Gen Mol, Oasi Maria SS (I.R.C.C.S.), Troina (Enna), Italy*
[3]*Dip S Neur Psic Età Evol Uni La Sapienza, Rome, Italy*
[4]*Centro Mal Metab Cong Policl Univ, Catania, Italy*

The hyperphenylalaninemias are a group of autosomal recessive diseases caused in more than 98% of cases by mutations in the phenylalanine hydroxylase (PAH). To date, were described more than 500 mutations causing disease (http://www.pahdb.mcgill.ca/). Although in all studies the molecular detection rate of mutations is generally very high, about 5–10% of alleles remain undetermined. Recent studies have shown that some of these alleles are carriers of deletions of one or more exons.

These alterations cannot be detected by molecular methods based on PCR analysis that are normally used, but are detectable by using a specific technique "Multiple ligation-dependent probe amplification" (MLPA) in all 13 exons of the gene simultaneously. In the present study were sought deletions/duplications of one or more exons in patients negative for 1 or both alleles followed in two Italian centers: Rome "La Sapienza" and the "Hospital of Catania".

Of the 802 alleles analyzed, 43 were negative for mutation. These 43 DNA were analyzed with MLPA and 13 resulted positive: one for duplication and 12 with 4 different deletions, 2 of them described for the first time. All the DNA positive to MLPA analysis were submitted to two different confirmatory tests: multiplex Dosage Comparative Analysis (CMDA) and Real-TimePCR. All alterations were confirmed except two.

Our study shows that in the Italian population 1.7% of PAH gene mutations is due to exon deletion/duplication, and that use MLPA increases the detection rate of mutations, but could cause false positives and should be confirmed using another method.

328-O

EFFICACY AND SAFETY OF TREATMENT WITH BH4 BEFORE THE AGE OF 4 YEARS IN PATIENTS WITH MILD PHENYLKETONURIA

Leuret O[1], Kuster A[2], Barth M[3], Eyer D[4], De Parscau L[5], Odent S[6], Gilbert-Dussardier B[7], Feillet F[8], Labarthe F[1]
[1]*Médecine Pédiatrique & INSERM U921, CHU Tours, France*
[2]*Réanimation Pédiatrique, CHU Nantes, France*
[3]*Génétique, CHU Angers, France*
[4]*Pédiatrie, CHU Strasbourg, France*
[5]*Pédiatrie et Génétique Médicale, CHU Brest, France*
[6]*Génétique, CHU Rennes, France*
[7]*Génétique, CHU Poitiers, France*
[8]*Centre de réf. maladies métaboliques, CHU Nancy, France*

Background: Sapropterin dihydrochloride, an EMEA-approved synthetic formulation of BH4, is available in France since 2009 for PKU-patients older than 4-years. We report 13 patients treated before the age of 4-years and demonstrate the safety and efficacy of this treatment.

Methods: PKU-patients treated with BH4 before the age of 4-years were screened in West and East regions of France.

Results: Thirteen patients (7 females) were enrolled in this retrospective study. Mean phenylalaninemia at diagnosis was 552±175 μM. A positive response to BH4 was assessed by (i)a 24 h-BH4 loading test (20 mg/kg/d), performed during the neonatal period (n=9) or before 1-year of age (n=4) and inducing a 78±13%-decrease of phenylalaninemia, (ii)and genotyping. Long-term therapy with BH4 was initiated during the neonatal period (n=5) or at the age of 13±13 months (n=8), with BH4 (Schircks., n=5) or Kuvan. (Merck-Serono., n=8). All patients are actually treated with Kuvan.. The mean duration of treatment was 27±25 months. BH4-therapy drastically improved diet phenylalanine tolerance (from 465±194 to 1525±621 mg/day, $p<0.0001$) and allowed to stop (or not start) phenylalanine-free amino acid supplementation in 11 patients. Additionally, in the 8 patients treated after few months of diet therapy, BH4 treatment improved metabolic control, significantly decreasing phenylalaninemia (331±76 to 243±75 μM, $p<0.05$) and increasing percentage of phenylalaninemia tests into therapeutic targets (120–300 μM, 67±17% with BH4 vs 37±21% before BH4, $p<0.05$). Finally, no side effects were reported.

Conclusion: BH4-therapy improved phenylalanine tolerance and metabolic control with no side effects in BH4-responder PKU-patients before the age of 4-years.

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PHENYLKETONURIA MANAGEMENT: TUNISIAN EXPERIENCE

Azzouz H[1], Ben Harrath M[1], Ben Turkia H[1], Ben Chéhida A[1], Ben Abdelaziz R[1], Bennour I[1], Khmir S[2], Tebib N[1], Kaabachi N[2], Abdelmoula Ms[1], Ben Dridi Mf[1]
[1]*Dep Pediatr and Metab Dis, Rabta Hosp, Tunis, Tunisia*
[2]*Biochemestry labo, Rabta Hosp, Tunis, Tunisia*

Background: Phenylketonuria is an autosomal recessive inborn error of phenylalanine metabolism resulting from deficiency of phenylalanine hydroxylase. It may be frequent in Tunisia because of the high rate of consanguinity. In the absence of an early management most children develop profound mental retardation.
Purpose: Study the epidemiological, clinical, paraclinical and evolution under phenylalanine restricted diet in patients with phenylketonuria.
Patients and Methods: It's a retrospective study on children with phenylketonuria who received dietary management from January 1998 to June 2009, enrolled in department of Pediatrics and metabolic disease in the Rabta hospital.
Results: We collected 39 patients with phenylketonuria, issued from 33 families. Parental consanguinity was found in 69% of cases. Nine patients were diagnosed by a screening of index cases with a median age of 20 days. Thirty patients were diagnosed on clinical signs at an average age of 29.7 months.
Physical examination was normal in all patients screened. Abnormalities were found on neurological examination in 60% of patients diagnosed on clinical signs with motor delay (20%), mental retardation (93.6%), seizures (33%), autism (50%) and restlessness (70%).
For patients screened, the evolution under controlled diet in phenylalanine was generally positive except for one patient. For patients diagnosed on clinical signs, prognosis was mainly conditioned by the age of onset of the diet but also by the initial intelligence quotient, the biological balance and a multidisciplinary management.
Conclusion: The diet controlled in phenylalanine allows normal intellectual development for patients treated early. However, it can be beneficial even for patients lately diagnosed.

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TETRAHYDROBIOPTERIN RESPONSIVENESS IN A PHENYLKETONURIC SPANISH COHORT

Serrano J[1], Blasco Alonso J[1], Navas VM[1], Gonzalo M[2], Yahyaoui R[3], Rueda I[3], Carazo B[1], Sierra C[1]
[1]*Ped Gastroenterol, Hosp Materno-Infantil, Málaga, Spain*
[2]*Endoc and Nutrition. Carlos Haya Hosp, Málaga, Spain*
[3]*Clinical Laboratory, Carlos Haya Hosp, Málaga, Spain*

Introduction: A loading test with BH4/sapropterin must be performed in order to evaluate the response profile and justify long-term treatment.
Material and Methods: Prospective intervention study to evaluate efficacy of the sapropterin/BH4 loading test in PKU patients during the 2006–2010 period. Inclusion criteria: PAH-deficient patients with usual Phe levels <360 μmol/l under Phe-restricted diet, signed informed consent, no acute disease during test. Loading test performed under a 3 days diet with neither natural protein restrictions nor Phe-free protein substitutes. Single 20 mg/kg sapropterin/BH4 dose at the third day. Urine and plasma neopterin/biopterin levels were determined in order to assess BH4 absorption. We defined as responders those whose Phe levels decreased at least a 30% 6–8 hours after BH4 dose.
Results: Eighteen cases included: 5 mild, 12 moderate and one severe PKU. Mean age was 12.0±9.5 years; mean Phe levels previous to test were 506.61±359.35 μmol/l and maximum Phe levels reached during test were 1412.58±410.26 μmol/l. Baseline Phe levels were 1206.54±383.59 μmol/l and mean Phe decrease 8 h post-BH4 was 23.35±15.65%. Maximum decrease was seen 9 h after BH4 (median of 6 h). Two patients were complete responders (one of them moderate PKU) and are nowadays under non-Phe-restricted diet. Three patients were early responders (4–6 h), now on sapropterin treatment, tolerating double amount of Phe.
Conclusion: Maximum Phe level at neonatal diagnosis seems to be a simple and reliable factor in order to predict BH4 response. In complete responders, continued treatment allows withdrawal of Phe-restricted diet in a high percentage of cases.

331-P

EVALUTION OF 42 PATIENTS WITH HYPERPHENYLALANINEMIA CAUSED BY A DEFECT IN TETRAHYDROBIOPTERIN METABOLISM

Kizilelma A[1], Tokatli A[1], Kalkanoglu-Sivri HS[2], Dursun A[1], Aydin HI[1], Blau N[2], Coskun T[1]
[1]*Div Metab Dis, Hacettepe Univ, Ankara, Turkey*
[2]*Div of Clinic Biochem, Univ Child Hosp, Zurich, Switzerland*

In this study, 42 out of 2009 patients with hyperphenylalaninemia with a defect in tetrahydrobiopterin (BH4) metabolism were evaluated dates between March 1980–March 2009. 71.4% (30/42) of the patients had DHPR deficiency, 19% (8/42) PTPS and 9.5% (4/42) GTP-CH deficiency. 57.1% (24/42) of the patients were female and 42.86% (18/42) were male. 40.47% (17/42) of patients were diagnosed via National screening program for phenylketonuria (PKU), 52.38% (22/42) of the patients based on their clinical findings and remaining 3/42 with sibling history. The mean age of the diagnosis was 28.29 months (0.3–224 months). The diseases were diagnosed significantly earlier in a group of patients who had been screened for BH4 metabolism defect when HPA was detected. The mean blood phenylalanine was 13.63 mg/dl at the time of diagnosis. 39/42 patients were products of consanguineous marriages while 68.4% of them were first cousins marriages and only 7.9% were not relatives. The most common clinical findings were growth retardation, developmental delay, mental and motor retardation as well as seizures.
As a result, we emphasize that all patients with HPA should be screened for BH4 metabolism defects. All parents should be informed about the seriousness of the illness and genetic counseling must be given. Consanguineous marriages should be prevented to decrease social, health and economical costs for both affected families and for the whole society.

332-P

A NEW INFANT PKU PROTEIN SUBSTITUTE WITH PREBIOTICS: IMPACT ON GASTRO-INTESTINAL MICROFLORA

MacDonald Dr A[1]
[1]*Metabolic Unit, Children's Uni Hospital, Birmingham, United Kingdom*

Background: Prebiotic oligosaccharides, an important component of breast milk, stimulate the growth of potentially health-promoting microflora (e.g. Bifidobacteria). They are now added to an infant phenylalanine-free protein substitute (PS) for PKU.
Aim: To study the effect of infant PS (PKU Anamix Infant; Nutricia) containing prebiotics on gastrointestinal microflora.
Methods: A 9-week pilot intervention study in PKU infants on dietary treatment. Infants were prescribed PS with added prebiotics for 8 weeks, and were evaluated at weeks –1, 0, 4 and 8. Stools samples, records of stool description and 3-day formula intake were collected.
Results: Nine infants (entry median age: 7.9 weeks; range 7.1–19.4) were enrolled. At study entry, in addition to PS, infants received the following phenylalanine-containing milk: breast milk (n=3); breast milk plus standard infant formula containing prebiotics (n=3), standard infant formula containing prebiotics (n=2), infant formula without prebiotics (n=1). Overall, there was no significant difference between baseline and study-end Bifidobacteria concentrations, with levels comparable to breast-fed infants. Gastrointestinal Bifidobacteria levels increased in infants with low baseline concentrations (patient 3 [breast fed] at study entry=6.6%; week 4=49.6%; week 8=35.6%; patient 8 [non-prebiotic standard infant formula] at study entry=3.6%; week 4=62.7%; week 8=58.4%). Significant reductions in stool mean pH were shown at week 4 compared with baseline (baseline mean=6.8 [range=6–7.8 p=0.05] vs week 4 mean=5.8 [range=4.7–7.6; p=0.05]), although this lowered by week 8 [mean=6.61, range=5.2–7.8]).
Conclusion: Gastrointestinal Bifidobacteria and stool pH concentrations in infants taking PS with prebiotics are similar to those reported for breast-fed infants.

333-P

EXPLORING THE PSYCHOLOGICAL PROFILE OF ADOLESCENTS WITH PKU

Mason B[1], Brown G[2], Abulhoul L[1], Bond K[1]
[1]*Metabolic Medicine, Great Ormond St Hosp, London, United Kingdom*
[2]*Royal Holloway, Uni of London, London, United Kingdom*

Background: Children with chronic health conditions are at greater risk of adjustment problems, internalising and externalising symptoms and low self concept (Lavigne & Faier Toutman, 1992). Research suggests that children/adolescents with PKU present with an increased risk of developing both psychological and social difficulties (Smith and Knowles, 2000; Weglage et al, 2009). However research to date has not utilized self-report measures of psychological wellbeing, or investigated the effects of 'feeling different', a common concern.
Method: 102 patients aged 11 to 16 years took part: 32 patients with PKU and 34 patients with a visible difference and 36 participants from a school control group. Well validated self report questionnaires were completed measuring anxiety, depression, self esteem, overall stress, and positive and negative effect. In addition a self-report questionnaire was used to measure feelings of general, external and internal shame, to help explore one aspect of how the two paediatric groups felt, and were affected by, their different medical conditions.
Results: Adolescents with PKU did not significantly differ from the medical or the school control groups on measures of psychological distress or shame, though some scores were elevated compared to population norms.
Conclusions: Adolescents with PKU attending Great Ormond Street Hospital were well adjusted with no greater psychological difficulties than their peers. Future research is required to explore the impact PKU has upon daily living, the effects of feeling different, and the mediating factors that determine adjustment and coping during childhood and adolescence.

334-P

DHA SUPLEMENTATION IN PKU- EVALUATION OF RESPONSE

Campistol J[1], Gutiérrez A[1], Vilaseca MA[1], Capdevila A[1], Vidal M[1], Alonso I[1], Lopez A[1], Colomer R[1], Artuch R[1]
[1]*Metabolic unit Hospital Sant Joan de Deu, Barcelona, Spain*

Background: Phenylketonuria is an autosomal recessive metabolic disease caused by a deficiency of phenylalanine hydroxylase. The dietary therapy for the effective management of PKU, in particular the restriction of high-protein animal-origin foods, compromise patients' intakes of fat and distort the ratio of n-3: n-6 essential fatty acids in the diet. PUFAS deficit can produce neurological and visual impairments.
Objectives: The aim of our study was to evaluate the effect of DHA supplementation on WM alterations, VEP latencies and neuropsychological profiles in a group of early and continuously treated PKU patients.
Material and Methods: A 21 PKU patients with early diagnosis (mean age 9–25 years old). All of them were on a Phe-restricted diet and supplemented with different PKU formulas Inclusion criteria were: a) low DHA concentrations in erythrocyte membrane phospholipids b) prolonged latencies of P100 wave in visual evoked potentials and/or presence of white matter hyperintensities on brain MRI (T2, FLAIR o DWI sequences). All patients were treated with DHA for a period of 12 months. At baseline and at 12 months of treatment, assessment was conducted include biochemical parameters; brain MRI, VEP, ophthalmologic evaluation and neuropsychological tests.
Results & Comments: All the patients concluded study with normalization of the DHA levels after suplementation. No significative improvement in the WM abnormalities, neuropsychological test and visual contrast tests were evident one year later. Moreover some improvement in the VEP (P-100 wave latencies) was noticeable. Our results don't support that the supplementation with DHA at these age, will improve neurological abnormalities.

335-P

COMPLIANCE WITH TREATMENT OF PATIENTS WITH PHENYLKETONURIA TREATED AT THE OUTPATIENTS CLINICS OF MEDICAL GENETICS SERVICE AT HOSPITAL DE CLINICAS DE PORTO ALEGRE, BRAZIL

Nalin T[1], Schweigert ID[2], Giugliani L[3], Vieira TA[3], Burin M[3], Guidobono R[3], Refosco L[3], Netto CB[3], Souza CFM[3], Schwartz IVD[3]
[1]*Pos Prog Med: Med Sciences, UFRGS, Porto Alegre, Brazil*
[2]*Depart Internal Med, UFRGS, Porto Alegre, Brazil*
[3]*Med Genetics Serv, HCPA, Porto Alegre, Brazil*

Background: Phenylketonuria is an inborn error of metabolism in which there is an increase in serum amino acid phenylalanine.
Objective: Assess compliance to treatment for patients with Phenylketonuria by deficiency of enzyme phenylalanine hydroxylase.
Material and methods: Cross-sectional study of patients with Phenylketonuria treated at the outpatients clinics. The parameters of compliance were considered the consumption of phenylalanine (criterion 1) and metabolic formula (criterion 2), direct questioning to patients/relatives (criterion 3) and median of the plasmatic phenylalanine in the last year (criterion 4).
Results: From the 45 patients studied, with a median age of 11 years, 51% are male. According to the criterion utilized, were considered compliance 16 (criterion 1); 27 (criterion 2); 33 (criterion 3) and 20 (criterion 4) patients, respectively. There was no correlation among the compliance criteria used. Differences were found when compared criteria 1 and 2 ($p = 0.027$), criteria 1 and 3 ($p = 0.002$) and criteria 3 and 4 ($p = 0.015$).
Conclusion: Compliance to treatment is hardly quantified by isolated parameters. The distinct perception of compliance to the treatment by patients, in relation to various criteria, supports the need for searching new strategies to promote compliance and studying methods to evaluate it.

336-P

CHARACTERIZATION OF THE HPA PATIENT COHORT OF ZURICH 1997–2006 IN VIEW OF FUTURE THERAPEUTIC USE OF SAPROPTERIN

Zimmermann M[1], Jacobs P[2], Fingerhut R[3], Torresani T[3], Baumgartner MR[1], Rohrbach M[1]
[1]*Div Metab Dis, Univ Child Hosp, Zurich, Switzerland*
[2]*Div Gastro & Nutrition, Univ Child Hosp, Zurich, Switzerland*
[3]*Swiss Neonatal Screening Laboratory, Zurich, Switzerland*

Hyperphenylalaninaemia (HPA) is an inborn error of metabolism caused by mutation of the phenylalanine hydoxylase (PAH) gene. Treatment is mainly limited to lifelong Phe-restricted diet; however some HPA individuals respond to oral, pharmacologic BH4-therapy (sapropterin dihydrochloride, Kuvan.), now available in the Unites States and Europe, which might partially replace the Phe-restricted diet.
To gain better insight into our HPA cohort all patients diagnosed from 1997 to 2006 were reviewed retrospectively for epidemiological data, age at diagnosis, PAH mutations, and BH4 responsiveness.
80 patients were entered into an anonymised registry: 41 (51.25%) had classical, 16 (20%) moderate, 10 (12.5%) mild PKU and 13 (16.25%) mild HPA. 50 patients (62.5%) had 48 hour BH4 loading test in the neonatal period; 2 patients with classical, 5 with moderate, and 2 with mild PKU showed a reduction of Phe levels >30%. Molecular testing in 32 patients (40%) revealed that all patients carrying mutations associated with low PAH residual activity were not responsive to BH4 whereas only 45.5% of patients with genotypes associated with high PAH residual activity were responsive to BH4. p.R261Q was the most frequent mutation found in 13/60 alleles.
Our results suggest that genotyping does not always correlate with neonatal BH4 loading. In order to better evaluate those patients that might benefit from sapropterin, retesting with an extended BH4 loading test will be necessary.

337-P

FROM HYPERPHENYLALANINEMIA TO MENTAL RETARDATION: THE KEY ROLE OF SEROTONIN

Pascucci T[1], Andolina D[1], Pittalà A[2], Meli C[2]
[1]S.Lucia European Centre Brain Research, Rome, Italy
[2]Metab Unit Policlinico University, Catania, Italy

Treatment of phenylketonuria (PKU) is an early restricted diet that requires exclusion of several natural foods. Consequently, compliance is often extremely difficult and many adolescents with phenylketonuria (PKU) do not adhere strictly to the diet with several neuropsychological consequences. Elevated blood phenylalanine (PHE) levels, known as hyperphenylalaninemia (HPA), promote cognitive deficits involving mainly the prefrontal cortex (pFC) and frontal/executive skills. The pressure created by these difficulties has renewed the interest in identifying alternative approaches, so requiring understanding of mechanisms involved in PHE-dependent cognitive deficits.

Data obtained in ENU2 mice, the genetic model of PKU, showed that cognitive deficits mainly depend on the PHE interference with serotonin (5-HT) synthesis by inhibition of brain tryptophan hydroxylase activity, and a correlation between PHE-induced interference on cerebral and peripheral 5-HT metabolism has been observed. Clinical data also showed that discontinuation of PHE-restricted diet determines a dramatic decrease the 5-HT metabolite, in the CSF of hyperphenylalaninemic adolescents.

Because of difficulty to investigate human cerebral serotonin metabolism, objective of our study was evaluation of 5-HT peripheral metabolism in HPA patients. Analysis of blood samples of HPA patients, although in a preliminary phase, suggested an interference PHE-dependent on 5-HT peripheral metabolism in HPA patients.

Since data from ENU2 mice showed ability to improve cognitive performance in PKU mice administering, during critical period of postnatal development, 5-hydroxytryptophan (5-HTP), the product of tryptophan hydroxylase that easily increase brain 5-HT levels, we retain that restoration of physiological 5-HT levels could become a new target for treatment of HPA patients.

338-P

NEUROPROTECTIVE EFFECT OF LIPOIC ACID AGAINST OXIDATIVE STRESS IN A MODEL OF HYPERPHENYLALANINEMIA IN RATS

Moraes TB[1], Coelho JG[2], Rosa AP[1], Dalazen GR[2], Mazzola PN[1], Wajner M[2], Dutra-Filho CS[2]
[1]PPG-Bioq, UFRGS, Porto Alegre, Brazil
[2]Dep Biochem, UFRGS, Porto Alegre, Brazil

Background: Hyperphenylalaninemia (HPA) is a pathological condition caused by a severe deficiency of phenylalanine hydroxilase (PAH), which converts Phe to tyrosine, accumulating phenylalanine (Phe) in biologic fluids. Main clinical features are psycomotor developmental retardation and intellectual impairment. Recent studies have shown the involvement of oxidative stress in the pathophysiology of the disease. Lipoic acid (LA) is a potent antioxidant that has been proposed as a good alternative to treat neurodegenerative disorders.

Objectives: The aim of this study was to evaluate the antioxidant effect of LA treatment in rats subjected to a chemically-induced HPA.

Material and Methods: Six-day-old Wistar rats received daily injections of LA (40 mg/Kg), α-methyl-phenylalanine (1,6 μmol/g), a PAH inhibitor, and Phe (2,1 μmol/g) for 8 days. Controls received saline instead. Twelve hours after last injection, animals were killed. The brain was removed and homogenated to measure the oxidative stress parameters (TBARS, protein carbonyl, DNA-PC, catalase, glutathione peroxidase and superoxide dismutase).

Results: It can be seen that lipoperoxidation, protein oxidation and DNA damage were increased along with altered antioxidant enzymatic defenses in hyperphenylalaninemic rats. On the other hand, none of these effects were observed when hyperphenylalaninemic rats received LA treatment.

Conclusion/Discussion: The present study demonstrated that LA can act as an efficient antioxidant to prevent the effects of oxidative stress on SNC caused by hyperphenylalaninemia in rats. If these results were observed in patients, it is possible that dietary supplementation of lipoic acid may be a therapeutic approach additionally to the usual treatment of PKU. (CNPq, CAPES, IBNnet, FAPERGS, and PROPESQ/UFRGS)

339-P

BRAIN FUNCTION IN INDIVIDUALS WITH PKU TREATED WITH KUVAN: EVIDENCE FROM FUNCTIONAL MAGNETIC RESONANCE IMAGING

Christ SE[1], Peck D[2], Moffitt A[1], Hillman R[2]
[1]Dept Psych Sci, U Missouri, Columbia, United States
[2]Dept Child Health, U Missouri Med School, Columbia, United States

Background: Phenylketonuria (PKU) is a genetic disorder characterized by inefficient metabolism of phenylalanine. Early and continuous dietary control prevents the severe neurologic and cognitive consequences once associated with PKU. Kuvan (sapropterin dihydrodichloride, BH4) represents a new supplemental pharmacologic treatment for PKU. In the present study, the researchers utilized functional MRI to examine neurocognitive functioning in individuals with and without PKU. The potential impact of Kuvan treatment on neural activity in PKU was also explored.

Methods: Brain imaging data was collected from 7 individuals with early-treated PKU (mean age = 21.9 years) immediately before treatment with Kuvan and then again after 4 weeks of Kuvan treatment. For comparison purposes, data was also collected from 5 non-PKU individuals (mean age = 20.0 years). At each timepoint, neural activity was recorded during performance of a working memory task.

Results: Analysis of the pre-treatment data revealed PKU-related irregularities in neural activation in prefrontal cortex (PFC) and other brain regions, $F(1, 10) > 5.53$, $p < .05$ FDR-corrected. At the 4-week evaluation, two participants had responded to Kuvan with a >20% reduction in phenylalanine levels. Both also showed improved activation for a region in orbitomedial PFC. Findings for other brain regions were mixed.

Conclusion: The present results provide evidence of brain dysfunction in individuals with early-treated PKU. Whereas the initial findings on Kuvan treatment are promising, additional data is needed to fully evaluate its benefits for brain function in PKU.

340-P

BH4-RESPONSIVENESS IN WIELKOPOLSKA REGION POLISH PKU PATIENTS

Kaluzny L[1], Bik-Multanowski M[2], Erenz-Surowy B[3], Siwinska-Mrozek Z[1], Cichy W[1]
[1]Dept of Gastroent and Metab, Med Univ, Poznan, Poland
[2]Chair of Pediatrics, Jagiellonian Univ, Krakow, Poland
[3]Screening Laboratory, Poznan, Poland

Phenylketonuria (PKU, OMIM 261600) is the most frequent inborn error of amino acid metabolism, caused by mutations in the phenylalanine hydroxylase (PAH) gene. Tetrahydrobiopterin (BH4) responsive PKU is a variant of PAH deficiency. Based on allele frequencies 18 to 95% patients from European countries is potentially responsive to BH4 (Poland—26%). The aim of the study was the evaluation of BH4-responsivness in Wielkopolska region PKU children. Diagnosis of BH4-responsiveness was performed by the newborn screening for PKU, followed by a BH4 loading test (20 mg/kg, 46 patients, F=27, M=19, all patients born since 2001). For children born before 2001 we used combined loading test with phenylalanine (100 mg/kg) and BH4 (20 mg/kg–22 children F=15, M=7). We defined BH4 responsiveness as more then 30% phenylalanine concentration reduction within 24 hours. Analysis of mutations was performed.

Results: The overall frequency of BH4 responsiveness was 19% (13 out of 67 patients). Frequency among children with classical phenylketonuria was 7% (4 out of 56), with mild phenyloketonuria 82% (9 out of 11). In newborn screening procedures we found one child with 6-PTPS deficiency. The most common BH4 responsive mutations were, like as in other publications, A403V and IVS10nt-11 g>a. In most cases non-responders genotype was R408W/R408W.

The result of our study suggest that incidence of BH4 responsiveness in children with PKU from Wielkopolska region of Poland is low. It can be caused by high frequency of non-responsive mutations.

341-P

SCREENING FOR BH4–RESPONSIVENESS IN PKU: RESULTS WITH A QUANTITATIVE METHOD

Garelli D[1], Pagliardini V[1], Ignaccolo MG[1], Mussa A[1], Porta F[1], Meli C[2], Ponzone A[1], Spada M[1]
[1]*Dept Ped, Univ Child Hosp, Torino, Italy*
[2]*Div Metab Dis, Univ Hosp, Catania, Italy*

Background: During the last 10 years, 10–85% of PKU patients were reported as BH4–responsive and candidates to the treatment with cofactor on the basis of either the lowering of plasma Phe after a BH4 challenge, or of the genotype, or of the increase of dietary Phe tolerance. Inconsistency of BH4–responsiveness after repeated cofactor challenges or within the same genotypes, and erroneous evaluation of Phe tolerance, however, makes questionable these findings.
Objectives: To test quantitatively the effects of BH4 in PKU patients.
Patients and methods: 15 PKU patients (severe: 3; mild: 5; benign: 7), already considered BH4–responsive on the basis of a BH4 loading test and of the genotype, were submitted to a quantitative, self-controlled procedure. The results of a simple oral Phe (100 mg/kg) and of a combined oral Phe (100 mg/kg) and BH4 (20 mg/kg) loading were compared. The tests were performed at normal basal Phe concentration and any additional Phe and Tyr intake was avoided. Plasma Phe, Tyr and biopterin concentrations were measured over 24 hours.
Results: In all patients the clearance of plasma Phe and Tyr production were unaffected by cofactor challenge but related to patients' genotype and phenotype. Biopterin concentration increased 6 times after simple Phe and 34 times after combined Phe + BH4 loading.
Conclusion: The administration of BH4 does not alter Phe and Tyr metabolism in PKU patient. The assessment of BH4-responsiveness by methods so far used is not reliable, and the occurrence of BH4– responsive forms of PKU still has to be definitely proven.

342-P

NEUROCOGNITIVE FINDINGS IN INDIVIDUALS WITH PHENYLKETONURIA AND TREATMENT WITH SAPROPTERIN DIHYDROCHLORIDE (BH4)

White DA[1], Grange DK[2], Christ SE[3]
[1]*Dept Psychology, Washington Univ, St. Louis, United States*
[2]*Dept Peds, Washington Univ, St. Louis, United States*
[3]*Dept Psych Sci, Univ Missouri, Columbia, United States*

Background: Phenylketonuria (PKU) is a disorder in which phenylalanine (Phe) metabolism is disrupted. The disorder is associated with dopamine disregulation and white matter abnormalities in the brain. Impairments in cognition (particularly executive abilities) are common, even in patients treated early/continuously with dietary Phe restriction. Sapropterin dihydrochloride (BH4) is a pharmaceutical agent that lowers Phe in BH4 responders. We are evaluating changes in brain and cognition that occur during BH4 treatment.
Methods: Brain and cognition are evaluated in PKU patients at baseline before BH4 treatment (20 mg/kg/day) using MRI/DTI (diffusion tensor imaging) and neuropsychological tests focused on executive abilities. For BH4 responders, follow-up evaluation is conducted after 6 months of BH4 treatment. Data collection is ongoing. At this time, participant ages range from 7 to 35 years (M=18; SD=8). Evaluation at baseline has been conducted with 19 PKU patients and 12 controls, and at follow-up with 5 PKU patients and 5 controls.
Results and Conclusions: Baseline findings to date indicate that executive performance is significantly poorer for PKU patients than controls across a range of tasks assessing abilities such as inhibitory control (go/no-go, p=.04; stimulus-response compatibility, p=.03), strategic processing (verbal fluency, p=.007; word list learning, p=.001), and working memory (2-back, p=.001). These results reflect specific and pervasive impairments in executive abilities prior to treatment with BH4. Follow-up findings provide evidence of improvement in executive abilities during treatment with BH4. At the conference, baseline findings from newly enrolled patients will be presented, as well as specific findings from follow-up neuropsychological assessment and MRI/DTI.

343-P

PKU MUTATION UPDATE AND ASSESSMENT OF THE POTENTIAL BENEFIT FROM BH4 SUPPLEMENTATION THERAPY IN SERBIA

Stojiljkovic M[1], Djordjevic M[2], Zukic B[1], Tosic N[1], Karan-Djurasevic T[1], Radmilovic M[1], Spasovski V[1], Pavlovic S[1]
[1]*IMGGE, Belgrade, Serbia and Montenegro*
[2]*Mother and Child Healthcare Institute, Belgrade, Serbia and Montenegro*

Mutations in the phenylalanine hydroxylase gene (PAH) cause phenylketonuria (PKU). Recently, it was shown that a number of PKU patients respond to tetrahydrobiopterin (BH4) supplementation treatment. Apparently, the effect of BH4 therapy is dependent on a specific PAH mutation. Therefore, we present the update on PAH mutations detected in Serbian PKU patients and the calculated frequency of patients who could benefit from BH4 use. In total, we performed genotype analysis of 44 unrelated patients by PCR-RFLP and 'broad range' DGGE/DNA sequencing analysis. Altogether, we identified 20 different mutations and only one, R243X, was not previously found in Serbian population. The most frequent mutations, L48S (27.3%), R408W (17%), P281L (8%) and E390G (7%), accounted for 59.3% of all mutant alleles, while remaining ones occurred at frequency less than 5%. Interestingly, increased number of analyzed PKU patients reinforced the status of L48S mutation as the most frequent one in Serbian population. The L48S mutation as well as 4 other mutations (E390G, R261Q, R158Q and R413P) were characterized as BH4-responsive ones in previous European studies. Accordingly, the total frequency of BH4-responsive mutations for Serbian population is 43.2%. Number of Serbian patients carrying at least one BH4-responsive mutation (including four L48S homozygotes) is 24. Thus, the calculated frequency of BH4-responsiveness in Serbian PKU population is 54.5%, quite close to the predicted average value for European populations. Since BH4 supplementation therapy is not available in Serbia, this is the first assessment of the potential benefit from tetrahydrobiopterin supplementation therapy implementation in our country.

344-P

SERVICE-USER SATISFACTION WITH THE GROUP CLINIC MODEL FOR MANAGEMENT OF PHENYLKETONURIA: A PILOT EVALUATION

Raymond K[1], Mumford N[1], Bond K[1], Skeath R[1], Stafford J[1], Abulhoul L[1]
[1]*Metabolic Medicine, Great Ormond St Hosp, London, United Kingdom*

Background: Since 2003, patients with Phenylketonuria (PKU) and their families have attended group clinics. Clinics have age-appropriate content, and are facilitated by the multi-disciplinary team (individual consultations remain available). This evaluation explored parental satisfaction with the group clinic model for PKU management. Findings were hoped to inform both service development, and further methods of evaluating clinics.
Method: The PKU team developed a questionnaire comprising of rating scales, and open and closed questions. Questionnaires were distributed to parents over four separate clinics; thirteen completed questionnaires were returned.
Results: The majority of participants indicated that they liked the clinics, found them helpful, and reported practicing the ideas discussed in clinic. Aspects that were considered beneficial can be categorized into the following themes: sharing experiences with other families, information/learning activities, and samples of new foods. Most families reported they would prefer a combination of group and individual clinics, 1–2 times each year. It should be noted that some parents (2/13) indicated their family did not like the group model, and felt uncomfortable sharing experiences in the group.
Conclusions: Overall, findings indicate families believe they benefit from the group model of PKU management, both in terms of learning, and having opportunities to meet other families affected by PKU. However, in order to draw firm conclusions, this evaluation should be replicated with a larger group, and young people with PKU should also be asked to provide feedback. Future research could include exploring the relationship between satisfaction with PKU clinics and clinical outcomes.

345-P

PLASMA 3-O-METHYL DOPA AS A POTENTIAL BRAIN FUNCTION BIOMARKER IN TREATED ADULT PKU PATIENTS

Rahman Y[1], Lumsden D[1], Turner C[2], Mundy H[1], Champion M[1], Dalton RN[2]
[1]*Dep Inh Metab Dis, GSTFT, London, United Kingdom*
[2]*Well Child Laboratory, Evelina Child Hos, London, United Kingdom*

Aim: To explore the potential role of plasma 3-O-methyl DOPA (3OMD) as a marker for neuropsychological function in adult PKU patients
Background: Previous works have shown a correlation between raised plasma phenylalanine, abnormal neuropsychological tests of higher cognitive function, and decreased plasma L-DOPA in patients with PKU. 3OMD is a major metabolite of L-DOPA and is considerably more stable with longer biological half life.
Methods: 19 plasma samples from 18 adult PKU patients (10 females; 10 off-diets) were collected, separated and stored at -80oC until analysis. Plasma phenylalanine concentrations were analyzed by ion-exchange high-pressure chromatography. Plasma 3OMD concentrations were analyzed (50ul) by liquid chromatography stable isotope dilution electrospray mass spectrometry-mass spectrometry (MSMS).
Results: Plasma 3OMD ranged from 24.5–92.6 (mean = 54.3) nmol/L with a trend to suggest a negative correlation between plasma phenylalanine and 3OMD (R2=0.09). Lower values of 3OMD were observed in all four symptomatic patients. A serial data in a case showed a reduction of blood phenylalanine with an improvement of clinical symptoms and an increased of 3OMD after dietary intervention. Three late diagnosed patients have 3OMD concentration above the mean value.
Conclusion: This cross-sectional data showed a better correlation between plasma 3OMD with clinical neuropsychological symptoms in adult PKU patients, compared with plasma phenylalanine alone. Plasma 3OMD could provide an additional value as biochemical marker to monitor adult PKU patients especially those who are off-diets. However larger studies with longitudinal data are required to better understand the effects of phenylalanine on brain function.

346-P

THE OUTCOME OF THE WHITE MATTER ALTERATION IN EARLY TREATED PHENYLKETONURIC (PKU) PATIENTS

Leuzzi V[1], Chiarotti F[2], Walter J[3], Mercante F[1], Burgard P[4]
[1]*Dept Child Neurol and Psych, La Sapienza, Roma, Italy*
[2]*National Health Institute, Roma, Italy*
[3]*Royal Manchester Children's Hospital, Manchester, United Kingdom*
[4]*University of Heidelberg, Heidelberg, Germany*

Background: Pathogenesis and clinical consequences of MRI white matter (WM) abnormalities in PKU patients are not completely known. Almost all the studies were designed as transversal observations, while no data are available on the progression, if any, of WM lesions.
Objective: To study WM alteration outcome by examining serial MRI exams in early treated patients.
Methods: 100 PKU patients (mean age 19.1 years, SD 7.42, range 6.4–52.1) were enrolled according to the following criteria: a) early diagnosis and treatment; b) two or more consecutive brain MRIs; c) blood Phe assessed before each exam. MRI T1-, T2-weighted and FLAIR images were scored in 329 scans: 9 were performed by the age of 10, 103 between >10 and 15 years, 81 between >15 and 20 years, 115 between >20 and 30, and 21 over the age of 30.
Results: The factors that independently influenced the variation of the WM severity score between pairs of subsequent examinations were: a) the age of the patient at the second examination (negatively related); b) the time gap between the examinations and c) the value of blood Phe at the second examination (both positively related); d) the country (UK < Italian patients); e) the sex (F>M). The increase of the score was significantly higher in patients aged 15 years or less in comparison with patients over the age of 30 years, when WM alterations stabilized or also slightly declined.
Conclusions: WM alterations are an age-specific and self-limiting consequence of the chronic exposure to high levels of Phe.

347-P

PHENYLALANINE HYDROXYLASE FUNCTION IN VITRO AND DRUG RESPONSE IN VIVO ARE DETERMINED BY BOTH PHENYLALANINE AND TETRAHYDROBIOPTERIN CONCENTRATIONS

Staudigl M[1], Gersting SW[1], Kemter KF[1], Woidy M[1], Messing DD[1], Danecka MK[1], Blau N[2], Muntau AC[1]
[1]*Molec Pediatr, Hauner Child Hosp, LMU, Munich, Germany*
[2]*Univ Child Hosp Zurich, Zurich, Switzerland*

Phenylalanine hydroxylase (PAH) is complexly regulated by L-Phenylalanine (L-Phe) and tetrahydrobiopterin (BH4). To understand the mutual impact of these substances on PAH kinetics, we developed an automated real-time PAH activity assay that enabled measurements of purified proteins at a wide range of L-Phe and BH4 concentrations. Depicted as activity landscapes, variant PAH showed severe shifts in the optimal range of activity upon varying L-Phe and BH4 concentrations and altered substrate and cofactor inhibition. As BH4 has been accepted as a pharmacological chaperone in the treatment of phenylketonuria and in light of high variations of Phe and BH4 levels in treated patients, we aimed to translate the mutual impact seen in activity landscapes to patient data from the literature. Data of patients bearing a homozygote or functional hemizygote genotype were analyzed with respect to BH4-responsiveness taking into account initial blood Phe concentrations and the BH4 dosage applied. Patients with the same genotype showed differences in BH4-responsiveness as a function of blood Phe. Individuals with different genotypes showed peak BH4-responses at different Phe concentrations, a shift of the range of Phe concentrations at which BH4-responsiveness occurs, and variances in BH4 dose response. These in vivo results substantiate our observations made by analyzing activity landscapes of the recombinant PAH protein and they support the hypothesis, that a patient's metabolic state has a much greater impact on the interplay of BH4 and the function of PAH than currently appreciated. This may have an impact on the design of future clinical evaluation procedures of BH4-responsiveness.

348-P

NEW INSIGHTS INTO INTERALLELIC COMPLEMENTATION OF PHENYLALANINE HYDROXYLASE IN PHENYLKETONURIA

Danecka MK[1], Gersting SW[1], Kemter KF[1], Lotz AS[1], Woidy M[1], Muntau AC[1]
[1]*Molec Pediatr, Hauner Child Hosp, LMU, Munich, Germany*

More than 530 mutations in the phenylalanine hydroxylase (PAH) gene have been identified in patients suffering from phenylketonuria (PKU). Extensive studies on single PAH variants contributed to the recognition of the molecular phenotype of specific mutations. However, a significant share of patients shows compound heterozygosity making genotype-phenotype correlations weak. To study the intracellular interplay of PAH variants in compound heterozygosity different cell culture based high-throughput techniques were applied.
A novel Bioluminescence Resonance Energy Transfer (iBRET) method enabled us to analyze the composition of mixed PAH oligomers derived from two different alleles. We analyzed protein stability by calculation of Bmax, a measure for the availability of variant PAH derived from one allele in the oligomer. In addition, determination of Kd allowed for analysis of the affinity of interacting PAH variants. This was substantiated by multi-well analyses of the respective protein amount of cells co-transfected with two PAH variants and by the determination of residual enzyme activity. Using this experimental setup, we observed both, positive and negative interallelic complementation. Moreover, we showed that the pharmacological chaperone, tetrahydrobiopterin, can modulate interallelic complementation and restore enzyme function.
Taken together, our results support the hypothesis that the PAH tetramer in vivo consists of subunits arising from both alleles and we gained further insights into how this phenotype is corrected by the pharmacological chaperone. This combined approach of in vivo techniques may aid in translating molecular findings into the patient's phenotype in PAH deficiency.

349-P

DOES METABOLIC CONTROL INFLUENCE DAILY NEUROPSYCHOLOGICAL FUNCTIONING IN ADULTS WITH PKU?

Brinkley A[1], Keating M[1], Lynch A[1], O'Regan M[1], Stenson C[1], Hayes A[1], Monavari A[1], Crushell E[1], Treacy E[1]
[1]*Nat Ctr for INH Metab Dis, Child Uni Hos, Dublin, Ireland*

Background: Mild neuropsychological impairments are described in adults with PKU despite early dietary treatment. Controversy persists as to the functional significance of these findings. In Ireland, a 'Diet for Life' policy for PKU is in place since 1965.
Objectives: To study cognitive and executive functioning in our adult PKU cohort, comparing good vs. poor metabolic control.
Methods: 27 patients are enrolled to date, ages 17–40 (M = 28) years, diagnosed by newborn screening who maintained satisfactory metabolic control to adolescence with normal range IQs. Patients were assigned to High or Low Phe group according to Phe levels < or >700 μmol/L at time of testing. A control group consisted of healthy adults. Cognitive and executive functioning were measured using the WASI, D-KEFS and the BRIEF-A.
Results: Although mean IQ in both groups was within the Average range, the High Phe group had significantly lower Full Scale IQ ($p < .001$). The High Phe group also had significantly decreased performance on a number of DKEFS subtests, although mean scores were within the normal range. Significant group differences were found on measures of cognitive flexibility ($p < .05$), set shifting ($p < .05$) and verbal inhibition ($p < .05$). There were no significant differences in self rated executive functioning.
Conclusion: Patients with PKU with poor metabolic control achieved significantly lower scores on tests of cognitive and executive functioning as compared to those with good metabolic control. Results suggest that poor dietary compliance in adult PKU can result in subtle neuropsychological deficits.

350-P

MEASUREMENT OF GROWTH IN CHILDREN WITH PKU: HOW WELL ARE CHILDREN WITH PKU GROWING ON CONVENTIONAL DIETARY TREATMENT?

Daly A[1], Neville C[1], MacDonald A[1]
[1]*Birmingham Children's Hospital, Birmingham, United Kingdom*

Introduction: Over the last half century the dietary treatment of PKU has improved, but there is little longitudinal growth data reported with contemporary diet.
Aim: To review the longitudinal growth (z-scores for weight and height) in children aged 1- 14y from one UK treatment centre.
Methodology: Growth data was collected on 83 children with PKU, diagnosed by newborn screening. There were 41 boys and 42 girls with a mean age of 8.1 y (range 3mth–15y). Median z-scores for weight and height were calculated at five ages: birth, 1y, 5y, 10y and 15y. They were all following a low phenylalanine diet, aiming to maintain blood phenylalanine concentrations within UK, MRC (1993) guidelines.
Results: The median weight and height z-score, increased with age. Weight z-score: birth, -0.39 (n=82); 1y, -0.05 (n=81); 5y, 0.37 (n=76); 10y, 0.28 (n=58); and 15y, 0.16 (n=24) with a median change of 0.16 between birth and 15y. Height z-score: birth, -0.42 (n=59) 1y, -0.16 (81); 5y, -0.14 (n=74); 10y, 0.29 (n=58); and 15y, 0.35 (24) with a median change of -0.14 between birth and 15y. Therefore, in the first 5 years of life children had a negative height z-score, but with improving growth throughout childhood. Weight z-score also improved throughout childhood, with peak weight z-score at 5 y.
Conclusion: Early growth data is similar to other European studies, indicating that early growth is sub-optimal. However, reassuringly, later catch–up growth is demonstrated without excessive weight gain, suggesting modern dietary management is associated with a satisfactory growth profile.

351-P

FIRST EXPERIENCES WITH TETRAHYDROBIOPTERIN (BH4) LOADING TEST IN PKU PATIENTS >4 YEAR OF AGE IN THE NETHERLANDS: CONCLUSIONS FOR REFINEMENT TESTING BH4 RESPONSIVENESS

van Spronsen FJ[1], van Rijn M[1], Heiner-Fokkema MR[2], Bosch AM[3], Modderman P[2], Abeling NG[4], Blau N[5]
[1]*Beatrix Child Hosp, Univ Med Center, Groningen, Netherlands*
[2]*Lab Metab Dis, Lab Cent, Univ Med Center, Groningen, Netherlands*
[3]*Emma Child Hosp, AMC, UVA, Amsterdam, Netherlands*
[4]*Lab Gen Metab Dis, Emma Child Hosp, AMC, Amsterdam, Netherlands*
[5]*Div Clin Chem & Biochem, Univ Child Hosp, Zurich, Switzerland*

Background: BH4 responsiveness can be defined as ≥20% or 30% decrease of Phe in BH4 loading test (BLT). The Dutch physicians metabolic diseases decided to perform the BLT in all PKU patients >4 years following a specific scheme.
Methods: Before BLT was performed, patients were asked to send 4 samples (at 2 days: 7–9 am; 5–6 pm) to establish Phe variation within a day. In patients with Phe <400 μmol/L, extra Phe as natural protein was started 6–8 days before BLT to achieve stable Phe ≥400 μmol/L at start of BLT (20 mg/kg BH4 at both T0 and T24; BH4 responsiveness: Phe decrease >30% compared to T0). Blood samples were taken at T=0, 8, 16, 24 and 48 hrs. In BH4 responsive patients BH4 was prescribed for a longer time period.
Results: 44 of 78 patients (57%) had a 30–82% Phe decrease compared to T0. In these 44, Phe variation within a day before BH4 was 2–48% In 32%, 19% and 3% of BH4 responsive patients, the response to BH4 decreased from >30% to <30%, <20% and <10%, respectively, taking Phe variation without BH4 into account; long-term BH4 response varied. In the non-BH4-responders, Phe variation without BH4 was 2–85% of the morning Phe. In 73%, and 91%, response to BH4 was <10%, and <20%, respectively, taking the Phe variation without BH4 into account.
Conclusions: Taking the Phe variation without BH4 into account may be more important than using 20 or 30% as the cut- off to decide on BH4 responsiveness.

352-P

CHANGES IN NEURO-PYSCHOMETRIC MEASURES IN A SAPROPTERIN RESPONSIVE ADOLESCENT PATIENT WITH PKU

Kearney S[1], MacDonald A[1], Vijay S[1], Chakrapani A[1]
[1]*Birmingham Children's Hospital, Birmingham, United Kingdom*

Introduction: In PKU, although Sapropterin dihydrochloride (6R-BH4) (Merck Serona) reduces blood phenylalanine concentrations, it is unknown if it improves the subtle deficits observed in executive function, speed processing and social and emotional difficulties.
Case Study: A boy aged 14y with well-controlled PKU (mutations F39L/IVS 12+1G>A), was compliant with dietary treatment, despite neophobia to low protein foods. He was thin and complained of frequent hunger pains. He had previous psychological intervention due to family disputes he caused about diet. A carefully controlled trial with 10 mg/kg/day Sapropterin demonstrated that his blood phenylalanine concentrations reduced by 40% by day 5, to consistently less than 350 μmol/l. His phenylalanine tolerance increased from 450 mg/daily to 1000 mg/daily. He had neuro-pyschometric testing pre and 4 weeks post-Sapropterin. The case study reported him to be 'calmer,' 'less hyper' and socially 'more normal' and his self-esteem improved. His carer reported mood changes; he was happier, more relaxed, no longer an 'angry, young man,' and improvements in attention and concentration were reflected both at home and school. On repeat psychometric testing, only 4 weeks post-Sapropterin, there was subtle improvements across indices of attention measures, speed of inhibition and switching, and immediate memory span. His energy intake increased from 1600 kcal/d to 2200 kcal/d.
Conclusions: In the short term, Sapropterin therapy appeared to result in subtle improvements in attention, executive function, mood and nutritional status in a previously well-controlled boy with PKU. Further longitudinal, controlled studies are required to study neurocognitive changes with Sapropterin.

353-P

ONE WEEK CAMP INTERVENTION DECREASES MARKER OF LIPID PEROXIDATION IN FEMALES WITH PHENYLKETONURIA

Singh RH[1], Quirk ME[2], Le NA[3]
[1]Emory Univ, Dept of Hum Genet, Decatur, United States
[2]Emory Univ, GDBBS, Atlanta, United States
[3]Emory Univ, Dept Endocrin, Atlanta, United States

Background: Patients with phenylketonuria (PKU) have been reported to have elevated lipid peroxidation markers as compared to their unaffected counterparts. It is unknown if improved management of the disorder modulates these markers.
Objective: To determine if females with PKU have changes in thiobarbituric acid reactive substances (TBARS), a marker of lipid peroxidation, corresponding to changes in plasma phenylalanine concentrations.
Methods: Females attending a weeklong metabolic camp in 2008 had their plasma phenylalanine and TBARS concentrations measured on the first and last day of camp. Changes in plasma phenylalanine and TBARS concentrations were evaluated using a paired t-test. Associations between TBARS and phenylalanine concentrations (pre- and post-camp, and changes in phenylalanine concentrations) were evaluated.
Results: Twenty-three females were assessed (age 24.2 ± 12.5 years). Plasma phenylalanine concentrations significantly decreased, from a median of 1285 μmol/L (range: 296–2297 μmol/L) to 417 μmol/L (range: 49–1363 μmol/L) (P <0.0001). TBARS also significantly decreased from a median of 0.87 TBARS equivalents/100 μL plasma on the first day of camp (range: 0.55–1.45) to 0.66 TBARS equivalents/100 μL plasma on the last day of camp (range: 0.38–1.84) (P = 0.03). Phenylalanine concentrations and change in phenylalanine concentrations were not associated with concurrent TBARS concentrations or change in TBARS.
Conclusions: With improved dietary compliance during the camp, significant reductions in both plasma phenylalanine and TBARS were achieved. While plasma phenylalanine concentrations were not associated with changes in TBARS, this study is the first to suggest the immediate reductions in oxidative status of PKU patients with greater compliance.

354-P

MOLECULAR AND PHENOTYPICAL ASPECTS IN A GROUP OF ROMANIAN PATIENTS WITH PHENYLKETONURIA

Vulturar R[1], Barecki M[2]
[1]Univ. of Med and Pharm, Ist Lab Gen Expl, Cluj-Napoca, Romania
[2]Univ Med and Pharm, Child Neurology Clin, Cluj-Napoca, Romania

Phenylketonuria (PKU) is a autosomal recessive disease that if is untreated will impaire postnatal cognitive development resulting from a neurotoxic effect of hyperphenylalaninemia (HPA); the associated clinical phenotype varies considerably. Its metabolic phenotype is accountable to multifactorial origins both in nurture, where the normal nutrition introduces L-phenylalanilne (Phe), and in nature, where mutations (>520 alleles) occur in the PAH gene. The allelic variation at the PAH locus yield greater or lesser risk of impaired cognitive development according to the degree of HPA.
The clinical evaluation of a group of 20 PKU patients (with different stage of treatment) from clinical sections of Cluj Children Hospital, Romania, was done according to The Munchen development scale for the children with mental age below 3 years, with The Simon-Binet test for children with mental age between 3–7 years and with The Raven's Progressive Matrices for those above 7 years.
Biochemical phenotypes associated were included in severe (classic PKU) when the level of Phe is above 1200 μmol/l, moderate PKU (Phe: 600–1200 μmol/l) or mild HPA for Phe: 120–600 μmol/l. The leukocyte DNA was isolated using classical method (phenol and chloroform) followed by polymerase chain reaction (PCR) with subsequent restriction analysis. In this group we identified patients homozygous for mutation R408W (for more than 50%), patients compound heterozygotes for R408W/L48S or R408W/R261Q mutations. Our data provide that the highest degree of concordance was found in patients with null/null genotypes and evidence that a simple genotype-phenotype correlation does exist in this group of patients.

355-P

METABOLIC RESPONSE IN 10 CHILEAN PHENYLKETONURICS WHO RECEIVED TETRAHYDROBYOPTERINE (BH4) DURING 48 HRS

Cornejo V[1], Cataldo X[1], Perez B.[2], Desviat Lr.[2], Ugarte M.[2], Raimann E[1]
[1]INTA, University of Chile, Santiago., Chile
[2]CEDEM, Universidad Autonoma, Madrid, Spain

Background: PKU is treated with phenylalanine (Phe) restricted diet, but due to the poor adherence, especially in adolescence, alternative treatment have been searched, being one of these the administration of BH4.
Objectives: to evaluate the metabolic response to BH4 in early diagnosed PKU.
Methodology: 10 PKU with Phe values at diagnosis < 20 mg/dl, present age in average 8.8±1.6 years were selected. BH4 20 mg/kg/day was given in 2 consecutive days in 1 dose. Blood Phe levels were measured every 8 hours (one day before BH4, at 24, and 48 hours with BH4, and a day after. Five patients maintained their normal Phe intake (basal blood Phe x 292±54 uM) and in 5 cases Phe intake was raised 50% (powder cow´s milk), with basal Phe levels x 268,2±89 uM.
Results: In 5 cases Phe levels decreased at 24 hrs in average from 278,9 uM to 150,6 uM (> 46% response). At 48 hours, 2 other cases were added with a decrease of Phe levels of average: from 354,6 uM to 218,1 uM (>38,5%)), the mutations of them are: pR243Q, pE390G, pY414C, pV388M. Only one case responded 32% at 24 hours and at 48 hours diminished to 16% and his mutation is IVS10int-11q>A.
Conclusion: 50% of PKU responded to BH4 at 24 hours, but 70% at 48 hours. The 7 BH4 responders have a mutation already known in this group. We can conclude that a single BH4 load for 24 hours does not permit to detect all PKU BH4 responders.

356-P

HIGH PLASMA PHENYLALANINE CONCENTRATIONS ARE CORRELATED WITH INCREASED PREVALENCE OF MOOD SWINGS AND INTROVERT BEHAVIOUR IN PKU PATIENTS OF VARIOUS AGES

Anjema K[1], van Rijn M[1], Heiner R[2], Bonnema J[1], van Spronsen FJ[1]
[1]*Section of Metab Dis, Beatrix Child Hosp, Groningen, Netherlands*
[2]*Lab of metab Dis, Beatrix Child Hosp, Groningen, Netherlands*

In PKU, knowledge about the relation of behavior (including being distractive, hyperactive), and plasma phenylalanine (Phe) is scarce. The aim of this study was to determine whether high Phe is associated with disturbed behavior and thereby is predictable. Participants were 51 early treated PKU patients (aged 11.0 ± 9.2 years). Mean Phe concentration was 365 ± 254 μmol/l in total population; and 288 ± 184 μmol/l in the 31 patients < 12 years of age. After sending blood samples patients, parents or partners were called to give the result of the Phe concentration. Before giving the Phe concentration, they were asked double-blind in a standardized questionnaire if they noticed hyperactivity, annoying behavior, mood swings and introvert or extravert behavior, and could predict the Phe level.

Results: Hyperactivity is negatively correlated with Phe levels in the total patient group (p=0.02), but not specifically in < 12 years (p=0.19). Annoying behavior was significantly correlated with high Phe levels in the < 12 years (p=0.03), but not in the total population (p=0.59). Mood swings were positively correlated in both the total group and <12 years (p=0.01 and 0.00). Introvert behavior was negatively correlated with high Phe in both the total group and <12 years (p=0.02 and 0.00). Respondents showed to be able to predict the category of Phe concentration (68% correct, p=0.00).

Conclusion: These data showed that Phe concentrations are related to behavior, and that patients/caregivers can more or less predict from the behavior whether the Phe concentration is too high.

357-P

PKU SLOW RESPONDER TO BH4 LOAD TEST

Beltran-García S[1], Caballero-Pérez V[1], Monge-Galingo L[1], Perez-Delgado R[1], Lopez-Pison J[1], García-Jimenez MC[1]
[1]*Div Metab Dis, Univ Hosp Miguel Servet, Zaragoza, Spain*

Introduction: Early dietary treatment is effective in hyperphenylalaninemia, but this phenylalanine (Phe) restricted diet has negative aspects. Treatment with saproterin has been shown to markedly reduce blood Phe levels in a substantial proportion of patients with PKU. A 24-hour protocol with 20 mg/kg BH4 is the most commonly used method but multiple administrations of BH4 and extension of the test to up to one week may detect some additional slow responders.CASE REPORT:A 22-year-old male hyperphenylalaninemia classic (PKU)affected, who had been diagnosed in the neonatal period with a Phe levels in the metabolic screening of 2700 nmol/ml. In the molecular study, L48S/I65T mutations were found. He was on a limited diet in natural proteins with Phe's levels <500 nmol/mL during 22 years. At the age of 16, the tetrahydrobiopterin (BH4) loading test (Phe 100 mg/kg + an only dose BH4 20 mg/kg) was performed and the response to the test was considered negative because there was not a reduction in blood Phe levels >30% 8 hours after the administration. At the age of 22, a long-term (1 week) loading test with sapropterin (8,5 mg/kg/day) was realized and Phe levels were reduced from 502 nmol/mL to 121 nmol/mL. Sapropterin was prescribed to the patient, which let him increase the ingestion of natural proteins.

Conclusions: The overload test prolonged for 3 or 7 days allows us to detect slow responders. We consider reasonable to realize it in patients whose response to the combined test is negative, because this treatment let them improve his quality life and liberate partially their diet.

358-P

BLOOD PHENYLALANINE CONTROL IN PATIENTS WITH CLASSICAL PKU: A PKU CLINIC AUDIT

Carol Hartnett CH[1], Eva Yap Todos EYT[1], Osman Ipsiroglu OI[1], Sylvia Stockler SS[1]
[1]*British Columbia Children's Hospital, Vancouver, Canada*

The intellectual outcome in PKU is directly related to the dietary control of blood phe levels. Limited data exists on how well blood phe control realistically is achievable in PKU patients.

We analysed all blood phe levels obtained between 1 month–1 year, 1 year–6 years, and 6 years–12 years of life, from 30 patients with classical PKU and phe tolerance <30 mg/kg, followed in our PKU clinic. Therapeutic phe levels are defined as 2–6 mg/dl. We expressed phe control as percentage of phe levels out of therapeutic range.

Within the first year of life, 17 / 30 (57%) patients had more than 50 % (range 51–76, median 65) of phe levels outside therapeutic range. In 3 patients, 30 % phelevels were higher than 10 mg / dl. Only 8 / 30 patients had less than 30% phelevels outside therapeutic range.

Between 1 year–6 years and 6 years to 12 years, 37% and 41% patients had more than 50% phelevels outside therapeutic range.

Optimal therapeutic control is difficult to achieve and involves individual phenylalanine tolerance, adherence to phenylalanine restricted diet and quality of medical care. New treatment strategies are needed to improve the overall outcome of this complex-to-treat condition.

359-P

PILOT STUDY TO EVALUATE THE EFFECTS OF KUVAN ON ADULT INDIVIDUALS WITH PHENYLKETONURIA WITH MEASURABLE MALADAPTIVE BEHAVIORS

Moseley KD[1], Azen C[2], Ottina MJ[1], Koch R[1], Yano S[1]
[1]*USC/Keck School of Med, Dept of Peds, Los Angeles, United States*
[2]*USC/Keck School of Med. CHLA, GCRC, Los Angeles, United States*

Background: We report 12 month data on a pilot study to evaluate changes in behavior while on Kuvan. (BH4). is a drug that is used for the treatment of PKU. Kuvan. is a co-factor for phenylalanine (phe), tyrosine, and tryptophan hydroxylases. BH4 may affect tyrosine and tryptophan hydroxylases in the brain and affect behavior without a reduction in blood phe levels.

Aim: To evaluate effects of Kuvan. on maladaptive behavior in patients with PKU.

Methods: Ten subjects (>18 years) with maladaptive behavior were enrolled in a 12-month study. Kuvan. was given at 20 mg/kg/day. Baseline and quarterly measures of plasma amino acids, as well as baseline, six-month and 12-month evaluation of the Vineland II Adaptive Behavior Scales (VABS-II) and a PKU Behavior Check List were obtained.

Results: Comparison of 12-month data to baseline showed no change in blood phe levels (p=0.33), but increased blood tyrosine levels (p=0.05) and decreased blood phe/tyrosine ratio (p=0.067). The VABS-II showed no change in communication, daily living skills, socialization, or motor skills, but significant improvement for internal behavior including anxiety, nervousness, and unexplained sadness (p=0.018). On the PKU Behavior Check List, subjects showed significant improvement in the sum of scores over the 15 negative behaviors (p<0.0001).

Conclusion: PKU subjects who did not respond to Kuvan in blood phe level, showed significant improvement in maladaptive behavior, may suggest effects of Kuvan in the CNS. Long term evaluation of CNS effects of Kuvan is warranted.

360-P

VITAMIN B6 AND B12 STATUS IN TURKISH CHILDREN WITH PHENYLKETONURIA

Buyuktuncer Z[1], Gokmen-Ozel H[1], Kucukkasap T[1], Koksal G[1], Kilic M[2], Dursun A[2], Kalkanoglu-Sivri HS[2], Tokatli A[2], Coskun T[2], Besler HT[1]
[1]*Hacettepe Univ, Dept Nutr Diet, Ankara, Turkey*
[2]*Hacettepe Univ, Dept Paed, Metab Unit, Ankara, Turkey*

Background: It has been known that patients with phenylketonuria on a restricted diet have an increased risk of vitamin B12 and probably vitamin B6 deficiencies. The knowledge on blood vitamin B6 and B12 levels of Turkish phenylketonuric patients is very limited. This study was designed to evaluate vitamin B6 and B12 status in phenylketonuric children and age-matched healthy controls.
Methods: Eighty-three children (40 boys, 43 girls) with phenylketonuria and 102 healthy age-matched controls (46 boys, 56 girls) were recruited. There were two exclusion criteria: (i) patients not on dietary treatment, (ii) patients using vitamin and/or mineral supplement, (iii) having mental retardation. The median age was 8 years (2 to 30 years) and 11 years (4 to 31 years) for phenylketonuric and control groups, respectively. The fasting plasma vitamin B6 (pridoxal-5-phosphate) level was analyzed using Clin Rep Chromatography kit in HPLC system. The fasting serum B12 level was analyzed using routine method.
Results: The plasma vitamin B6 (median= 178.6 mg/l; range: 31.9 to 540.5 mg/l and median= 81.1 mg/l; range=28.3 to 443.6 mg/l, respectively) and serum B12 levels (median= 423 pg/mL; range=101 to 1013 pg/mL and median=355 pg/mL; range=101 to 875 pg/mL, respectively) were significantly lower in the control subjects compared to phenylketonuric patients ($p<0.001$). Eight percent of the phenylketonuric patients' and 9% of the control subjects' serum vitamin B12 levels were below the normal ranges.
Conclusions: The present study suggests that vitamin B6 in particular; B12 deficiencies are common both in our patient population and healthy controls.

361-P

BLOOD PHENYLALANINE CONTROL IN TURKISH PHENLYKETONURIC CHILDREN

Gokmen-Ozel H[1], Buyuktuncer Z[1], Koksal G[1], Kilic M[2], Dursun A[2], Kalkanoglu-Sivri HS[2], Tokatli A[2], Coskun T[2]
[1]*Hacettepe Univ, Dept Paed, Metab Unit, Ankara, Turkey*

Background: Only limited data are available comparing blood phenylalanine control achieved among dietary treated patients with phenylketonuria in Turkey. In this single centre, retrospective study, the aim was to compare the blood phenylalanine control achieved in dietary-treated patients with phenylketonuria of all age groups.
Methods: Three hundred and ninety children (214 boys, 176 girls) with phenylketonuria were recruited. The median age was 4.6 years (1.7 to 17.9 years). All patients followed a strict low-phenylalanine diet comprising: (i) a dietary phenylalanine allocation using a 15 mg phenylalanine exchange system; (ii) a phenylalanine-free protein substitute; and (iii) special low-protein foods permitted in usual quantities. Patients were excluded if they were: adults not on dietary treatment; pregnant; lost to follow-up or had hyperphenylalaninemia and did not require dietary treatment. The latest measured phenylalanine levels were included.
Results: In the early years of life (0–4 years), the median blood phenylalanine concentrations achieved in patients appeared to be well controlled (median= 348 mmol/L; range= 5.4 to 1234 mmol/L). From school age years (4–10 years), blood phenylalanine concentrations increased by 49% in teenage (11–17 years) years (median= 607 mmol/L; range= 3.6 to 3114 mmol/L and median= 904 mmol/L; range= 206 to 1838 mmol/L, respectively). The median blood phenylalanine concentrations increased with age ($r=0.465$, $p=0.000$).
Conclusions: Improvements in blood phenylalanine control can be achieved by intense and continuing education, regular and frequent blood testing, and recording of food intake.

362-P

THE IMPACT OF ETHNICITY ON PHENYLALANINE CONTROL IN PHENYLKETONURIA

Skeath R[1], Mumford N[1], Stafford J[1], Abulhoul L[1]
[1]*Great Ormond St. Hospital for Children, London, United Kingdom*

Background: Optimal phenylalanine (phe) control is the aim of treatment of Phenylketonuria (PKU). Ethnic inequalities have been observed in chronic diseases but to our knowledge this has not been examined in patients with PKU.
Methods: Median phenylalanine levels of 158 patients were calculated. Patients were divided into three age groups; <5 years, 5–10 years and >10 years and in each group by ethnicity. The acceptable range of phenylalanine was based on the Medical Research Council Working Party 1993 recommendations.
Results: In the <5 years (n=37) 80% of white British children (20/25) had good control (phe 120–360 μmol/l) compared with 58% of all the ethnic minorities (7 /12). In the 5–10 year group (n=54) 75% of white British patients (30/40) had good control (phe 120–480 μmol/l), 79% of all the ethnic minorities (11/14) and 40% of the Turkish patients (2/5). In the >10 year group (n=68) 50% white British children (30/60) had good control (phe 120–480 μmol/l) and 38% (3/8) of the ethnic minorities. However if phe<700 μmol/l target range was used for >10 years 88% of white British children (53/60) had good control compared with 63% of the ethnic minorities (5/8) and 33% of the Turkish patients (1/3).
Conclusion: Phenylalanine control worsens with increasing age however this appears to be worse in our ethnic minority patients particularly our Turkish patients. A change of clinical approach is required to reduce the ethnic disparities of phenylalanine control and better meet the individual needs of our diverse population. As our patient numbers are small more collaborative studies are required to improve understanding of this problem.

363-P

BRAIN ANTIOXIDANT RESPONSES INDUCED BY NEOPTERIN

Latini A[1], Oliveira KG[1], Remor AP[1], Rial D[1], Prediguer DS[1], Oliveira S[2], León C[2], Gottfried C[3], Barbeito L[2]
[1]*Universidade Federal de Santa Catarina, Florianópolis, Brazil*
[2]*Institut Pasteur de Montevideo, Montevideo, Uruguay*
[3]*Universidade Federal do Rio Grande do Su, Porto Alegre, Brazil*

Neopterin (Neo), an endogenous pteridine, is found at increased levels in cerebrospinal fluid of patients with brain inflammation and neurodegeneration. However, the role of this compound is virtually unknown. Therefore, the effect of Neo on behavioral and neurochemical parameters was investigated in Swiss male mice and gial cells. Thirty-day-old animals received intracerebroventricularly crescent Neo doses (15 and 150pmol). Thirty minutes after Neo administration, animals were submitted to the open field test. Four hours later, mice were killed and the cerebral cortex was dissected and handled for measuring thiobarbituric acid-reactive substances (TBA-RS) and non-proteic sulfhydryl groups (NPSH) levels, the activities of glutathione peroxidase (GPx), glutathione reductase (GR) and the respiratory chain complexes I-IV. In addition, C6 lineage astroglial cells and striatal primary astrocytes were exposed to Neo (50 and 500nM) alone or in combination with H2O2 (0.1 and 1 mM) or azide (5 mM) for 3 hours, in order to assess free radical production (2′,7′-dichlorofluorescein oxidation). The results demonstrated a significant increment in the central locomotor activity indicating a Neo-induced anxiolytic-like effect. Reduction of TBA-RS and increment of NPSH levels and increased GPx activity was observed in cortical preparations from Neo-treated animals. Similarly, reduction in free radical generations was observed in both, lineage and primary Neo-exposed glial cells. The rest of the parameters remained unaltered. These data indicate that Neo might possess central antioxidant properties and could suggest that increased Neo levels in brain disorders might represent a protection to neuronal injury possibly by inhibiting free radical production.

364-P

CARBAMOYLPHOSPHATE SYNTHASE I (CPS 1) DEFICIENCY: TREATMENT WITH CARGLUMIC ACID (CARBAGLU®)

Williams M[1], Huijmans JGM[2], van Diggelen OP[2], van der Louw EJTM[1], de Klerk JBC[1], Haeberle J[3]
[1]*Dept of Ped Erasmus MC-Sophia Child Hosp, Rotterdam, Netherlands*
[2]*Dept Clin Gen Erasmus MC, Rotterdam, Netherlands*
[3]*University Children's Hospital, Zurich, Switzerland*

Patient: A girl, born in 2001 was seen on the 3 rdday of life because of foodfusal, tachypneu and lethargy. There was respiratory alkalosis. Blood ammonia level was 354 µmol/l. Plasma glutamine and alanine levels were increased, citrulline and arginine levels were very low.
Diagnosis: Liver enzymatic investigations (2003) showed CPS deficiency: 74 nmol/hr/mg (nl 310–1000). OTC activity was 33.400 nmol/hr/mg (nl 18.500–64.800). Two DNA mutations were found in the CPS-gene: a new mutation H243P (exon 8:c.728 A>C) and a deletion c.621–712del90bp on the CPS transcript (frame deletie exon 7).
Follow up: The patient was treated with citrulline (250 mg/kg per day), sodiumbenzoate (250 mg/kg per day), carnitine (50 mg/ kg per day), a protein restricted diet (1 g/kg per day) and aminoacid suppletion. Her psychomotor development is normal. In 2007 with a gradual increase in protein intake (1.7 g/kg per day), hyperammonemia occurred. An increase in glutamine and low levels of arginine were observed. Arginine suppletion (250 mg/kg per day) was started. Ammonia levels normalized.
Objectives: Because of the CPS residual activity, activation by carglumic acid could lead to an increase in the quality of life (decrease in medication and increase in protein intake).
Method: In a week, after introduction of carglumic acid 200 mg, 5 times daily, the sodiumbenzoate treatment and arginine suppletion could be stopped.
Conclusion: With the introduction of carglumic acid medication intake has decreased and protein intake has increased (1.5 g protein/kg per day).

365-P

DIETARY TREATMENT IN CITRULLINAEMIA: ESSENTIAL AMINO ACID MIXTURES DO NOT COVER MICRONUTRIENT REQUIREMENTS

Meyer U[1], Goedecke K[1], Illsinger S[1], Janzen N[1], Das AM[1]
[1]*Dept, Paed., Hannover Medical School, Hannover, Germany*

Background: Dietary treatment in citrullinaemia consists of restricted intake of natural protein supplemented with an amino acid mixture containing essential amino acids and micronutrients.
Objective: The aim of this study was to assess the supply of micronutrients, energy and amino acids in patients with citrullinaemia.
Patients and methods: Dietary protocols from three patients without tube-feeding (age 2–3 years) treated in our clinic were analyzed. The results were compared to the recommendations of the German-Austrian-Swiss dietary association (DACH).
Results: While dietary requirements for amino acids and energy are fully met only about 50% of recommended requirements for micronutrients like calcium, iron, selenium, zinc, folic acid and vitamin B 12 are covered. Patients undergoing tube feeding with commercial products have a better supply with micronutrients.
Conclusion: Children suffering from citrullinaemia which undergo dietary treatment show incomplete coverage of micronutrients in their diet despite supplementation with special amino acid mixtures. Micronutrients have to be added as single substances alternatively the composition of amino acid mixtures used for urea cycle defects has to be adapted.

366-P

UREA CYCLE DISORDERS IN THE NETHERLANDS: A PATIENT SURVEY

Salkovic D[1], de Klerk JBC[2], Huijmans JGM[3], van Spronsen FJ[4], Bosch A[5], Visser G[6], Mulder MF[7], Morava E[8], Rubio-Gozalbo E[1], Boelen C[9], Chakrapani A[10], Williams M[2]
[1]*Dept of Ped Univ Med Center, Maastricht, Netherlands*
[2]*Dept of Ped Erasmus MC-Sophia Child Hosp, Rotterdam, Netherlands*
[3]*Dept Clin Gen Erasmus MC, Rotterdam, Netherlands*
[4]*Dept of Ped Univ Med Center, Groningen, Netherlands*
[5]*Dept of Ped Univ Med Center, Amsterdam, Netherlands*
[6]*Dept of Ped Univ Med Center, Utrecht, Netherlands*
[7]*Dept of Ped VU Med Center, Amsterdam, Netherlands*
[8]*Dept of Ped Univ Med Nijmegen, Nijmegen, Netherlands*
[9]*DEpt of Ped Univ Med Center, Leiden, Netherlands*
[10]*Dept of Inher Metab Dis, Birmingham, United Kingdom*

Background: In 2009 Chakrapani performed a first survey, collecting retrospective data by internet of UCD patients and treatment in The United Kingdom. Although many data were obtained this survey unfortunately lacked dietary data, and information on weight and growth. As individual country UCD patient numbers are relatively small, European data are necessary to get better insight in this patient group.
Objectives: Feasibility study for the creation of a European UCD database: A similar survey as performed in the UK was performed in the Netherlands. We thus hope to get more information on UCD patients, how they are treated in the Netherlands and finally in Europe. Overall goal is to improve treatment, and thus improve outcome.
Material and methods: All UCD patient files in the Netherlands were studied on location using the same data provided by Chakrapani. Additional data on weight growth and diet were included. All university centers were visited.
Results: There is a similar distribution and number of UCD patients as in the UK.
Treatment (medication) once instituted is not easily changed when new treatments come available. Not all patients receive aminoacids mixtures when on a protein restricted diet. Urea cycle patients' body length is below the average.
Conclusions: It is possible to perform patient surveys in countries. As the studies are retrospective not all data are available. Therefore more protocolled follow up is necessary to improve insight in patient treatment but also to improve treatment.
This work was supported by a grant from Orphan Sweden

367-P

EXPERIENCE OF UREA CYCLE DISORDERS IN THE UNITED KINGDOM

Chakrapani A[1], Champion M[2], Grunewald S[3], Lachmann R[4], Shortland G[5], Williams M[6], Morris AAM[7]
[1]*Birmingham Children's Hosp, Birmingham, United Kingdom*
[2]*Evelina Children's Hosp, London, United Kingdom*
[3]*Great Ormond Street Hosp, London, United Kingdom*
[4]*National Hospital for Neurology, London, United Kingdom*
[5]*Univ Hosp of Wales, Cardiff, United Kingdom*
[6]*Erasmus Medical Center, Rotterdam, Netherlands*
[7]*St. Mary's Hospital, Manchester, United Kingdom*

Objectives: To establish an initial impression of UCD incidence, prevalence, survival rates and treatment strategies in the UK.
Methods: Retrospective data collection via questionnaire to all major metabolic centers in the UK; data on diagnosis, presentation, treatment, mortality and morbidity on follow up obtained.
Results: A total of 215 cases were reported from 6 centers. The overall frequency of UCDs was: OTC Deficiency 45%, Arginiocuccuic aciduria 21%, Citrullinaemia 20%, Argininaemia 13%, CPS deficiency 5%, NAGS deficiency 2%, and HHH syndrome 1%. Neonatal presentations accounted for 79 cases (47%) and carried an overall mortality of 36%, but of those who presented with a peak ammonia of >1000micromol/l. A total of 184 (86%) were currently followed up. 116 patients were on drug treatment and 23 were not on any drugs. Arginine (72%), Benzoate (64%) and phenylbutyrate (31%) were the most frequently used medications. 78% of neonatal presenters and 42% of late presenters had significant neuro-disability at follow up.
Conclusions: The distribution of UCDs in the UK showed differences from previously published data from elsewhere. However, a more recent survey in the Netherlands revealed similar numbers and distribution of patients. A multinational longitudinal study is essential to strengthen this data and help establish optimal management strategies.

368-P

A CASE OF LETHAL NEONATAL TYPE CARBAMOYL PHOSPHATE SYNTHETASE 1 DEFICIENCY WITH R233C MUTATION

Kalkan Ucar S[1], Basol G[2], Calkavur S[3], Habif S[2], Bayindir O[2], Coker M[1]
[1]*Div Metab Dis, Ege Univ Child Hosp, Izmir, Turkey*
[2]*Div Bioch, Ege Univ Hosp, Izmir, Turkey*
[3]*Div Neonat, Behcet Uz Child Hosp, Izmir, Turkey*

Carbamoyl phosphate synthetase 1 deficiency (CPS1D) is an autosomal recessive disorder of the urea cycle which causes hyperammonemia. Two forms of CPS1D are recognized: a lethal neonatal type and a less severe, delayed onset type. Neonatal CPS1D cases often present their symptoms within the first days of life. We report a case of CPS1D in a girl who developed hyperammonemia (639 μg/dl), lethargy and seizures at third postnatal day. She was treated with haemodiafiltration, sodium benzoate and N-carbamylglutamate. Plasma glutamine, glycine, and ornithine levels were elevated; citrulline and arginine levels were low. Urinary orotate was undetectable. She was diagnosed as CPS1 (homozygous for R233C). On day 90, the patient was discharged with global developmental delay, grade 2 hydronephrosis and cardiopathy (VSD, PDA, aortic insufficiency). However, at the age of six months she died from septic shock. In respect of accumulation of clinical data helping to reveal the clinical presentation of this rare disorder the patient was presented.

369-P

EATING PATTERNS IN PATIENTS WITH UREA CYCLE DISORDERS: IMPACT ON DISEASE MANIFESTATIONS

Gardeitchik T[1], Humphrey M[1], Nation J[1], Boneh A[1]
[1]*Metabolic Service, MCRI and RCH, Melbourne, Australia*

Background: Urea cycle disorders (UCD) are caused by defects in the ammonia detoxification pathway. In theory, both high protein and low energy/protein intake can cause metabolic deterioration in patients with UCD.
Objectives: To identify dietary habits and eating patterns that might impact on disease presentation and frequency of metabolic decompensations in patients with UCD and to identify problems that might require specific attention in the care for these patients.
Methods: We conducted a retrospective analysis of all clinical, dietary and laboratory data of all UCD patients (n=90: 8 CPS1-, 64 OTC-, 11 ASS-, 7 ASL- deficiencies) attending the metabolic service over 25 years. Diagnosis was made following symptomatic presentation (44 patients), cascade screening (41) and newborn screening (5). Eighteen patients died in the neonatal period.
Results: Protein aversion was noted in 31 of 49 patients on whom information regarding dietary protein intake was available. At most recent follow-up 9/19 patients on a diet did not meet their daily prescribed protein intake. A precipitating factor was recorded in only 90 admissions: an infection (76; mostly associated with reduced energy/protein intake), too low energy/protein intake (9) and excessive protein intake (5).
Conclusion: Protein aversion is common in patients with UCD and may serve as a clinical diagnostic clue. Metabolic decompensations are more frequently related to low energy/protein intake than to high protein intake in these patients. Special attention is required to protein aversion, which leads to eating patterns that make it hard for many patients to reach their prescribed daily protein intake.

370-P

UNRECOGNIZED CITRULLINEMIA MIMICKING ENCEPHALITIS IN A 14 YEAR-OLD BOY—THE ROLE OF A STANDARDIZED LUMBAR PUNCTURE PROTOCOL

Karall D[1], Haberlandt E[1], Albrecht U[1], Rostasy K[1], Haberle J[2], Scholl-Burgi S[1]
[1]*University Children´s Hospital, Innsbruck, Austria*
[2]*University Children´s Hospital, Zurich, Switzerland*

Introduction: Citrullinemia is a urea cycle disorder caused by deficiency of argininosuccinate synthetase. Late onset forms can go undiscovered until a decompensation episode that can resemble encephalitis.
Patient: Herein we report a fourteen year old patient with an acute episode with reduced level of consciousness and confusion interpreted as encephalitis. EEG mainly showed bilateral slowing with some spikes plus spike waves and was interpreted as suspicious for encephalitis. Brain MRI was normal. Leucocytes in CSF were slightly elevated. Treatment for a CNS infectious disease was begun with aciclovir and ceftriaxone. Because symptoms did not resolve and there were some episodes of confusion, a repeat lumbar puncture was performed this time according to a standardized protocol including a metabolic screening with amino acid profile. An elevation of citrulline in CSF was found, which ultimately led to the diagnosis of a late onset citrullinemia.
Conclusion: The establishment of this diagnosis will protect the patient from the sequelae of unrecognized and thus untreated episodes of hyperammonemia. Thus, following a standardized lumbar puncture protocol is essential to detect patients with otherwise unrecognized underlying disorders.

371-P
ORNITHINE TRANSCARBAMYLASE DEFICIENCY IN PREGNANCY: CASE REPORT

Rokicki D[1], Teliga-Czajkowska J[2], Kornacka MK[3], Kowalik A[1], Sykut-Cegielska J[1]
[1]Dept Metab Dis, Endo, Diabet CMHI, Warsaw, Poland
[2]Dept Obst Gynecol, Med Univer, Warsaw, Poland
[3]Dept Neonatol, Med Univer, Warsaw, Poland

Women carrying mutation in ornithine transcarbamylase (OTC) gene may be at risk for hyperammonaemia in the postpartum period.
We present the case of a pregnant woman with OTC deficiency (IVS7–2nt mutation), diagnosed at the age of 5 years. Since then she was treated with protein restricted diet with arginine supplementation. No hyperammonaemic crisis had been observed. At the age of 23 the patient became pregnant. During pregnancy and labor she was doing well. She avoided eating protein even in prescribed amounts, so constant decrease of branch chain amino acids was observed and increased plasma glutamine level. The female newborn was born by cesarean section with weight 3560 g and Apgar score 10. Since the first day of postpartum period, the mother was treated aggressively with protein restricted diet (0.5 g/kg/d), arginine (0.2 g/kg/d), intravenous sodium benzoate and phenylbutyrate. Hyperammonaemia was reavealed once (176 μg%) with a short period of irritation and generalized fatigue. After full recovery the patient returned to her normal diet with protein 1 g/kg/d and the addition of essential amino acids. The daughter inherited the mutation from her mother, but during 12 months of observation develops normally, with no clinical or biochemical signs of hyperammonaemia until now.

372-P
OTC GENE MUTATIONS ASSOCIATED WITH FATAL HYPERAMMONEMIA IN PREVIOUSLY HEALTHY ADULT PATIENTS

Cavicchi C[1], Morrone A[2], Parini R[3], Guido C[2], Pochiero F[1], Rigoldi M[3], Billi P[4], Morelli O[5], Gentiloni Silveri N[6], Colasante A[7], Guerrini R[1], Donati MA[1]
[1]Div Metab Dis, Meyer Child Hosp, Florence, Italy
[2]Dep Woman & Child Health, Florence Univ, Florence, Italy
[3]Div Metab Dis, San Gerardo Hosp, Monza, Italy
[4]Div Gastroenterology, AUSL Bologna, Bologna, Italy
[5]Gastroenterology Inst, Univ of Perugia, Perugia, Italy
[6]Intensive Care Unit, Catholic Univ, Rome, Italy [7]
Intensive Care Unit, ASL Salerno 2, Eboli, Italy

Background: OTC gene mutations result in the X-linked Ornithine Transcarbamylase deficiency (OTCD). The phenotype is highly heterogeneous, ranging from acute neonatal hyperammonemic coma to asymptomatic adults.
Objectives: Clinical and molecular characterization with reference to environmental factors of 5 OTCD adult patients with fatal initial hyperammonemia.
Patients: Four males and one female, aged 21 to 66 years, developed sudden and acute hyperammonemia. The rapidly fatal course started with nausea, vomiting and consciousness disturbances and death occurred within a few days of admission to the critical care unit.
Results: Post-mortem OTC gene sequencing identified the novel c.314G>A (p.Gly105Glu), c.119G>T (p.Arg40Leu) substitutions and the known p.Arg40His, p.Arg277Trp, p.Ala208Thr late onset OTCD mutations. The p.Gly105Glu seems to interfere in the assembly of the trimer leading to a partially functional protein (patient's OTC activity 10%). The change from basic to hydrophobic residue due to p.Arg40Leu, may alter the net charge of the protein and lead to residual enzymatic activity. Interestingly, 3/5 mutations (p.Arg40Leu/His and p.Arg277Trp) affect CpG dinucleotides which represent mutational hot spots. A retrospective analysis of the patients' history revealed the probable triggering events: diet changes, cortisone therapy, chemotherapy and hormone therapy for in vitro fertilization.
Conclusions: In the critical care setting, OTCD should be considering an important cause of acute neurologic decline and fatal hyperammonemia in previously healthy adults. The disease's expression may be influenced by a combination of genetic and environmental factors; the latter may induce significant catabolic events able to upset the patient's homeostasis and interfere with residual OTC activity.

373-P
HYPERARGININEMIA PRESENTATION WITH SPEECH DISORDER IN TWO SIBLINGS

Soyucen E[1], Ozudogru S[2], Cetin K[2], Altay S[1], Onal H[3], Adal E[3], Aydin A[1]
[1]Dep Ped Metab Dis, Cerrahpasa Med Fac, Istanbul, Turkey
[2]Cerrahpasa Med Fac, Istanbul, Turkey
[3]Dep Ped Metab Dis,Bakirkoy Mat Child Hos, Istanbul, Turkey

Hyperargininemia is caused by a defect in the arginase 1 enzyme, which is involved in the last step of the urea cycle. It presents in childhood between 2 and 4 years of age with progressive cognitive deficits, epilepsy, and progressive spastic diplegia. In this report, Case 1 is an 8-year-old girl with non-specific liver function enzyme elevation, cerebral atrophy and speech disorders. She suffered from mental retardation and difficulties in speaking and walking. Case 2 was a 2-year-old girl, the sister of the 8-year-old diagnosed with hyperargininemia. She suffered only from speaking difficulties. The following was revealed by a Denver II Developmental Screening Test: social contact: 24 months old; fine motor skills: 20 months old; language: 8 months old; and gross motor skills: 23 months old. Given the observations from these cases, this report aims to show that speech disability was the first neurological symptom of hyperargininemia in our patients.

374-P
RAPID HPLC ESI-MS/MS METHOD FOR THE DIAGNOSIS OF UREA CYCLE DEFECTS

Rizzo C[1], Boenzi S[2], Goffredo BM[1], Benedetti S[1], Inglese R[1], Deodato F[2], Martinelli D[2], Bernardini C[1], Muraca M[1], Dionisi-Vici C[2]
[1]Metab. Laboratory, Bambino Gesù Hospital, Rome, Italy
[2]Div. Metabolism, Bambino Gesù Hospital, Rome, Italy

If not promptly recognized and treated, urea cycle defects cause severe neurological damage and/or death. It is therefore essential to obtain a rapid an accurate laboratory diagnosis once hyperammonemia has been detected. We describe a new MS/MS method which allows simultaneous quantitative determination in urine of urea cycle-related aminoacids (arginine, citrulline, glutamine, homocitrulline, ornithine, and argininosuccinate) and of orotic acid. HPLC separation of compounds was obtained with a column GEMINI-NX 3micron C-18 (Phenomenex, Italy) using two buffer gradient (H2O/MeOH+0.2%FOA) and subsequent detection by MS/MS, with a total time for sample preparation and analysis of 20 minutes. The method was linear for all metabolites measured in the range of 0.05–10 μmol/l (R^2>0.9996), with the only exception of citrulline for which the linearity range was 0.05–5.0 μmol/l. The detection limits were: citrulline 0.05 μmol/l, arginine and orotic acid 0.5 μmol/l, glutamine and ornithine 1.0 μmol/l. The limits of quantification were 0.1 μmol/l for citrulline and 1.0 μmol/l for the remaining metabolites. The determination of argininosuccinate was only qualitative because of co-elutation of the chromatographic peak with the corresponding anhydride(s). The intraday and interday coefficients of variations were below 7% for all metabolites in the respective concentration ranges. Clinical reference values were obtained from 50 control urine samples. The validity of the method and its clinical reliability was confirmed by retrospective analysis on samples obtained from patients with known urea cycle defects (OTC, AL, and AS defects and HHHs).

375-P

NEUROLOGICAL OUTCOME OF PEDIATRIC PATIENTS WITH UREA CYCLE DISORDERS

Arnoux JB[1], Dupic L[2], Barbier V[1], Boddaert N[3], Ottolenghi C[4], Rabier D[4], Valayannopoulos V[1], de Lonlay P[1]
[1]C Ref Maladies Metab, Hop Necker, APHP, Paris, France
[2]Réa Ped, Hop Necker, APHP, Paris, France
[3]Sce Radio Ped, Hôp Necker, APHP, Paris, France
[4]Lab Bioch Metab, Hop Necker, APHP, Paris, France

Background: Urea cycle disorders (UCD) are severe metabolic disorders leading to hyperammonemia related brain damage. The objective is to precise the neurological outcome of UCD patients and to definite predictive factors for poor neurological outcomes in neonatal-onset patients.
Method: We reviewed the data of 58 patients diagnosed with UCD and followed in our department between 1995 and 2008 (35 neonatal-onset, 23 late-onset patients).
Results: 3 patients died during the neonatal period. In neonatal-onset patients, we observed more developmental delay (29%) than in late onset patient (22%), with mean developmental quotients respectively at 93 and 81,and more neurological deficiencies (71% of patients vs 53%, p=0.0159). The main impairments were (neonatal-onset vs. late-onset, % of patients) speech (29% vs. 11%), drawing (59% vs. 22%) and hyperactivity attention disorders (37% vs. 16%).
For neonatal hyperammonemic coma, a correlation was found between the neurological outcome and i) the value of the highest ammonemia ($p<0,05$), also ii) with the intensity of the ammonemia intoxication (hyperammonemia during > 90 h; area under the curve of hyperammonemia > 14,000 μmol.h/L, $p<0,05$) but not with the initial plasmatic glutamine, ammonemia, pH, neither with the delay between first symptoms of intoxication and the diagnosis.
Conclusion: This study confirms the high rate of developmental delay and subsequent neurological impairments in both neonatal-onset and late-onset UCD patients. Very high ammonemia and prolonged exposition to hyperammonemia during the revealing coma are associated with a higher risk of neurological impairments for neonatal-onset UCD patients.

376-P

GYRATE ATROPHY IN A GIRL WITH OTC DEFICIENCY

Abulhoul L[1], Skeath R[1], McSweeney M[1], Thompson D[1], Russell-Eggitt I[1], Saunders D[1], Dixon M[1]
[1]Great Ormond St. Hospital for Children, London, United Kingdom

Background: Ornithine transcarbamylase deficiency (OTC) is a urea cycle disorder that is associated with hyperammonemia. To our knowledge gyrate atrophy has not previously been described in OTC deficiency.
Case Report: We present a 13 year old girl with OTC deficiency who has mild learning difficulties. She was diagnosed at 4 years of age following an encephalopathic episode. Diagnosis was made both biochemically and mutation analysis. Treatment was commenced with arginine and low protein diet but due to poor metabolic control she required nitrogen scavengers. Unfortunately, medical compliance was poor. At 12 years, a routine optician review, found retinal abnormalities. Fundus examination showed localized areas of chorio-retinal atrophy in the peripheral retina as seen in ornithine delta-aminotransferase deficiency (OAT). An electro-oculogram measure of retinal pigment epithelium and photoreceptor inter-action was just subnormal [Arden index 1.7 cf lower limit of normal 1.8–2.0]. The patient had normal plasma ornithine and persistently low arginine concentrations. Brain MRS demonstrated a low creatine peak. Urine creatine and guanidinoacetate to creatinine ratio were both low.
Discussion: The pathophysiology of OAT deficiency is not fully understood but reduced creatine synthesis is one possible hypothesis. We propose the gyrate atrophy seen in our patient may be due to creatine deficiency secondary to prolonged arginine deficiency.

377-P

MEASUREMENT OF OROTIC ACID IN PLASMA FOR THE DIAGNOSIS OF UREA CYCLE DISORDERS

Turner C[1], Dalton RN[2]
[1]WellChild Lab, Evelina Children's Hosp, London, United Kingdom
[2]Wellchild Lab, King's College London, London, United Kingdom

Background: The measurement of urinary orotic acid is mandatory for the differential diagnosis of hyperammonaemia. Since orotic acid is efficiently cleared by the kidney, blood levels are relatively low, representing an analytical challenge. Improvements in sensitivity of LC-MSMS instrumentation mean that plasma or dried blood spot (DBS) orotic acid can be measured, raising the possibility of adding this biomarker to neonatal screening & acute diagnostic protocols. We have therefore developed an assay for plasma/DBS orotic acid.
Method: Plasma (5 μl) or DBS (3 mm) were extracted with 150 μl methanol/water containing stable isotope labeled internal standards. This preparation allows measurement of amino-acids, acylcarnitines & other metabolites from separate injects in positive or negative ion mode. 5 μl of supernatant was injected onto a Chirobiotic T column (Sigma) and data collected in negative ion MRM mode (ABSciex API5000, Orotate m/z 154.9/111.1, 15N13C Orotate m/z 156.9/113.1) .
Results: Inter-assay imprecision was 0.5% at 102 μmol/L, 4.3% at 19.8 μmol/L & 21.6% at the limit of quantitation; set at 0.5 μmol/L). In 96 patients under investigation for metabolic disease without urea cycle defects, plasma orotate was <0.5 μmol/L, including 1 patient with severe hyperammonaemia secondary to MMA. In a male infant with OTC on day 1 plasma orotate was 62.5 μmol/L, in 2 patients presenting acutely with citrullinaemia, 146 & 46.2 μmol/L and in 2 unwell female patients with partial OTC, 41.1 & 2.4 μmol/L.
Conclusion: Orotic acid can be measured conveniently in plasma/DBS, allowing rapid differential diagnosis of hyperammonaemia. Further studies are required to evaluate this biomarker in newborn screening.

378-P

ORNITHINE TRANSCARBAMYLASE DEFICIENCY IN A GIRL- CASE REPORT

Mikhaylova SV[1], Mathina IA[1], Baydakova GV[1], Zakharova EY[2], Bologov AA[1]
[1]Russian Children Clinical Hospital, Moscow, Russian Federation
[2]Lab INH Met Dis, Res Cen Med Gen, Moscow, Russian Federation

Ornithine transcarbamylase deficiency (OTCD) is an X-linked disorder of urea cycle. A deficiency of the enzyme leads to hyperammonemia. In boys the disease is often manifest in the neonatal period, but heterozygous girls frequently have late onset. In a number of inherited metabolic diseases the hepatopathy is main clinical manifestation, in OTCD liver involvement is seen less frequently and may be misdiagnosed.
We describe the girl first brought to medical attention after acute respiratory infection at 1y 6 month. During illness she developed vomiting, gait disturbance, aggressive behavior and hepatomegaly (+ 3 sm). Laboratory studies showed transaminase elevations (ASAT 1331 U / l, ALAT 1080 U / l) and anemia (Hb 89 g / l). After the infusion therapy she become more active, transaminases level were decreased. Metabolic evaluation found hyperammonemia, increased urinary excretion of orotic acid -1086 mM / Mcreatinine, but level of amino acids in blood were normal. De novo mutation Pro305Leu has been found in the OTC gene in heterozygous state. These findings suggested the diagnosis of OTC deficiency. The administration of a low-protein diet (1 g/kg/day), L-arginine, and L-carnitine led to improvement of hepatic function, normalization of the ammonia level and other biochemical parameters, including the transaminases. This case report suggests the importance to perform orotic acid analysis in all patients with hepatopathies and acute encephalopathy.

379-P

INVESTIGATION IN PATIENTS WITH SUSPECTED INBORN ERRORS OF BILE ACID METABOLISM

Ferrer I[1], Garrido M[1], García MJ[1], Merinero B[1], Pérez-Cerdá C[1], Ugarte M[1], Ruiz-Sala P[1]
[1]*CEDEM, CIBERER, UAM, Madrid, Spain*

We studied 186 patients with suspected bile acid (BA) synthesis defects over a 4 year period. Most of the patients presented with liver disease, 3 patients were suspected to have cerebrotendinous xanthomatosis (CTX) and 11 were suspected of having a peroxisomal disorder (PD). Free and conjugated BA in plasma and conjugated BA in urine were determined by HPLC/MS/MS.

Three patients showed an elevation of cholenoic acids conjugated with glycine and/or sulfate and not detectable primary BA. This profile was consistent with a 3beta-hydroxy-Delta5-steroid dehydrogenase deficiency, to date, genetically confirmed only in one patient. Slight excretion of these unsaturated BA joined to primary BA was found in other 5 patients, with cause still unknown.

In one patient, high excretion of oxocholenoic acids (98% of the total BA) was compatible with a 3-oxo-Delta4-steroid-5beta-reductase deficiency, but mutations in the AKR1D1 gene were not found. In another 17 patients, less excretion of these acids was considered secondary to liver disease. Only one patient suspecting CTX had elevation of glucuronides of bile alcohols in plasma and urine. Mutation analysis confirmed the disease. All the patients with suspected PD had increases of plasma trihydroxycholestanoic acid and urine tauro-tetrahydroxycholestanoic acid. Ten patients had peroxisome biogenesis disorders and one patient was deficient in bifunctional protein.

Sixty-nine patients showed elevations of only primary BA, secondarily to the liver disease.

These analyses enable to diagnose BA synthesis diseases and contribute with useful data for other disorders. However, more evidence and research are needed to understand all the information provided.

380-P

HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA (FH) IN GREECE: EXPERIENCE FROM 33 PATIENTS

Drogari ED[1], Progias PP[1], Mavroidis NM[1], Laios EL[1], Koniari EK[1], Skouma AS[1], Mollaki VM[1]
[1]*Univ Child Hosp, Athens, Greece*

FH is an autosomal dominant disease with a frequency of 1:500 and 1:106 in its heterozygous and homozygous form respectively. It is mainly mutations in the Low Density Lipoprotein Receptor gene (LDLR) that cause high LDL and total cholesterol levels. Homozygous patients show a 6 to 8-fold increase in LDL levels, premature atherosclerosis and myocardial infarction before the 3 rd decade of life. Therefore, it is critical to identify and diagnose the disease in early childhood. The aim of the study was the clinical and molecular diagnosis of homozygous FH children and adults in Greece.

Forty clinically diagnosed homozygous FH patients from 33 families were examined. The molecular diagnosis was performed with direct DNA sequencing of the LDLR gene or restriction fragment length polymorphism analysis. The patients showed 480 2000 mg/dL total cholesterol, 4 of them had corneal arcus and all had tendom xanthomas. We found 8 Val408Met homozygotes, 6 Gly528Asp homozygotes, 4 Ser265Arg homozygotes, 1 Cys6Trp homozygote, and 1 Cys313Stop homozygote,. We also found 7 compound heterozygotes Ser265Arg/Val408Met, 2 compound heterozygotes Gly528Asp/Ser265Arg and 4 compound heterozygotes Gly528Asp/Ser265Arg, Gly528Asp/Gln233Pro, Ser265Arg/Cys152Arg, Cys6Trp/Ile420Asn, Ser265Arg/Cys313Stop, and Val408-Met/Cys313Stop, respectively.

The number of homozygous patients confirms that the disease is underdiagnosed, at least in Greece, since consanguinity is very uncommon. The lipid levels were different among the homozygotes carrying the same mutation(s), suggesting that there are other genetic and/or environmental factors affecting cholesterol metabolism.

381-P

A NEW INBORN ERROR OF BILE ACID SYNTHESIS BILE ACID-CoA LIGASE DEFICIENCY

Chong CPK[1], Mills PB[1], McClean P[2], Clayton PT[1]
[1]*UCL Institute of Child Health, London, United Kingdom*
[2]*St James's Hospital, Leeds, United Kingdom*

So far, the only gene shown to be mutated in children with a bile acid amidation defect is the BAAT gene encoding bile acid-CoA: amino acid N-acyl transferase. A female infant, born at 27 weeks gestation to parents of Pakistani origin required a prolonged period of parenteral nutrition. She developed conjugated hyperbilirubinaemia which persisted until the age of 12 mo. A liver biopsy showed portal to portal bridging fibrosis. By 18 mo liver function tests had returned to normal.

Analysis of urine bile acids by ESI-MS/MS showed unamidated cholic acid and chenodeoxycholic acid (m/z 407 and 391), their glucuronides (583, 567) and chenodeoxycholic acid sulphate (471). Analysis of plasma bile acids (PBA) by GC-MS, with and without de-amidation, showed the PBA were 89% unamidated (normal < 20%). No mutations were found in the BAAT gene. We therefore investigated the bile acid-CoA ligase encoded for by the SLC27A5 gene. Sequence analysis showed that the patient was homozygous for a mutation in this gene—His338Tyr; c.1012c>t. This mutation was confirmed by a restriction enzyme test and was not present in 50 ethnically matched DNA samples; the parents were shown to be heterozygotes. Sequence alignment showed that the mutation is in a highly conserved area of the gene suggesting that it is important for enzyme activity. This is the first reported mutation in the SLC27A5 gene. Parenteral nutrition may have been needed because of fat malabsorption but PN may also have contributed to the cholestatic liver disease.

382-P

RESPONSE TO CHENODEOXYCHOLIC ACID THERAPY IN AN INFANT WITH OXYSTEROL 7ALPHA-HYDROXYLASE DEFICIENCY

Chong CPK[1], Mills PB[1], McClean P[2], Clayton PT[1]
[1]*St James's Hospital, Leeds, United Kingdom*

Mutations in the CYP7B1 gene encoding oxysterol 7alpha-hydroxylase have been described in two infants with fatal liver failure and in older children and adults with recessive hereditary spastic paraplegia with loss of vibration sense and proprioception. Attempts to treat the liver disease with ursodeoxycholic acid and with cholic acid have been unsuccessful.

An infant of Pakistani descent presented at the age of 3 weeks with (mostly unconjugated) hyperbilirubinaemia. At 3 mo he developed a coagulopathy, hypoalbuminaemia, irritability and episodic hypoglycaemia. ALT was mildly elevated and GGT normal. On ursodeoxycholic acid treatment, his condition continued to deteriorate. Analysis of a (pre-treatment) urine sample by ESI-MS/MS showed that the major urinary bile acid was 3beta-hydroxy-5-cholenoic acid, present principally as the taurine conjugate (m/z 480), and the sulphate (453). Analysis of plasma by GC-MS showed the presence of increased amounts of 27-hydroxycholesterol and 3beta-hydroxy-5-cholestenoic acid. These findings have only been described in patients with oxysterol 7alpha-hydroxylase deficiency.

When started on treatment with chenodeoxycholic acid, his condition improved within 3 days. A liver biopsy (undertaken a week later) showed a giant cell hepatitis with marked fibrosis (probably cirrhosis) and micro- and macro-vesicular steatosis.

At the age of 3y, on chenodeoxycholic acid (8 mg/kg/d), he is thriving with normal liver function tests.

Ursodeoxycholic acid, cholic acid and chenodeoxycholic acid have different properties with regard to activation of receptors (such as FXR) and feedback inhibition of the bile acid synthesis pathways. They are not therapeutically equivalent.

383-P

NEWBORN SCREENING FOR CONGENITAL ADRENAL HYPERPLASIA (21-CAH) IMPROVED BY LC-MS/MS STEROID PROFILING

Janzen N[1], Sander S[2], Terhardt M[2], Illsinger S[1], Peter M[2], Das AM[1], Sander J[2]
[1]*Clin Pediatrics, Hannover Medical School, Hannover, Germany*
[2]*Screenng-Laboratory Hannover, Hannover, Germany*

Background: Immuno assays for 17-hydroxyprogesterone are the standard procedure for 21-CAH screening. Due to cross reactions a high number of false positives are generated especially in preterms even when cut offs are corrected for gestational age.
Objective: The aim of this study was to improve 21-CAH screening applying a more sensitive tandem mass spectrometric second tier method and to reduce the high false positive rate.
Patients and methods: During 2009 we tested 158.851 samples by time resolved immuno assay for 17-hydroxyprogesterone. All samples of preterms and all positives were additionally analysed by LC-MS/MS for 17- hydroxyprogesterone, 11-deoxycortisol, 21-deoxycortisol, cortisol and androstendione.
Results: There were 13 confirmed cases of 21-CAH (0.008%). Based on immuno assays, 886 positive results were obtained requiring retesting of newborns (PPV: 1.47 %). LC-MS/MS profiling resulted in 65 positives (PPV: 20%). In 5.304 samples 22–34 weeks gestational age the percentage of positives was 7.1 with immuno assay and 0.77 with LC-MS/MS, the percentages were 0.78 and 0.03 at 35–38 weeks and, 0.2 and 0.01 % for gestational weeks 39–44. Analysis of 21-desoxycortisol as a pathognomonic parameter improved the detection of 21-CAH significantly. The remaining steroids showed a typical pattern in stressed newborns.
Conclusion: LC-MS/MS profiling for steroids is able to reduce the rate of false positives considerably. There are only very few false positives in term neonates. In preterms false positives are reduced by a factor of 9.

384-P

FATTY LIVER AND DYSLIPIDEMIA AS IMPORTANT CLUES FOR DIAGNOSIS OF CHOLESTEROL ESTER STORAGE DISEASE

Kalkan Ucar S[1], Church H[2], Savage W[2], Habif S[3], Coker M[1]
[1]*Div Metab Dis, Ege Univ Child Hosp, Izmir, Turkey*
[2]*Willink Lab, Gen Med, Manchester, United Kingdom*
[3]*Div Bioch, Ege Univ Hosp, Izmir, Turkey*

The key role of the enzyme lysosomal acid lipase/cholesteryl ester hydrolase, responsible for the intralysosomal metabolism of cholesteryl esters and triglycerides, is illustrated by two lysosomal storage diseases inherited as autosomal recessive traits: Wolman and Cholesteryl ester storage disease (CESD). Wolman disease is associated massive intracellular substrate accumulation and is always fatal in early infancy. However, CESD is characterized by very low levels of enzymic activity sufficient to allow survival of the affected patients into adulthood.
A 15-year-old boy was referred for persistence of elevated serum alanine aminotransferase and aspartate aminotransferase, combined with hyperlipidemia (Total cholesterol 323, LDL-Cholesterol 159 mg/dl) after the experience of hepatitis B. All family members had normal lipid profile and liver function tests. His physical examination revealed a slight hepatomegaly. Infectious and autoimmune causes of elevated liver enzymes were ruled out. Low acid lipase activity was demonstrated (33 nmol/h/ per mg protein, normal 350–2000) leading to diagnosis of CESD. He had elevated chitotriosidase and atypical fatty liver disease in the absence of overweight, convenient with CESD.

385-P

CLINICAL OBSERVATIONS, MOLECULAR GENETICS ANALYSIS AND TREATMENT OF SITOSTEROLEMIA IN INFANTS AND CHILDREN

Niu D[1], Chong K[1], Hsu J[2], Wu J[2], Yu H[2], Huang C[2], Lo M[2], Kwok C[3], Kratz LE[4], Ho L[5]
[1]*Inst Clin Med, Ntl Yang-Ming Univ, Taipei, Taiwan*
[2]*Dep Pedia, Taipei Veterans General Hosp, Taipei, Taiwan*
[3]*Medicine, Tpe Veterans General Hosp, Taipei, Taiwan*
[4]*Bio Gen Lab, Kennedy Krieger Institute, Baltimore, United States*
[5]*Med Res & Edu, Veterans General Hosp, Taipei, Taiwan*

Background: The clinical observation and treatment of young children with sitosterolemia has rarely been reported. While ezetimibe is highly effective in adults presenting with sitosterolemia, its efficacy in young children has rare been described.
Patients' profiles, methods and therapeutic outcomes: We report clinical, biochemical and molecular genetic observations, and the treatment outcomes of 5 Chinese children presenting with sitosterolemia, from 4 separate families, in whom we identified 2 new (Y329X, G269R) and 3 known (R446X, N437K, R389H) mutations in ABCG5 gene. R389H mutation was found in 50% of the alleles. In this study, a female infant, despite on complete breast feeding from birth and 4 months of ezetimibe administration had the serum cholesterol rising up to 654 mg/dl at 7 months old. Although she failed to respond to ezetimibe at infant period, she started to have a fair response to this medicine at 2 years of age. Another 23-month-old female patient also had a slower response to treatment with ezetimibe than elder patients.
Conclusions: The response to ezetimibe in very young sitosterolemia children (< 2 years) may not be as well as the elder patients.

386-O

NEUTRAL LIPID STORAGE DISEASE PRESENTS WITH SEVERE DILATED CARDIOMYOPATHY AND DEFECTED ADIPOSE TRIGLYCERIDE LIPASE (ATGL) GENE

Rahman Y[1], Olpin S[2], Desphande C[3], Coassin S[4], Kronenberg F[4], Carr-White G[5]
[1]*Dep Inh Metab Dis, GSTFT, London, United Kingdom*
[2]*Dpt Clin Chem, Sheffield Child Hosp, Sheffield, United Kingdom*
[3]*Dep Clinical Genetic, GSTFT, London, United Kingdom*
[4]*Div Genetic Epi, Innsbruck Med Uni, Innsbrukc, Austria*
[5]*Cardiology Dep, GSTFT, London, United Kingdom*

Neutral lipid storage disease (NLSD) is a group of rare, autosomal recessive disorders characterized by an excessive accumulation of triglyceride-containing cytoplasmic droplets in multiple tissues. Two genes, adipose triglyceride lipase (ATGL/PNPLA2) and comparative gene identification-58 (CGI-58/ABHD5) have been shown to cause NLSD. CGI-58/ABHD5 encodes for a protein that co-activates a newly identified lipase, ATGL that hydrolyses triacylglycerides (TAG) for phospholipid and free fatty acid production. Even though mutations in both genes produce similar changes (Jordan's anomaly) in a peripheral blood smear, the resulting clinical manifestations are very different. Here we report a case of 19-year-old male of consanguineous South Asian parents, who has been referred from our Cardiology Department with a severe dilated cardiomyopathy and generalized weakness, complicated by a small cardio-embolic infarct in the right parietal and cerebellum areas. Ejection fraction on the echocardiogram was 14%. No history of ichthyosis was reported in the patient or the family. General metabolic cardiomyopathy screening was negative apart from Jordan's anomaly observed on leucocyte histology suggestive of NLSD. Tritium-labelled oleate uptake study on the cultured-fibroblast showed increased accumulation compared with normal controls. Subsequent ATGL gene analysis showed a homozygous mutation D166G (c.497A>G). The residue D166 belongs to the DXG/A motif which has been reported to be critical for ATGL activity in murine cells. This is the first mutation being described affecting the catalytic domain in man. This case adds further understanding to the basic biology of fat storage and its clinical implications.

387-P

X-LINKED CHONDRODYSPLASIA PUNCTATA CPDX2: A RELIABLE BIOCHEMIST APPROACH WITH PERPLEXING GENETICS

Giros M[1], Cañueto J[2], de Unamuno P[2], Gonzalez-Sarmiento R[3], Artigas M[4], Ciria S[3], Pi-Castan G[5], Garcia-Dorado J[2], Torrelo A[6], Hernandez-Martin A[6], Martin E[7], Garcia-Patos V[8], Vendrell T[9], Fernandez-Burriel M[10], Metzenberg A[11], Pintos-Morell G[12]
[1]*Inborn Errors Metabolism, Hosp Clinic, Barcelona, Spain*
[2]*Dep Dermatology, Hosp Univ, Salamanca, Spain*
[3]*Medicina Molecular, Univ Salamanca, Salamanca, Spain*
[4]*Serv Genética, Hosp Virgen del Camino, Pamplona, Spain*
[5]*Serv Pediatría Hospital de la Ribera, Alzira-Valencia, Spain*
[6]*Serv Dermatología, Hosp Niño Jesús, Madrid, Spain*
[7]*Unit Mitocon-IEM, Hosp 12 Octubre, Madrid, Spain*
[8]*Serv Dermatol Hosp Univ Vall Hebron, Barcelona, Spain*
[9]*Serv Genetica, Hosp. Vall de Hebrón, Barcelona, Spain*
[10]*Unit Genetic, Sev Anal Clin, Hosp Merida, Badajoz, Spain*
[11]*Depar Biology, California Univ, Northridge, United States*
[12]*Pediatrcs Hosp Germans Trias i Pujol, Badalona, Spain*

X-linked dominant chondrodysplasia punctata (CDPX2; MIM302960) is a rare inherited disease mainly characterized by bone dysplasia, ichthyosis and cataracts. Intrafamiliar phenotype variation is a known feature of CPDX2 probably due to skewed X-inactivation. It is caused by mutations in the emopamil-binding protein, gene EBP, which encodes the delta8, delta7-sterol isomerase. The biochemical hallmark is plasma and tissue cholesterol and 8-dehidrocholesterol (8DHC) increase. We have studied 13 CDPX2 Spanish patients from 9 families. The diagnosis was made by the typical clinical signs in 10 children and after family studies in 3 adult mothers. All patients presented EBP mutations, 4 novel and 5 previously described. Sterol analysis was performed by gas-liquid chromatography in 10 cases. All presented altered ratio of 8DHC+cholestenol/ cholesterol (0.71 %-17.7%, normal range <0.2%). No phenotypic-genotypic correlation was recognized, but we found the highest sterol ratios in the more severe neonatal cases (17.7% and 8%) without age dependence. Despite CDPX2 being an inherited disease, sporadic cases are frequent. In familiar cases, anticipation, phenotypic variation and incomplete penetrance are common. Mutations were inherited in 33% of Spanish families with a very mild mother's phenotype in 2. Different prevalence of mutation transmission was found in other populations (5%, 27% and 50% USA, Europe, and Japan respectively). We postulate that in our population CDPX2 might be underestimated due to the very mild presentation. The reliable sterol analysis could be useful to establish the true frequency of this perplexing disease.

388-P

METABOLIC CORRECTION INDUCED BY LEPTIN SUBSTITUTION THERAPY IN CONGENITAL GENERALIZED LYPODISTROPHY

Blasco Alonso J[1], Serrano J[1], Navas VM[1], López Siguero JP[2], Ortiz Pérez P[1], Carazo B[1], Sierra C[1], Cochran EK[3], Gorden P[3]
[1]*Ped Gastroenterol, Hosp Materno-Infantil, Málaga, Spain*
[2]*Endocrinol Unit. Hosp Materno-Infantil, Málaga, Spain*
[3]*Clin Endocrin Branch, NIH Bethesda, Maryland, United States*

Congenital generalized lypodistrophy (CGL) is a dramatic rare disease, characterized by absence of adipose tissue, leading to marked hypertriglyceridemia and abnormal fat storage, explaining its severe complications associated (diabetes, liver steatosis, cirrhosis, insulin resistance and atherosclerosis). There is no known curative treatment so far. We describe the case of a Spanish patient suffering from CGL and treated with a novel therapy in agreement with a NIH Endocrinology Unit in the USA.

A 5 year-old male boy with weight and height at 10 and 25 percentiles, with no fat in cheeks, hepatomegaly, no splenomegaly and acanthosis nigricans. Fasting hyperglycemia (174 mg/dl) and two hours after oral overload (213 mg/dl), insulin level of 190μU/ml, low fasting leptin levels (0.7 ng/ml), normal leptin receptor (45U/ml), low adiponectin (0.3 μg/ml) AST 141 IU/lL ALT 302 IU/L, GGT 324 IU/l, hypertriglyceridemia (2984 mg/dl), creatinine 0.3 mg/dl, total cholesterol 170 mg/dl with low HDL cholesterol (25 mg/dl), very low C peptide (23 ng/ml). He also needed high metformine doses (1500 mg/day) with glycosylated haemoglobin (HbA1c) 10.6%. After poor metabolic control, we got in touch with Bethesda's NIH (USA), and started daily subcutaneous human recombinant leptin (r-metHuLeptin), 1.6 mg/day, tapering it up to 2 mg/day. 12 months later, the glycemic control improved significantly (HbA1c 5.3%, normal glucose and insulin) as well as the triglyceride levels (254 mg/dl), allowing reduction in metformine dose. An abdominal ultrasound showed hepatomegaly but now almost normal texture of the liver.

Substitutive treatment with recombinant leptin is effective in insulin resistance management, allowing a better triglyceride control and reducing liver steatosis.

389-P

GLOBOTRIAOSYLCERAMIDE EXPRESSION IN HUMAN PLACENTAL CAPILLARIES

Hulkova H[1], Elleder M[1], Smid F[2], Ledvinova J[1], Kuchar L[1]
[1]*Inst Inherit Metab Dis First Fac Med, Praha, Czech Republic*
[2]*Inst Clin Bioch Lab Diagn First Fac Med, Praha, Czech Republic*

A series of placentas at term were examined using immunohistochemical in situ analysis of globotriaosylceramide (Gb3Cer), lactosylceramide, and GM1 ganglioside, the latter with cholera toxin-B subunit. Results of in situ analyses were correlated with extra situ biochemical analysis and with tandem mass spectrometry. Immunohistochemical study showed uniform distinct staining for Gb3Cer at the apical membrane of fetal villous capillary endothelial cells. The lipid was not detectable in endothelial cells of umbilical vessels and in capillaries in somatic structures (heart, skin, skeletal muscle) of neonates and adults. Endothelial cells in the maternal uteroplacental vessels in the basal plate displayed variable positivity for Gb3Cer. Detailed comparison with capillaries in non-pregnant endometrium is planned. The so far results (limited amount of samples) showed absence of Gb3Cer in proliferative endometrium. Besides that Gb3Cer was detected in heparinocytes and in stromal cells of placental villi. Trophoblast, amnionic epithelium and vascular smooth muscle cells were negative. In situ correlation of Gb3Cer with other glycosphingolipids showed coexpression with GM1 ganglioside only in endothelial cells of fetal villous capillaries. Lactosylceramide was restricted to Hoffbauer cells. The finding points to specific glycolipid microdomains of endothelial cells of placental villous capillaries which may reflect specific function.

390-P

SMITH-LEMLI-OPITZ SYNDROME; THE BRISTOL EXPERIENCE

Brown A Y[1], Sawyer H[2], Greenslade M[2], Burton-Jones S[2], Murdoch-Davis C[1], Williams M[2], Kemp H J[1]
[1]Biochemical Genetics, Southmead Hospital, Bristol, United Kingdom
[2]Bristol Genetics Lab, Southmead Hospital, Bristol, United Kingdom

Smith Lemli Opitz syndrome (SLOS) is an autosomal recessive congenital multiple malformation disorder caused by a deficiency of 7-dehydrocholesterol reductase (DHCR7), the last enzyme of cholesterol synthesis. Pathogenic mutations in the DHCR7 gene exhibit a broad clinical phenotype expressing severity from intrauterine lethality to mild dysmorphism/mental impairment. Over 130 DHCR7 mutations are described and most individuals are compound heterozygotes.

The carrier frequency is estimated at 3% in Caucasian populations however the UK incidence of SLOS estimated at 1/15,000–1/60,000 births is lower than expected, probably due to under-diagnosis (particularly in females) and lethality of severe cases in utero.

Diagnosis of SLOS by direct analysis of 7-dehydrocholesterol (sterol extraction, derivitisation, identification and quantitation by GC/MS) in plasma or tissue has been available in Bristol since 1995 identifying 30 diagnoses from 671 analyses (4.5%). DHCR7 gene testing introduced in September 2009, using direct bi-directional sequencing (ABI3730) of coding exons 3 to 9, now enables provision of a comprehensive SLOS service facilitating diagnosis, clarification of difficult cases, carrier identification and rapid pre-natal diagnosis.

We present a review of patients biochemically tested to date and describe four, including 1 still birth*, in detail.

i) c.964–1G>C/c.452G>A,p.Trp151X*,
ii) c.964–1G>C/c.1022 T>C,p.Leu341Pro
iii) c.461C>T,p.Thr154Met/ c.461C>G,p.Thr154Arg
iv) c.1210C>T,p.Arg404Cys/c.564G>A,p.Trp182X**

All four are compound heterozygotes, two exhibit the common mutation, c.964–1G>C (an intron 8 variant) and in one case a novel variant** (c.564G>A,p.Trp182X), predicted to result in a truncated protein and possible mRNA nonsense-mediated decay, was identified.

391-P

THE MOLECULAR GENETICS ANALYSIS OF 21-HYDROXYLASE DEFICIENCY IN CZECH POPULATION

Vrzalova ZV[1], Hruba ZH[1], Stahlova Hrabincova ESH[1], Fajkusova LF[1]
[1]Cen Mol Biol, Univ Hosp and Fac Medic, Brno, Czech Republic

Background: Congenital adrenal hyperplasia (CAH) is a group of inherited diseases, in which cortisol secretion is impaired. About 90% of CAH cases is associated with mutations in the CYP21A2 gene encoding the steroid 21-hydroxylase.

Objective: Molecular genetic analysis of CAH was performed in 1266 Czech individuals from 505 unrelated families. The 21-hydroxylase deficiency was confirmed in 267 probands.

Methods: We have used a long-range PCR based approach which permits differential amplification of the CYP21A2 and CYP21A1P genes, followed by direct probing for presence of known mutation sites in a secondary PCR analysis, further sequencing and MLPA method.

Results: In 267 Czech probands with 21-hydroxylase deficiency were identified 30 different CYP21A2 mutant alleles. The most frequent mutation, a chimeric CYP21A1P/CYP21A2 gene, was found in 33,7% of mutant alleles. Small DNA rearrangements of the CYP21A2 gene, derived and non-derived from CYP21A1P, were present in 56,8% and 2,4% of mutant alleles, respectively. Total deletions of CYP21A2 were detected in 4,9% of CYP21A2 mutant alleles whereas duplications of CYP21A2 associated with a mutation on both copies (p.Ser97fsX12 and p.Gln318X) were less frequent (0,4% of mutated alleles). To date, no mutations were determined in 4,0% of CYP21A2 mutant alleles.

Conclusions: Our genotyping approach allowed accurate identification of CYP21A2 gene mutations in 21-hydroxylase deficiency patients and their families and can be used for final confirmation of diagnosis and for the prenatal diagnostics.

392-P

MUTATION ANALYSIS IN ROMANIAN GAUCHER DISEASE PATIENTS

Drugan C[1], Catana C[1], Drugan T[1], Grigorescu-Sido P[1], Caillaud C[2]
[1]Univ Medicine Pharmacy, Cluj-Napoca, Romania
[2]Institut Cochin, Univ Paris V, Paris, France

Background: Gaucher disease, caused by an inherited deficiency of the enzyme glucocerebrosidase, is the most prevalent lysosomal storage disease in Romanian patients. We investigated the distribution of frequent mutations in the acid beta-glucocerebrosidase gene and the genotype-phenotype correlations in 55 patients of Caucasian, non-Jewish ethnicity.

Methods: Clinical phenotypes were determined for each patient: 53 patients were confirmed with type 1 disease, and 2 patients had type 3 disease. The point mutations N370S, L444P, R463C and 84GG were screened by PCR amplification and restriction enzyme digestion, whereas the recombinant alleles recNciI and recTL were detected by direct sequencing of the amplified fragments.

Results: Our results indicate a high prevalence of the N370S allele (53.6%), followed by the mutations L444P (16.4%), recNci I (3.6%), R463Q (3.6%) and R463C (1.8%). Sporadic mutations accounted for 20.9% of the disease producing alleles. Genotype phenotype correlations were similar to those reported for other Caucasian populations. The presence of the N370S allele excluded the development of neurological symptoms, while the L444P allele was generally associated with a more severe course of the disease. Type 3 patients were either homozygous for the L444P mutation, or compound heterozygous for this allele and an uncharacterised, private mutation. However, a high degree of clinical variability was observed among patients with the same genotype.

Conclusions: Our study confirms the previously reported genotype-phenotype correlations, as well as the predominance of frequent mutations, representing 79.1% in our patients.

393-P

THE FIRST LARGE DELETION DESCRIBED IN NIEMANN-PICK TYPE C DISEASE

Macías-Vidal J[1], Rodríguez-Pascau L[2], Lluch M[1], Dalmau J[3], Vilageliu L[2], Grinberg D[2], Coll MJ[1]
[1]Inst Clin Bioch, Hosp Clínic, CIBERER, Barcelona, Spain
[2]Dep Genet, Barcelona Univ, IBUB, CIBERER, Barcelona, Spain
[3]Dep Nutr & Metab, Child Hosp La Fe, Valencia, Spain

Background: Mutations in NPC1 or NPC2 genes are responsible of Niemann-Pick type C (NPC) disease (OMIM #257220), an autosomal recessive neurodegenerative lysosomal storage disorder caused by an incorrect regulation of intracellular lipid trafficking.

Case report: We examined cDNA and genomic DNA of a classical NPC biochemical phenotype patient. The first analysis of samples from patient revealed homozygosity for mutation p.T1066N. However, while the father was heterozygous for this change, the mother did not carry the mutation. The analysis of six polymorphisms (p.Y129Y, p.H215R, p.M642I, p.I858V, p.N931N and p.R1266Q) within the NPC1 gene in the patient and his parents suggested a deletion of the maternal allele.

Objectives: To confirm the presence of a deletion in one allele and to determine its limits.

Methods: 1) Relative quantification of the NPC1 DNA using the Comparative Ct (ΔΔCt) method in a real-time PCR system. 2) Analysis by PCR amplification and sequencing of 71 polymorphisms located in NPC1 and in the flanking genes CABLES1, C18orf45, C18orf8, ANKRD29, LAMA3, TTC39C, OSBPL1A, IMPACT and ZNF521.

Results: Quantitative PCR analyses revealed that the patient and the mother have half of the amount of NPC1 gDNA. The polymorphism analysis indicated that the centromeric limit of the deletion was within intron 13 of C18orf45 and the telomeric limit was at intron 11 of OSBPL1A. Therefore, the deletion expands, at the most, 1 Mb approximately.

Conclusion: We describe a large deletion in one Niemann-Pick type C patient, which to our knowledge it is the first described in this disease.

394-P

AUTOPHAGOSOME MATURATION IS IMPAIRED IN FABRY DISEASE

Chévrier M[1], Brakch N[2], Lesueur C[1], Genty D[3], Moll S[4], Djavaheri-Mergny M[5], Brasse-Lagnel C[1], Laquerrière A[3], Barbey F[6], Bekri S[1]
[1]Biochimie Médicale CHU Rouen EA4309, Rouen, France
[2]Div. Vascular Med, CHUV Lausanne, Lausanne, Switzerland
[3]Anat. Cytol. Path., CHU Rouen EA4309, Rouen, France
[4]Service de Pathologie HU Genève, Genève, Switzerland
[5]INSERM U916 Vinco, Bordeaux, France
[6]Transplantation Center CHUV Lausanne, Lausanne, Switzerland

Fabry disease is a lysosomal storage disorder (LSD) caused by a deficiency in α-galactosidase A. The disease is characterized by severe major organ involvement, but the pathologic mechanisms responsible have not been elucidated. Disruptions of autophagic processes have been reported for other LSDs, but have not yet been investigated in Fabry disease. Renal biopsies were obtained from 5 adult male Fabry disease patients before and after 3 years of enzyme replacement therapy (ERT) with agalsidase alfa. Vacuole accumulation was seen in renal biopsies from all patients compared with control biopsies. Decreases in the number of vacuoles were seen after 3 years of ERT primarily in renal endothelial cells and mesangial cells. Measurement of the levels of LC3, a specific autophagy marker, in cultured cells from Fabry patients revealed increased basal levels compared to cells from non-Fabry subjects and a larger increase in response to starvation than seen in non-Fabry cells. Starvation in the presence of protease inhibitors did not result in a significant increase in LC3 in Fabry cells, whereas a further increase in LC3 was observed in non-Fabry cells, an observation that is consistent with impaired autophagic flux in Fabry disease. Overexpression of LC3 mRNA in Fabry fibroblasts compared to control cells is consistent with an upregulation of autophagy. Furthermore, LC3 and p62/SQSTM1 (that binds to LC3) staining in renal tissues and in cultured fibroblasts from Fabry patients supports impairment of autophagic flux. These findings suggest that Fabry disease is linked to a deregulation of autophagy.

395-O

THE APPLICATION OF MULTIPLEXED, MULTI-DIMENSIONAL ULTRA HIGH PRESSURE LIQUID CHROMATOGRAPHY/ TANDEM MASS SPECTROMETRY TO THE HIGH THROUGHPUT SCREENING OF LYSOSOMAL STORAGE DISORDERS IN NEWBORN DRIED BLOODSPOTS

Herman JL[1], Shushan B[2], De Jesus VR[3], Kasper DC[4]
[1]ThermoFisher Scientific, Franklin, MA, United States
[2]Clinical Mass Spec Consultants, Toronto, OR, Canada
[3]NBS & Mol Bio, CDC, Atlanta, GA, United States
[4]Med Univ of Vienna, Dept of Ped & Ad Med, Vienna, Austria

Lysosomal storage disorders (LSDs) are characterized by low activities of particular enzymes found in the lysosome. Newborn screening assays for the five most common LSDs have been reported using tandem mass spectrometry (MS/MS) to measure the formation of product from incubations of dried blood spots (DBS) (1,2). Both on-line and off-line solid phase extraction (SPE) have been used to reduce matrix effects from the extracts from incubated DBS samples. The off-line SPE is time consuming but allows one minute per sample run times. The on-line SPE reduces sample preparation time but the MS/MS run is four minutes per sample, given that it requires a liquid chromatography step to separate all products and internal standards present in each sample. There is also interference from the substrate with the on-line SPE method that may not be completely removed by the methodology described.

We describe an on-line clean-up method for LSD newborn screening enzymatic analysis using Turbulent Flow Chromatography (TFC) and Ultra High Pressure Liquid Chromatography (UHPLC). The proposed sample preparation method allows direct injection of the incubated DBS samples without further purification. UHPLC separates all the substrates from all the products removing matrix effects from the incubation. The use of multiplexing technology allows sample to sample run times of 75 seconds. The method was validated against pure standards and DBS quality control materials. A set of sample retains from the existing off-line methodology was analyzed for comparison. The data is clinically relevant and superior to existing methodologies.

396-P

AGALSIDASE ALFA AND AGALSIDASE BETA HAVE SIMILAR EFFECTS ON FABRY OUTCOMES RESULTS FROM THE CANADIAN FABRY DISEASE INITIATIVE

Sirrs SM[1], Bichet DG[2], Casey R[3], Clarke JTR[4], Flowerdew G[5], Lemoine K[6], West ML[6]
[1]Adult Metabolic Diseases Clinic, Vancouver, Canada
[2]Department of Medicine, Univ Montreal, Montreal, Canada
[3]Department of Pediatrics Univ Calgary, Calgary, Canada
[4]Dep Peds Univ Hosp Sherbrooke, Sherbrooke, Canada
[5]Dept Comm Health Epi Dalhousie Univ, Halifax, Canada
[6]Department of Medicine Dalhousie Univ, Halifax, Canada

Background: The Canadian Fabry Disease Initiative (CFDI) is a nationwide study of all patients in Canada with Fabry disease between ages 5 and 85 (Clinical Trial Registration protocol # NCT 00455104).
Methods: All Canadian citizens with FD are eligible for inclusion. This analysis looks at the two CFDI cohorts of enzyme replacement (ERT) treated subjects: Cohort 1a—Subjects on ERT when the CFDI began who maintained their baseline treatment assignment; Cohort 1b—subjects newly meeting criteria for ERT and randomized 1:1 to agalsidase-beta 1.0 mg/kg q2wks or agalsidase-alfa 0.2 mg/kg q2wks. We compare the effects of agalsidase alfa and agalsidase beta on a composite clinical outcome consisting of renal (dialysis, transplant, or reduction in GFR by 50%), cardiac (admission for cardiac event), neurologic (stroke or sudden unilateral hearing loss) or death.
Results: 84 subjects in Cohort 1a and 54 subjects in Cohort 1b are enrolled. Median follow up was 23.0±8.1 months. Clinical features at baseline in the two treatment arms of each cohort did not differ so the data on composite outcome is combined for analysis. In cohorts 1a and 1b combined, 24 subjects met the composite outcome, 15 in the agalsidase alfa subgroup and 9 in the agalsidase beta subgroup (HR alfa vs beta 1.548 p=0.74). Serious adverse events were similar between the two treatments.
Conclusions: Agalsidase alfa and agalsidase beta produce equivalent Fabry-related outcomes through 23 months of therapy.

397-P

NOVEL HOMOZYGOUS ASAH1 MUTATION IN FARBER LIPOGRANULOMATOSIS TYPE 1 IN A CROATIAN BOY WITH LATE PRESENTATION AND EARLY DEATH

Cvitanovic-Sojat C[1], Gjergja Juraski R[1], Sabourdy F[2], Fensom AH[3], Fumic K[4], Paschke E[5], Levade T[2]
[1]Dept of Ped, U H Sestre milosrdnice, Zagreb, Croatia
[2]Dept of Metab Bioch and INSERM U858, Toulouse, France
[3]Genet Cent, Guy's Hosp, London, United Kingdom
[4]Lab for Metab Dis U H Centre Rebro, Zagreb, Croatia
[5]Lab of Metab Dis U of Graz, Graz, Austria

Background: We report a boy with an unusually late presentation of Farber lipogranulomatosis type l. The symptoms of classical type I Farber lipogranulomatosis usually appear between 2 weeks and 4 months after the birth, consisting of painful and swollen joints and hoarseness
Case report: In our patient the first symptoms appeared at the end of the first year of life in the form of joint swelling; other symptoms such as cherry-red spot, hoarseness, subcutaneous nodules appeared much later. The history of the disease, from the first symptoms till his early death, lasted 26.5 months. The neuronal dysfunction accompanied by the rapid neurological deterioration with seizures and myoclonias, rather than the general dystrophy, seemed to limit the duration of disease in our patient and provoked his early death. Diagnosis was confirmed by analysis of ceramide metabolism in cultured fibroblasts and of the ASAH1 gene, which indicated homozygosity for a novel point mutation.
Conclusion: The deficient activity of acid ceramidase correlated well with poor prognosis of the disease in our boy, in contrast to late appearance of dermal nodules and the subsequent severe clinical course with fatal outcome. Farber lipogranulomatosis should be suspected in children with joint swelling as the first and only symptom of disease. In order to advance our knowledge towards establishing genotype-phenotype correlations in Farber disease, detailed analysis of the ASAH1 gene is needed.

398-P

THE NATURAL HISTORY AND GENOTYPE—PHENOTYPE CORRELATIONS IN SPANISH PATIENTS WITH JUVENILE NEURONAL CEROID LIPOFUSCINOSIS

Perez Poyato MS[1], Montero Sanchez R.[1], Mila Recasens M[2], Cusi Sanchez V[1], Ferrer Abizanda I[3], Coll MJ[4], Domingo Jimenez R[5], Pineda Marfa M[1]
[1]*Hosp Sant Joan de Deu, Barcelona, Spain*
[2]*IDIBAPS Hosp Clinic, Barcelona, Spain*
[3]*Hosp Bellvitge, Barcelona, Spain*
[4]*Institut Bioquimica Clinica, Barcelona, Spain*
[5]*Hosp Virgen Arrixaca, Murcia, Spain*

Background: Patients with Juvenile neuronal ceroid lipofuscinosis (JNCL) show blindness, epilepsy, mental retardation and ataxia. The most frequent mutation is a 1.02 kb deletion in the CLN3 gene and mutations in the CLN1 gene produce a variant form clinically similar.
Objectives: To describe the clinical evolution, pathologic and genetic findings, looking for phenotype / genotype correlations in Spanish JNCL patients.
Materials and methods: The clinical records of 25 Spanish patients were reviewed from 1975 to 2010 and a clinical data base was created. Enzymatic assays for Palmitoyl—protein thioesterase (PPT1) activity, ultrastructural studies, and gene testing have been performed. The SPSS was used for statistical analyses.
Results: We differentiated two major JNCL group: group I (n=12) / variant (vJNCL) with mutations in CLN1 gene and group II (n=13) / classic (cJNCL) with mutations in CLN3 gene. The regression of developmental milestones occurred at median age earlier in vJNCL than cJNCL patients. vJNCL patients suffered from mental decline at median age of 5 years old and cJNCL at ten years of age. vJNCL patients developed epilepsy before the age of nine years and cJNCL patients at ten years. The visual failure appeared at a median age of six years, the time course to blindness was more rapid in heterozygous patients.
Conclusion: The evolution of the disease is more severe in vJNCL than cJNCL patients. The progression to blindness was shorter in CLN3 heterozygous patients whom showed a delay clinical course. We reported novel mutations and clinical variability within CLN1 families.

400-O

RNASET2-DEFICIENT CYSTIC LEUKOENCEPHALOPATHY IS A NEW LYSOSOMAL DISORDER

Henneke M[1], Diekmann S[1], Haud N[2], Alia A[3], Hurlstone A[2], Gärtner J[1]
[1]*Dep Ped & Ped Neuro, Georg August Univ, Gottingen, Germany*
[2]*Faculty of Life Sciences, University, Manchester, United Kingdom*
[3]*Leiden Inst of Chemistry, Leiden Univ, Leiden, Netherlands*

Background: We recently described those human RNASET2-deficiency results in cystic leukoencephalopathy (Nat Genet 2009; 41:773–775). Clinical phenotype and brain magnetic resonance (MR) imaging pattern cannot be differentiated from neonatally asymptomatic congenital cytomegalovirus brain infection. Patients manifest with psychomotor impairment, micro- or normocephaly, spasticity and epilepsy. Brain abnormalities include multifocal white matter lesions, bilateral cysts in anterior temporal lobes, enlarged inferior horns and calcifications.
Methods: We characterized a cohort of 39 patients with unclear cystic leukoencephalopathy using genetic analyses and cell biology methods. Disease pathogenesis was studied in human cell lines and rnaset2-deficient zebrafish.
Results: We identified 5 RNASET2-mutations in 7 subjects. RNASET2 encodes glycoprotein RNASET2, the only human member of the Rh/T2/S family of RNases. RNASET2 enters into the secretory pathway and is endowed with catalytic activity hydrolyzing single-stranded RNA. Expression experiments revealed a secretion defect for missense mutation C184R. Immunofluorescence experiments showed retention of the C184R-mutant within the endoplasmic reticulum whereas wild-type RNASET2 was partially colocalized with lysosomes. All mutations result in loss of protein function. Zebrafish embryos with rnaset2-deficiency develop engorged lysosomes in neurons containing undigested ribosomal RNA (rRNA) and leading to axonal degeneration. Adult rnaset2-mutants exhibit white matter lesions on high-field MR microimaging resembling lesions seen in RNASET2-deficient patients.
Conclusion: Human RNASET2-deficiency extends the spectrum of lysosomal storage disorders with rRNA accumulation most likely interfering with brain development through axonal degeneration. The zebrafish rnaset2-mutants represent the first zebrafish model for a childhood-onset white matter disorder serving as preclinical model for further elucidating disease mechanisms and therapeutic approaches.

401-P

AUTOMATED QUANTIFICATION OF FILIPIN FLUORESCENCE USING A HIGH CONTENT MICROSCOPE PLATFORM FOR THE DIAGNOSIS OF NIEMANN-PICK TYPE C DISEASE

Rigat BA[1], Fladd CA[1], Clarke JTR[1], Mahuran DJ[1], Callahan JW[1]
[1]*The Hospital for Sick Children, Toronto, Canada*

Background: Diagnosis of Niemann-Pick disease type C (NP-C) requiring demonstration of abnormal intracellular cholesterol homeostasis using biochemical or cytological methodologies remains difficult. We evaluated quantification of filipin fluorescence as a more rapid and reliable clinical test than qualitative filipin testing.
Methods: We developed an assay for the quantification of the fluorescence intensity arising from the binding of filipin to the non-esterified cholesterol stored in NP-C patient fibroblasts. The filipin staining is performed in conjunction with a live cell dye and analyzed using a high content microscope platform. The major advantage of the assay arises from the ability to automatically record the separate images for each dye within individual cells using an advanced optical imaging system, and then quantification of the cumulative fluorescence intensity using a sophisticated image analysis software, based on a rapid image-analysis algorithm designed to provide generic spot analysis.
Results: Validation of the assay: cell density played a major role in the quality of the results; titration of the two dyes provided the best working concentrations. Robustness of the assay diagnosis values was ascertained by comparing human control fibroblasts (n>5), to known NP-C patients (n>12) and patients from other lysosomal storage disorders.
Conclusion: The automated quantification of filipin fluorescence is a simple, rapid and reliable assay to generate an accurate diagnosis for NP-C disease that could be easily set up in most hospital laboratories. Additionally, this assay could represent a valuable tool to search for and then monitor novel therapeutic compounds. (Supported by CIHR and NPC Foundation)

402-P

OSTEOPENIA IN GAUCHER DISEASE DEVELOPS EARLY IN LIFE: RESPONSE TO IMIGLUCERASE ENZYME THERAPY IN CHILDREN, ADOLESCENTS AND ADULTS

Mistry PK[1], Weinreb NJ[2], Kaplan P[3], Cole JA[4], Gwosdow AR[4], Hangartner T[5]
[1]*Yale University School of Medicine, New Haven, United States*
[2]*University Research Foundation, Coral Springs, United States*
[3]*Children's Hospital of Philadelphia, Philadelphia, United States*
[4]*Genzyme Corporation, Cambridge, United States*
[5]*Wright State University, Dayton, United States*

Objective: Osteopenia is a common consequence of type 1 Gaucher disease (GD1). We analyzed the bone mineral density (BMD) of children, adolescents and adults with GD1 at baseline and following imiglucerase treatment.
Methods: Imiglucerase-treated GD1 patients aged 5- 50 yrs from the ICGG Gaucher Registry were included. Lumbar spine BMD was expressed as Z-scores. BMD data at baseline and up to 10y on imiglucerase were analyzed. Results were correlated with hemoglobin, platelet counts, spleen and liver volumes, bone pain and bone crises. Descriptive statistics were used to calculate mean Z-scores. Non-linear mixed effects models were used to analyze Z-scores over time.
Results: Baseline hematological and visceral manifestations in the four age groups were similar. The most common GBA1 genotype of patients with bone manifestations was N370S/Other. At baseline, Z-scores were below ≤-1 in 44% of children (≥5 to <12 years; n=43), 76% of adolescents (≥12 to <20 years; n=41), 54% of young adults (≥20 to <30 years; n=56) and 52% of adults (≥30 to <50 years; n=171). Among children with Z-scores below -1 at baseline (n=19), regression models revealed the median Z-scores improved from baseline -1.38 (95% CI -1.73 to -1.03) over 6 yr of imiglucerase to -0.73 (95% CI -1.25 to -0.21). Similar responses were observed in adolescents and young adults; in older adults response was attenuated.
Conclusion: Low bone density is prevalent in all age groups with GD1 with most pronounced effect in adolescence. Imiglucerase results in amelioration of osteopenia in all age groups, with largest effect in younger patients.

403-P

INTRAEPIDERMAL NERVE FIBER DENSITY IN RELATION TO SMALL FIBER FUNCTION AND PAIN IN FABRY DISEASE

Biegstraaten M[1], Hollak CEM[1], Binder A[2], Maag R[2], Baron R[2], Bakkers M[3], Faber CG[3], van Schaik IN[1]
[1]*Academic Medical Center, Amsterdam, Netherlands*
[2]*Universitätsklinikum Schleswig-Holstein, Kiel, Germany*
[3]*Maastricht University Medical Center, Maastricht, Netherlands*

Background: Fabry patients often suffer from pain and pain attacks. The pathophysiology of pain in Fabry disease is not fully understood, but small fiber damage has been proposed as a possible mechanism.
Methods: To get a better understanding of the relation between small fiber neuropathy (SFN) and pain in Fabry disease, intraepidermal nerve fiber density (IENFD) was assessed in a large cohort of Fabry patients to determine the extent of structural damage of small fibers in Fabry disease. Subsequently, relations between structural abnormalities, functional impairment and pain severity were investigated. We also explored correlations between concentrations of deacylated Gb3 (globotriaosylsphingosine, lyso-Gb3) and IENFD, small fiber function and pain.
Results: Forty-three patients (61% of the Dutch Fabry population) agreed to have a skin biopsy. We found a decreased IENFD in all male patients and in 57% of the female patients. More severe loss of intraepidermal nerve fibers was associated with more severe small fiber function loss. No association between IENFD and pain was found. Lifetime exposure to lyso-Gb3 was associated with functional impairment of small nerve fibers in male hemizygotes.
Conclusions: Most Fabry patients have a decreased IENFD which is associated with functional impairment, but a direct relationship with pain was not found. We propose that peripheral sensitization and the disappearance of pain in a subset of patients both play a role in the complex relationship between pain and SFN. Furthermore, our results point to a role of lifetime exposure to lyso-Gb3 in the pathogenesis of SFN in male patients.

404-P

WHAT DO YOU THINK OF ENZYME REPLACEMENT THERAPY AND NEWBORN SCREENING FOR MUCOPOLYSACCHARIDOSES? OPINIONS FROM PATIENTS AND FAMILIES OF PATIENTS IN JAPAN AND KOREA

Tao-Nishida Eriko[1], Seo Joo-Hyun[2], Sohn Young-Bae[3], Yotsumoto Junko[2], Kosuga Motomichi[1], Tanaka Toju[1], Omori Mika[2], Kawame Hiroshi[2], Jin Dong-kyu[3], Okuyama Torayuki[1]
[1]*National Center for Child Health, Tokyo, Japan*
[2]*Ochanomizu University, Tokyo, Japan*
[3]*Samsung Medical Center, Seoul, Republic of Korea*

Background: Newborn screenings (NBS) for Mucopolysaccharidoses (MPS) are now under consideration for some types of MPS. Although the clinical trials of enzyme replacement therapy (ERT) for those MPS have demonstrated amelioration of some clinical manifestations, ERT also has raised numerous practical, ethical, and even medical issues especially when there has been no clear benefit to the patients.
Objectives: To clarify the issues in ERT and NBS for MPS by assessing the opinions of individuals with MPS and of their parents in Japan and Korea.
Methods: A questionnaire, including hypothetical clinical scenarios about ERT and NBS for MPS, was sent to members of MPS support groups in Japan and Korea. A part of the questionnaire was generated based on the results of previous study (Hayes IM, et al., Clin Genet 2007).
Results: The questionnaire was completed by 264 MPS support group members in Japan and Korea. More than 80% of Japanese and 90% of Korean were in favor of ERT where severe physical and intellectual problems are well established. The majority of respondents gave an affirmative for NBS, and they expect an early treatment with an early diagnosis. However, in untreatable cases, approximately 20% of respondents had negative opinions about an early diagnosis before appearance of any symptoms.
Conclusion: We have to carefully-examine about NBS for MPS (including what type of MPS would be introduced into NBS). It is necessary to establish the system to provide a chance for pre- and post-counseling and the system to obtain informed consent.

405-P

MULTIPLE OPERATIONS IN INDIVIDUALS WITH MUCOPOLYSACCHARIDOSIS TYPE II (MPS II): DATA FROM THE HUNTER OUTCOME SURVEY (HOS)

Giugliani R[1], Bodamer O[2], Burton B[3], De Meirleir L[4], Harmatz P[5], Jones S[6], Lampe C[7], Malm G[8], Parini R[9], Steiner R[10], Mendelsohn N[11], on behalf of the HOS Investigators.[12]
[1]*HCPA/UFRGS, Porto Alegre, Brazil*
[2]*Univ Children's Hospital, Salzburg, Austria*
[3]*Northwestern Univ Feinberg School Med, Chicago, IL, United States*
[4]*Dept of Paed Neur, Univ Hospital AZK-VUB, Brussels, Belgium*
[5]*Children's Hospital, Oakland, CA, United States*
[6]*St Mary's Hospital, Manchester, United Kingdom*
[7]*Children's Hospital, Univ of Mainz, Mainz, Germany*
[8]*Karolinska Univ Hospital, Stockholm, Sweden*
[9]*Ped Dept, San Gerardo Hospital, Monza, Italy*
[10]*Oregon Health & Sci Univ, Portland, OR, United States*
[11]*Children's Hospitals & Clinics Minnesota, MN, United States*
[12]*HOS, Oxford, United Kingdom*

Objectives: To determine the prevalence of multiple/repeat operations in patients with MPS II.
Methods: Data on surgical intervention from HOS—a multinational observational database on patients with MPS II—were analyzed.
Results: The study population comprised 527 patients aged 0.1–48.4 years at last visit for which data about surgical intervention had been reported in HOS on/before 23 July 2009. In this population, 83.7% of patients underwent 2232 operations in total. Among patients who had operations (n = 441), a median of 4.0 operations were performed per patient: 14.3% underwent two operations, and 72.1% underwent three or more operations. One patient had 26 operations. The types of operations most commonly performed were tympanostomy (271 patients; 622 operations); hernia repair (264 patients; 419 operations); adenoidectomy (261 patients; 335 operations); tonsillectomy (187 patients; 219 operations); and operations for carpal tunnel syndrome (96 patients; 127 operations). Tympanostomy, hernia repair and adenoidectomy were repeated in 34.0%, 24.0%, and 12.2% of patients who underwent operations, respectively, and in some individuals these operations were performed more than seven times. Of the 441 patients who had operations, 18.6% underwent two different types, and 62.1% underwent three or more types; the most common combination was tympanostomy and hernia repair.
Conclusions: Undiagnosed patients with manifestations suggestive of MPS II who require multiple and/or repeat operations should be strongly suspected of having the disease and referred for diagnostic tests. As individuals with MPS II are likely to require operations, healthcare teams should be prepared for this possibility.

406-P

GLUCOCEREBROSIDASE ACTIVITY & PARKINSONISM—POTENTIAL PATHOGENIC MECHANISMS

Heales SJ R[1], Manwaring V[1], Mills K[1], Berry A[2], Allen G[2], Heywood W[1], Burke D[3]
[1]*UCL Institute of Child Health, London, United Kingdom*
[2]*UCL Institute of Neurology, London, United Kingdom*
[3]*Enzyme & Metabolic Unit, Great Ormond St, London, United Kingdom*

Background: Gaucher disease arises as a result of mutations affecting glucocerebrosidase (GBA). GBA deficiency is also documented as a risk factor for developing Parkinson's disease (PD). Currently, the mechanisms responsible for developing PD are not known.
Objectives: In view of the potential role of glial—neuronal interactions in the pathogenesis of some lysosomal disorders and PD, GBA activity was compared in human neuronal (SH-SY5Y) and astrocytic (1321 N1) cells. A proteomic approach was employed to identify proteins that were differentially expressed as a result of impaired GBA activity.
Methods: GBA was assayed in SH-SY5Y and 1321 N1 cell homogenates. Activity of the lysosomal enzyme, beta-galactosidase was also determined as a reference enzyme. Conduritol-beta-epoxide (CBE) was used to inhibit GBA. Quadrupole time of flight mass spectrometry was used to characterise protein expression.
Results: GBA activity was found to be significantly ($p<0.005$) greater (2.5 fold) in neuronal cells. Beta-galactosidase activity was comparable in the two cell types. For neuronal cells, CBE exposure resulted in increased expression of actin cytoplasmic 1 (ACTB).
Conclusion: Greater GBA activity in neuronal cells may point to a particular role in neuronal cells. Increased expression of ACTB is of interest as alterations in brain ACTB have been implicated in dystonia.

407-P

AUTOPHAGIC IMPAIRMENT IN MUCOLIPIDOSIS II AND III SKIN FIBROBLASTS

Otomo T[1], Higaki K[2], Nanba E[2], Ozono K[1], Sakai N[1]
[1]*Dept Pediatrics, Osaka Univ, Suita, Japan*
[2]*Div Functional Genomics, Tottori Univ, Yonago, Japan*

Background: Mucolipidosis (ML) II and III are autosomal recessive diseases caused by GlcNAc-phosphotransferase deficiency and clinically overlaps with mucopolysaccharidoses. Autophagy is a lysosomal protein degradation pathway. It not only provides nutrients during fasting but also maintains cellular turnover by degrading misfolded proteins and damaged organelles including mitochondria. In ML, since lysosomal enzymes lack mannose-6-phosphate residues and are not targeted to the lysosome, the lysosomal autophagic degradation pathway could be impaired. We thus examined autophagy in ML cells.
Methods: Skin fibroblasts were obtained from normal, ML-II and ML-III patients. We investigated several autophagic and degradation markers such as LC-3, beclin-1, p62 and ubiquitinated protein by Western blotting and immunocytochemistry. Mitochondrial morphology and membrane potential were examined with Mitotracker and JC-1 staining, respectively.
Results: Immunoblots showed that membranous LC-3 II protein was remarkably increased in ML cells, while beclin-1 was not increased. Autolysosome amassment was observed by immunocytochemistry. Western blotting also showed ubiquitinated protein and p62 accumulation. Mitochondrial structure was fragmented and membrane potential activity was impaired in ML cells. These impairments were temporarily rescued by blocking autophagy with 3-methyladenine (3-MA) for 16 hours. The above phenotypes were milder in mucolipidosis III compared with mucolipidosis II.
Conclusions: In this study, we demonstrated autophagic impairment especially the clearance of autolysosomes in ML cells. The result of 3-MA treatment suggests direct damage to mitochondria by increased autophagosome formation. These findings raise the possibility of exploring new therapeutic options by modulating autophagy in ML II and ML III patients.

408-P

CHOLESTERYL ESTER STORAGE DISEASE (ACID LIPASE DEFICIENCY): ANOTHER FACTOR FOR EARLY ATHEROSCLEROSIS. LONG TERM FOLLOW-UP FROM 19 GREEK PATIENTS AND THEIR FAMILIES

Drogari ED[1], Manolaki NM[1], Progias PP[1], Koniari EK[1], Christomanou HC[2]
[1]*Univ Child Hosp, Athens, Greece*, [2]*Lys Neur Dis Inst, Athens, Greece*

Cholesteryl ester storage disease (CESD) is due to acid lipase deficiency (adult form) with autosomal recessive inheritance. We describe our experience from 17 families with CESD.
All families were screened for three consecutive generations. They originate from different areas of Greece and parents are unrelated. Families were identified after referral to the Lipid Clinic for high lipid levels and liver disease.
Lipid levels (total cholesterol, triglycerides, HDL, VLDL, LDL), enzyme activity in blood and liver tissues from 19 children-patients, 34 parents, 20 siblings, 24 grandparents and 32 uncles/aunts were screened. All 19 patients had liver biopsies and 10 had skin biopsies where enzyme activity was measured. Liver CT scan showed fatty liver with fibrosis and calcificated areas in all patients. Histochemistry in liver biopsy showed adipose of cholesterol ester with fibrosis. Total cholesterol levels ranged from 280–450 mg/dl, triglycerides from 420–1100 mg/dl, HDL from 19–38 mg/dl, and LDL from 180–395 mg/dl in homozygotes. Hepatic enzymes were elevated 4 to 10-fold compared to normal. Enzyme activity in blood, liver and skin fibroblasts was 0–2% of control values for homozygotes and 35–48% for heterozygotes. Increased lipid levels and early ischemic heart disease was reported in 72% of adult family members. Patients were homozygotes and compound heterozygotes (5 mutations identified in 17 patients). Treatment started after diagnosis using low fat diet, statins and Ezetimibe. There was considerable improvement of lipid levels and liver enzymes (40–90% reduction) during a follow-up of 10–20 years.
CESD contributes to premature atherosclerosis in homozygote and heterozygote patients and should be further investigated.

409-P

FABRY DISEASE: GLA GENE VARIANT ALLELES LEADING TO NORMAL ALPHA GALACTOSIDASE ACTIVITY

Ferri L[1], Guido C[1], la Marca G[2], Malvagia S[2], Cavicchi C[2], Fiumara A[3], Parini R[4], Antuzzi D[5], Zampetti A[5], Guerrini R[2], Giglio S[6], Genuardi M[6], Donati MA[2], Morrone A[1]
[1]*Dept for Woman and Child's Health, Florence, Italy*
[2]*Metab and Musc Unit, Meyer Child Hosp, Florence, Italy*
[3]*Dept of Paediatrics, Univ of Catania, Catania, Italy*
[4]*San Gerardo Hospital, Monza, Italy*
[5]*Catholic University, Rome, Italy*
[6]*Med Genet Unit, University of Florence, Florence, Italy*

Background: While absence or reduced alpha-Galactosidase A (alpha-Gal A) lysosomal enzyme activity can be easily detected in male Fabry patients, heterozygous females need to be recognized at the molecular level. A few GLA intronic polymorphic variants have been identified. It is currently unknown whether specific combinations of such variants can determine phenotypic manifestations of Fabry disease, especially in suspected female patients presenting enzyme activity in the normal range.
Objectives: To determine the distribution of GLA nucleotide variants in the Italian population and to shed light on their possible pathogenic effect.
Materials and methods: Determination of alpha-Gal A enzyme activity on dried blood spots by LC/MS/MS and direct GLA sequence analysis on genomic DNA in 110 healthy males and several females referred as suspected Fabry patients. In the latter MLPA analysis was also performed.
Results: Normal alpha-Gal A activity was detected in all controls. By direct sequence analysis we found new and known polymorphic intronic variants that were combined in five different haplotypes. Such haplotypes were also detected in several females and males referred as suspected Fabry patients in whom molecular investigation had not identified any clearcut pathogenic alteration in the GLA gene.
Conclusion/Discussion: The identification of haplotypes carrying combinations of polymorphic nucleotide variants in a control population provides evidence against a potential correlation between these polymorphisms and the development of phenotypic manifestations of Fabry disease.

410-P

HIGH PLASMA CHITOTRIOSIDASE ACTIVITY IN AN INFANT WITH RAPIDLY PROGRESSIVE WOLMAN'S DISEASE AND NOVEL MUTATION IN LIPA GENE

Juras KJ[1], Fumić KF[2], Calandra SC[3], Verheijen FWV[4], Huljev Frković SHF[5], Vuković JV[5], Rajić LjR[5], Sarnavka VS[5], Petković Ramad-a DPR[5], Jelaić DJ[6], Barić IB[5]
[1]*Univ School of Med, Zagreb, Croatia*
[2]*Clin Inst for Lab Diag, Univ Hosp Center, Zagreb, Croatia*
[3]*Depart of Biomed Sc, Univ of Modena, Modena, Italy*
[4]*Depart of Cl Genet, Eras Med C, Univ Hos, Rotterdam, Netherlands*
[5]*Depart of Ped, Univ Hosp Cent, Zagreb, Croatia*
[6]*Depart of Path&Cyt, Univ Hosp Cent, Zagreb, Croatia*

Wolman's disease is a rare, autosomal recessive lysosomal storage disease (LSD) which presents as an infantile form of acid lipase deficiency. The deficiency leads to massive accumulation of cholesterol esters and triglycerides in macrophages throughout the viscera, causing diarrhea, massive hepatosplenomegaly, failure to thrive, calcifications of adrenal glands and liver failure. Although uniformly lethal, there is some variation in disease progress. The only life saving therapy so far is early umbilical cord blood or bone marrow transplantation. Chitotriosidase is an enzyme selectively activated in tissue macrophages which, overloaded with lipids, secrete chitotriosidase in large amounts. It is highly elevated in Gaucher's and Niemann-Pick's type A/B disease. Mild to moderate elevation can be considered as a positive predictive test for other LSD's, as well. Our patient was a girl with rapidly progressive course who died at age of 87 days before transplantation could be done. Her acid lipase activity was 49,8 nmol/h/mg (normal 125–480). She was homozygous for a novel point mutation in exon 4 of LIPA gene (c.419G>A, p.W140X). Her plasma chitotriosidase activity was 6610 and 8805 mU/mL at age of 47 and 61 days, respectively (normal ≤150) indicating a severe mutation. To our knowledge, this is the highest chitotriosidase activity observed in Wolman's disease so far. Our findings suggest that plasma chitotriosidase could provide useful information on the severity of Wolman's disease thus facilitating decision making in managing the patient. It could also be considered as a possible marker for monitoring future enzyme replacement therapy in Wolman's disease.

411-P

EFFECTIVENESS OF IDURSULFASE FOR HUNTER SYNDROME IN EUROPEAN PATIENTS ENROLLED IN THE HUNTER OUTCOME SURVEY

Guillen-Navarro E[1], DeMeirleir L[2]
[1]*Med Genet Unit, Hosp Univ Virgen Arrixac, Murcia, Spain*
[2]*Univ Ziekenhuis Bruss, Brussel, Belgium*

Background: The Hunter Outcome Survey (HOS) is a global database used to characterize the natural history of the Hunter syndrome as well as to evaluate the safety and effectiveness of enzyme replacement therapy (ERT) with idursulfase (Elaprase., Shire HGT, Cambridge, MA, USA).
Methods: The HOS database was queried to assess the effectiveness of ERT in prospective (i.e., alive at HOS entry) European patients who had results both prior to starting ERT and after at least 6 months of ERT (median duration=24.3 months).
Results: As of January 2010, 259 prospective European patients had received idursulfase. The age distribution at start of ERT was: 22.8% <5 years, 45.2% 5 to <12 years, 18.2% 12 to <18 years, 13.9% ≥18 years (overall median age at start of ERT=8.8 years). The median age at onset of symptoms and diagnosis was 1.8 and 3.5 years, respectively. Urine glycosaminoglycan excretion (μg/mg creatinine) decreased from a median 245 at baseline to 106 after at least 6 months of ERT (n=108). Palpable liver size (cm) decreased from a median 6 at baseline to 3 after at least 6 months (n=63). Functional benefits were also seen, including increases in forced vital capacity (median 0.15 L after 1 year) and 6-minute walk test distance (median 39 m after 1 year). Reductions in left ventricular mass were observed. Three patients experienced a total of 5 serious infusion-related reactions. Ten deaths occurred, but none were considered treatment-related.
Conclusions: Evidence of beneficial effects of idursulfase was observed in European patients enrolled in HOS.

412-P

SAFETY AND EFFICACY OF VELAGLUCERASE ALFA IN PATIENTS WITH TYPE 1 GAUCHER DISEASE PREVIOUSLY TREATED WITH IMIGLUCERASE: ONGOING EXTENSION OF STUDY TKT034

Pastores G[1], Zimran A[2], Tylki-Szymanska A[3], Mehta A[4], Mardach R[5], Heisel-Kurth M[6], Eng C[7], Smith L[8], Harmatz P[9], Charrow J[10], Zahrieh D[11], Grabowski G[12]
[1]*NYU Sch Med, New York, United States*
[2]*Shaare Zedek Med Center, Jerusalem, Israel*
[3]*Child Memorial Health Inst, Warsaw, Poland*
[4]*Royal Free Hosp, London, United Kingdom*
[5]*Kaiser Permanente, Los Angeles, United States*
[6]*Child Hosp of Minnesota, Minneapolis, United States*
[7]*Baylor College of Med, Houston, United States*
[8]*Child Mercy Hosp, Kansas City, United States*
[9]*Child Hosp & Res Cent Oakland, Oakland, United States*
[10]*Child Memorial Hosp, Chicago, United States*
[11]*Shire Human Genetic Therapies, Cambridge, United States*
[12]*Cincinnati Child Hosp Med Center, Cincinnati, United States*

Background: Velaglucerase alfa (1) is an ERT for type 1 Gaucher disease produced by gene activation in a human cell line.
Methods: TKT034 was an open-label, multicenter, 12-month, switch trial in which type 1 Gaucher disease patients (age ≥2 years), with stable hemoglobin concentration and platelet count, received velaglucerase alfa equal to their prior imiglucerase dose (≥15–60U/kg every other week). Patients were eligible to enroll in an ongoing extension study. Follow-up data to 18 months are now available.
Results: Of 40 patients (age 9–71 years) who received ≥1 dose of velaglucerase alfa in TKT034, 38 continued into the extension. Velaglucerase alfa was generally well tolerated with most reported AEs of mild or moderate severity. No patient experienced a life-threatening AE. At baseline (prior to velaglucerase alfa dose), median hemoglobin concentration was 13.8 g/dL [range:10.7–16.5] and median platelet count was 162x10^9/L [range:29–399]. Following 12 months' velaglucerase alfa treatment, clinical parameters (hemoglobin concentration, platelet counts, spleen and liver volume) remained stable. At 18 months, mean change from baseline for hemoglobin concentration and platelet count were negligible (-0.1 g/dL [95% CI:-0.5,0.3], and 9% [95% CI:-1%,19%], respectively). Follow-up spleen and liver volumes will be measured at Month 24.
Conclusion: Patients with type 1 Gaucher disease previously treated with imiglucerase successfully transitioned to velaglucerase alfa, maintaining hematological parameters at therapeutic levels through 18 months' treatment. Liver and spleen volumes were maintained through 12 months (follow-up data available at Month 24).
(1)velaglucerase alfa is approved in the USA; it is an investigational product in Europe.

413-O

CARDIAC MANIFESTATIONS IN PATIENTS WITH MUCOPOLYSACCHARIDOSIS I

Parini R[1], Chyung YH[2], Cox GF[2], Jones S[3], Wraith JE[3]
[1]*Ospedale Nuovo San Gerardo dei Tintori, Monza, Italy*
[2]*Genzyme Corporation, Cambridge, MA, United States*
[3]*Genetic Medicine, St. Mary's Hospital, Manchester, United Kingdom*

Objectives: An analysis was conducted to characterize the prevalence, type, and chronology of cardiac manifestations among mucopolysaccharidosis I (MPS I) patients.
Methods: Echocardiographic data were reviewed from 873 patients enrolled in the MPS I Registry, an international observational database of MPS I patients. The age of onset and frequency of cardiomyopathy, congestive heart failure, cor pulmonale, and valvular disease were assessed.
Results: Heart valve abnormalities were the earliest and most commonly reported cardiac manifestation, found in 74% of Hurler, 86% of Hurler-Scheie, and 93% of Scheie patients and presenting at mean ages of 2.4, 7.2, and 16 years, respectively. Mitral valve abnormalities were the most common (regurgitation and stenosis in 76% and 31% of patients respectively) followed by tricuspid (regurgitation and stenosis in 52% and 4% of patients respectively), aortic (regurgitation and stenosis in 43% and 17% of patients respectively), and pulmonary (regurgitation and stenosis in 20% and 5% of patients respectively). Approximately 20% of patients from all phenotypes had cardiomyopathy, with the earliest age of onset in Hurler patients (mean age 2.8 years). Among all patients, the most commonly reported cardiomyopathy was hypertrophic (49%) followed by dilated (22%, mainly in Hurler patients), mixed (9%), restrictive (3%), and unknown (10%). Congestive heart failure was reported in 5%, 9%, and 8% of Hurler, Hurler-Scheie, and Scheie patients, respectively. Cor pulmonale was observed in less than 2% of all patients.
Conclusions: Cardiac manifestations occur commonly and at an early age in MPS I.

414-P

SOURCE DOCUMENT VERIFICATION OF DATA FROM THE MPS I REGISTRY

Wijburg F[1], Clarke J[2], Correo JN[3], Guffon N[4], Martins AM[5], Whitley CB[6], Wraith JE[7]
[1]*Academic Medical Center, Amsterdam, Netherlands*
[2]*Div Clin Metab Genet, Hosp Sick Child, Toronto, Canada*
[3]*Universidade Federal de São Paulo, São Paulo, Brazil*
[4]*Femme Mère Enfant Hospital, Lyon, France*
[5]*Pontificia Universidade Católica Campina, São Paulo, Brazil*
[6]*Univ Minnesota Gene Therapy Center, Minneapolis, MN, United States*
[7]*Genetic Medicine, St. Mary's Hospital, Manchester, United Kingdom*

Objectives: The MPS I Registry is an international observational database that tracks the natural history and outcomes of patients with mucopolysaccharidosis I. Since all information is reported voluntarily, we sought to assess overall data accuracy through source data verification at several sites.
Methods: Registry data were compared against source documents at 6 key sites in Europe, Latin America and North America that collectively enroll almost half of the >800 patients currently in the Registry. Three patients were randomly selected for source document verification at each site among all patients enrolled ≥18 months and ever receiving laronidase. The following parameters central to MPS I and its treatment were examined from the baseline and the last available visit/assessment: signed information/authorization/consent forms; birth date; gender; initial diagnosis date; genotype; α-L-iduronidase activity; treatment information; chronic otitis media; grommet tube placement/replacement; cardiomyopathy; congestive heart failure; cardiac valve abnormalities and valve replacement; echocardiogram; pulmonary function testing; supplemental oxygen use; episodes of pneumonia/year; height and weight; and urinary glycosaminoglycan levels. Both data that were not verifiable (missing from source documents) as well as data discrepancies were counted as errors.
Results: Overall, 1,754 items were checked. The mean error rate was 4.9%, which compares favorably to the 9.75% error rate compiled from published literature on registries and clinical trial databases. Most discrepancies were due to missing source documents or data entry errors, often involving dates or transposed numbers. No systemic errors were found.
Conclusion: These results demonstrate robust data integrity in the MPS I Registry.

415-P

ACUTE HYDROCEPHALUS REVEALING INFANTILE ONSET OF POMPE DISEASE

Dobbelaere DD[1], Jissendi PJ[2], Cuisset JMC[3], Mention KM[1], Soto Ares GSA[2]
[1]*Reference Center For Inherited Metabolic, Lille, France*
[2]*Neuroradiology, Lille, France*
[3]*neuropediatric service, Lille, France*

Background: Pompe disease is a rare autosomal recessively inherited lysosomal storage disorder caused by mutations of the gene coding for acid α-glucosidase. Patients with the classic early onset usually come to attention in early life with marked muscular hypotonia and heart failure caused by hypertrophic cardiomyopathy. Without treatment, Pompe disease is fatal within the first year, mostly from cardiorespiratory failure. Central nervous system complications associated with this disorder are rarely reported. We describe here a case of a 4 months and a half-old girl with diagnosis of Pompe disease after an acute hydrocephalus.
Case report: The clinical history starts with feeding difficulties and fatigue reported by the mother. The pediatrician noticed a systolic murmur and referred the infant for cardiac examination which revealed hypertrophic cardiomyopathy. The following day and before clinical deterioration, the infant was admitted to the pediatric emergency where a full anterior fontanel was found. Additional clinical examination revealed an associated increased head circumference and axial hypotonia. Cerebral computed tomography showed a severe hydrocephalus with dilatation of the four ventricles. The infant underwent an acute placement of a ventriculoperitoneal shunt. Post-surgery surveillance biological tests showed slightly increased levels of CK (518 Ul/L) and increased levels of transaminases (ALT = 176 Ul/L, AST = 116 Ul/L). A metabolic disorder was evoked and Pompe disease was diagnosed. ERT was started immediately after exhaustive work-up. Despite ERT, this girl develops diffuse and evolutive leucopathy on MRI with evidence of dysmyelinisation on spectro MRS.

416-P

A CLINICAL SCALE FOR THE STUDY OF PROGRESSIVE MYELOPATHIES

Castilhos RM[1], Blank D[2], Giugliani R[2], Fernandes LNT[3], Jardim LB[2]
[1]*PPGCM, UFRGS, Porto Alegre, Brazil*
[2]*Med Genet Serv, Hosp Clin, Porto Alegre, Brazil*
[3]*Dep of Int Med, UFRGS, Porto Alegre, Brazil*

Progressive myelopathies can be secondary to some inborn errors of metabolism (IEM) such as Mucopolysaccharidoses (MPS), mucolipidoses (ML), and adrenomyeloneuropathy (AMN). The available scales were only validated to degenerative vertebral diseases.
Aims: to propose a new clinical scale addressed to progressive myelopathies.
Methods: A new scale was built, called "Progressive Mielopathy Severity Score System" (PROMSS). PROMSS varied between zero to 100, and covered the following domains: motor disability (50% of the scale), sphincter dysfunction (20%), spasticity (10%), and sensitive losses (20%). Interrater and intrarrater reliability were tested. External validation was tested by applying JOA, EDSS, the Barthel index and the Osame Motor Disability Score.
Results: 37 patients with myelopathy accepted to participate. There were 17 AMN, 3 MPS I, 3 MPS IV, 2 MPS VI, 2 ML and 9 patients with HTLV-1 infection. The mean (sd) PROMSS score was 70.5 (11.4). Strong construct validity (Spearman's rank test between PROMSS and: JOA: $r = 0.84$, $p < 0.0001$; EDSS: $r = -0.83$, $p < 0.0001$; Barthel: $r = 0.56$, $p < 0.002$; Osame: $R = -0.94$, $p < 0.0001$) and reliability (intra-rater: $r = 0.83$; $p < 0.0001$; inter-rater: $r = 0.94$, $p < 0.0001$) were demonstrated. Discussion: several clinimetric requirements were met. Responsiveness was lacking; it needs longer periods between assessments. Since PROMSS has a wide range and because it covers all aspects of medullary diseases (motor, sensory and sphincter functions), we hope it will be useful for follow-up studies on IEM myelopaties.

417-P

FUNCTIONAL PROFILE OF BRAZILIAN PATIENTS WITH MUCOPOLYSACCHARIDOSIS TYPE II

Ruas N.[1], Schwartz I.[2], Guarany F.[3], Muñoz-Rojas V.[1], Netto C.[4], Pinto L.[4], Souza C.[4], Vieira T.[4], Giugliani R.[2]
[1]*Post Graduation in Pediatrics –UFRGS, Porto Alegre, Brazil*
[2]*Genetics Department-UFRGS, Porto Alegre, Brazil*
[3]*Phyisiatry Service-HCPA, Porto Alegre, Brazil*
[4]*Medical Genetics Service HCPA, Porto Alegre, Brazil*

Introduction: Mucopolysaccharidosis II is a genetic disease caused by the deficiency of the enzyme idutonate-2-sulfatase, which causes the accumulation of glycosaminoglycans in the tissues. This compromises the function at skeleton, causing limitations and dependence in the fulfillment of daily activities. This study aimed to characterize the functional profile of a sample of Brazilian patients with MPS II.
Methods: The sample consisted by twelve patients followed at the ambulatory of Mucopolysaccharidoses of the Medical Genetic Service of Hospital de Clínicas de Porto Alegre (HCPA), Rio Grande do Sul state, Brazil. The assessment of functional independence was performed using Pediatric Evaluation of Disability Inventory (PEDI) for children under 8 years and Functional Independence Measure (FIM) for patients over that age.
Results: The patients, all male, had a mean age of 9 (SD 7.30) years. Of these, 7 had the severe form of the disease and 5 presented a more attenuated. The patients assessed by the protocol FIM (severe form= 5/7) had a needed help in 50% of activities, including the self-care activities and mobility. The activities of daily life that require more assistence are eating, bathing, dressing, walking and social interaction.Five patients were evaluated by the PEDI: it was observed that all patients showed a lower development scores, mainly in the area of self-care and mobility.
Conclusions: These results suggest the activities of self-care and mobility are the most affected by MPS II. The functional capacity evaluation of MPS II patients is important because through it is possible to identify limitations that could be improved by a therapeutic approach.

418-P

MEASUREMENT OF CAROTID INTIMA-MEDIA THICKNESS IN PATIENTS WITH MUCOPOLYSACCHARIDOSES

Wang RY[1], Covault KK[1], Dauben RD[2], Chang AC[3]
[1]*Div. Metab Dis, CHOC Children's, Orange, United States*
[2]*Div. Neurology, St. Joseph's Hospital, Orange, United States*
[3]*Div. Cardiology, CHOC Children's, Orange, United States*

Background: Potentially life-threatening cardiac manifestations such as coronary intimal proliferation and luminal stenosis have been reported in ERT-treated mucopolysaccharidosis (MPS) patients due to progressive glycosaminoglycan storage. A pilot study was conducted to explore the feasibility of carotid artery intima-media thickness (C-IMT), an established noninvasive adult cardiovascular disease marker, as a cardiac risk discriminant in MPS patients.
Methods: Following informed consent, measurements of bilateral C-IMT via neck ultrasound and coronary artery diameter via echocardiogram were obtained. Intra-observer variation was minimized by using the same echocardiogram/ultrasound technicians and interpreters.
Results: Eleven patients with MPS (4 MPS I, 4 MPS II, 2 MPS III, 1 MPS VI) and three control patients have participated in the study. Values are reported as mean ± SD. Mean values were compared with Student's t-test, assuming unequal variance.
Mean MPS and control patient ages were 8.9±4.6 and 9.1±1.5 years, respectively (p=0.96). Mean MPS and control C-IMT were 5.4±0.7 and 4.9±0.2 mm (p=0.077). Mean MPS and control left main coronary diameters were 2.7±0.6 and 2.6±4 mm (p=0.76), while right coronary diameters were 2.3±0.7 and 2.7±0.2 mm (p=0.36). No significant correlations were found between C-IMT and either coronary artery diameter.
Conclusion: In a comparison approaching statistical significance, C-IMT in MPS patients is increased compared to controls, possibly reflecting arterial intimal glycosaminoglycan accumulation. However, coronary diameters of MPS patients were not significantly different from controls. Enrollment for this study continues; prospective studies should be performed to determine if MPS patients with thicker C-IMTs are at higher risk of cardiac events.

419-P

CHIMERISM OF BONE MARROW REDUCES THE GLYCOLIPID STORAGE IN FABRY DISEASE MICE

Yokoi T[1], Kobayashi H[1], Fukuda T[2], Eto Y[3], Ida H[4], Ishige N[5], Kitagawa T[5], Otsu M[6], Nakauchi H[6], Ohashi T[1]
[1]*Dep Gene Ther, Inst DNA Med, Jikei Univ, Tokyo, Japan*
[2]*Div Neuropath, Dep Neurosc, Jikei Univ, Tokyo, Japan*
[3]*LSD Res Cent/ Inst Genet Dis, Jikei Univ, Tokyo, Japan*
[4]*Dep Ped, Jikei Univ, Tokyo, Japan*
[5]*Tokyo Health Service Assosiation, Tokyo, Japan*
[6]*Div SCT, Inst Med Sci, Univ Tokyo, Tokyo, Japan*

Objective: Fabry disease (FD) is a lysosomal storage disease, characterized by deficient activity of lysosomal enzyme, Alpha-galactosidase A. This results in glycolipid storage in various organs. Hematopoietic stem cell-based gene therapy is a strong candidate to offer the permanent correction for FD. However, the minimum requirement of enzyme activity to reduce the glycolipid storage is still unknown. In this study we have used a mouse model of FD to establish what level of engraftment and enzyme activity is required to reduce the accumulation of substrates.
Method: Lethally irradiated FD mice (12 weeks old) were transplanted with normal bone marrow cells (C57BL/6.Ly5.1) mixed with those of FD mice (C57BL/6.Ly5.2) at various ratios. 8 weeks after transplantation, chimerism and differentiation of peripheral blood cells were measured. Enzyme activity and the accumulation of substrates (globotriaosylceramide: GL3) in each organ was determined.
Result: Chimerism remained stable at 8 weeks after transplantation and differentiation of transplanted cells to granurocytes, monocytes, B cells and T cells were equally. Enzyme activity increased significantly and GL3 decreased significantly in spleen by 10% chimerism and in heart by 50% chimerism. In liver enzyme activity was not increased significantly, but GL3 was decreased significantly by 50% chimerism.
Discussion: The results indicate the possibility of developing effective and efficient conditioning protocols for LSDs. Stem cell gene therapy may require only a low level of normal or gene-corrected cells for a permanent and beneficial therapeutic outcome.

420-P

NIEMANN-PICK TYPE C DISEASE IN BRAZIL: A MULTICENTER RETROSPECTIVE STUDY OF 28 PATIENTS

Lourenco CM[1], Souza FTS[2], Vlaskova H[3], Dvorakova L[3], Van der Linden V[4], Albuquerque RCAP[5], Santos MLSF[6], Ribeiro E[7], Souza CF[2], Giugliani R[2], Elleder M[3], Marques Jr W[1]
[1]*Neurogenetics Unit, Univ of Sao Paulo, Ribeirao Preto, Brazil*
[2]*Hospital de Clinicas de Porto Alegre, Porto Alegre, Brazil*
[3]*Institute for Inherited Metabolic Disord, Prague, Czech Republic*
[4]*Hospital Barão de Lucena, Recife, Brazil*
[5]*Hospital de Base, FAMERP, S. J. do Rio Preto, Brazil*
[6]*Hospital Pequeno Príncipe, Curitiba, Brazil*
[7]*Hospital Albert Einstein, Fortaleza, Brazil*

Background: Niemann-Pick disease type C (NPC) is a brain lysosomal storage disorder with a wide clinical variability even ranging from fulminant presentations with hydrops fetalis to adult onset neurologic presentation.
Objectives: To characterize the clinical features of Brazilian patients with NPC and to identify possible genotype-phenotype correlations.
Methods: A clinical retrospective study and review of exams were carried out of 28 patients diagnosed with NPC.
Results: 17 patients were confirmed to have NPC by Filipin staining (eleven patients required molecular analysis of NPC1 gene). Regarding clinical form, 6 were perinatal and 10 were infantile. Most of the patients with perinatal/infantile forms carry at least one alelle with a deletion in NPC1 gene. All patients had prolonged jaundice and five of them were diagnosed with "neonatal hepatitis". The first symptom of neurological disease in the infantile group was developmental delay in all but one patient; vertical supranuclear gaze paralysis (VSGP) was a common finding but not universal (86% of patients). Brain MRI performed showed cerebral and cerebellar atrophy with leukoencephalopathy.
Conclusions: The clinical course in NPC disease in our cohort was similar among affected patients and is characterized by a relentless neurodegenerative course that leads to premature death. The onset of neurological presentation of the disease varied among the patients but showed a linear progression of deterioration regardless the clinical form. Filiping staining was an important tool to diagnose NPC patients, however inconclusive tests should be carefully evaluated by the clinician before rule out a NPC diagnosis.

421-P

SUBSTRATE REDUCTION THERAPY IN THE TREATMENT OF NEUROLIPIDOSES: NIEMANN-PICK TYPE C AS A PARADIGM

Lourenco CM[1], Van der Linden V[2], Camelo JS[1], Santos MLSF[3], Albuquerque RCAP[4], Ribeiro E[5], Marques Jr W[1]
[1]*Hospital Barão de Lucena, Recife, Brazil*
[2]*Hospital Pequeno Príncipe, Curitiba, Brazil*
[3]*Hospital de Base, FAMERP, S. J. do Rio Preto, Brazil*
[4]*Hospital Albert Einstein, Fortaleza, Brazil*

Background: Neurolipidoses are a complex group of lysosomal storage disorders of the brain. The course of such disorders is relentless leading to death in premature age after the onset of the neurological symptons. One of the best known neurolipidoses is Niemann-Pick type C disease (NPC), characterized by a defect in cholesterol esterification causing lipid storage; Substrate Reduction Therapy (SRT) has been proposed as a treatment for NPC and other neurolipidoses based on study of iminosugars effects in the brain.
Objective: To study a cohort of Brazilian NPC patients treated with SRT.
Material/Methods: Thirteen patients with clinical, biochemical and/or molecular diagnosis of NPC were evaluated with a clinical protocol/physical examination and complementary exams (abdominal ultrasound, brain MRI, EEG, NCV/EMG) during one year of treatment with the imino sugar N-butyldeoxynojirimycin (NB-DNJ)
Results: All patients but one had started on an inhibitor of glycosphingolipid storage after the onset of the neurological symptons. All patients with hepatomegaly have showed some decrease of liver size, although various degree of splenomegaly still remains. Stabilization of mental deterioration was seen in 10 patients with neurological symptons—one of the patients with the juvenile form had remarkable improvement of neurological features, but two patients had progress of the disease with seizures and cognitive decline. Electrophysiological studies did not demonstrate any peripheral neuropathy.
Conclusions: SRT seems to be a reasonable approach to treat lysosomal storage disorders, especially those disorders with brain involvement. Nevertheless, it is necessary to develop better biomarkers to follow the treatment response in such patients.

422-P

REDUCTION OF ELEVATED PLASMA GLOBOTRIAOSYLSPHINGOSINE FOLLOWING ENZYME REPLACEMENT THERAPY IN PATIENTS WITH CLASSIC FABRY DISEASE

Linthorst GE[1], van Breemen MJ[2], Rombach SM[1], Dekker N[2], Zwinderman AH[3], Breunig F[4], Wanner C[4], Aerts JMFG[2], Hollak CEM[1]
[1]*Dept of endocr and metab, Acad Med Centr, Amsterdam, Netherlands*
[2]*Dept of Med Biochemistry, Acad Med Cent, Amsterdam, Netherlands*
[3]*Dept of clin epidem, Acad Med Centr, Amsterdam, Netherlands*
[4]*Dept of int med/nephrol, Univ. Wurzburg, Wurzburg, Netherlands*

Background: Elevated plasma globotriaosylsphingosine (lysoGb3) is a hallmark of classical Fabry disease. The effect of enzyme replacement therapy (ERT) on Gb3 and lysoGb3 levels in classical Fabry disease patients was studied.
Methods: Fabry disease patients (22 males and 21 females) with aGalA mutations associated with classical phenotypes were investigated during one year of treatment. Patients received agalsidase alfa at 0.2 mg/kg (7 M/7F), agalsidase beta at 0.2 mg/kg (6 M/5F), or 1.0 mg/kg (9 M/9F).
Results: In males, plasma lysoGb3 and Gb3 concentrations decreased after 3 months of ERT, but stabilized and remained elevated in all treatment groups. Males often developed antibodies (AB+), but was seen less frequent in those receiving agalsidase alfa (43%) compared to 83% at 0.2 mg/kg and 89% at 1.0 mg/kg agalsidase beta, respectively ($p<0,05$). The average reduction in plasma lysoGb3 at t=12 months was smaller in AB+ hemizygotes receiving agalsidase alfa ($p<0,05$) or beta (not significantly) at a dose of 0.2 mg/kg (-43% and -50%, respectively, as compared to AB+ hemizygotes treated with agalsidase beta 1.0 mg/kg (-73%) or AB- hemizygotes (-71%). In females no antibody formation was noted and Gb3 levels were normal. The average reduction in plasma lysoGb3 did not differ significantly between treatment groups at 12 months: -42% in heterozygotes receiving agalsidase alfa and -29% and -50 % in those treated with 0.2 mg/kg and 1.0 mg/kg agalsidase beta, respectively.
Conclusions: ERT reduces plasma lysoGb3, regardless of the recombinant enzyme used. Reductions of lysoGb3 are influenced by agalsidase antibodies and dose.

423-P

MUCOLIPIDOSIS TYPE IV IN A TURKISH CHILD ASSOCIATED WITH A NOVEL MCOLN1 MUTATION

Tuysuz B[1], Goldin E[2], Metin B[3], Korkmaz B[3], Yalcinkaya C[3]
[1]*Dep Ped Genet, Ist Uni Cerrahpasa Med, Istanbul, Turkey*
[2]*NIH Med Genet, Nat Hum Gen Res, Bethesda, United States*
[3]*Dep Ped Neurol, Ist Uni, Cerrahpasa Med, Istanbul, Turkey*

Mucolipidosis IV is caused by mutations in MCOLN1, a gene encoding mucolipin-1, a cation channel of the TRP family. Mucolipin-1 is responsible for maintaining lysosomal function. Patients with MCOLN1 mutations have a defective development of the corpus callosum and motor function is affected. As a result, patients cannot speak or ambulate. In addition, clouding of the cornea and progressive retinal degeneration causes visual impairment. Mucolipin-1 is directly responsible for stomach acid secretion, which is absent in patients, resulting in hypergastrinemia. The majority of known MLIV patients are Ashkenazi Jews, and most have a splice IVS3-2 A > G, or a 6.4 kb deletion mutation in MCOLN1. Here we present a Turkish patient who, in addition to the typical neurological and visceral characteristics of MLIV, also demonstrates defects in the posterior limb of internal capsule by MRI, micrognathia, and clinodactyly of the fifth fingers. Direct sequencing of his DNA revealed a homozygous c.1364C>T (S456L) mutation in MCOLN1, which was heterozygous in both consanguineous parents. This mutation, like several previously described, changes the protein sequence in the channel pore domain of the protein. Serine 456 is conserved in mucolipin proteins throughout evolution, therefore the mutation is considered as causative for the severe phenotype of this patient.

424-P

TWO PATIENTS WITH MUCOLIPIDOSIS TYPE III: CLINICAL OVERLAP WITH JUVENILE RHEUMATOID ARTHRITIS AND PROGRESSIVE PSEUDORHEUMATOID DYSPLASIA

Arcagok B[1], Kivilcim M[1], Oktay G[1], Bursali A[2], Kasapcopur O[3], Arisoy N[3], Tuysuz B[1]
[1]*Dep Ped Ortop, Baltalimani Hosp, Istanbul, Turkey*
[2]*Dep Ped Rom, Ist Uni Cerrahpasa Med, Istanbul, Turkey*

Mucolipidosis (ML) II and III reflect multiple deficiencies of many lysosomal hydrolases that require post-translational processing to form the recognition site that permits their cellular uptake. The fundamental defect is related with N-acetylglucosaminyl-1-phosphotransferase. Patients with ML II have complete deficiency of this enzyme, while ML III has varying amounts of residual activity of the enzyme. Clinical manifestations of ML III include joint stiffness and limitation of motion, coarse face, corneal opacity and aortic regurgitation. We present 9 years old boy and 7 years old girl with mild coarse facial features, corneal clouding, and limitation of elbow, wrist, interphalangeal joints and mild findings of dysostosis multiplex radiologically. The enzyme analyses were compatible with ML II or III. Due to absence of hypopigmentation and of congenital disease onset, the diagnosis of ML II was easily ruled out. The clinical phenotype of ML III often overlaps with juvenile rheumatoid arthritis and progressive pseudorheumatoid dysplasia. The second patient had been diagnosed and treated as juvenile romatoid arthritis for a short time. ML III shows genetic heterogeneity. Two genes encode three subunits of the enzyme α/β and γ. The clinical features of ML III gamma are similar to but milder than those observed in individuals with ML III alpha/beta. Late onset disease and mild clinical findings suggest that our patients have gamma phenotype.

425-P

SIALIDOSIS TYPE II, INFANTILE FORM WITH RENAL INVOLVEMENT IN A BOY

Kivilcim M[1], Arcagok B[1], Ozek E[1], Caliskan S[2], Sever L[2], Tuysuz B[1]
[1]*Dep Ped Nephrol, Ist Uni, Cerrahpasa Med, Istanbul, Turkey*

Sialidosis is a lysosomal storage disease caused by the deficiency of alpha-N-acetyl neuraminidase-1. The lack of this enzyme results in an abnormal accumulation of complex carbohydrates known as mucopolysaccharides, and of fatty substances known as mucolipids. Sialidosis is classified into two main clinical variants: type I and type II. Type II may be subdivided into three forms: congenital, infantile, juvenile. We present a twelve years old boy with mental retardation, severe joint stiffness, kyphoscoliosis, myoclonic seizures, coarse and atypical face, optic atrophy, and cherry red spot at macula, inguinal hernia as well as aortic and mitral regurgitation and deafness. Increased levels of protein and oligosaccharides were detected in urine analysis. Foamy cells were seen in bone marrow. Skeletal radiographies showed dysostosis multiplex. Nephromegaly was demonstrated in urinary ultrasonography. Cytoplasmic swelling and vacuoles shown by kidney biopsy were compatible with storage disease. The leukocyte enzyme analyses revealed neuraminidase deficiency. His fibroblast culture findings were consistent with the diagnosis of sialidosis. He died at age of 17 from chronic renal disease. Sialidosis type I is the milder form, whereas type II is the more severe form with an earlier onset and is also known as the 'dysmorphic' type. The patient was diagnosed with sialidosis type II and infantile form due to severe dysmorphic findings and clinical manifestations that had developed in first year of life.

426-P

MUCOPOLYSACCHARIDOSIS TYPE I: PHENOTYPE-GENOTYPE CORRELATIONS AND EVALUATION OF THE RESPONSE TO ENZYME REPLACEMENT THERAPY

Alp Z[1], Cimen S[1], Bertola F[2], Aydin A[3], Tuysuz B[1]
[1]*Dep Mol Genet, Milano Uni, Milano, Italy*
[2]*Dep Ped Metab, Ist Uni Cerrahpasa Med, Istanbul, Turkey*

Mucopolysaccharidosis type I (MPS I) has three variants, differing widely in their severity, with Hurler (H) syndrome being the most severe, Scheie (S) syndrome the mildest and Hurler-Scheie (HS) syndrome giving an intermediate phenotype. We have evaluated the phenotype-genotype correlations of six patients with MPS I and responses of four patients with MPS I-HS and S who have been receiving enzyme replacement therapy (ERT). Two boys with MPS I-H carried homozygous mutation of IVS4–1G>A and the treatment was not initiated because of mental retardation. First patient was diagnosed at the age of 15 months and died at the age of 9 due to cardiac failure. The other patient was diagnosed at the age of 4 months and the clinical findings have deteriorated progressively by the age of five. Though not severe, all the clinical signs except mental retardation were observed in two patients with MPS I HS. One of them was 14,5 year-old boy who had compound heterozygous mutation of IVS12+6 T>A/ p.Q70X. He has been receiving ERT for 7 years. Especially symptoms of sleep apnea, decreased effort capacity and joint complaints were recovered significantly and coarse face was markedly improved. Other patient with MPS I HS was 4,5-year-old girl who was homozygous for the mutation c.46_57del12. Two sibs aged 23 and 33 were diagnosed with MPS I-S. Both had mild joint symptoms and had homozygous p.E276K mutation. The last three patients have been receiving ERT for 7 months but did not show significant clinical improvement.

427-P

MUCOPOLYSACCHARIDOSIS TYPE VII (SLY DISEASE): TWO DIFFERENT CLINICAL PRESENTATIONS

Kalkan Ucar S[1], Koroglu O[2], Yalaz M[2], Kultursay N[2], Coker M[1]
[1]Div Metab Dis, Ege Univ Child Hosp, Izmir, Turkey
[2]Div Neonat, Ege Univ Child Hosp, Izmir, Turkey

Sly syndrome, or Mucopolysaccharidosis type VII (MPS VII), is a very rare autosomal recessive lysosomal storage disorder (LSD). Sly syndrome is due to β-glucuronidase deficiency and shows a wide range of severity and system involvement heterogeneity similar to MPS I and II, with phenotypic extremes from the very severe fetal hydrops to the oligosymptomatic variant. Here, we present two cases with MPS VII, the first with slowly progressive, later onset, normal intelligence, oligosymptomatic mild variant [β-glucuronidase activity measured as 0.7 nmol/hour/mg protein (N : 90–140 nmol/hour/mg protein)], and second severe type with hydrops fetalis, facial dysmorphism, hepatosplenomegaly, and skeletal dysplasia [β-glucuronidase activity measured as 0 nmol/hour/mg protein (N : 129±45.8 nmol/hour/mg protein)].

428-P

FABRY DISEASE PATIENTS AMONG BRAZILIAN HAEMODIALYSIS SUBJECTS

Muller KB[1], Rodrigues MDB[1], Pereira VG[1], Martins AM[1], D'Almeida V[2]
[1]Dep Ped, Univ Federal de São Paulo, São Paulo, Brazil
[2]Dep Biosci, Univ Federal de São Paulo, Santos, Brazil

Background: Fabry Disease (FD) is a X-linked lysosomal storage disorder caused by the deficiency of alpha-galactosidase A (α-galA), which results in the intralysosomal accumulation of globotriaosilceramide (Gb3). This accumulation in vascular endothelium leads to a multisystemic disease, with acroparesthesia, hypohydrosis, angiokeratomas, corneal opacity, cold and exercise intolerance, together with cardiocerebrovascular and renal disease. In the absence of a family member who has already received a diagnosis of FD, many cases are not identified until adulthood, making FD an under diagnosed disease. Screening among dialysis patients is important because it allows the detection of FD in their family members and also ensures the correct treatment with enzyme replacement therapy. The aim of this work was to identify patients with FD among Brazilian male dialysis patients.
Methods: 6597 dried blood spots on filter paper (DBS) samples were received from 76 cities of Brazil since November 2005. Among them, 320 samples were excluded according to the study criteria, leaving a total of 6277. We performed the α-galA activity using a fluorescent assay. Values below 2.5 μmol/Lblood/h were considered compatible with FD and had the leukocyte activity measured to confirm the diagnosis. ,
Results: Until now 23 patients were identified with FD by DBS and leukocyte enzyme activity assays.
Conclusion: We found that nearly 0.37% of our sample was composed of FD patients. These results are in accordance with others FD screenings among dialyzed patients and confirm the fact that FD is a under recognized disorder.
Supported by: FAPESP, CNPq, AFIP, IGEIM, Genzyme do Brasil.

429-P

EXPERIENCE IN TREATING VERY YOUNG MUCOPOLYSACCHARIDOSIS VI PATIENTS WITH ERT

Ribeiro E[1], Acosta A[2], Giuliani L[3], Horovitz D[4], Bezerra K[1], Magalhães T[4], Palhares D[3], Cardoso L[2], Vieira T[5], Giugliani R[5]
[1]Faculdade de Medicina Christus, Fortaleza, CE, Brazil
[2]Medical Genetics Service/HUPES/UFBA, Salvador, BA, Brazil
[3]Department of Pediatrics/UFMS, Campo Grande, MS, Brazil
[4]Medical Genetics Center/IFF/FIOCRUZ, Rio de Janeiro, RJ, Brazil
[5]Medical Genetics Service/HCPA/UFRGS, Porto Alegre, RS, Brazil

Background: Mucopolysaccharidosis VI (MPS VI) is caused by a deficiency of the lysosomal enzyme N-acetylgalactosamine-4-sulphatase. Enzyme replacement therapy (ERT) showed clinical benefits for patients older than 6 years, but experience with treatment of small children is still limited.
Objective: To report four unrelated MPS VI patients who started ERT under one year of age.
Methods: Patients were followed in different Brazilian centers, where data was collected before first infusion (BL) and around 6/12/26 and 52 weeks of ERT. A structured formulary was used to collect data.
Results: Patient 1: female, age at diagnosis (AD) 8mo, started ERT at 10mo; BL presented pectus carinatum, joint stiffness, cardiopathy and skeletal abnormalities; with ERT glycosaminoglycans(GAGs) decreased; spinal cord compression was diagnosed after 6mo. Patient 2: male, diagnosed prenatally, first infusion at 5 days, no abnormalities at BL; during the 5th infusion presented O2 desaturation with perioral cyanosis; picture was mild, episode not considered IAR; difficulties with venous access associated to social issues impacted compliance; Patient 3: male, AD 4mo, started ERT at 6mo, no abnormalities at BL; with ERT decrease on GAGs. PATIENT 4: male, AD 2mo started ERT at 4mo; at BL presented typical facial features and skeletal abnormalities; with ERT GAGs decreased.
Conclusions: ERT was well tolerated by these very young MPS VI patients, and led to a decrease in urinary GAGs. Although disease progression signs were observed in some patients, we believe that starting ERT in young patients can improve the clinical outcome.

430-P

OXIDATIVE STRESS PARAMETERS IN GAUCHER DISEASE

Rodrigues MDB[1], Guariniello LD[2], Martins AM[1], D'Almeida V[3]
[1]Dep Ped, Univ Fed São Paulo, São Paulo, Brazil
[2]Dep Psychobiol, Univ Fed São Paulo, São Paulo, Brazil
[3]Dep Biosc, Univ Fed São Paulo, São Paulo, Brazil

Background: Oxidative stress may play an important role in many metabolic disorders. Gaucher Disease (GD) is a lysosomal storage disorder caused by the deficiency of the enzyme ß-glucosidase. Its deficiency leads to accumulation of glycosylceramide inside the cells and to an altered redox status in GD fibroblasts. In addition, treated GD patients present different activity levels of some antioxidant erythrocytes enzymes when compared to healthy subjects.
Objectives: To evaluate oxidative stress parameters in plasma and macrophages cultures obtained from healthy volunteers (HV) and treated GD patients.
Methods: Thirty milliliters of heparinized blood was collected from 5 treated GD patients and 15 HV. Plasma was obtained in order to determine paraoxonase 1 activity assay (arylesterase activity). Monocytes were obtained by centrifugation with Ficoll and were incubated at 37°C/5% CO2 in RPMI 1640 with 10% fetal bovine serum until complete adherence. Monocytes were cultured for 7 days to allow their differentiation into macrophages. In the 7th day we evaluated reactive oxygen species (ROS) formation with dichloro-dihydrofluorescein diacetate reagent. These results were normalized with a viability assay (MTT).
Results: GD patients macrophages cultures presented higher production of ROS in comparison to HV (t test, p=0.02). Although no difference was observed in plasma arylesterase activity of paraoxonase 1 (t test, p=0.17), GD patients seem to present lower activities of this antioxidant enzyme.
Conclusion: Oxidative stress seems to be an important phenomenon involved in GD physiopathology.
Financial support: FAPESP, CNPq, CAPES, AFIP and IGEIM.

431-P

SPERMATOGENESIS AND SEMINIFEROUS EPITHELIUM INTEGRITY IN A MURINE MODEL OF MUCOPOLYSACCHARIDOSIS I

Pereira VG[1], Moreira CM[2], Aguiar Jr O[2], D'Almeida V[2]
[1]*Dep Ped, Univ Fed São Paulo, São Paulo, Brazil*
[2]*Dep Biosc, Univ Fed São Paulo, Santos, Brazil*

Background: Recent studies have demonstrated that the accumulation of undegraded molecules in lysosomal storage disorders may alter some complex processes of cellular signaling in different tissues, which could lead to cellular damage and death. The aim of this study was to evaluate the spermatogenesis and seminiferous epithelium integrity in a mucopolysaccharidosis type I (MPS I) murine model.
Methods: Testes were removed from six male Idua -/- and six Idua +/+ 6 months-old mice. Paraffin sections were stained with hematoxylin-eosin for histopathological analysis under a light microscope.
Results: Testes from MPS I animals presented 20% of reduction in the number of tubules and an elevated number of atrophied (or Sertoli cells-only) tubules compared to controls. In addition, exfoliated germ cells were visualized in the lumina of some seminiferous tubules. Functional units with normal diameter were also seen.
Conclusions: Our results demonstrate morphological/functional alterations in the seminiferous epithelium of MPS I mice. Considering that endocytic activity of Sertoli cells is essential for maintaining homeostasis during spermatogenesis, it seems reasonable to suppose that the accumulation of glycosaminoglycans may affect the endocytic capacity of Sertoli cells, leading to the observed epithelial disassembly.
Financial support: FAPESP, CNPq, CAPES, AFIP and IGEIM.

432-P

NATURAL HISTORY, DETAILED ANTHROPOMETRIC DATA AND JOINT RANGE OF MOTION OF PATIENTS WITH MAROTEAUX-LAMY SYNDROME (MUCOPOLYSACCHARIDOSIS TYPE VI)

Jurecka A[1], Rozdzynska A[1], Marucha J[1], Czartoryska B[2], Tylki-Szymanska A[1]
[1]*Dep Metab Dis, CMHI, Warsaw, Poland*
[2]*Dep Genet, Inst Psych Neurol, Warsaw, Poland*

Objectives: Our goal was to describe the natural history, clinical manifestations, detailed anthropometric features and joint range of motion (ROM) in 4 living patients with Maroteaux-Lamy syndrome before introduction of enzyme replacement therapy.
Results: 1. Clinical features of MPS VI varied among our patients. During the course of the disease, patients developed short stature, skeletal malformations, restricted ROM, corneal clouding, hearing impairment, and cardiac abnormalities. 2. All patients had similar characteristics at the time of birth but showed significant difference when compared with the healthy population. 3. Analysis of ROM showed impairments at multiple joints in all patients. Restriction in upper extremities ROM was observed since the first year of life. These limitations were particularly visible in the shoulder joint. Gross and fine motor delays were present in all children at the time of assessment, and were most evident in locomotor abilities. These limitations intensified and became more severe with the patients' age, making patients' self-care more difficult.
Conclusions: 1. Polish MPS VI patients presented with clinical phenotypes within a broad spectrum, ranging from severe to relatively mild. 2. Anthropometric features of patients with MPS VI significantly differed from the healthy population. Growth patterns were associated significantly with MPS VI at birth. Children with MPS VI grow considerably slower, and differences between healthy and affected children increase with age. 3. MPS VI patients, similarly to patients with other MPS disease, require introduction of a proper active physical rehabilitation program as early as possible to prevent joint restrictions.

433-P

NATURAL HISTORY OF NIEMANN—PICK B IN TUNISIA

Ben Turkia H[1], Vanier MT[2], Ben Chehida A[1], Azzouz H[1], Abdelmoula MS[1], Tebib N[1], Ben Dridi MF[1]
[1]*Paediatric Department /La Rabta Hospital, Tunis, Tunisia*
[2]*Maladies Métaboliques, INSERM U 82, BRON, France*

Background: Natural history of Niemann-Pick B was studied in Caucasian populations but there is no data concerning NPB in the Maghrebian population where genotypic homogeneity was found.
Aim: To characterize natural history of NPB in Tunisian population.
Methods: Tunisian patients with deficiency in acid sphingomyelinase were included. An assessment of visceral, hematological, neurological, lipid and bone involvement were performed at the start and at the end of the study.
Results: Thirty four patients with NPB (30 children and 4 adults) were diagnosed over a period of 21 years (1988–2008). After a mean follow up period of 13 years; 25 patients were assessed. Patients were mostly originated from the center of Tunisia. Stable hepatomegaly and splenomegaly were seen in 90 and 93% of patients, respectively. All adults have short stature. Chest interstitial infiltrate was observed in 96% of patients. Restrictive syndrome was found in 9/13 patients investigated. Osteopenia was demonstrated on DXA in 80% of patients. Proportion of patients with anaemia decreased whereas leucopenia and thrombocytopenia frequencies increased. High cholesterol and triglyceride level were found respectively in 25 and 31% of patients and low HDL CT in 94%. Homozygosity for delR608 mutation was found in 21 of 22 families.
Conclusion: Tunisian NPB phenotype is moderate. Lipid abnormalities are less frequent with the exception of low HDL CT. Persistant short stature was more frequent in affected adults.

434-P

DILATED CARDIOMYOPATHY REVEALING AN INFANTILE FORM OF POMPE DISEASE IN A 17-MONTH-OLD GIRL

Soule N[1], Perez T[2], Abou Ezzi K[2], Bonnefoy R[1], Paoli F[1], Froissart R[3], Chantepie A[1], Labarthe F[1]
[1]*Médecine Pédiatrique, CHU Tours, France*
[2]*Unité Pédiatrique de Soins Intensifs, CHU Tours, France*
[3]*Laboratoire de Maladies Métaboliques, CHU Lyon, France*

Introduction: Patients with infantile form of Pompe disease (PD) classically exhibit a severe hypertrophic cardiomyopathy associated with myopathy leading to death in the first year of life. We report the case of a 17-month-old girl affected by PD revealed by a chronic heart failure with dilated cardiomyopathy.
Case Report: This girl was the first child of non-consanguineous parents, without specific familial history. A moderate hypotonia, associated with psychomotor delay, muscle weakness and failure to thrive was reported from 9-month-old. At 17-month-old, she was admitted for a severe cardiorespiratory failure. Deep tendon reflexes were absent and routine biochemical investigations displayed a 8 fold-increased CK levels. Echocardiography showed a major left ventricle dilatation (54 mm, Z-score +9.9SD), with severe contractile dysfunction (ejection fraction 23%, normal value [nv]55–75%) and slight hypertrophy, which was not evocative of PD. Brain Natriuretic Peptide level was very high (>5000 ng/ml, nv<30), according to heart failure. Plasma lactate level, acylcarnitine profile and urinary organic acid profile were normal. Electrocardiogram, showing short PR intervals and high voltage QRS complexes, suggested glycogen storage disease. Finally, PD was confirmed by acid maltase deficiency in lymphocytes (0 μkt/kg prot, nv 2.6–10) and increased urinary excretion of tetraglucose. Physical condition progressively improved after 7-months of enzyme replacement therapy and conventional cardiac therapies, but with persistence of a dilated cardiomyopathy.
Conclusion: Dilated cardiomyopathy maybe the revealing symptom of infantile form of PD, possibly reflecting the late stages of disease progression. In such condition, electrocardiogram pattern can orient diagnosis.

435-P

ENZYME ASSAY AND CLINICAL ASSESSMENT IN SUBJECTS WITH A CHINESE HOTSPOT LATER-ONSET FABRY MUTATION (IVS4+919G→A)

Yu H[1], Niu D[1], Lin H[2], Huang C[1], Chong K[2], Hsu Ju-Hui[1], Lee P[1], Cheng K[1], Chiang C[3], Ho H[4], Kao S[4], Chen S[5], Lin P[5]
[1]*Dep Pedia, Taipei Veterans General Hosp, Taipei, Taiwan*
[2]*Inst Clin Med, Ntl Yang-Ming Univ, Taipei, Taiwan*
[3]*Neonatal Scr Cente, Chinese Found Health, Taipei, Taiwan*
[4]*Sec Newborn Scren, Tpe Inst of Pathology, Taipei, Taiwan*
[5]*Dep Ophthalmology, Veterans Gen Hosp, Taipei, Taiwan*

Background and Purpose: Newborn screening for Fabry disease in Taiwan revealed a high incidence of the later-onset GLA mutation IVS4+919G→A (~1 in 1,500–1,600 males). We evaluated the enzyme assay and clinical manifestations in adult subjects with this mutation.
Methods: Enzyme activity assay, echocardiography, urine examinations for microalbumin, creatinine, and ophthalmologic examinations were performed in 94 subjects with Fabry mutation IVS4+919G→A (22 males and 72 females).
Results: Plasma α-galactosidase A activities were analyzed in all 94 subjects, which showed 1.29 ± 1.39 nmol/h/mL for 22 males and 6.03 ± 2.42 nmol/h/mL for 72 females (normal range: 12.4 ± 2.25 nmol/h/mL). Ninety subjects received echocardiographic examinations and showed that 19 subjects had left ventricular hypertrophy (LVH) (14 males and 5 females). Urine examinations for microalbumin and creatinine were performed on at least two occasions in 86 subjects, which revealed that 17 subjects had microalbuminuria (5 males and 12 females). Fifty-two subjects received ophthalmologic examinations, and 41 subjects were found at least one item of ocular manifestations of Fabry disease. For subjects over 40 years of age, we found male subjects were more likely to have LVH than females ($p < 0.001$). Female subjects over 40 years of age were more likely to have LVH than those under 40 ($p < 0.005$).
Conclusion: This study showed a high prevalence of cardiovascular, renal, and ocular manifestations in Taiwan Chinese subjects with later-onset Fabry mutation IVS4+919G→A, and will help to recognize its natural history more precisely and determine the optimal timing for therapeutic intervention.

436-P

GROWTH & DEVELOPMENT IMPAIRMENT IN A CHILD WITH HUNTER SYNDROME: WHEN IS THE RIGHT TIME TO GIVE ENZYME REPLACEMENT?

Anzar J[1], Sjarif DR[2], Tanjung C[2]
[1]*Pediatric Dept, Sriwijaya Univ, Palembang, Indonesia*
[2]*Pediatric Dept, University of Indonesia, Jakarta, Indonesia*

Background: Hunter syndrome is an X-linked disorder that results from deficiency of iduronate-2-sulfatase. The only available therapy is enzyme replacement (ERT) (Elaprase.), the cost of ERT is very expensive and not expected to cure all symptoms, especially if there is cerebral involvement.
Objectives: To report one case of Hunter Syndrome that occurred in Indonesia and the limitation of enzyme replacement therapy due to cost.
Case Report: A 6-year-old Javanese boy with the normal 1st and 2nd brothers from middle socio-economic. No consanguinity. He starts to lose the ability of writing and walking normally in this past one year. He has good performance in school but now he refuses to write. His face became coarse with more hair and enlarged skull. His height is 110 cms (P10 NCHS) and weight 20 kgs (P25 NCHS). Head circumference 55 cms (>2SD). Coarse facies, thick eye brow, flat nasal bridge, frontal bossing, umbilical hernia, short and wide phalanges with joint stiffness
Laboratory. Iduronate-sulfatase (leukocyte) 0,29 nmol/mg Prot/4 hours.
Discussion: Growth in Hunter syndrome is usually impaired, since there is the accumulation of dermatan sulfate in the body, accumulation results in myxomatous valvular changes. The same problem happens with the development. Because heparan sulfate is an essential component of nerve cell membranes, the accumulation results in progressive mental deterioration. The report of ERT for the cerebral involvement is not satisfactory, so the ERT should be done soon since there is still no significant cerebral involvement in this patient. The problem is about the cost.

437-P

ENZYMATIC SCREENING IN DRIED BLOOD SPOTS FOR GLYCOGEN STORAGE DISEASE TYPE II USING POLYCLONAL ANTIBODIES TO LYSOSOMAL ACID α-GLUCOSIDASE

Kitagawa TK[1], Suzuki KS[1], Owada MO[1], Tanaka AT[2], Keutzer JK[3]
[1]*Tokyo Health Service Association, Tokyo, Japan*
[2]*Osaka City University School of Medicine, Osaka, Japan*
[3]*Genzyme Corporation, Cambridge, United States*

Background: Early diagnosis and treatment of glycogen storage disease (GSD) II is essential for maximum efficacy of the enzyme replacement therapy. Previously two diagnostic procedures were proposed to identify GSD II, we evaluated the diagnostic accuracy of the two methods, one is Chamoles's method (A) and other is fluorogenic immune capture assay, using polyclonal antibodies to lysosomal α-glucosidase (GAA) (B).
Materials: The dried blood spots (DBS) were obtained from 8 GSD II patients and 308 healthy control subjects.
Method A: DBS from individuals with GAA activity <25%, neutral α-glucosidase /GAA ratio >25, GAA inhibition by acarbose >75% were referred for a confirmatory whole-blood sampling.
Method B: Purified recombinant human GAA was obtained from Genzyme Co. A rabbit anti- GAA polyclonal antibody was produced and purified by usual manner.
To measure GAA activity, we immobilized anti-GAA antibody on microtiter plates to capture the enzyme and measured its activity with 4-MUα-glucopyranoside.
Results: The GAA activity calibration curves were linear over the range 0–1000 pg/well in method B. GAA activities in DBS from 8 patients with previously diagnosed GSD II were almost zero.
Of the 307 newborns screened, the 5 newborns showed the same abnormal levels. After detail examination, 4 cases were diagnosed with pseudo GAA deficiency; another case seemed to be a heterozygote by DNA analysis. The screening results were quite similar in both methods.
Conclusion: The high sensitivity and specificity of the both methods, suggested that the immune capture assay would be suitable for newborn mass-screening to detect GSD II.

438-P

NEWBORN SCREENING FOR POMPE DISEASE IN JAPAN

Oda EO[1], Tanaka TT[1], Kosuga MK[1], Osawa MO[2], Okuyama TO[1]
[1]*Center for Lysosomal diseas NCCHD, Tokyo, Japan*
[2]*Tokyo Women's Medical University, Tokyo, Japan*

Pompe disease is caused by a deficiency of acid alpha-glucosidase (GAA) and resulting in the accumulation of glycogen primarily in muscle tissue. Previous reports show successful newborn screening (NBS) has already initiated in Taiwan. In contrast, comparatively high frequency of "pseudodeficiency" makes NBS for Pompe disease more complicated in Japan. "Pseudodeficiency" is a group of individuals who has sequence variants p.G576S in GAA gene. They have low GAA activity, but show no symptoms of the disease.
We obtained dried blood spots (DBS) samples from 496 Japanese healthy controls and 29 Japanese Pompe disease patients and 5 obligate carriers. To investigate the feasibility of screening for Pompe disease in Japan, we assayed GAA with following conditions: (1) tGAA measured at pH 3.8 (2) GAA measured at pH 3.8 in the presence of acarbose and (3) Neutral glucosidase activity (NAG) measured at pH 7.0 without acarbose. For our screening, we calculated "%inhibition; (tGAA – GAA) / tGAA" and ration of "NAG/GAA." And we also analyzed sequence variants p.G576S in GAA gene using genomic DNA extracted from DBS. For screening, we use a method with following program: Samples with GAA < 8% of the normal mean, % inhibition > 60%, and NAG/GAA ratio > 30 were detected as positive.
There was 2 false positive. One was compatible with "Pseudodeficiency". The false- positive rate from this study was approximately 0.37%, suggesting that newborn screening for Pompe disease in Japan is feasible.

439-P

MUCOPOLYSACCHARIDOSES IN THAILAND: SIRIRAJ EXPERIENCE

Wasant P[1], Sathienkijkanchai A[1], Vattanavicharn N[1], Liammongkolkul S[1], Keeratichamroen S[2], Ketudat-Cairns JR[2], Svasti J[2], Kolodny EH[3]
[1]*Fac Med Siriraj Hosp, Mahidol Univ, Bangkok, Thailand*
[2]*Chulaborn Research Institute, Bangkok, Thailand*
[3]*New York University, New York, United States*

Background: Mucopolysaccharidoses (MPS) are a group of lysosomal storage disorders caused by deficiency of lysosomal enzymes needed to degrade glycosaminoglycans (or mucopolysaccharides); which are stored in various tissues and excreted in large amount in the urine. Storage in tissue results organ dysfunction and change in morphogenesis. We described here a group of patients with MPS identified in past 20 years at Siriraj Hospital in Thailand.
Methods: Screening using urine GAG followed by electrophoresis/ chromatogram in all patients suspected clinically to have MPS, the only center doing the test in Thailand. Confirmatory test using white blood cells enzyme analysis and molecular analysis was carried out only in MPS I & II.
Results: There are 54 patients identified by urine GAG; however only- 14 patients were confirmed by leucocytes enzyme analysis. Molecular analysis was done only in patients with MPS I-H, MPS I-HS, MPS I-S and MPS II. There were 5 patients with MPS I (9.3 %); 17 cases of MPS II, (31.5 %); 7 cases of MPS III (12.9 %); 10 cases of MPS IVA (18.5 %); 7 cases of MPS IV B (12.9 %); 4 cases of MPS VI (7.4 %) , one case of MPS VII (1.8 %), which was the first reported from Thailand and 3 cases of unidentified MPS (5.5 %). All patients were diagnosed late with multi-organ involvement. None of the MPS patients received enzyme replacement therapy or bone marrow transplantation.
Conclusion: The delayed diagnosis and unavailability of enzyme replacement therapy led to adverse outcome.

440-P

SIGNIFICANT RESPIRATORY IMPROVEMENT IN A HUNTER PATIENT UNDER ERT WITH IDURSULFASE

Del Toro M[1], Moreno A[2], Riudor E[3], Coll MJ[4], Domínguez MC[5]
[1]*Pediatric Neurology, Hosp Vall d'Hebron, Barcelona, Spain*
[2]*Pediatric Pneumology, Hosp Vall d'Hebron, Barcelona, Spain*
[3]*Metabolic Laboratory, Hosp Vall d'Hebron, Barcelona, Spain*
[4]*Institut de Bioquimica Clinica, Barcelona, Spain*
[5]*Bioch Mol Biol Res Cent, H Vall d'Hebron, Barcelona, Spain*

Background: Hunter syndrome (mucopolysaccharidosis II), is an X-linked disorder caused by a deficiency of iduronate-2-sulfatase. Accumulation of glycosaminoglycans is responsible of signs and symptoms of the disease and can be partially reverted under treatment with idursulfase.
Objective: To report a Hunter patient with a significant improvement in respiratory disease with idursulfase.
Clinical Report: We report a thirteen-year-old patient diagnosed of Hunter disease at the age of 8 months. The first manifestation of the disease was an acute respiratory syndrome at the age of 6 months which progressed rapidly to a severe pneumopathy requiring traqueostomy at the age of 7 months. He was diagnosed of Hunter disease (R443X mutation). At the age of ten he showed typical Hunter characteristics with very mild psychomotor retardation. During the last months before admission he suffered frequent lower air tract infections and progressive worsening of respiratory distress. He was admitted at the PICU unit because of severe dyspnoea. A thoracic scanner was performed showing severe tracheal and bronchial stenosis with bilateral pulmonary bronchiectasis. Fibroscopy showed important mucopolysaccharides deposits in trachea and bronchi. The patient required aggressive mechanical ventilation parameters and weekly fibroscopic dilatations of the respiratory tract. Treatment with idursulfase was introduced and two months later he was discharged from hospital under nocturnal mechanical ventilation. After 4 years of treatment he is stable. Fibroscopy shows a progressive reduction of the deposits and a tracheomalacia which is being evaluated for surgery.
Conclusion: Idursulfase has been very effective in improving obstructive air way disease in our patient.

441-P

CHARACTERIZATION OF THE SUMF1 MOLECULAR DEFECTS IN MULTIPLE SULFATASE DEFICIENCY

Sabourdy F[1], Baeza E[1], Guffon-Fouilhoux N[2], Delrue MA[3], Froissart R[4], Megarbane A[5], Dusser A[6], Caillaud C[7], Levade T[1]
[1]*Lab Biochimie Métabolique, IFB Purpan, Toulouse, France*
[2]*Unité Maladies Métaboliques, CHU Herriot, Lyon, France*
[3]*Genétique Médicale, CHU Pellegrin, Bordeaux, France*
[4]*Centre de biologie et Pathologie, Bron, France*
[5]*Unité de Génet Méd, Univ Saint Joseph, Beirut, Lebanon*
[6]*Neuropédiatrie, CHU de Bicêtre, Kremlin-Bicêtre, France*
[7]*Lab Génétique Médicale, Institut Cochin, Paris, France*

Multiple sulfatase deficiency (MSD) is a very rare autosomal recessive disorder, which is caused by deficient activity of the Formylglycine-Generating Enzyme (FGE), a critical protein involved in post-translational activation of sulfatases. Patients with MSD exhibit a defective activity of all sulfatases, and combine symptoms from several individual sulfatase deficiency disorders such as metachromatic leukodystrophy, mucopolysaccharidosis and X-linked ichthyosis. Here, we report 5 unrelated families in which clinical examination and lysosomal enzyme analyses led to MSD diagnosis. Age of onset varied from birth to 7. Mutational analysis of the SUMF1 gene or cDNA encoding FGE allowed the identification, for each patient, of the underlying mutations. Except for the A279V, R349W and G263V substitutions, the other mutations have not been described so far. Site-directed mutagenesis studies were used to investigate the outcome of these mutations on the expression and function of the FGE protein. In addition to provide insights into genotype-phenotype correlations, molecular diagnosis of MSD allowed prenatal diagnosis.

Patient	Origin	Mutation	Affected Exon	Mutant Protein	Abnormal Function
J. A	France	c.836C>T; c.836C>T	6	A279V	Larger side chain, clash with A283 and F275
F.M.	Lebanon	c.706C>T; c.1045C>T	5 and 9	R236X, R349W	Five side chain interactions lost (E9
M.I.K	Pakistan	c.788G>T; c.788G>T	6	G263V	Larger side chain, clash with T263 and T270
E.A.	France	IVS5+1G>C, [c.776A>G; c.1018 T>C]	5, 6 and 9	Exon 5 skipping, N259S, Y340H	Ca2+ binding impairment (E6); affected catalytic site ?(E9)
M.D.	France	c.520–954dup	4 to 7	unknown	

(Supported by INSERM and VML)

442-P

CLINICALLY SIGNIFICANT HEMOGLOBIN RESPONSE OBSERVED WITHIN 3 MONTHS FOLLOWING TREATMENT WITH VELAGLUCERASE ALFA IN PATIENTS WITH TYPE 1 GAUCHER DISEASE

Gonzales DE[1], Ben Dridi M-F[2], Lukina E[3], Kisinovsky I[4], Ben Turkia H[2], Elstein D[5], Zahrieh D[6], Crombez E[6], Bhirangi K[6], Zimran A[5]
[1]*Sanatorio Español, Asunción, Paraguay*
[2]*La Rabta Hospital, Tunis, Tunisia*
[3]*National Research Center for Hematology, Moscow, Russian Federation*
[4]*Your Health S.A., Buenos Aires, Argentina*
[5]*Shaare Zedek Medical Center, Jerusalem, Israel*
[6]*Shire Human Genetic Therapies, Cambridge, United States*

Background: Velaglucerase alfa, an ERT for type 1 Gaucher disease (GD1) produced by gene activation in a human cell line, was recently approved in the US.
Methods: In study TKT032, 25 treatment-naïve GD1 patients, aged 4–62 years, with intact spleens, were randomized to receive intravenous velaglucerase alfa 60 U/kg (n=12) or 45 U/kg (n=13) every other week for 1 year. The primary endpoint was change in hemoglobin concentration in the 60U/kg group. Other clinical parameters were assessed as secondary endpoints and time to first hemoglobin response (≥1 g/dL increase in hemoglobin concentration) was a tertiary endpoint.
Results: By 1 year, mean hemoglobin concentration increased in both groups (60 U/kg [baseline median, 10.8 g/dL, range 7.1–12.3]: 23.3% increase, +2.4±0.3 g/dL, p<0.0001; 45 U/kg [baseline median, 10.9 g/dL, range 8.5–12.9]: 23.8% increase, +2.4±0.5 g/dL, p=0.0001). Clinically significant improvements were also seen in platelet counts, and liver and spleen volumes. In both groups, three-quarters of patients achieved ≥1 g/dL increase in hemoglobin concentration by Week 15; in the 60 U/kg group, all patients achieved ≥1 g/dL increase by Week 27 vs Week 37 for the 45 U/kg group.
Conclusion: In this treatment-naïve GD1 patients, velaglucerase alfa administered at 60 U/kg and 45 U/kg demonstrated clinically meaningful improvements in clinical parameters. Both doses were associated with rapid improvement in hemoglobin values, with the majority of patients responding as early as 15 weeks.
Footnote: velaglucerase alfa is approved in the US; it is an investigational product in Europe.

443-P

SCREENING FOR FABRY AND POMPE DISEASE IN HIGH RISK POPULATIONS BY ENZYME ASSAY IN DRIED BLOOD SPOTS

Parkes O[1], Church H[1], Cooper A[1]
[1]*Biochemical Genetics, Genetic Medicine,, Manchester, United Kingdom*

Background: Fabry and Pompe disease can present with atypical or late onset disease. The conditions result from deficient activity of the lysosomal enzymes α-galactosidase A and α-glucosidase respectively. Atypical Fabry patients may present with left ventricular hypertrophy, isolated kidney disease or unexplained stroke. Late onset Pompe Disease normally presents with unexplained, muscle weakness.
In an attempt to identify variant forms of these disorders, dried blood spots have been collected from renal, cardiac, neurology and neuromuscular clinics.
Once collected, dried blood spots can be stored desiccated at -200 and shipped in batches, at ambient temperature, by first class post.
Methods: Received, dried blood spots were stored desiccated at -20 degrees C until analysis. 3 mm spots were punched from the cards into 96 well plates and enzyme proteins extracted into detergent on an orbital shaker. α-galactosidase A and α-glucosidase activities was measured using fluorimetric substrates based on 4-methylumbelliferone. Assays for the enzymes were validated by analysis of control and known affected samples.
Results: 519 samples were assayed for Fabry disease and 97 for Pompe. 15 patients were identified with Pompe disease including 11 infantile onset patients from the Middle East. 3 male Fabry patients were identified.
Conclusions: Assay of these enzymes in dried blood spots allows reliable diagnosis of Fabry and Pompe Disease and simplifies transport of samples over long distances.

444-P

PHARMACOLOGICAL TREATMENT FOR PULMONARY HYPERTENSION IN A PATIENT WITH MUCOLIPIDOSIS III

Vairo F[1], Netto CBO[1], Bittar CM[1], Souza CFM[1], John A[2], Cury G[1], Schwartz IVD[1]
[1]*Medical Genetics Service, HCPA, Porto Alegre, Brazil*
[2]*Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil*

Introduction: Mucolipidosis III (ML III) is a rare autosomal recessive lysosomal storage disorder (LSD) resulting from decreased activity of Glc-N-Acetyl phosphotransferase. One of the main associated clinical signs is cardiac involvement, typically manifesting as aortic and mitral valvular insufficiency.
Case-report: We report a 16 years-old boy with ML III (weight: 22 kg; height: 108 cm), presenting skeletal disease since he was 3 y. He has no cognitive impairment. Clinical diagnosis of LSD was made at 10 years and ML III was biochemically confirmed at 16 y. Mutations analysis of GNPTAB gene is still in progress, but he was found to be heterozygote for the c.503_3504delTC mutation. He had sleep disturbance, apneas, choking and fatigue. Somnography showed obstructive sleep apnea and echocardiography revealed left ventricular hypertrophy, and tricuspid, mitral and aortic valvar thickness as well as insufficiency. Systolic pulmonary artery pressure (sPAP) was 54–59 mmHg, suggesting the diagnosis of pulmonary hypertension. CPAP and sildenafil citrate (12.5 mg, three times a day) were prescribed. After three months of treatment, the patient reported significant clinical improvement, with choking and apneas reduction and decrease of sPAP to 44–49 mmHg. No adverse event was reported.
Conclusion: There is a paucity of data about pharmacological treatment of ML III patients in the literature. We brought this case to show that phosphodiesterase inhibitors, as sildenafil citrate, may improve symptomatology associated to PH in these patients. Support: CNPq

445-P

A NOVEL MUTATION IN RAPIDLY PROGRESSIVE INTERMEDIATE NEURONOPATHIC GAUCHER DISEASE

Illingworth M[1], O'Connor B[1], Stewart F[2], Vellodi A[3], Jones S[4], Peake D[1], Hughes J[1]
[1]*Royal Belfast Hospital for Sick Children, Belfast, United Kingdom*
[2]*Dept of Clinical genetics, BCH, Belfast, United Kingdom*
[3]*GOSH NHS Trust, London, United Kingdom*
[4]*Willink Biochemical Genetics Unit, Manchester, United Kingdom*

Background: Lysosomal storage disorders represent a group of at least 41 genetically distinct, biochemically related, inherited diseases. Gaucher disease is the most prevalent inherited lysosomal storage disorder. It is subclassified into 3 types; type 1 non-neuronopathic, type 2 acute neuronopathic Gaucher Disease (NGD) and type 3 subacute NGD. NGD has a reported incidence of 1 in 100,000. An intermediate late-onset rapidly progressive NGD has been described. We present the clinical course of a compound heterozygote (F213I/G377C) with intermediate NGD.
Methods: Case study of a 17 month old boy who presented with a 5 month history of developmental regression and bilateral V1 nerve palsies. Examination revealed multiple cranial nerve signs (bilateral V1, 1X, X1, X11) with splenomegaly, myoclonus and an extrapyramidal movement disorder. Of note he was the first born to non-consanguineous parents who had 8 previous miscarriages.
Results: Intermediate NGD was confirmed with low leukocyte glucocerebrosidase activity and chitotriosidase level of 8260 μmol/l/hr. Genetic testing identified 2 missense mutations in the GBA gene. F213I has been associated with NGD; G377C has not been previously described. He commenced Enzyme Replacement Therapy with improvement in visceromegaly. Neurlogical decline has been rapidly progressive, with increasing stridor, pharmaco-resistant cortical and subcortical myoclonus and extrapyramidal movement disorder.
Conclusion: This case and novel mutation, further contributes to the understanding of a phenotypic continuum in NGD. Further research is warranted, to aid identification of potential treatment responsive genotypes in NGD, working towards development of rational treatment protocols and guiding prognostic and genetic counseling.

446-O

IDENTIFICATION OF POTENTIAL BIOMARKERS AND MODIFIERS OF CLN3 DISEASE

Lebrun AH[1], Moll-Kosrawi P[1], Storch S[1], Pohl S[1], Kilian D[1], Streichert T[2], Mole SE[3], Ullrich K[1], Kohlschutter A[1], Braulke T[1], Schulz A[1]
[1]*Child Hosp, Univ Med Center Hamburg, Hamburg, Germany*
[2]*Array Serv Cent, Univ Med Center Hamburg, Hamburg, Germany*
[3]*Lab Mol Cell Biol, Univ College London, London, United Kingdom*

Background: Mutations in the CLN3 gene lead to juvenile neuronal ceroid lipofuscinosis, a pediatric neurodegenerative disorder characterized by visual loss, epilepsy and psychomotor deterioration. Although most CLN3 patients carry the same 1 kb deletion in the CLN3 gene, their disease phenotype can be variable. The aims of this study were (1) to study the clinical phenotype in CLN3 patients with identical genotype, (2) to identify genes that are dysregulated in CLN3 disease regardless of the clinical course that could be useful as biomarkers, and (3) to find modifier genes that affect the progression rate of the disease.
Patients and Methods: 25 CLN3 patients homozygous for the 1 kb deletion were classified into groups with rapid, average, or slow disease progression using an established clinical scoring system. Genome-wide expression profiling was performed in 8 CLN3 patients with different disease progression and matched controls.
Results: The study showed high phenotype variability in CLN3 patients. Five genes were dysregulated in all CLN3 patients, of which DUSP2 was also validated in an acutely CLN3-depleted cell model. These genes might be useful as biomarkers of the disease. Thirteen genes were up-regulated in patients with rapid and down-regulated in patients with slow disease progression. Among these potential modifier genes, RAPGEF1 and SPIB were validated in an acutely CLN3-depleted cell model.
Conclusions: These findings indicate that differential perturbations of distinct signaling pathways might alter the disease progression and provide insights into the molecular alterations that underlie neuronal dysfunction in CLN3 disease and neurodegeneration in general.

447-P

AN UNUSUAL PRESENTATION OF POMPE DISEASE

Bainbridge K[1], Broomfield A[2], Burke D[1], Heales S[1]
[1]*Enzyme Unit, Gt Ormond St Hosp, London, United Kingdom*
[2]*Met Med, Gt Ormond St Hosp, London, United Kingdom*

Background: Pompe disease is an autosomal recessive muscle disorder of glycogen metabolism resulting from a deficiency of lysosomal alpha-glucosidase (GAA). Presentation may be from infancy to late adulthood. Symptoms predominately relate to the accumulation of glycogen in muscle tissue with subsequent loss of function. Here we report an unusual initial presentation of Pompe disease with symptoms and response to carbohydrate loading more typical of a liver glycogen storage disorder (GSD).
Case report: Hepatomegaly with persistent transaminitis was investigated in a 5 years old boy following admission to hospital with pneumonia. A history of poor growth and excessive tiredness particularly between meals and after exercise was noted. No evidence of heart failure was evident. Apparent symptomatic relief with increased exercise tolerance in response to cornstarch loading was reported. Initial investigations focused on suspected liver GSD. Enzymology for GSD III, VI, and IX was unremarkable. Liver biopsy revealed lysosomal glycogen accumulation. Liver GAA activity was undetectable and low activity was confirmed in leucocytes and blood spots.
Discussion: This is an unusual presentation of Pompe disease with liver dysfunction and excessive fatigue predominate features. Clinical response to cornstarch loading supports a possible role of GAA in glucose homeostasis.

448-P

JUVENILE TAY SACHS DISEASE IN A 5 YEAR OLD CYPRIOT BOY

Ourani S[1], Drousiotou A[2], Mavrikiou G[2], Georgiou T[2], Stylianou I[1], Hadjiloizou S[3], Christofidou-Anastasiadou V[1]
[1]*Paed. Dep. Hosp. Archbishop Makarios III, Nicosia, Cyprus*
[2]*Bioch Genetics, Cyprus Inst of Neur&Gen, Nicosia, Cyprus*
[3]*Cyprus Paed Neurology Inst, Nicosia, Cyprus*

Background: Tay Sachs disease (TSD) is an autosomal recessive neurodegenerative disease caused by deficiency in α subunit of β-Hexosaminidase A (Hex A) which results in the accumulation of GM2 ganglioside within the lysosomes of nerve cells. TSD is a clinically heterogeneous entity.
Case report: We report the first case of Tay Sachs Disease in the Cypriot population. The boy was referred, at the age of 5 years, because of developmental regression. Initial concerns arose at four years due to poor fine motor skills. Thereafter, he appeared gradually clumsier, developing an unsteady gait and stuttering. Behavior and cognitive performance was inconsistent with his age. His neurological exam revealed no focal semiology and there were no dysmorphic features. Prenatal and perinatal history is reported normal. He is the second child of healthy, non-consanguineous parents of Greek Cypriot descent and with negative family history. Metabolic workup revealed a low percentage of Hex A activity (5% in plasma, normal 52–78% and 12% in leucocytes, normal 49–70%). Low activity of Hex A was also found in the patient's fibroblasts. Sequencing of the HEX A gene identified a known, non-sense mutation (p.Trp26X) on one allele. The second mutation was not identified.
Conclusions: Juvenile TSD is an infrequent disorder and the diagnosis is often missed or delayed, especially in non Jewish populations. We suggest that this entity should be considered in patients presenting with developmental delay and/or regression even in the absence of a typical picture. Accurate diagnosis of the proband permits appropriate genetic counseling.

449-P

STRUCTURE OF TRIPEPTIDYL-PEPTIDASE I (TPP1) PROVIDES INSIGHT INTO THE MOLECULAR BASIS OF LATE INFANTILE NEURONAL CEROID LIPOFUSCINOSIS

Krätzner R[1], Pal A[2], Grune T[2], Grapp M[1], Schreiber K[1], Gärtner J[1], Sheldrick GM[2], Steinfeld R[1]
[1]*Ped Neurol, Univ Gottingen, Gottingen, Germany*
[2]*Struct Chem, Univ Gottingen, Gottingen, Germany*

Late infantile neuronal ceroid lipofuscinosis is a fatal neurodegenerative disease of childhood and is caused by mutations in the TPP1 gene that encodes tripeptidyl-peptidase I. We show that purified TPP1 requires at least partial glycosylation for in vitro autoprocessing and proteolytic activity. We crystallized the fully glycosylated TPP1 precursor under conditions that implied partial autocatalytic cleavage between the prosegment and the catalytic domain. X-ray crystallographic analysis at 2.35 angstroms resolution reveals a globular structure with a subtilisin-like fold, a Ser475-Glu272-Asp360 catalytic triad, and an octahedrally coordinated Ca2±binding site that are characteristic features of the S53 sedolisin family of peptidases. In contrast to other S53 peptidases, the TPP1 structure revealed steric constraints on the P4 substrate pocket explaining its preferential cleavage of tripeptides from the unsubstituted N terminus of proteins. Two alternative conformations of the catalytic Asp276 are associated with the activation status of TPP1. 28 disease-causing missense mutations are analyzed in the light of the TPP1 structure providing insight into the molecular basis of late infantile neuronal ceroid lipofuscinosis.

450-P

POMPE DISEASE: DIAGNOSTIC DILEMMAS

Bainbridge K[1], Broomfield A[2], Burke D[1], Heales S[1]
[1]*Enzyme Unit, Gt Ormond St Hosp, London, United Kingdom*
[2]*Met Med, Gt Ormond St Hosp, London, United Kingdom*

Background: Pompe disease is a muscle disorder of glycogen metabolism resulting from a deficiency of lysosomal acid alpha-glucosidase (GAA). GAA activity is commonly measured in leucocytes or dried blood spots using acarbose as an inhibitor of interfering alpha-glucosidases. We present 2 infantile cases with bloodspot GAA activity suggestive of Pompe disease but with equivocal leucocyte alpha-glucosidase results.

Case report: 2 cases both presenting in the neonatal period with cardiomyopathy and failure-to-thrive were investigated for Pompe disease by measuring leucocyte and bloodspot GAA activity (with acarbose) and total acid alpha-glucosidase activity (without acarbose). Clinically, neither patient was typical of infantile Pompe disease with patient A exhibiting clotting abnormalities and patient B having skeletal abnormalities. Very low bloodspot GAA activity and a ratio of bloodspot GAA/total acid alpha-glucosidase highly suggestive of Pompe disease was found in both patients. However, high residual leucocyte GAA activity, significantly higher than typically seen in infantile Pompe disease, was a consistent finding in both patients. Vacuolated lymphocytes were detectable in patient A and Pompe disease was subsequently confirmed by muscle GAA activity and molecular analysis. To date, Pompe disease has not been confirmed in patient B and we are awaiting results from genetic analysis.

Discussion: Blood GAA assays in leucocytes, and more recently in bloodspots, are now in common use for the diagnostic investigation of suspected Pompe disease. However, the interpretation of leucocyte and bloodspot GAA results are not always straight forward and the potential for false positives and negatives exist.

451-P

FREQUENCY OF THE 24-bp DUPLICATION IN THE CHITOTRIOSIDASE GENE IN THE CYPRIOT POPULATION: COMPARISON OF TWO LOCATIONS WITH DIFFERENT MALARIA ENDEMICITY IN THE PAST

Mavrikiou G[1], Petrou P[1], Georgiou Th[1], Drousiotou A[1]
[1]*Biochem Genet Dept, Cyprus Inst Neur Gen, Nicosia, Cyprus*

Human chitotriosidase belongs to the family of chitinases and is secreted by activated macrophages. Chitotriosidase is elevated in several lysosomal storage disorders but more markedly in Gaucher disease, where it is used as a marker for disease progression and for monitoring the efficacy of treatment. A recessively inherited 24-bp duplication in exon 10 of the chitotriosidase gene (CHIT1), resulting in the loss of enzyme activity, is frequently encountered in different populations. The objectives of our study were: a) to determine the normal chitotriosidase levels in the Cypriot population, b) to establish the frequency of the 24-bp duplication and c) to compare the frequency of the 24-bp duplication in two locations, one at high altitude and one at sea-level with low and high malaria endemicity in the past, respectively. We measured plasma chitotriosidase activity and determined the genotype of 114 healthy unrelated individuals originating from various parts of Cyprus. We also genotyped 95 individuals from a village at an altitude of 880 m; and 100 individuals from an area at the sea-level. The normal range of chitotriosidase activity (for homozygous wild type individuals) was found to be 9.5–44.0 nmol/hr/ml (mean 26.77). We found a heterozygote frequency for the 24-bp polymorphism of 35% and a homozygote frequency of 7% (mutant allele frequency 0.25). These results are similar to those of other Mediterranean countries (mutant allele frequency 0.13–0.27). No statistically significant difference was found between the frequency of the mutant allele in the high altitude village (0.384) and the frequency in the lowland area (0.305).

452-P

CLINICAL VARIABILITY IN A MILD MPS II FAMILY WITH 16 AFFECTED MALES

Piazzon FB[1], Grinberg H[2], Rebelo CC[1], Furquim IM[1], Avila M[2], Benvenucci LF[2], Vieira MLC[2], Giugliani R[3], Kim CA[1]
[1]*Genetic Unit, Instituto da Crianca FMUSP, São Paulo, Brazil*
[2]*Instituto do Coracão, INCOR/FMUSP, São Paulo, Brazil*
[3]*Laboratório de Genética Médica, HCPA, Porto Alegre, Brazil*

MPS type II is a rare X-linked multisystemic lysosomal storage disorder caused by a deficiency of the enzyme iduronate-2-sulfatase (IDS). Some of its major features are coarsening face, myocardial enlargement with valvular dysfunction and neurologic involvement. There are 2 forms: the severe one (IIA) which presents progressive mental retardation and physical disability, leading to death before the age of 15; the mild one (IIB) has few symptoms and is compatible with survival until adulthood.

We report on a family of 16 male members affected (from 1 to 74 years) with MPS IIB confirmed by IDS dosage and mutation identification. One of them was a 23 years old male, who died 2 days after the first cardiac transplantation for MPS II. All others show normal intelligence and no serious health problems. His older brother at 28yo has just mild aortic insufficiency. One other affected echocardiography was normal at 19yo. One ancestral affected member died at 74yo. Among 12 asymptomatic female heterozygote carriers, one died at 94yo and the others are alive.

We discuss the surprisingly clinical variability, showing the differences in the presentation and evolution of the disease in the family members. No affected adults received the enzyme replacement therapy. Only 2 members (an 1-year-old twin boys) are undergoing the ERT with recombinant human iduronate-2-sulfatase. In this family, we had dilemma in indicating the enzyme replacement therapy.

453-P

SWALLOWING DIFFICULTIES AND SPEECH PROBLEMS ARE COMMONLY SEEN IN LONG TERM SURVIVORS WITH CLASSIC INFANTILE POMPE DISEASE TREATED WITH ENZYME THERAPY

Van Gelder CM[1], van Capelle CI[1], de Gier HHW[2], van den Hout JMP[1], van der Ploeg AT[1]
[1]*Dep Ped, Erasmus MC Univ Med Centr, Rotterdam, Netherlands*
[2]*Dep ENT, Erasmus MC Univ Med Centr, Rotterdam, Netherlands*

Background: Pompe disease is a lysosomal storage disorder caused by deficiency of alpha-glucosidase, which mainly affects skeletal muscle and heart. Patients with the infantile form present shortly after birth and die without therapy within the first year of life. Enzyme therapy has improved survival and motor outcome significantly, but has also revealed physical problems that require specific attention. This study focuses on the occurrence of swallowing difficulties and speech problems in patients with classic infantile Pompe disease.
Methods: Fiberoptic endoscopic evaluation of swallowing (FEES) was used to visualize the various stages of swallowing in six patients with classic infantile Pompe disease. In patients older than 24 months FEES was combined with a standardized articulation test.
Results: A FEES was performed at a mean age of 45 months (range 8–119 months). Four patients had difficulties with normal food intake. Dysphagia was present in five patients. The major problems noted were delayed swallowing and the presence of pharyngeal food residues. Micro-aspirations were observed in three patients. Speech assessments were performed in three patients at a mean age of 72 months (range 46–119 months). Articulation abnormalities were observed in all three patients and comprised active and passive articulator compensation and hypernasal resonance and reduced speech intelligibility.
Conclusions: Swallowing difficulties are commonly seen in long term survivors with classic infantile Pompe disease treated with enzyme therapy and pose patients at an increased risk of aspiration and development of pneumonias. Since many infants develop abnormal articulation early start of speech therapy is mandatory.

454-P

DICARBOXYLIC ACIDURIA IN WOLMAN'S DISEASE

Anderson G[1], Burke D[2], Krywawych S[2], Leaky J[2]
[1]*Dept Histology, GOSH, London, United Kingdom*
[2]*Chem Path Metabolic and Enzyme Unit GOSH, London, United Kingdom*

A two month infant presented with failure to thrive and hepatosplenomegaly. The analysis of trimethylsilyl derivativates of organic acids by Gas Liquid Mass Spectrometry, on different two urine specimens revealed a significantly increased excretion of pimelate (95, 133—normal 20 < micromol/mmol creatinine), azelate (141, 228—normal 20 < micromol/mmol creatinine), suberate (135, 190—normal 50 < micromol/mmol creatinine) and 2-hydroxysebacate (60—normal 1 < micromol/mmol creatinine). Furthermore, in these urine specimens the other organic acids, including all the non mentioned dicarboxylic acids were excreted in normal amounts and the 3-hydroxybutyrate or acetoacetate excretion was not raised. The infant was not receiving any unusual dietary formula.
Histological investigations concluded that the features seen in the blood and bone marrow films were typical of Wolman's disease. The blood demonstrated the presence of vacuolated lymphocytes with lipid droplets and no acid esterase activity and similarly, aspirated bone marrow showed lipid laden foamy storage cells.
The excretion of this combination of dicarboxylic acids has not been described previously. Further urine organic acid investigations will be conducted on other patients diagnosed with Wolman's disease to ascertain the consistency of this finding. The excretion of these three dicarboxylic acids of carbon chain length 7, 8 and 9 may prove to be a useful addition diagnostic marker of this disease and also, offer further insight into further metabolic interactions occurring in this condition.

455-P

BMT IN JUVENILE MLD: LESSONS FROM URINARY SULPHATIDE PROFILING AND LEUKOCYTE ASA MONITORING IN THE COURSE OF TREATMENT

Kuchar L[1], Poupetova H[1], Hlavata J[1], Honzik T[2], Fialova M[1], Ledvinova J[1], Zeman J[2]
[1]*Inst Inherit Metab Dis, Charles Univ, Prague, Czech Republic*
[2]*Dept Paed Inst Inher Met D, Charles Univ, Prague, Czech Republic*

Background: Metachromatic leukodystrophy (MLD, OMIM 250100) is caused by the deficiency of arylsulphatase A (ASA) resulting in lysosomal storage of sulphatides. Three main forms of MLD (late infantile, juvenile and adult) can be distinguished by clinical manifestation. There is no specific treatment and the only option is bone marrow transplantation (BMT) in cases with later onset.
Objectives: To monitor effect of BMT on ASA activity in leukocytes, urinary sulphatides excretion and sulphatide isoform profiling in patient with juvenile MLD
Methods: ASA activity in leukocytes was determined using 4-nitrocatechol sulphate at 37°C and 0°C. Urinary sulphatides were quantified by tandem mass spectrometry (MS/MS) in negative ion mode and isoform ratio was calculated.
Results: Patient´s ASA activity (residual ASA 1.5%) reached the control range in 3 months after BMT and its value remained normal during the whole examination period (20 months).
Quantitative and qualitative MS/MS analysis of urinary sulphatides before BMT was in accordance with the MLD status (excretion 8x elevated). After BMT, short period of decreased sulphatide excretion and isoform profile improvement was evident. After 6 months of treatment, however, sulfatide excretion increased again to levels before BMT and isoform profile reverted to original pathology. This corresponded to clinical progression of the disease that restarted after short time stabilization.
Conclusion: Measurement of ASA activity is necessary to monitor restored enzyme production in the bone marrow. However, urinary sulphatide concentration and especially isoform profile correlate better with the actual clinical situation after BMT.
Support: grant project MSM 0021620806, grant GAUK 259039–19509/2009

456-P

A MULTICENTER, MULTINATIONAL, LONGITUDINAL CLINICAL ASSESSMENT STUDY OF SUBJECTS WITH MUCOPOLYSACCHARIDOSIS IVA (MORQUIO SYNDROME)

Harmatz P[1], Chang M[2], Decker C[2], Burton B[3], Guffon N[4], Hendriksz C[5], Hollak C[6], Jones S[7], Lin S[8], Mengel E[9], Mitchell J[10], Parini R[11], Valayannopoulos V[12], Vellodi A[13]
[1]*Children's Hospital Oakland, Oakland, United States*
[2]*BioMarin Pharmaceutical Inc., Novato, United States*
[3]*Children's Memorial Hospital, Chicago, United States*
[4]*Hopital Femme Mère Enfant, Lyon, France*
[5]*Birmingham Children's Hospital, Birmingham, United Kingdom*
[6]*Academic Medical Center, Amsterdam, Netherlands*
[7]*Central Manchester University Hospital, Manchester, United Kingdom*
[8]*MacKay Memorial Hospital, Taipei, Taiwan*
[9]*University of Mainz, Mainz, Germany*
[10]*McGill University Health Centre, Montreal, Canada*
[11]*Az. Ospedaliero S. Gerardo, Monza, Italy*
[12]*Hôpital Necker-Enfants Malades, Paris, France*
[13]*Great Ormond Street Hospital, London, United Kingdom*

Background: MPS IVA is a disorder characterized by deficient activity of N acetylgalactosamine 6 sulfatase (GALNS) causing excessive lysosomal storage of keratan sulfate (KS). This excessive storage causes systemic skeletal dysplasia, short stature, and joint abnormalities, all of which limit mobility and endurance. Odontoid process hypoplasia and ligamentous laxity cause instability of the cervical spine that may lead to cord compression and neurologic impairment. The objective of the study is to quantify endurance and respiratory function in subjects with MPS IVA and to better characterize the spectrum of symptoms and biochemical abnormalities in MPS IVA.
Methods: Interim results of key clinical assessments will be summarized including measures of endurance (6MWT and 3MSCT) and measures of respiratory function. Results of biomarker testing including plasma KS concentration; urine KS concentration (normalized to creatinine) and inflammatory cytokines and biochemical markers of bone and cartilage metabolism will be described. Other information presented will include demographics and anthropometric measurements.
Discussion: Characterization of clinical impairments across a large subject population is expected to contribute significantly to the understanding of MPS IVA disease. In addition, this information will facilitate selection of appropriate clinical and biomarker endpoints for future therapeutic clinical studies.
Conclusion: MorCAP is expected to enroll 200–300 subjects in approximately 15 centers in multiple countries. This will be the first large clinical assessment study based on direct observation and testing of subjects with MPS IVA

457-P

EFFECTS OF INTERRUPTION OF ERT IN RENAL FUNCTION IN FABRY DISEASE PATIENTS

Netto C[1], Vairo F[1], Bittar C[1], Pereira MSS[1], Jardim L[1], Giugliani R[1]
[1]*Medical Genetics Service, HCPA, Porto Alegre, Brazil*

Fabry disease (FD) is an X-linked lysosomal disorder due to the deficiency of α-galactosidase A that causes storage of globotriaosylceramide (Gb3). Disease progression leads to vascular disease secondary to the involvement of kidney, heart and the central nervous system. The current treatment for FD is Enzyme Replacement Therapy (ERT), which prevents the deposition in the kidney and heart, or reverts, at least partially, the vascular pathophysiology.
We are reporting our experience regarding to the interruption of ERT in one group of FD patients (n=5). We have been treating 4 male and 1 female patients from 3 different families, for over 8 years. During this period patients had two intervals of ERT interruption (18 and 8 months), when glomerular filtration rate (GFR-Cr EDTA) was analyzed. Data shows that a patient, the oldest one, showed important decline in renal function after ERT interruption. We believe this is a unique opportunity to show whether ERT interruption might cause worsening of renal function in FD patients.

458-O

A PHASE 1/2, MULTICENTER, OPEN-LABEL, DOSE-ESCALATION STUDY TO EVALUATE THE SAFETY, TOLERABILITY, AND EFFICACY OF BMN 110 IN SUBJECTS WITH MUCOPOLYSACCHARIDOSIS IVA (MORQUIO SYNDROME)

Hendriksz C[1], Vellodi A[2], Jones S[3], Capponi M[4], Decker C[4]
[1]*Birmingham Children's Hospital, Birmingham, United Kingdom*
[2]*Great Ormond Street Hospital, London, United Kingdom*
[3]*Royal Manchester Children's Hospital, Manchester, United Kingdom*
[4]*BioMarin Pharmaceutical Inc., Novato, United States*

Background: This study examined weekly, 4 to 5 hour infusions of the recombinant version of N acetylgalactosamine 6 sulfatase (BMN 110) in a small group of subjects with MPS IVA between 5 and 18 years of age. The safety of increasing doses of BMN 110 every 12 weeks for 3 dose levels will be described, and the impact of treatment on reducing the elevated KS substrate as reflected by measurement of plasma and urinary KS will be reported. Results of key clinical assessments will be presented including measures of endurance (6 minute walk test and 3-minute stair climb test) and measures of respiratory function (including forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), and maximum voluntary ventilation (MVV)). Results of biomarker testing including inflammatory cytokines and biochemical markers of bone and cartilage metabolism will be described. Other information presented will include demographics and anthropometric measurements.
Discussion/Conclusion: Enzyme replacement therapy (ERT) is a potential new treatment option for MPS IVA. ERT is expected to reduce KS storage in affected tissues, potentially leading to improvement in clinical measures of endurance and respiratory function.

459-P

I-CELL DISEASE: DIFFERENCES FROM HURLER SYNDROME AND FOLLOW-UP OF THE PATIENTS

Kalkan Ucar S[1], Akaslan A[1], Kagnici M[1], Coker M[1]
[1]Div Metab Dis, Ege Univ Child Hosp, Izmir, Turkey

I-cell disease (also called mucolipidosis II α/β, ML-IIα/β) is an autosomal recessive disorder that results as a consequence of defective targeting of lysosomal hydrolases to the lysosomes. The disorder is so called because fibroblasts from afflicted patients contain numerous phase-dense inclusion bodies in the cytosol. I-cell disease patients present with symptoms earlier than do Hurler syndrome patients and in addition, I-cell patients do not exhibit mucopolysaccharidurıa. We present three patients (five and two years, and ten months old) with I-cell disease, all characterized by severe psychomotor retardation, coarse facial features, craniofacial abnormalities, and severe skeletal abnormalities (kyphoscoliosis, widening of the ribs, lumbar gibbus). A clinically and laboratory unique feature seen in all our I-cell disease patients that allows easy distinction from Hurler syndrome were a striking gingival hyperplasia and lack of mucopolysaccharidurıa. The disease progresses rapidly with developmental delay and failure to thrive. Psychomotor retardation was evident in the first and second patient. The first patient died at the 5 years of age. Hematopoietic stem cell transplantation (HSCT) has proven effective therapy for individuals with adrenoleukodystrophy, metachromatic leukodystrophy or globoid cell leukodystrophy. Presenting these patients we are going to discuss the protocol for HSCT of I-cell disease.

460-P

ADULT-ONSET POMPE DISEASE PRESENTING WITH SEVERE FATIGUE AND SELECTIVE INVOLVEMENT OF TYPE 1 MUSCLE FIBERS

Van den Berg L[1], De Vries J[2], Reuser A[3], van der Ploeg A[1], van Doorn P[2]
[1]Dep of Ped, Erasmus MC Univ Med Cen, Rotterdam, Netherlands
[2]Dep of Neurol, Erasmus MC Univ Med Cen, Rotterdam, Netherlands
[3]Dep of Clin Gen, Erasmus MC Univ Med Cen, Rotterdam, Netherlands

Background: Pompe disease is an inherited metabolic myopathy caused by deficiency of lysosomal acid alpha-glucosidase. Limb girdle weakness is the most frequent and prominent first sign in adults with Pompe disease.
Case Report: We present a case of adult-onset Pompe disease with an uncommon clinical presentation with severe fatigue and myalgia prior to the onset of limb girdle weakness. Remarkably, muscle biopsy clearly demonstrated selective involvement of only type 1 muscle fibers.
Discussion: These findings may indicate that muscle-fiber-type-specific pathology might be related with the symptomatology in Pompe disease.

461-P

MOLECULAR DIAGNOSIS OF MUCOLIPIDOSIS TYPE II AND TYPE III: GENOTYPE-PHENOTYPE CORRELATIONS AND PRENATAL DIAGNOSIS

Sabourdy F[1], Latorre V[1], Baeza E[1], Bonnefoy Y[1], Levade T[1]
[1]Lab Biochimie Métabolique, IFB Purpan, Toulouse, France

Mucolipidosis II and III (MLII and MLIII) are autosomal recessive lysosomal storage disorders due to N-acetylglucosaminyl 1-phosphotransferase deficiency. This enzyme is involved in the mannose-6-phosphate-dependent targeting of lysosomal enzymes to the acidic compartments. In MLII and III, lysosomal hydrolases are excreted in extracellular fluids instead of being addressed to lysosomes. Mucolipidosis can present with various degrees of clinical severity. While the most severe forms (MLII) are associated with dysmorphism, mental and motor retardation, marked failure to thrive, dysostosis multiplex, and cardiorespiratory complications leading to early death, the less severe forms (MLIII) exhibit a later onset and allow patients to survive into adulthood.
Two genes, GNPTAB and GNPTG, encode for N-acetylglucosaminyl 1-phosphotransferase subunits. Several mutations in the GNPTAB gene were shown to be responsible for MLII and MLIIIA. To further investigate genotype-phenotype correlations (Tappino et al, 2009 ; Cathey et al, 2009), we analyzed 10 new cases diagnosed in our laboratory. Four novel mutations are presented. Our results confirm the severity of the phenotypes associated with the frequent and already reported p.L1168QfsX5 mutation in a homozygous context. They also support the in silico prediction that the p.R334Q alteration, despite being a missense mutation, is linked to a poor prognosis. In addition to provide insights into genotype-phenotype correlations, the molecular diagnosis of mucolipidosis allowed to perform two prenatal diagnoses.
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A PHASE 4 TWO DOSE LEVEL STUDY OF GALSULFASE IN MUCOPOLYSACCHARIDOSIS VI INFANTS

Harmatz P[1], Guffon N[2], Garcia P[3], Cheng S[4], Lagan K[4], Decker C[4]
[1]Children's Hospital Oakland, Oakland, United States
[2]Hopital Femme Mère Enfant, Lyon, France
[3]Hospital Pediátrico de Coimbra, Coimbra, Portugal
[4]BioMarin Pharmaceutical Inc., Novato, United States

Objectives: Mucopolysaccharidosis type VI (MPS VI) results from deficient N-acetylgalactosamine 4 sulfatase enzyme activity. Safety and efficacy of enzyme replacement therapy (ERT) was studied in infants.
Methods: Four patients aged 3.3–12.7 months received weekly ERT doses 1.0 or 2.0 mg/kg for 52 weeks. Efficacy assessments were glycosaminoglycans (GAG); gross/fine motor, cardiac, vision; hearing. Safety evaluations included adverse events; blood/urine analyses; physical, cardiac exams; antibody titers.
Results: One patient showed improved hair texture, facial appearance while the rest were unchanged from baseline. Skeletal radiographs remained abnormal. Growth stayed normal. At Week 52, baseline GAG was decreased by 75%. All developed antibody to drug, but %GAG decrease was unrelated to antibody titer. Abnormal baseline hearing improved in all; two developed corneal clouding. Developmental and cardiac assessments were unchanged. All experienced at least 1 Adverse Event (AE) and 1 serious AE. Five total drug-related AEs were reported in 2 patients, none severe or serious.
Conclusions: Fifty-two weeks of ERT showed a favorable safety profile in MPS VI infants. Maintenance of normal growth, improved physical appearance and hearing, absence of decline in development and cardiac status, and corneal clouding progression indicate that early initiation of ERT may affect some but not all MPS VI features. Small study sample and short study duration preclude definitive conclusions about early ERT's effect on disease progression. Further investigation is warranted to provide more complete information on early ERT's potential benefits, including long-term growth outcomes.

463-P

PRENATAL DIAGNOSIS OF ADENYLOSUCCINATE LYASE DEFICIENCY: OUR EXPERIENCE

Marie S[1], Nassogne MC[1], Vincent MF[1]
[1]UCL, Cliniques Universitaires St-Luc, Brussels, Belgium

Adenylosuccinate lyase (ADSL) deficiency is an autosomal recessive disorder of purine synthesis pathway. It results in the accumulation in the body fluids of the two succinylpurines, SAICA-riboside and succinyladenosine, the dephosphorylated derivatives of the two substrates of the enzyme. The clinical presentation of this defect is quite variable, with a psychomotor retardation usually very severe, but which can be moderate to mild in some patients. It is frequently accompanied by seizures, autistic features, and/or muscular hypotonia. The ADSL gene is located on chromosome 22 and consists of 13 exons, encoding a protein of 484 aminoacids. To date, 47 mutations have been reported in the gene (Spiegel et al. 2006 , Mouchegh et al. 2007 , Jurecka et al. 2008, Gitiaux et al. 2009, Christensen et al. 2010, Sempere et al. 2010, Chin Chen 2010). The molecular analysis of fetal DNA is the only possible prenatal diagnosis for ADSL deficiency (Marie et al. 2000). Analysis of succinylpurines in the amniotic fluid has been tested only once and turned to be not reliable enough, because of the presence of too small amount of these compounds. We performed the prenatal diagnosis for 7 families, resulting in 13 molecular analyses. Six of these pregnancies had to be interrupted because of the presence of the two mutated alleles in the fetal DNA. Unfortunately, the analysis of amniotic fluid could not be performed on these cases.

464-P

FUNCTIONAL CHARACTERIZATION OF TWO NOVEL SUMF1 MUTATIONS LEADING TO A MILD PHENOTYPE IN MULTIPLE SULFATASE DEFICIENCY

Schlotawa L[1], Radhakrishnan K[2], Schmid R[3], Schmidt B[2], Dierks Th[4], Gärtner J[1], Baumgartner M[5]
[1]Dep Ped Neurology, Georg- August- Univ, Goettingen, Germany
[2]Dep Biochem II, Georg- August- Univ, Goettingen, Germany
[3]Child Hosp, Winterthur, Switzerland
[4]Dep Biochem I, Univ Bielefeld, Bielefeld, Germany
[5]Univ Child Hosp, Div Metab, Zuerich, Switzerland

Multiple Sulfatase Deficiency (MSD) is a rare inborn error of posttranslational modification of sulfatases. Mutations in the sumf1 gene encoding the formylglycine-generating enzyme (FGE) lead to instable mutant FGE protein with reduced FGE activity resulting in impaired sulfatase activities. The clinical phenotype comprises symptoms of different single sulfatase deficiencies and can be divided into neonatal very severe forms of disease, and late infantile severe and mild forms. Disease severity is determined by the stability as well as the residual activity of mutated FGE protein. We report a patient with MSD displaying a mild course of disease. The patient is compound heterozygous for two yet undescribed mutations p.C52fsX57 and p.E130D. mRNA of the nonsense mutation is not detectable in patient fibroblasts whereas p.E130D is expressed and correctly localized in the endoplasmic reticulum. The residual activity of FGE E130D reaches 50% of wild type FGE activity. Protein stability is drastically impaired leading to complete degradation of FGE p.E130D in three hours. Sulfatases in patient fibroblasts show mildly reduced activities. The functional consequences of the new FGE mutation p.E130D confirms and extends the previous observation that instable mutant protein with high residual activity lead to a mild clinical phenotype. Therapeutic approaches with influence on the stability of this highly active mutant protein are likely to have deep impact on the course of disease in MSD.

465-P

RAPID AND EFFECTIVE APPROCH FOR LYSOSOMAL STORAGE DISORDERS NEWBORN SCREENING

la Marca G[1], Malvagia S[2], Casetta B[3], Pasquini E[2], Donati MA[2], Zammarchi E[4]
[1]Dept Pharmacology, Florence Univ, Florence, Italy
[2]Metab and Musc Unit, Meyer Hospital, Florence, Italy
[3]AB SCIEX, Monza, Italy
[4]Dep Woman and Child Health Florence Univ, Florence, Italy

Background: The lysosomal storage diseases (LDSs) are a heterogeneous group of over 40 inherited genetic disorders. They have a devastating impact on patients, on their families and on the public health. Since effective therapies for several of them are currently available, an early diagnosis can affect the disease process.
Objectives: To set-up a robust method suitable for large-scale studies with a minimized preparation process and with reduced costs for the characterization of five LSDs.
Methods: A new MS/MS-based method to quantify 5 enzyme activities on dried blood spots (DBS) is reported. Each enzyme reaction was carried out separately. After incubation, all 5 reaction mixtures were stopped, combined together and centrifuged. The cleaning-up of the injected mixture was performed through a fast two-dimensional chromatography preceding the mass-spectrometer measurement. The method takes only 4 minutes as analysis run-time and without any sample purification step following the enzymatic reaction.
Results: We assessed the effectiveness of this approach in assaying the enzymatic activities on DBS for Pompe, Gaucher, Fabry, Niemann-Pick A/B and Krabbe diseases. The normal range of activity has been determined on 10.000 healthy newborns and 500 healthy adults. Enzymatic activities on DBS from all affected patients showed values below the normal range.
Conclusion: The low costs, the rapid analysis and a greatly facilitated sample preparation offer a completely different approach to simultaneous determination of enzymatic activities, which should provide an excellent applicability for a newborn screening program.

466-P

LYSOSOMAL STORAGE DISEASES IN NORTHERN SASKATCHEWAN: A GENETIC AND MASS SPECTROMETRIC ANALYSIS

Lehotay DC[1], Fitterer B[2], Hall P[2], Antonishyn N[2], Eichhorst J[2], Etter M[2], Gravel R[3], Casey R[3], Gelb MH[4]
[1]University of Saskatchewan, Saskatoon, SK, Canada
[2]Saskatchewan Disease Control Laboratory, Regina, SK, Canada
[3]Alberta Children's Hospital, Calgary, AB, Canada
[4]University of Washington, Seattle, United States

Dry blood spots (DBS) were used for the screening and diagnosis of lysosomal enzyme activities by tandem mass spectrometry. Reference ranges for normals and affected patients were established for 5–6 commonly measured treatable enzyme deficiencies. In addition, we also developed a method for measuring hexosaminidase, the enzyme responsible for Sandhoff's disease, the incidence of which is high in northern Saskatchewan (SK). The assay was tested using DBS from patients with Sandhoff disease, as well as unaffected controls. Population normal ranges, age-related differences in enzyme activity, and loss of enzyme activity in stored DBS over time were determined. The ability of the assay to differentiate genetically confirmed heterozygotes from wild type was also measured. This new, MS/MS based assay allows for rapid detection of hexosaminidase enzyme deficiencies for both Sandhoff's and Tay-Sach disease in DBS, making screening of newborns as part of a newborn screening program possible for the first time.
Genetic sequencing of the HEX-B gene from an affected individual from northern SK revealed a novel mutation. Experiments were performed to assess the allelic frequency of the mutation in the affected population. A real-time PCR assay was developed to detect wild type and mutant alleles present in DBS. A 96-well semi-automated DNA extraction and PCR set-up were utilized to facilitate rapid screening of a novel mutation for Sandhoff's disease in Saskatchewan for the first time. The heterozygote frequency for infantile Sandhoff's disease in northern Saskatchewan appears to be more than 10 times higher than in the general population

467-P

PRELIMINARY DATA FROM AN INTERNATIONAL DISEASE REGISTRY FOR NIEMANN-PICK DISEASE TYPE C

Pineda M[1], Mengel E[2], Wraith JE[3], Wijburg FA[4], Vanier MT[5], Schwierin B[6], Muller A[6], Silkey M[6], Giorgino R[6], Patterson MC[7]
[1]*Hospital Sant Joan de Déu, Barcelona, Spain*
[2]*Villa Metabolica, University of Mainz, Mainz, Germany*
[3]*St Mary's Hospital, Manchester, United Kingdom*
[4]*University of Amsterdam, Amsterdam, Netherlands*
[5]*INSERM Unit 820, Lyon, France*
[6]*Actelion Pharmaceuticals Ltd, Allschwil, Switzerland*
[7]*Mayo Clinic, Rochester, United States*

Background: A disease registry was started in Europe in September 2009 to evaluate the long-term disease course of Niemann-Pick disease type C (NP-C) in clinical settings.
Methods: All patients with a diagnosis of NP-C are eligible for inclusion irrespective of treatment. Demographics, disease characteristics and treatment data are collected. Patients are monitored using a disability scale (Pineda et al. Mol Genet Metab 2009;98:243–9) that evaluates ambulation, manipulation, language and swallowing; modified by rating from 0 (best) to 1 (worst).
Results: 18 patients (median age [range] 13.8 [1.9–46.3] years; 50% female) were enrolled as of March 2nd 2010. Seventeen patients were confirmed as receiving miglustat therapy (mean exposure 1.83 years). Early visceral involvement was recorded in 4/9 (44%) evaluable patients. Most patients (15/18 [83%]) were diagnosed by biochemical or biochemical/genetic testing. Age at diagnosis ranged from 1.7–44.2 years. All patients had neurological manifestations at enrolment. Median ages at first neurological manifestation were: 0.4 years in early-infantile (aged 6 months to <2 years, n=1), 4.9 years in late-infantile (2 to <6 years, n=3), 10.1 years in juvenile (6 to <15 years, n=10), and 39.6 years in adolescent/adult patients (≥15 years, n=3). The median (95%CI) composite disability score among 15 evaluable patients was 0.35 (0.23–0.55) at enrolment. Low numbers of patients had normal ambulation (2/15 [13%]), language (3/15 [20%]) and manipulation (3/16 [19%]) at enrolment; 7/16 (44%) had normal swallowing.
Conclusions: This registry will provide valuable information on the long-term progression of functional neurological impairments and treatment outcomes in NP-C.

468-P

THE EFFECT OF LONG-TERM IMIGLUCERASE TREATMENT ON CHILDREN WITH GAUCHER DISEASE

Zaman T[1], Moradian R[1]
[1]*Metab Unit, Tehran Univ, Tehran, Islamic Republic of Iran*

Methods: This is a retrospective study of 31 children with Gaucher disease (GD) receiving imiglucerase during10 years (2000–2009). Type 1:21 (67.76%), IIIB: 7 (22.58%), IIIA: 3 (9.66%).Male:61.3%.Age at diagnosis: < 0 to 20 years, 25 % up to 18 months, 75 % up to 5.7y. Age at first infusion: 1.25y to 22 y,75 % up to: 5.8y. Age at last follow up 2–24 yr, mean: 7.2y. Duration of therapy: 10 months to 9.5y; 9 cases ≥ 3y.Growth retardation; 63.15%.At the dignosis;anemia: n=29;(72.7%),thrombocytopenia: 23(65.2%).At the most recent assessment; normal Hb:(85.9%), normal PLT:(88.88%).At diagnoses: splenomegaly: n=30:severe (MN> 15):43.3%, moderate (MN>5 to ≤ 15): 33%. Hepatomegaly n=30: mild or none (≤1.25):18(60%),severe (1.25 to 2.5):7(23.3%).After imiglucerase for 24–48 month in 11 cases: volume reduction for spleen 50–66% in 8, liver:20–33% in 6, 66.6% in 2.Bone pain ; (8/31): 25.8%, prior bone crises: 3 (9.67%), erlenmeyer flask deformity; 10/15 (66.66%), avascular necrosis: 5 (33.33%), marrow in filtration; 3 (20%),z-score: n=12:mild (>-1) or none 4 (33.3%), moderate (≤-1 to -2.5) 5(42.7%).Psychomotor retardation; squint and convulsion at age 12–24 month in 7 (IIIB), most of them have been resolved in 3 patients with high dose imiglucerase after 5 years. Most frequent genotypes for all disease types: n= 40, leu 483 pro/leu 483 pro in 47.5%in all types and 80% in neuropatic GD as well as GD with avascular necroris.
Conclusion: Enzyme replacement therapy with IV imiglucerase was effective in GD type I as well as mild IIIB.

469-O

MORBIDITY AND MORTALITY FOLLOWING HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR THE TREATMENT OF MUCOPOLYSACCHARIDOSIS VI

Pederson T[1], Rizzo D[1], Orchard P[2], Bonfim C[3], Al-Seraihy A[4], Nicely H[5], Turbeville S[5]
[1]*CIBMTR, Minneapolis, United States*
[2]*Marrow Transplant Program, Unv. of MN, Minneapolis, United States*
[3]*Hospital de Clinicas UFPR, Curitiba, Brazil*
[4]*King Faisal Specialist Hospital, Riyadh, Saudi Arabia*
[5]*BioMarin Pharmaceutical Inc., Novato, United States*

Background: A retrospective study was performed using aggregate data reported between 1982–2007 to the Center for International Blood and Marrow Transplant Research (CIBMTR) to gain better insight into patient mortality and morbidity following HSCT for the treatment of MPS VI.
Methods: Analysis assessed patient demographics, morbidity and overall survival calculated using Kaplan-Meier method.
Results: 45 MPS VI patients received an allogeneic HSCT between 1982–2007. Median age at transplant was 5 years (range <1–22 years). Sixteen patients (36%) were transplanted after 1999. Thirty-nine (87%) received a transplant between the ages of 0–9 years. The most common conditioning regimen was cyclophosphamide + busulfan (N=30; 67%). Bone marrow was the most common graft type (74%), followed by cord (24%) and peripheral blood (2%). Most received a HSCT from an unrelated donor (60%) while 33% were from an HLA-identical sibling. The cumulative incidence of acute GvHD at 100 days was 36% (95% CI: 21–53%) and chronic GVHD was 13% (95% CI: 4–28%) at 1 year, and 17% (95% CI: 6–33%) at 2 years. Probability of overall survival at 100 days was 78% (95% CI: 65–89%); at 1 year 66% (95% CI: 52–79%); and remained at 67% (95% CI: 52–79%) after 3 years with a median follow-up of 52 months (range, 3–267).
Conclusion: The 45 patients described represent the largest available cohort of MPS VI patients to undergo HSCT to date providing clinicians with the estimates of morbidity and mortality associated with HSCT for the treatment of MPS VI.

470-P

ONE YEAR TREATMENT WITH MIGLUSTAT IN INFANTILE NIEMANN-PICK TYPE C

Bandeira A[1], Morais L[2], Santos M[3], Martins E[1]
[1]Metab Unit, Centro Hospitalar do Porto, Porto, Portugal
[2]Pneumol Unit, Centro Hospitalar Porto, Porto, Portugal
[3]Neurop Unit, Centro Hospitalar Porto, Porto, Portugal

Background: The clinical course of Niemann Pick Type C disease (NPC) is very heterogeneous with systemic and neurological involvement presenting from perinatal period to adulthood. The experience with Miglustat in severe infantile neurological form is recent.
Methods: We present the evolution and side effects of one year therapy in a case of severe infantile form.
Results: A three years old boy that with splenomegalia since age of 4 month and catalepsy episodes since the age of six months. At his second year of life a decline of is motor function appeared, with feeding and respiratory problems. When first seen at 28 months, he was ataxic with frequent catalepsy episodes, pyramidal syndrome, and bell shaped chest. Clinical diagnosis of NPC was supported on enzymatic and molecular study. He started miglustat at 34 months of age.
One year after, episodes of catalepsy and seizures became more frequent but paralysis of downward eye movement, cerebellar signs and dystonia remained stable. EEG revealed a more diffuse paroxistic activity. After an initial improvement in organomegalies and feeding problems they reappeared after one year of therapy with no gaining in weight. There was a stabilization of pulmonary function with no need of hospitalization because of respiratory infections. There were no side effects.
Conclusions: In the present case, treatment was started two years after the beginning of the disease. After an initial stabilization in the first 6 months, the disorder continued to progress. There was not a clear benefit with Miglustat treatment in this patient.

471-P

GENOTYPE-PHENOTYPE CORRELATIONS IN TURKISH PATIENTS WITH ALPHA GALACTOSIDASE A DEFICIENCY

Koca S[1], Ezgu F[1], Okur I[1], Biberoglu G[1], Tumer L[1], Hasanoglu A[1]
[1]Dep Ped Metab and Nut, Gazi Univ Med Fac, Ankara, Turkey

Objectives: Fabry disease results from deficient activity of the enzyme α-galactosidase and progressive lysosomal deposition of globotriaosylceramide in cells throughout the body. Although there are reports about the genotype-phenotype correlations from various countries, there has been no study from Turkey.
Materials and Methods: Selective screening for α-galactosidase deficiency and mutations of the GLA gene among the members of 6 unrelated families which have been confirmed to have at least one confirmed patient was initally carried out. A total of 11 patients were included in the study. Possible correlations were sought between the clinical and laboratory features and the genotype of these patients. Enzyme assays and DNA sequence analysis were carried out in Gazi University Laboratory of Pediatric Metabolism.
Results: 2 patients were found to have R220X and 6 patients were found to have the R227X. Also three patients were found to be homozygous for three separate missense mutations; G258R ,D266N ,M42V. Males with Fabry disease had disease signs and report symptoms earlier. Heterozygous females were all symptomatic except two patients. The patients with nonsense mutations seemed to have more significant system involvement.
Discussion: Family screening is a very important tool to determine the other carriers of GLA mutations if exists a previously confirmed patient. As α-galactosidase levels could fall into normal range, the diagnostic method of choice should be mutation screening of the GLA gene in heterozygote females. There seems to be no direct correlation between the residual enzyme levels and the extent of clinical involvement.

472-O

ELIGLUSTAT TARTRATE, AN INVESTIGATIONAL ORAL THERAPY FOR GAUCHER DISEASE TYPE 1 (GD1): PHASE 2 RESULTS AFTER 2 YEARS

Lukina E[1], Peterschmitt J[2], Watman N[3], Arreguin EA[4], Pastores G[5], Lastrebner M[6], Dragosky M[6], Rosenbaum H[7], Phillips M[8], Kaper M[2], Singh T[2], Puga AC[2]
[1]National Research Center for Haematology, Moscow, Russian Federation
[2]Genzyme Corporation, Cambridge, MA, United States
[3]Hospital Ramos Mejia, Buenos Aires, Argentina
[4]Inst Mex Seguro Social Hosp Especialid, Col. La Raza, Mexico
[5]New York University, New York, NY, United States
[6]Inst Argentino Diagnostico Tratamiento, Buenos Aires, Argentina
[7]Rambam Medical Center, Haifa, Israel
[8]Sha'are Zedek Medical Center, Jerusalem, Israel

Background: Eliglustat tartrate (Genz-112638) is a novel, oral inhibitor of glucosylceramide synthase under investigation for the treatment of GD1.
Objective: Report the efficacy and safety of eliglustat tartrate after 2 years of treatment.
Methods: This ongoing, open-label, uncontrolled, multicenter, Phase 2 clinical trial enrolled 26 untreated adults with GD1 who received eliglustat tartrate (50 or 100 mg bid). Efficacy assessments included changes from baseline in spleen and liver volumes, hemoglobin and platelet levels, biomarkers, and bone mineral density (BMD) and other skeletal parameters (reviewed centrally).
Results: At 2 years, mean hemoglobin level improved by 2.1±1.5 g/dL and platelet count by 81.5±56.0%; mean spleen volume decreased by 52.4±10.7% and liver volume by 23.9±12.8%. Median chitotriosidase and CCL18 levels decreased by 75.9% and 74.0% respectively. No bone crises occurred. Available femur MRI data showed improved dark marrow signal in 8 patients and stability in 10 others. There were no new lytic lesions or bone infarcts. Existing lytic lesions remained stable; of 7 existing infarcts, 1 improved and 6 remained stable. Lumbar spine BMD improved continuously: DXA Z-scores increased by 0.060±0.69 and DXA T-scores by 0.56±0.78. Eliglustat tartrate was well tolerated. The most common adverse events were viral infections (6 patients), and urinary tract infections, increased blood pressure, and abdominal pain (3 patients each). Eight drug-related adverse events, all mild, occurred in 6 patients.
Conclusions: Eliglustat tartrate has shown promising efficacy and safety as a potential oral substrate-reduction therapy for GD1 with continued improvements in hematologic, visceral, and bone parameters after 2 years.

473-O

PRELIMINARY LONG-TERM SAFETY, TOLERABILITY, AND ASSESSMENTS OF RENAL FUNCTION OF ADULT FABRY PATIENTS RECEIVING TREATMENT WITH AT1001 (MIGALASTAT HYDROCHLORIDE), A PHARMACOLOGICAL CHAPERONE, FOR UP TO 3 YEARS

Hughes D[1], Adera M[2], Castelli J[2], Bragat A[2], Marsden DL[2], Boudes PB[2]
[1]*Div Haem, Royal Free Hosp, UCL, London, United Kingdom*
[2]*Amicus Therapeutics, Cranbury, United States*

Background: AT100 is an orally administered, small molecule pharmacological chaperone that selectively binds lysosomal alpha-galactosidase A (alpha-Gal A), increasing enzyme stability and lysosomal trafficking. In pre-clinical studies, AT1001 significantly increased enzyme activity and decreased tissue globotriaosylceramide (GL-3) in Fabry transgenic mice. In Phase 1 studies, AT1001 was generally safe and well-tolerated and increased leukocyte alpha-Gal A activity in healthy human volunteers.
Objectives: To assess safety and efficacy of AT1001 in phase 2 clinical trials.
Methods: Four open-label Phase 2 trials were completed. 27 subjects (18 male, 9 female) ages17 to 65 years, with various genotypes, were enrolled, and received different doses and dose regimens. Twenty-six subjects completed 12 or 24 weeks treatment; and 23 subjects (14 male, 9 female) entered extension studies to evaluate long-term safety and efficacy.
Results: Fifteen subjects have received AT1001 for over 2 years and 8 more than 3 years. Nineteen subjects continue on AT1001. Previously, for subjects treated with AT1001 for 1 to 2 years it was reported that treatment with AT1001 was generally safe and well-tolerated, good responders (in vitro or in vivo increases in alpha-Gal A activity) demonstrated reduction in kidney GL-3, and preliminary renal function data was encouraging. Long-term assessments now demonstrate maintained renal function in 23 subjects receiving AT1001 for up to 3 years.
Conclusions: eGFR in subjects treated with AT1001 for up to 3 years compares favorably to published eGFR literature in untreated and ERT treated Fabry patients. AT1001 is potentially an important new option for treatment of Fabry patients.

474-P

DIVERSE CLINICAL PRESENTATIONS IN NIEMANN-PICK DISEASE TYPE C: ARE THERE RED FLAGS FOR EARLY DIAGNOSIS?

Haliloglu G[1], Gurakan F[2], Yuce A[2], Topcu M[1]
[1]*Ped Neurol, Hacettepe Child Hosp, Ankara, Turkey*
[2]*Ped Gastroeneterol, Hacettepe Child Hosp, Ankara, Turkey*

Background: NPC is a progressive neurovisceral lysosomal storage disease resulting from mutations in two genes NPC1 and NPC2 that play a role in intracellular cholesterol and lipid trafficking.
Patients and Results: Clinical presentation and age at diagnosis and follow-up in 15 patients (9 boys and 6 girls) with molecularly confirmed (NPC1 14 patients, NPC2 1 patient. There was first-degree consanguinity in all families except one. The first group presented with non-immune hydrops fetalis, neonatal cholestasis, hepatosplenomegaly and fetal ascites (n=7) between the ages of 1 month–17 months. The second group presented with isolated splenomegaly (n=1) at the age of 5 y, with progressive behavioral and extrapyramidal involvement and death within the proceeding 13 years. The third group of patients presented with initial diagnoses of cerebral palsy and/or epilepsy. Mean age of the patients in this group at presentation was 6y (2–11y). Clinical findings were motor and mental developmental delay, hypotonia, gaze palsy, narcolepsy and splenomegaly, tremor, head drops and pseudobulbar involvement with extrapyramidal and cerebellar findings and vertical supranuclear gaze palsy (VSGP). Diagnosis was done by bone marrow aspiration and skin fibroblast cultures. Two of these patients are currently on miglustat treatment.
Conclusions: Early diagnosis of NPC is crucial in the era of substrate reduction therapy for neurological stabilisation. Hepatosplenomegaly, fetal ascites and neonatal cholestasis in the perinatal and infancy period, hypotonia, ataxia and gaze palsy after the age of 2 years, extrapyramidal movement disorder, VGSP in late adolescence are red flags for the diagnosis.

475-P

ENZYME REPLACEMENT THERAPY IN 10 PATIENTS WITH MPS TYPE-6

Gul E[1], Payas A[1], Yilmaz S[1], Aydin A[2], Tuysuz B[1]
[1]*Dep Ped Genet, Ist Uni Cerrahpasa Med, Istanbul, Turkey*
[2]*Dep Ped Metab, Ist Uni Cerrahpasa Med, Istanbul, Turkey*

Mucopolysaccharidosis type-6 is associated with deficiency in N-acetylgalactosamine-4-sulfatase which results in accumulation of dermatan sulfate within lysosomes in connective tissues and characterized by osseous, corneal and visceral changes without intelligence impairment. Enzyme replacement therapy (ERT) with Naglazyme was initiated in four patients with MPS type-6 on November 2006. We treated ten patients (six male and 4 female) with MPS type 6 with ERT for 4 years. Clinical examination, growth parameters, ophthalmologic and hearing examinations, echocardiography, joint flexion range of motion, sleep quality, 3 minute stair climbing and 12 minute walking capacity were assessed at the baseline and every 6 months after weekly infusion of 1 mg/kg Naglazyme.
ERT starting age ranged from 3.5 to 9 with mean age of 8,1 year. Mean therapy period was 2.2 years (8 months to 4.5 years). Joint flexion range of motion and sleep apnea/hypopnea index as well as 3 minute stair climb test and 12 minute walk test scores were improved markedly in all patients. Some patients showed improvement in visual acuity. Patients who were treated more than 2 years, coarse face were regressed. In most of the patients liver volume was decreased. Ophthalmologic, hearing examinations and echocardiographic findings showed no difference. Two patients (8 and 9 years) with severe form were died after 1.5 years of ERT due to complication of anesthesia and lower respiratory tract infection. We conclude that symptoms were reduced at least after 2 years treatment with ERT.

476-P

IS GENISTEIN EFFECTIVE? EXPERIENCE WITH THE MPS IIIA MOUSE MODEL

Montaño AM[1], Carvalho T[1], Tomatsu S[1]
[1]*Dpt of Pediatrics, Saint Louis Univ., Saint Louis, United States*

Mucopolysaccharidosis (MPS) type IIIA is caused by deficiency of heparan-N-sulfatase and leads to accumulation of heparan sulfate (HS) in glial and neuron cells. Clinical symptoms are severe mental defect with cognitive problems and mild somatic features. No effective treatment is present. Recently, Genistein, a specific inhibitor of protein tyrosine kinase, has been found to inhibit GAG synthesis reducing GAG concentration in fibroblast cultures from MPS patients. We evaluated efficacy of Genistein in reducing HS accumulation in MPS IIIA mouse model. Genistein was daily administered for one month by oral gavage at different doses (50, 100 or 200 mg/kg/day) to mice with different ages (13, 25 and 75 days) in the first day of treatment. For mice of 13 days old, we administered 200 mg/kg/day of Genistein for 3 months. Urinary GAG levels reduced after one month of treatment for all doses when the treatment started at the youngest age. Urinary HS concentration was lower in mice treated with Genistein at 200 mg/kg/day during 3 months. GAG deposits in liver decreased for some animals treated for 3 months at 13 days of age. We observed a clear tendency of reduction in GAG storage for MPS IIIA mouse model when high doses of Genistein were used and treatment started at early stages. No impact of Genistein was observed when treatment was given at older ages. This suggests an age-related effect of Genistein. The subtle effect of Genistein in MPS IIIA mouse model encourages its use in a combined therapeutic MPS protocol.

477-O

COGNITIVE OUTCOME IN 14 MPS II PATIENTS TREATED WITH IDURSULFASE

Valayannopoulos V[1], Arnoux JB[1], Kossorotoff M[1], Chabli A[2], Caillaud C[3], Lemoine M[4], Lyonnet S[5], Lemerrer M[5], Cormier-Daire V[5], de Lonlay P[1]
[1]*Ref Center IEM, Necker Enfants-Malades H, Paris, France*
[2]*Biochem Lab, Necker Enfants Malades Hosp, Paris, France*
[3]*Biochem Lab, Cochin Hosp, Paris, France*
[4]*Phys Medicine, Necker-Enf Malades Hosp, Paris, France*
[5]*Genetics Dep, Necker-Enfants Malades Hos, Paris, France*

Background: Mucopolysaccharidosis II (MPSII) is an X-linked lysosomal disorder caused by deficiency of iduronate-2-sulfatase (I2S). Recently, enzyme replacement therapy (ERT) with recombinant human I2S (idursulfase) has been approved for the treatment of MPS II patients.
Patients and methods: 14 MPS II patients aged from 2 months to 10.5 years were treated from 12/2006, by idursulfase weekly infusions. Among them, 6 patients were less than 5 years at the onset of treatment. 4/6 of less than 5 years patients and 7/8 of older patients presented mild to severe psychomotor retardation with behavior disturbance. All patients were annually assessed by cognitive tests and Conner's behavior scale and underwent yearly brain MRI.
Results: At this stage of treatment (12 to 42 months) all patients with cognitive impairment displayed a deterioration of their performances and behavior. One patient lost all motor and cognitive skills. Among the 3 patients with normal cognitive development, 2 remained unaffected: one 8 year patient and 1 patient treated at age 2 months (despite a family history of a brother with severe delay) and 1 developed abnormal behavior. MRI abnormalities were found in all patients with temporal lobe lesions in the most severe patients. All patients improved their visceral symptoms.
Conclusion: Our data show no efficacy of idursulfase on the cognitive impairment of MPS II patients including patients less than 5 years. However the good outcome in one patient who started ERT very early raises the question of the impact of prompt treatment on the cognitive development of these patients.

478-P

JUVENILE NEURONAL CEROID LIPOFUSCINOSIS IN A PATIENT WITH COMPOUND HETEROZYGOUS CLN3 MUTATIONS: A 9-YEAR FOLLOW-UP

Al-Thihli K[1], Matsuba C[2], Roland E[3], Stockler-Ipsiroglu S[1], Mercimek-Mahmutoglu S[1]
[1]*Div Biochemical Dis, Dep Ped, Univ BC, Vancouver, Canada*
[2]*Dep Ophthalmology, Univ of BC, Vancouver, Canada*
[3]*Div Neurol, Dep Ped, Univ BC, Vancouver, Canada*

Background: The juvenile neuronal ceroid lipofuscinosis (JNCL) is an autosomal recessive lysosomal storage disease characterized by loss of vision, seizures, dementia and Parkinsonism. Premature death occurs around 30 years.
Case Report and Results: This 17-year-old male was presented with a rapidly progressive reduction of his visual acuity at age 8 years. Fundus examination revealed marked retinal pigmentary changes. An ERG was suggestive of severe rod-cone dystrophy and was non-recordable. He became blind 2 years later. His peripheral smear revealed cytoplasmic vacuolation of lymphocytes. Two known pathogenic missense mutations were found in the CLN3 gene confirming the diagnosis of JNCL. At age 10 years, he developed generalized seizures which responded well to Clobazam. His cranial MRI showed mild delayed myelination in periventricular area and mild cerebral atrophy. He developed cognitive decline at age 13 years and parkinsonian signs at age 15 years. Unified Parkinson's Disease Rating Scale (UPDRS) deteriorated within 1.5 years (increased from 15 to 34). He had no response to Selegiline and Levodopa/Carbidopa therapy. L-carnitine and polyunsaturated fatty acids were started. Child Behaviour Checklist for a standardized behavior rating scale showed improvement in his behavioral and emotional functioning on this therapy. His total 'Kohlschutter Score' showed deterioration in his vision, intellect, language, motor function and epilepsy in 9-year follow-up (decreased from 11 to 5).
Conclusion: Clinical rating scales emphasize the importance of multiple clinical parameters in evaluation of disease burden and progression in chronic progressive neurodegenerative disorders.

479-P

FEMORAL HEAD AVASCULAR NECROSIS AND STROKE-LIKE LESIONS IN A GAUCHER TYPE I PATIENT HETEROZYGOUS FOR FACTOR V LEYDEN: JUST A COINCIDENCE?

Vairo F[1], Netto CBO[1], Dornelles A[1], Segal SL[1], Bittar CM[1], Vedolin L[2], Schwartz IVD[1]
[1]*Medical Genetics Service, HCPA, Porto Alegre, Brazil*
[2]*Hospital Moinhos de Vento, Porto Alegre, Brazil*

Introduction: Gaucher Disease (GD) affects mainly the liver, spleen, bone marrow and skeleton. Clinical and radiological evidence of skeletal involvement occurs in the majority of patients. While enzyme replacement therapy (ERT) is highly effective in reversing visceral and haematological manifestations, its precise impact on the bone disease is unknown. GD splenectomized patients, especially those ones who are carriers of thrombophylic mutations, appear to be at a higher risk to develop avascular necrosis (AVN) but no other types of thromboembolic events.
Case Report: We report a 33 year-old male patient with type-I GD (p/N370S/L444P) who is heterozygous for factor V Leiden (FVL) mutation. His symptoms started when he was 16 years old, with hepatosplenomegaly and bone changes. He underwent splenectomy at age 25. The diagnosis of GD was performed at 27 years-old just after an episode of AVN. ERT with imiglucerase started in 2004 and total hip arthroplasty was performed in 2008. In 2010, he presented blurred vision and weakness of both hands. MRI showed multiple stroke-like lesions. No other risk factor for stroke is present.
Discussion/Conclusion: Antithrombotic therapy is usually not recommended for asymptomatic carriers of FVL neither for GD patients presenting AVN. Our case emphasizes that GD patients who are carriers of FVL may be are at higher risk to develop not only AVN, but also other types of thromboembolic events; so, the use of antithrombotic therapy should be considered in these patients.

480-P

TRIGGER FOR INITIATING ENZYME REPLACEMENT THERAPY (ERT) TREATMENT IN CHILDREN WITH FABRY DISEASE

Ramaswami U[1], Hendricksz C[2], Wijburg F[3], Bouwman M[3], Linhart A[4], Pintos Morell G[5], Kalkum G[6], Parini R[7], Beck M[6]
[1]*Paed Metab Unit, Addenbrookes Hosp, Cambridge, United Kingdom*
[2]*Clin Inher Met Disease, B'ham Child Hosp, Birmingham, United Kingdom*
[3]*Acad Med Clinic, Amsterdam, Netherlands*
[4]*Cardio & Int Med, Charles Uni, Prague, Czech Republic*
[5]*Dep Paed Uni Hosp Germans Trias, Barcelona, Spain*
[6]*Childrens Hosp, Johannes Gutenberg Uni, Mainz, Germany*
[7]*Dep Paed, Univ Milan Bicocca, Milan, Italy*

Background: Anderson Fabry Disease (AFD), an X-linked lysosomal disorder presents in early childhood although life threatening cardiac, renal and neurological complications usually occur in adulthood. Despite availability of national guidelines, optimal timing to start enzyme replacement therapy (ERT) is currently unclear. We performed an audit to identify the triggers to treat children with AFD in leading European centers.
Method: 10 European paediatric metabolic centers were sent a two part anonymised questionnaire. Part A aimed to identify the most likely trigger to start ERT at first presentation and in Part B the clinicians ranked from a list of fabry related manifestations, the common triggers to start ERT in individual patients at subsequent visits.
Results: 7 Part A and 32 Part B forms were returned. In part A, no geographical differences were noted, with overt cardiac, renal and neurological manifestations being important triggers to start ERT at first presentation. In part B however, symptoms that actually triggered ERT included commonly reported symptoms i.e. gastrointestinal, fatigue, heat intolerance and fever pain crises. Interestingly, positive correlations between trigger to start ERT and genotype; tortuous retinal vessels; acroparaesthesia and family history were noted.
Conclusion: Despite increasing knowledge of suboptimal responses to ERT in adults presenting with end organ disease, the immediately identified potential triggers to treat children was overt end organ damage. However, following subsequent assessments, common early onset Fabry specific childhood symptoms were the triggers. Increasing the awareness of early symptom progression in AFD is important to help with timely treatment strategies.

481-P

FOUR CASES OF NIEMANN-PICK TYPE C DISEASE PRESENTED WITH EARLY ONSET CHOLESTASIS

Kucukcongar A[1], Okur I[1], Ezgu FS[1] , Tumer L[1] , Dalgic B[2], Hasanoglu A[1]
[1]*Gazi University Div of Pediatric Metab, Ankara, Turkey*
[2]*Gazi Univ Div of Pediatric Gastroentr, Ankara, Turkey*

Niemann-Pick disease type C (NP-C) is lipid storage disorder characterized by progressive neurological deterioration leading to premature death. It has a wide spectrum of clinical phenotypes. Initial manifestations can be hepatic, neurologic, and psychiatric. Cholestasis associated with hepatosplenomegaly, is generally the most common sign. Here, four patients who had presented with cholestasis and eventually diagnosed with NP-C will be discussed.
Case 1: Female infant who is the first child of healthy, unrelated parents. On physical examination hepatosplenomegaly was found at the age of 10 days. Cholestasis findings were demonstrated on laboratory tests.
Case 2: Ten months old girl was noticed to have hepatosplenomeagly during a physical examination. Later on she was also found to have cholestasis.
Case 3: Male patient who is the second sibling of healthy, consangineous parents. At the age of 3 years he presented to his paediatrician with complaints of vomiting, diarrhea. He was found to have hepatosplenomaly and icterus as well as cholestasis.
Case 4: Male infant was born at premature to consanguineous parents. Abdominal distention was realized at the age of one month. Hepatosplenomegaly with cholestasis was noticed.
Bone marrow examination revealed presence of storage cells suggestive of NP-C in all of the cases. Lysosomal enzyme activities from leucocytes for beta-glucosidase and acid-sphingomyelinase were normal. Intracellular accumulations of cholesterol were observed in skin fibroblasts by Filipin-staining method and confirmed the diagnosis of NP- C.
With this report the differential of diagnosis NP-C disease was emphasized in patients with hepatosplenomegaly and cholestasis.

482-P

ABDOMINAL LYMPHADENOPATHY DURING ENZYME REPLACEMENT THERAPY: AN EMERGING CHALLENGE OF GAUCHER DISEASE?

Sarajlija A[1], Djordjevic M[1], Kecman B[1], Djuricic S[2], Ristic G[3]
[1]*Metab Div, Moth and Child Health Inst, Belgrade, Serbia and Montenegro*
[2]*Pathol Div, Moth and Child Health Inst, Belgrade, Serbia and Montenegro*
[3]*Immunol Div, Moth and Child Health Inst, Belgrade, Serbia and Montenegro*

Background: Several investigators reported occurrence of abdominal lymphadenopathy in children with Gaucher disease (GD) during the course of enzyme replacement therapy (ERT). Age of abdominal lymphadenopathy onset ranged from 3 to 8 years and it was mainly encountered in type III of GD.
Objective: To present patient with unusual complication of GD.
Case Report: A boy from northern Serbia was diagnosed with GD at 15 months age. Presence of hepatosplenomegaly, pancytopenia and horizontal gaze palsy were in accordance with GD type III. Diagnosis was confirmed by enzymatic and genetic analysis (compound heterozygousity for L444P/D409H mutations in GBA gene). At 4 years of age ERT was initially started with 60 IU/kg every two weeks with dose being doubled after two years. During treatment, we observed normalization of organ volumes and hematologic parameters with slowly progressive neurological manifestations. After 5 years of ERT, an enlargement of mesenteric and prevertebral lymph nodes was verified by clinical, ultrasound and CT exams. During following months progression of abdominal lymphadenopathy was observed whilst boy remained without symptoms attributable to these changes. Surgical biopsy of enlarged lymph node was performed and histopathology revealed numerous Gaucher cells surrounded by reactive histiocytic proliferation diffusely filling central and peripheral sinusoids. Progression of lymphadenopathy was correlated to 50% increase in chitotriosidase level and development of marked hypergammaglobulinemia.
Conclusion: Mounting evidence of abdominal lymphadenopathy occurrence in pediatric Gaucher patients on ERT opens new questions regarding GD pathophysiology and treatment strategies.

483-P

DIAGNOSIS OF POMPE DISEASE USING DRIED BLOOD SPOTS (DBS) ON FILTER PAPER AT A BRAZILIAN REFERENCE CENTER FOR INBORN ERRORS OF METABOLISM

Kyosen SO[1], Muller KB[2], Martins AM[1], D'Almeida V[2]
[1]*CREIM, UNIFESP, Sao Paulo, Brazil*
[2]*LEIM, UNIFESP, Sao Paulo, Brazil*

Background: The LEIM is a reference center for the diagnosis of Pompe disease DBS and began to offer the GAA assay using this technique in 2006.
Method: The assay of GAA activity was performed in DBS according to the technique described by Chamoles et al (2004) which results are expressed both in a Neutral Glucosidase /Acid Glucosidase ratio (N/A) and by the percentage of inhibition of Neutral Glucosidase (% INH) using acarbose.
Results: Over a period of 3 years we received 457 samples of possible Pompe disease patients. Thirty three (7.2%) samples were in the affected range, 14 were from patients below 1y of age, 6 between 1 and 18y and 13 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: 76.5±18.6 and 86±21.6, 68.3±22 and 92.5±1.4, 59.2±34.6 and 90.6±3.5. In the negative samples, the N/A (mean±SD) and the % INH (mean±SD) of the samples below 1y of age, between 1 to 18y and above 18y were respectively: 11.7±4.3 and 63.9±10.3, 13.5±4.9 and 67.5±9.7, 14.4±4.9 and 74.24±6.8. The N/A could clearly differentiate the positive and negative samples without overlapping. However, the %INH presented an overlap between positive and negative samples only of individuals below 1y.
Conclusion: Taken together, the determination of N/A and %INH as a diagnostic procedure is sensitive, accurate, time-saving and suitable for establishing the definite diagnosis of Pompe disease.
Financial support: FAPESP, CNPq, CAPES, AFIP and IGEIM.

484-P

A TREATMENT EXPERIENCE OF TYPE 3 GAUCHER DISEASE: PIRACETAM AND MIGLUSTAT THERAPY IN PROGRESSIVE MYOCLONIC EPILEPSY

Onal H[1], Adal E[1], Ersen A[2], Aydin A[3]
[1]*Metab Dis, Bakirkoy Moth and Child Hosp, Istanbul, Turkey*
[2]*Kasimpasa Military Hospital, Istanbul, Turkey*
[3]*Div Metab Dis, Cerrahpasa Med Fac, Istanbul, Turkey*

The neurologic manifestations of Gaucher disease (GD) patients have to date been refractory to any treatment approach.
A 21-month-old boy was diagnosed GD with beta-glucocerebrosidase activity in leucocytes: 0,33 mmol/g.h. (normal: 1–5) and L444P/L444P mutation. Enzyme replacement therapy was initiated and considerably improved the manifestations of cytopenia and liver enlargement. After 4 years of age, neurologic deteriorations were recognized as seizures, gait stiffness, speech disturbance,dystonia and nocturnal twitching. Whereas ERT dose was increased (120 IU/kg/month), general neurologic clinical picture was worsen with myoclonic, tonic-clonic seizures and status epilepticus. Neurologic condition was not influenced by oxcarbazepine, topiramate, levetiracetam, clonazepam, phenobarbital, clobazam; only midazolam infusion was effective in seizures and patient couldn't be discharged from hospital for 3 months. We decided to use 'piracetam' for seizures and titrated dose of 12 g/day (500 mg/kg) by increasing 1 gr in every two days. Midazolam therapy was stopped by decreasing day by day. The frequency of seizures were decreased (1–2 seizures in one week) and the patient was discharged. Enzyme replacement therapy was discontinued after 3 months and miglustat (200 mg/day) was initiated. Piracetam and miglustat therapy was effective in seizures and dysarthria, improved walking ability and any significant adverse effects weren't observed during the nine month follow-up.
The stabilization of the clinical course in our patient is noteworthy. Though further evidence is needed in order to draw any definite conclusions, our data suggest that combined piracetam and miglustat therapy may be beneficial for neuronopathic forms of GD.

485-P

NIEMANN-PICK C1 DISORDER—A DIAGNOSTIC CHALLENGE IN DEVELOPING COUNTRIES

Boy R[1], Paiva I[1], Guardin L[1], Carvalho J[1], Azevedo A[2], Souza FT[3], Giugliani R[3]
[1]*Ped Dept, UERJ, Rio de Janeiro, Brazil*
[2]*Hematol Dept,UERJ,, Rio de Janeiro, Brazil*
[3]*Medical Genetics Service,HCPA, Porto Alegre, Brazil*

Objectives: To report the clinical manifestations and diagnostic work-up on two early symptomatic cases of NPC.
Material/Methods: Case 1:22mo old male patient was referred presenting hepatosplenomegaly, fever and pancytopenia. He was the second son of a nonconsanguineous couple, with mild developmental delay; Case 2:5ys old female was evaluated for neurological regression, spastic paraparesia and vertical supranuclear ophthalmoplegia. She presented hepatosplenomegaly and diarrhea at day two and evolved with mild developmental delay. Seizures began at 2ys and neurological deterioration started soon thereafter. NPC was suspected and a BM biopsy was perfomed.The gap between symptoms' onset and the NPC diagnosis was 5ys.
Results: Hepatic biopsies (case 2)–at 1mo–inconclusive, and at 4mo-micronodular hepatic cirrhosis. The BM histology of both patients showed foam cells. Plasma chitotriosidase (case 1) showed increased activity.Acid sphingomyelinase (NPA/B) and beta-glucosidase (Gaucher) were normal. Filipin test in fibroblasts was positive on both cases. Sequencing of NPC1 gene revealed two pathogenic mutations (c.3467A>T and c.3662delT) on case 1(Prof. M.Elleder, Prague); molecular analysis on case 2 in progress.
Conclusions: NPC has an extremely heterogeneous clinical presentation and the symptoms are not specific to the disease.The confirmation of a diagnosis requires biochemical and/or molecular testing in reference centres.The BM biopsy, although not mandatory for NPC diagnosis, was useful to discard other diseases. Considering the emerging SRT for NPC and its beneficial effects on neurological disease progression, precise and early diagnosis is very important. This stresses the need of reference services to provide access to rare disease diagnosis for patients evaluated under the public health system umbrella, still scarce in developing countries.

486-P

EFFICACY OF RECOMBINANT HUMAN ARYLSULFATASE B (GALSULFASE) ON RESTRICTED RANGE OF MOTION AND ACTIVITIES OF DAILY LIVING IN PATIENTS WITH MAROTEAUX-LAMY SYNDROME (MUCOPOLYSACCHARIDOSIS TYPE VI): IMPROVEMENT AFTER 24 WEEKS OF TREATMENT

Jurecka A[1], Marucha J[1], Rozdzynska A[2], Czartoryska B[3], Tylki-Szymanska A[1]
[1]*Dep Metab Dis, CMHI, Warsaw, Poland*
[2]*Antrop Lab, CMHI, Warsaw, Poland*
[3]*Dep Genet, Inst Psych Neurol, Warsaw, Poland*

Objectives: Our goal was to describe the improvement in range of motion (ROM) and daily living activities in 3 patients with Maroteaux-Lamy syndrome following 24 weeks of therapy with galsulfase.
Methods: Passive and active ROM was measured by a goniometer and assessed by the same physiotherapist using International Method of Measuring and Recording Joint Motion. Functional status was assessed by age–appropriate Health Assessment Questionnaire (HAQ). All assessments were performed at baseline and after 24 weeks of galsulfase therapy.
Results: Baseline ROM analysis showed significant restrictions in passive and active ROM in multiple joints in all patients. These limitations were particularly visible in the shoulder joint. Differences among subjects were observed. The youngest patient in our study, who seems to have more severe course of the disease when compared to other subjects, had the greatest degree of impairment at baseline. After 24 weeks of galsulfase therapy, all patients displayed improvement of ROM in various joints, especially in upper limbs. The youngest patient had the most marked improvement in the shoulder among the study subjects. All patients displayed improvements in functional status (investigator's observation and HAQ assessment).
Conclusions: We observed improvement in ROM in all patients following 24 weeks of galsulfase therapy. Because MPS VI is progressive and joint impairment appears early in the stage of the disease it is important to initiate enzyme replacement therapy as early as possible. In order to help optimize patients' outcomes, physical therapy should be added and adjusted to the patient's efficiency and capabilities.

487-O

A PHARMACOGENETIC APPROACH TO THE SELECTION OF FABRY PATIENTS FOR PHARMACOLOGICAL CHAPERONE THERAPY

Benjamin ER[1], Wu X[1], Katz E[1], Mascioli K[1], Della Valle MC[1], Chang H[1], Greene D[1], Schiffmann R[2], Lockhart DJ[1], Valenzano KJ[1]
[1]*Amicus Therapeutics, Cranbury, United States*
[2]*Baylor Research Institute, Dallas, United States*

Backgrounds: Fabry disease is an X-linked lysosomal storage disorder caused by mutations in the gene (GLA) that encodes α-galactosidase A (α-Gal A). More than 600 mutations have been reported, of which more than 60% are missense. The iminosugar AT1001 (1-deoxygalactonojirimycin), a pharmacological chaperone under clinical development as a therapy for Fabry disease, selectively binds α-Gal A, increasing physical stability, lysosomal trafficking, and total cellular activity.
Methods: A systematic approach to select patients that may be candidates for AT1001 therapy was developed. Each of 426 Fabry disease-causing missense and small in-frame insertion or deletion mutations were expressed in HEK-293 cells and tested for response to AT1001.
Results: Increased α-Gal A levels were seen for a majority of the mutant forms. To identify the subset most likely to respond to AT1001 in vivo, criteria were developed that consider the magnitude of the enzyme response to a clinically-relevant AT1001 concentration. Mutant forms that met the in vitro criteria were generally found to be responsive in vivo as measured in white blood cells from male patients orally administered AT1001 during Phase 2 clinical trials. Those that did not meet the in vitro criteria showed very limited or no in vivo α-Gal A response.
Conclusions: These results suggest that a pharmacogenetic reference table comprised of the AT1001-responsive α-Gal A mutant forms and the corresponding GLA mutations can be used to select male and female Fabry patients for pharmacological chaperone therapy with AT1001 based on genotype.

488-P

INTERNATIONAL EXPANSION OF THE MPS-BRAZIL NETWORK

Giugliani R[1], Federhen A[1], Martins T[1], Pinto L[1], Burin M[1], Leistner-Segal S[1], Matte U[1], Toralles M[2], Amorin T[2], Acosta A[2], Llerena Jr J[3], Horovitz D[3], Ribeiro M[4], Boy R[5], Kim C[6], Pina Neto J[7], Steiner C[8], Martins A[9], Ribeiro E[10], Silva L[11], Valadares E[12], Duarte A[13], Lacerda E[14], Santos ML[15], Schwartz I[1]
[1]*Medical Genetics Service/HCPA/UFRGS, Porto Alegre, RS, Brazil*
[2]*Medical Genetics Service, HUPES-UFBA, Salvador, Brazil*
[3]*Medical Genetics Center, IFF-FIOCRUZ, Rio de Janeiro, Brazil*
[4]*Clinical Genetics Service, UFRJ, Rio de Janeiro, Brazil*
[5]*Mother-Child Department, HUPE, Rio de Janeiro, Brazil*
[6]*Medical Genetics Unit, IC-HC-USP, São Paulo, Brazil*
[7]*Medical Genetics Sector, HCRP, Ribeirão Preto, Brazil*
[8]*Department of Medical Genetics, UNICAMP, Campinas, Brazil*
[9]*UNIFESP-EPM, São Paulo, Brazil*
[10]*Hospital Geral César Cals, Fortaleza, Brazil*
[11]*Department of Physiology, UFPA, Belém, Brazil*
[12]*Department of Pediatrics, FM-UFMG, Belo Horizonte, Brazil*
[13]*Medical Genetics Service, IMIP, Recife, Brazil*
[14]*Department of General Biology, ICB-UFG, Goiânia, Brazil*
[15]*Hospital Pequeno Príncipe, Curitiba, Brazil*

Objectives: The MPS BRAZIL-NETWORK (MBN) was created to improve the diagnosis and management of MPS diseases in Brazil. Since then, physicians from many countries have contacted the MBN to request support for the investigation of patients with suspected MPS disease.
Methods: The contact with MBN has been performed through the website www.redempsbrasil.ufrgs.br or the email redempsbrasil@ufrgs.br. Customers could download informative materials and instructions for sample collection and shipment, as well as the educational material on MPS prepared by MBN. Services from several countries sent biological samples, usually dried blood spots, to the MBN headquarters, located at the Medical Genetics Service of Hospital de Clínicas de Porto Alegre, Brazil (MGS-HCPA), where the laboratory tests were performed.
Results: From April 2004 to April 2010, 177 foreign patients with MPS suspicion were investigated. The MPS diagnosis was confirmed in 146/177 (82.5%) patients. The most frequent type of MPS diagnosed was MPS II, confirmed in 68/146 (46.6%) of the MPS patients, followed by MPS VI (24.6%) and MPS I (18.5%). Most of these patients are from Latin American countries (119/146, or 81.5%). However, there is an increasing referral rate from other continents, with significative numbers coming from Middle East.
Conclusions: The MPS-BRAZIL NETWORK is improving the diagnosis of MPS not only in Brazil, but also in other countries where access to diagnosis is not easily available. These results highlight the importance of providing access to diagnosis of these diseases, especially now that treatment is possible for many of these conditions.

489-P

SLEEP EVALUATION IN UNTREATED MPS VI PATIENTS

John AB[1], Fagondes SC[1], Schwartz IV[2], Azevedo ACM[2], Barrios PM[3], Dalcin PT[1], Menna Barreto SS[1], Giugliani R[2]
[1]Pulmonary Diseases Service/HCPA, Porto Alegre, Brazil
[2]Medical Genetics Service/HCPA, Porto Alegre, Brazil
[3]Cardiology Service/HCPA, Porto Alegre, Brazil

Background: In MPS VI, partially degraded GAGs accumulate in tissues, such as the upper airways (UA), which leads to the development of obstructive sleep apnea (OSA).
Objective: To estimate the prevalence of OSA in a group of South American patients with MPS VI who were not treated before with ERT/BMT, and the association of OSA with clinical and echocardiographic findings.
Methods: Twenty-eight MPS VI patients (mean age of evaluation: 98.5 mo) were evaluated, and data about history, PE, transthoracic Doppler echocardiogram and overnight polysomnography (PSG) were collected.
Results: The most frequent clinical signs during sleep were snoring and witnessed apnea. Three patients (10.7%) had already undergone adenotonsillectomy and 6(21.4%) adenoidectomy. Physical examination revealed that 82.1% had pectus carinatum and 78.6% macroglossia. Polysomnography results showed that 23/27 patients(85.1%)had OSA;the disorder was mild in 4,moderate in 5,and severe in 14 patients. Central apneas occurred rarely. Echocardiograms showed evidence of pulmonary hypertension(PH) in 14 patients(50%).Lower SpO2(p=0.037)and nadir(p=0.007)were positively associated with PH. Witnessed apnea was the most important predictor of PH in this sample(p=0.016).
Conclusions: The prevalence of OSA in untreated MPS VI was high, and the level of desaturation was positively correlated with PH. Witnessed apnea during sleep was the most important variable to predict PH. Symptoms during sleep were not associated with PSG findings, suggesting that this population should undergo routine PSG even before symptoms of airway obstruction are noticed during sleep. This study is useful for the evaluation of MPS VI specific respiratory outcomes in patients.
(Acknowledgements: Lara Kersten for PSG studies, MPS-Brazil-Network and BioMarin for support).

490-P

SPONTANEOUS REVERSAL OF HYPERTROPHIC CARDIOMYOPATHY IN POMPE DISEASE

Spada M[1], Pagliardini V[1], Garelli D[1], Ignaccolo MG[1], Porta F[1], Perfetto F[1], Filocamo M[2], Riggi C[3], Pagliardini S[4], Ponzone A[1]
[1]Dept Ped, Univ Child Hosp, Torino, Italy
[2]Gaslini Institute, Genova, Italy
[3]Div Ped Card, Univ Child Hosp, Torino, Italy
[4]Newborn Screening Unit, Univ Child Hosp, Torino, Italy

Pompe disease is caused by the deficiency of the lysosomal enzyme acid-alpha-glucosidase (GAA) that leads to lysosomal glycogen accumulation in skeletal, cardiac and smooth muscle resulting in two different clinical phenotypes: the severe infantile-onset form, in which the massive deposition of glycogen causes early hypotonia and fatal hypertrophic cardiomyopathy, and the late-onset variant, which usually appears as slowly progressive myopathy with respiratory insufficiency in absence of cardiac involvement.
Here we report on a patient who presented with neonatal hypertrophic cardiomyopathy (IVSs: 6.5 mm at 5 days, 7.9 mm at 25 days), progressive muscular weakness and elevated serum CK levels. At the age of 4 months the patient underwent a muscle biopsy that showed vacuolar myopathy and very low GAA activity (0.4 nmol/h/mg of protein; vn 2.7–14.0). The patient was, finally, proven to be a compound heterozygous at the GAA locus carrying the common "late-onset" IVS1 c.-32-13 T>G leaky splicing mutation in association with the c.1224 M>G (p.M408V) missense mutation. Surprisingly, a spontaneous reversal of cardiac hypertrophy occurred and the cardiomyopathy, closely monitored at echocardiography, fully reverted between 9 and 12 months of age despite progressive muscular weakness and permanent elevation of CK levels.
As to our knowledge this appears to be the first report of spontaneous and early reversal of the hypertrophic cardiomyopathy in Pompe disease, further experience is needed to elucidate if this could be a considered a common cardiac outcome in late-onset variants or if present genotype is predictive of this peculiar cardiac issue in GAA deficiency.

491-P

DIAGNOSTIC DELAY IN MUCOPOLYSACCHARIDOSIS (MPS I) PATIENTS: THE PICTURE IN LATIN AMERICA

Giugliani R[1], Ospina S[2], Villalobos J[3], Guerra P[4], Sanchez L[5], Bay L[6]
[1]Medical Genetics Service/HCPA/UFRGS, Porto Alegre, RS, Brazil
[2]Universidad del Rosario, Bogota, Colombia
[3]Universidad Central de Venezuela, Caracas, Venezuela
[4]Universidad San Sebastián, Puerto Montt, Chile
[5]Hospital de Especialidades, Nuevo León, Mexico
[6]Hosp. Nac. de Pediatría J. P. Garrahan, Buenos Aires, Argentina

The delay between symptom onset, diagnosis, and treatment in MPS I patients from Latin America (LATAM) was compared to the rest of the world (ROW) among patients enrolled in the MPS I Registry, an international observational database.
As of February 2010, the Registry contained data from 888 patients in over 30 countries, including 82 patients from Brazil and 50 from the rest of LATAM. Among LATAM patients, the median age at first symptom was 0.8y for Hurler patients, 1y for Hurler-Scheie patients, and 5.1y for Scheie patients. For ROW, these ages were similar: 0.5y for Hurler, 2.2y for Hurler Scheie, and 5.5y for Scheie. Among patients from LATAM, the gap between the median age of first symptom and median age of diagnosis was 0.9y for Hurler, 3y for Hurler-Scheie, and 4.6y for Scheie; among ROW patients, this gap was 0.3y for Hurler, 1.6y for Hurler-Scheie, and 3.9y for Scheie. The interval between median age at diagnosis and median age at first treatment in LATAM was 2.6y for Hurler, 4.6y for Hurler-Scheie, and 5.6y for Scheie; in ROW this interval was 0.5y for Hurler, 4.8y for Hurler-Scheie, and 7.9y for Scheie. Despite early signs and symptoms, diagnosis of MPS I is often delayed. This delay was more prolonged in LATAM than in ROW for all phenotypes. After diagnosis, most patients experience a further delay before beginning treatment. These results highlight the need for better disease recognition and access to diagnostic tests, as well better access to and speeder initiation of treatment.

492-P

EXPERIENCE OF SWITCHING ENZYME REPLACEMENT THERAPY (ERT) PRODUCTS IN PATIENTS WITH ANDERSON FABRY DISEASE: A SPECIALIST NURSE PERSPECTIVE

Thompson L[1], Bleakley C[1], Hallows L[1]
[1]Salford Royal NHS Foundation Trust, Manchester, United Kingdom

Background: Anderson Fabry disease (α-galactosidase A deficiency) is a rare, x-linked Lysosomal storage disorder that can cause early death from renal, cardiac and cerebrovascular involvement. Two licensed treatment products are available in the UK in the form of a 2 weekly infusion. During a Fabrazyme. supply problem we set out to maintain all patients on therapy. A clinical decision to maintain patients on the licensed dose of Fabrazyme. 1 mg/kg and Replagal. 0.2 mg/kg commenced discussions.
Methods: At the time 94 patients were receiving Fabrazyme. and 4 Replagal.. A scoring system was developed to aid clinical decisions making. The allocation of Fabrazyme. was calculated to provide a 1 mg/kg dose for only 30 of the 94 patients. A total of 64 patients were offered Replagal. at 0.2 mg/kg We will describe the considerations and communications framework
Results: All 64 patients that were offered Replagal, accepted and transferred without an interruption in treatment. With the exception of 1 male who discontinued due to the discomfort of reactions, the remaining 63 patients have transferred products uneventfully. The aim was achieved.
Conclusion: It is possible to safely manage a large number of Fabry patients to provide a seamless treatment regime within a crisis and maintain patient confidence when holistic care is provided in a specialist centre where dedicated time from specialist nurses, doctors, and MDT members are working together for the best interest of the patient and their families.

493-P

SEVERITY PROFILE OF MUCOPOLYSACCHARIDOSIS I (MPS I) IN BRAZIL AND LATIN AMERICA

Giugliani R[1], Ospina S[2], Villalobos J[3], Guerra P[4], Sanchez L[5], Bay L[6]
[1]*Medical Genetics Service/HCPA/UFRGS, Porto Alegre, RS, Brazil*
[2]*Universidad del Rosario, Bogota, Colombia*
[3]*Universidad Central de Venezuela, Caracas, Venezuela*
[4]*Universidad San Sebastián, Puerto Montt, Chile*
[5]*Hospital de Especialidades, Nuevo León, Mexico*
[6]*Hosp. Nac. de Pediatría J. P. Garrahan, Buenos Aires, Argentina*

Objectives: To evaluate the severity profile of MPS I disease in MPS I patients from Brazil and Latin America (LATAM) and the rest of the world (ROW).
Methods: The MPS I Registry is an ongoing, international, observational program intended to track clinical outcomes of patients with MPS I over time. Demographics, phenotype distribution, genotype information, symptom frequency and chronology, and age at death were determined for Brazilian patients and LATAM patients as a whole and compared to data from ROW.
Results: As of February 2010, the Registry contained data from 888 patients in over 30 countries, including 82 patients from Brazil and 50 from the rest of LATAM. In Brazil, among the 64 patients with a reported phenotype, 30% were Hurler, 53% were Hurler-Scheie, and 17% were Scheie. Among the 36 non-Brazilian LATAM patients with a reported phenotype, the proportion was 61% Hurler, 36% Hurler-Scheie, and 3% Scheie, which was closer to the ROW phenotype distribution of 65% Hurler, 23% Hurler-Scheie, and 12% Scheie. By phenotype, the age at onset of symptoms in various organs was similar in Brazil and LATAM to ROW, with coarse facies, corneal clouding, and hepatomegaly reported in over 80% of all patients. The median age of death among the 9 deceased Brazilian patients was 8.3 years versus 4.9 years among the 170 deceased patients in ROW.
Conclusion: The smaller proportion of Hurler patients in Brazil and the higher median age at death in suggests that patients from Brazil have a more attenuated phenotype than ROW.

494-P

OUR EXPERIENCES OF SWITCHING PATIENTS FROM FABRAZYME TO REPLAGAL

Hallows L[1], Waldek S[1]
[1]*Salford Royal NHS Foundation Trust, Manchester, United Kingdom*

Introduction: Fabry disease is an X-linked, inherited Lysosomal Storage Disease. A deficiency of the enzyme alpha galactosidase A causes a glyoclipid (globtriaosylceramide) to accumulate within the blood vessels, other tissues and organs.
Methods: We reviewed our group of patients switched from Fabrazyme. to Replagal. identifying 3 patients for presentation as case studies. We describe the medical history, the reason for changing Enzyme Replacement Therapy (ERT) and outcome.
Case Study 1: 36 year old male on Fabrazyme for 2 years, then experienced infusion related reactions (skin irritation, urticaria, deteriorating to rigors, dyspnoea and coughing) despite administration of antihistamines and corticosteroids. After switching to Replagal, the infusion reactions ceased with no further need for antihistamines or corticosteroids.
Case Study 2: 42 year old male on Fabrazyme with occasional minor reactions (mild chills) hence requiring an infusion time of over three hours. Due to a shortage of Fabrazyme the patient was switched to Replagal. The reduced infusion time of forty minutes considerably improved their quality of life.
Case Study 3: 54 year old male on Fabrazyme for 11 months, switched to Replagal. The patient then began to experience infusion related reactions (dyspnoea and presyncope) after starting each infusion. Symptoms were not alleviated with oxygen or antihistamines. The patient discontinued Replagal treatment due to these reactions.
Conclusion: These case studies illustrate the differences we have experienced with switching patients from Fabrazyme. to Replagal. and highlights that switching therapy may be a valuable tool in managing adverse effects and improving the patients' quality of life.

495-O

LONG TERM CARDIAC EFFECTS OF NAGLAZYME® (GALSULFASE) THERAPY (nrx) IN MPS VI PATIENTS

Braunlin E[1], Rosenfeld H[2], Kampmann C[3], Johnson J[2], Beck M[3], Giugliani R[4], Guffon N[5], Ketteridge D[6], Sá Miranda CM[7], Scarpo M[8], Schwartz I[4], Teles EL[9], Wraith JE[10], Barrios P[4], Dias da Silva E[7], Richardson M[6], Gildengorin G[2], Imperiale M[11], Schatz A[11], Decker C[11], Harmatz P[2]
[1]*University of Minnesota, Minneapolis, MN, United States*
[2]*Children's Hospital & Research Center, Oakland, CA, United States*
[3]*Centre for Lysosomal Storage Diseases, Mainz, Germany*
[4]*Medical Genetics Service/HCPA & INAGEMP, Porto Alegre, Brazil*
[5]*Hôpital Femme Mère Enfant, Lyon, France*
[6]*Women's and Children's Hospital, Adelaide, Australia*
[7]*Unidade de Biologia do Lisossoma e Perox, Porto, Portugal*
[8]*University of Padova, Padova, Italy*
[9]*Hospital de Sao Joao,, Porto, Portugal*
[10]*Royal Manchester Children's Hospital, Manchester, United Kingdom*
[11]*BioMarin Pharmaceutical, Novato, CA, United States*

Objectives: Evaluate change in cardiac structure and function during NRx.
Methods: Cardiac ultrasound data was reviewed retrospectively from 53 MPS VI patients (16 M, 37F; age 6–29Y) who participated in NRx clinical trials. Measurements of left ventricular (LV) end diastolic size, LV posterior wall and septal diastolic wall thickness, shortening fraction, mean mitral valve gradient (MMV), peak aortic valve gradient (PAV), mitral (MR) and aortic regurgitation (AR) scores were obtained at baseline and week 96.
Results: All patients had normal baseline LV size and function; LV wall thickness z-scores were moderately increased. Average PAV was normal (9.25 mm Hg) while MMV was slightly > normal (6.25 mm Hg); MR score (1.5) was > AR (0.5). During therapy no significant changes were seen in LV size or function, valve gradients or MR; but LV septal thickness decreased ($p<0.007$) and AR increased significantly $p=0.003$) from baseline to 0.9. Mitral valve replacement occurred in 2 subjects before and one during NRx. There were no significant differences in LV size or function or valve regurgitation scores by gender or age. PAV and MMV were significantly greater ($p<.0001$) for those ≥12 years compared to <12 years.
Conclusions: LV function is preserved. Septal thickness decreases and is possibly related to removal of GAG. AR increased but lack of placebo data limits interpretation; this change may represent the natural history of disease. NRx does not appear to improve or prevent progression of valve disease. It is possible earlier initiation of NRx will limit development of cardiac valve disease

496-P

ENZYME REPLACEMENT THERAPY IN 25 MUCOPOLYSACCHARIDOSIS TYPE VI BRAZILIAN CHILDREN UNDER AGE FIVE YEARS

Ribeiro EM[1], Ribeiro EM[2], Magalhães TSPC[3], Horovitz D[3], Acosta A[4], Giuliani L[5], Palhares D[5], Kim CA[6], Paula AC[6], Kerstenestzy M[7], Pianovski MAD[8], Costa MIF[9], Santos FC[10], Martins AM[11], Aranda CS[11], Soares N[4], Cardoso Jr L[4], Llerena Jr. JC[3], Bruno CA[1]
[1]*Univ Fed Rio Grande do Norte, Natal, Brazil*
[2]*Hospital Albert Sabin, Fortaleza, Brazil*
[3]*C Gen Med, Inst. Fernandes Figueira, Rio de Janeiro, Brazil*
[4]*Universidade Federal da Bahia, Salvador, Brazil*
[5]*Dep Ped, Univ Fed Mato Grosso do Sul, Campo Grande, Brazil*
[6]*Inst Crianca da Universidade de S. Paulo, S. Paulo, Brazil*
[7]*Hospital Barão de Lucena, Recife, Brazil*
[8]*H Clínicas Univ Fed Paraná, Paraná, Brazil*
[9]*Centro de Reabilitacão Infantil, Natal, Brazil*
[10]*Hospital Universitário do Maranhão, S Luis, Brazil*
[11]*IGEIM Univ Fed São Paulo, São Paulo, Brazil*

Background: Mucopolysaccharidosis VI is caused by deficient arylsulfatase B. Due to study restrictions and specific endpoints to be evaluated, clinical trials included patients over five years. The early introduction of treatment is expected to be more effective in avoiding complications and disease progression. Following such rationale, ERT is being administered to MPS VI Brazilian children before 5 years.
Methods: 25 patients (15 M/10F) are described. They started ERT between 5 days and 58 months of age (average 38 months), with treatment duration varying from 1 to 32 months (average 15 months).
Results: On baseline, only the 5 day-old patient was symptom-free; 24 patients had radiological abnormalities; 13, heart involvement and 22, coarse facial features, visceromegaly and joint stiffness. No significant drug-related adverse events were observed. Less upper airway infections, better sleep pattern and decreased urinary GAG excretion were documented. In the 4 patients that underwent MRI, all had compressive myelopathy; two underwent surgery, one is being prepared for surgery and the other is still under evaluation.
Conclusions: In the published data available from older patients, ERT has shown improved functional status and endurance. In very young children, there were improvements in growth charts and developmental milestones. The treatment with Galsulfase seems to be safe in young patients and to decrease the disease progression. Longer term follow-up reports and clinical studies in young patients must be encouraged in order to gather substantial data on treatment efficacy.

497-A

ENZYME REPLACEMENT THERAPY IN MPS VI: EARLY TREATMENT WITH GALSULFASE IN THREE SIBLINGS

Ribeiro EM[1], Ribeiro EM[2], Bezerra KRF[3], Albuquerque SMP[1], Bruno CA[2]
[1]*Univ Fed Rio Grande do Norte, Natal, Brazil*
[2]*Faculdade de Medicina Christus, Fortaleza, Brazil*

Background: MPS VI is a lysosomal storage disease with functional impairment, normal cognitive status and shortened lifespan. With ERT, a better outcome is expected. Age and clinical severity in the beginning of therapy can influence treatment results. Reports of siblings are of special interest to evaluate treatment response as they usually share the same mutations and environment.
Methods: Case report.
Results: 3 children from consanguineous parents. Sib 1 and 2, a girl and a boy, diagnosed at age 5 years and 19 months, respectively. They started ERT 1 year later. Sib 3, boy, diagnosed prenatally, started therapy with 5 days. Sib 1 had upper airway obstruction, occasional episodes of bronchial spasms, sleep apnea, cardiac disease, hepatomegaly, umbilical hernia, joint restriction, skeletal deformities and unusual gait. Sib 2 presented the same manifestations of sib 1, Mongolian spots and gibus. Sib 3, born with 4200 g and 49 cm, presented Mongolian spots. Skeletal radiographies, Echocardiogram and ECG were normal. After 18 months under ERT, sibs 1 and 2 show joint improvement, regression of visceromegaly and decreased the number of upper airway infections. Sib 3 has normal facial appearance, growth and development, no corneal clouding or visceromegaly but presents hernia and vertebral abnormalities. Infusion reactions occurred only in the sibling 1.
Conclusions: The 3 reported cases reinforce galsulfase. ERT safety in young patients and the better outcome when start the treatment early.

498-P

DISTRIBUTION OF MUSCLE WEAKNESS, RATE OF DECLINE AND RESPONSE TO THERAPY IN ADULTS WITH POMPE DISEASE

Van der Ploeg AT[1], van der Beek NA[2], de Vries JM[1], van Doorn PA[2]
[1]*Div Metab Dis, ErasmusMC Univ Med Cent, Rotterdam, Netherlands*
[2]*Div Neurology, ErasmusMC Univ Med Cent, Rotterdam, Netherlands*

Background: Pompe disease is a metabolic myopathy caused by deficiency of alpha-glucosidase required for degradation of lysosomal glycogen. Patients with the classic infantile form present shortly after birth with a cardiomyopathy and generalized muscle weakness and die within the first year of life. Children and adults may show first symptoms at any age and present with proximal muscle weakness. No exact data are available on the distribution of muscle weakness, the rate at which individual muscle groups lose their strength and how individual muscle groups respond to therapy.
Methods: Manual muscle testing and hand held dynamometry were applied to assess the muscle strength of individual muscle groups at baseline and on regular time points before and after start of therapy.
Results: Muscle strength was measured of 14 different muscle groups with manual muscle testing and 9 with hand held dynamometry in 94 adult patients with Pompe disease. The regular assessment of strength of individual muscle groups enabled us to draw a picture on the distribution of muscle weakness in adults with Pompe disease. A distinction could be made between muscles that were affected early and late in the disease course and the percentage of patients that experienced weakness. We could also show the rate of loss of strength of individual muscle groups and how individual muscle groups responded to therapy.
Conclusion: The results of the study give more insight in loss of muscle strength in untreated adults with Pompe disease and the response to therapy of individual muscle groups.

499-P

THROMBOCYTOPENIA IN HUNTER DISEASE: REPORT OF TWO CASES

Amartino H[1], Sosa P[1], Richard L[1]
[1]Hospital Universitario Austral, Pilar, Argentina

Although splenomegaly is a common manifestation in MPSII, thrombocytopenia has not been reported as a clinical symptom of the disease. We report two MPS II patients presenting severe thrombocytopenia during course of disease analyzing its response to ERT.

Patients: Case 1: GFC, 14 years old. boy, mild MPS II diagnosed since neonatal period. At age 2 years old he received unrelated BMT. He responded with temporary normalization of I2S enzymatic activity. Five years later, graft failure was clear. He presented hepatosplenomegaly, dysostosis, joint rigidity and coarse face. He had severe thrombocytopenia (<10000 platelets/mm3) without anemia or leukopenia. Bone marrow biopsy showed diffuse infiltration by storage cells. He had recurrent episodes of spontaneous bleeding and petechiae. At age 9 years old he started idursulfase (0.5 mg/kg/dose/weekly). Platelets count (PC) increases to 50000/mm3 after one year and more than 100000/mm3 within second year. Now it is stable around 140000/mm3.

Case 2: FB, 9-years-old, severe form of MPS II (R468Q) diagnosed at age 2 years old. He was referred to hospital at 6 years old presenting petechiae. Severe thrombocytopenia (9000 plts/mm3) was found. With initial diagnosis of immune thrombocytopenic purpura (ITP) he was treated with IgG i.v. 1 gr/kg x 2 doses. The response was poor (PC above 30000/mm3). Simultaneously, anemia and neutropenia were observed. At 7 years old he started idursulfase. Normalization of PC and WBC was seen within first 2 months of ERT.

Conclusion: Thrombocytopenia should be taken in consideration as an unusual but severe clinical manifestation of MPS II which has shown good response to ERT

500-P

HUNTER SYNDROME: ENZYME REPLACEMENT THERAPY WITH IDURSULFASE IN 3 PATIENTS UNDER 5 YEARS OF AGE IN ARGENTINA

Amartino H[1], Marchione D[2], Perichon G[2], Richaudeau A[1], Caysials A[2], Rozenfeld P[3]
[1]Hospital Italiano, Buenos Aires, Argentina
[2]LISIN, Facultad de Ciencias Exactas, UNL, La Plata, Argentina

The approval of idursulfase (ElapraseTM) as specific treatment for MPSII was based in a clinical trial with 2 primary endpoints -6 MW test and changes in predicted FVC- which excluded children under 5 years of age. Therefore, safety and efficacy of idursulfase have not been yet established for young children.

Aim: To report our experience with idursulfase replacement therapy in 3 young MPSII patents. Case 1: Male, 1 y.o., neonatal diagnosis (familial history of MPS II). Mutation: c683C>A/p.P228Q. First symptoms: hepatosplenomegaly and recurrent upper respiratory infections. ERT started at 14 months.

Case 2: Male, cousin of case 1, diagnosed at 4y 9/12 m. He presented coarse face, hypoacusia, organomegaly, sleep disturbances and dysostosis. ERT started at 5 y 2/12 m

Case 3: Male, 4 y.o, diagnosed at 16 months. Mutation: p.R468Q. First symptoms: Gibbus, coarse face and hepatomegaly. ERT started at 30 months

Results: Idursulfase was been provided in standard dose (0,5 mg/kg/step-wise over 3 hours, weekly) during 32 months for cases 1 and 2 and 24 consecutive months for case 3. From 4th to 7th infusions, case 3 developed skin rash, which resolved by decreasing infusion rate and adding antihistaminic pre-treatment. No other adverse event was noticed. All the cases showed normalization of urinary GAG excretion in, as well as decreased rate of annual respiratory events and improvement of organomegaly.

Conclusions: We conclude that idursulfase was effective and safe in these 3 young patients. Long-term studies will be necessary to accurately evaluate safety and efficacy in this population

501-P

SCREENING FOR FABRY DISEASE IN JAPAN

Nakamura K[1], Hattori K[1], Matsumoto S[1], Mitsubuchi H[1], Endo F[1]
[1]Dept Pediatrics, Kumamoto Univ, Kumamoto, Japan

Background: 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.

Methods: Enzyme-deficient infants were retested, and infants who were double-screening positive were diagnostically confirmed by enzymatic activity and mutation analyses.

Results: 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.

Conclusions: 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.

502-P

A NEW MUTATION OF FABRY DISEASE IN A TURKISH FAMILY

Oneli-Mungan N[1], Ozbek M[1], Temiz F[1], Topaloglu K[1], Yuksel B[1], Deprez RHL[2]
[1]Dep Ped Metab Cukurova Univ Hosp, Adana, Turkey
[2]Div Genetics, Amsterdam Univ Hosp, Amsterdam, Netherlands

Background: Fabry disease is a rare, multisystem storage disease, caused by deficiency of the lysosomal enzyme α-galactosidase. The gene coding this enzyme is located in region Xq21.33-X22. Enzyme deficiency leads to ceramidetrihexoside accumulation in the blood vessels, cardiac myocytes, autonomic spinal ganglia, and glomeruli and tubule of the kidney. Clinical features are acroparesthesia, unexplained boots of fever, hypohydrosis, and corneal opacities. Untreated cases die early from cardiac, central, and renal complications. Enzyme replacement therapy is the only treatment choice for this disease. Mutation analysis, and carrier detection is invaluable, as there is a genetic heterogeneity.

Patients and Methods: A 9 year-old boy was admitted to our clinic with complaints of red populous and moderate pain in extremities. His skin biopsy revealed angiokeratomas. An enzyme study was consistent with Fabry disease with absent α -galactosidase activity. His cardiac, and renal functions, and eye examination were completely normal. After detection of this index case we investigated his large family for α-galactosidase levels, and found seven new cases.

Results: The patient and the family have the mutation at c.785G>T in the GLA gene. This mutation leads an amino acid change at position 262 of the protein (p.Trp262Leu) and was not described before.

Discussion: Fabry disease is difficult to diagnose due to nonspecific symptoms and findings. Many cases are diagnosed late, usually in adolescence in this disease. And, there is no firm phenotype-genotype correlation. It is therefore important to identifying mutations, not only for making correct diagnosis but also for genetic counseling.

503-P

CARDIAC EVALUATION IN MUCOPOLYSACCHARIDOSIS PATIENTS UNDERGOING ENZYME REPLACEMENT THERAPY

Brands MMMG[1], Hagemans MLC[1], Hop WC[2], Helbing WA[3]
[1]*Div Metab Dis Erasmus MC Univ Hosp, Rotterdam, Netherlands*
[2]*Div Biostatistics ErasmusMC Univ Hosp, Rotterdam, Netherlands*
[3]*Div Cardiology Erasmus MC Univ Hosp, Rotterdam, Netherlands*

Background: Cardiac involvement (e.g. regurgitation, stenosis, morphologic changes of the cardiac valves and cardiac hypertrophy) has been reported in all types of mucopolysaccharidosis (MPS). It is still unclear if enzyme replacement therapy has an effect on cardiac parameters.
Patients and Methods: Twenty-three patients (1–18 years) with MPS I (n=7), MPS II (n=6) and MPS VI (n=10) receiving enzyme replacement therapy were systematically evaluated by electrocardiography (ECG) and echocardiogram. For every individual, change over time was assessed using linear regression with weeks of treatment as the independent variable. To this end, outcomes were logarithmically transformed (SPSS 15.0).
Results: 143 echocardiograms were performed during a median follow-up time of 98 weeks (range: 48–312). Five patients had a normal echocardiogram at baseline. The ECG conduction times of all patients were normal. More than half of the patients showed cardiac abnormalities at baseline. After enzyme therapy 4 out of the 12 patients with an abnormal left ventricular mass index (LVMI) at baseline showed a significant improvement in LVMI. Three patients showed a significant improvement in left ventricular posterior wall in diastole (LVPWd). No significant change in interventricular septal thickness at diastole (IVSd) was found in patients with abnormal baseline values. No improvement was seen on valve regurgitation.
Conclusion: Valve regurgitation and cardiac hypertrophy are common findings at the start of enzyme therapy in our patients with MPS. Enzyme therapy seems to have an effect on LVMI and LVPWd in individual cases, but no substantial changes were seen in other echo parameters.

504-P

NEUROLOGIC ASSESSMENT IN PATIENTS WITH FABRY DISEASE BEFORE AND AFTER ENZYME REPLACEMENT THERAPY (ERT) WITH AGALSIDASE BETA

Mendes CSC[1], Rand MH[1], Kyosen SO[1], Martins AM[1]
[1]*CREIM, UNIFESP, Sao Paulo, Brazil*

Background: Fabry disease (FD) is an X- linked lysosomal disorder caused by deficiency of alpha-galactosidase A, resulting in accumulation of globotriaosylceramise (Gb3).This accumulation affect both peripheral and central nervous system. The ERT has been shown to improve neuropathic pain and sensory disturbances.
Methods/patients: Five patients (4 M/1F) were evaluated prior and one year after agalsidase beta ERT with the standard dose of 0.9 to 1.1 mg/kg biweekly. Patients were inquired about achroparesthesia and performed clinical assessments to detect alterations of motor function, cranial nerves, miotatic and superficial reflexes, cerebelar signs, thermic and tactile sensory.
Results: Patients started ERT with a mean age of 36.2y (average 15y to 58y). Before ERT, 3 patients referred achroparethesia, 1 presented miotatic hyperreflexia, 4 presented thermic sensory alterations, none of the patients presented any alteration regarding motor function, cranial nerves and cerebelar and one patient, the youngest one, presented normal neurologic exam. After 1 year of ERT, 3 patients referred improvement of achroparesthesia, in one patient the thermic alteration disappeared and in the other 3 it improved. There was no change regarding the myotatic hyperreflexia presented by one patient. None of the patients presented new neurologic alterations after one year of ERT.
Conclusion: Although this is a limited number of cases our data show that the ERT has improved the function of the peripheral nervous system in 4 patients, with improvement of the cold and warm sensation.
Financial Support: IGEIM

505-P

PATIENT EXPERIENCE OF DOSE REDUCTIONS OF FABRAZYME(R)

Cousins A[1], Fondo A[1], Murphy E[1], Lachmann R[1]
[1]*Ch Dent Met Unit, the Nat Hosp Neurology, London, United Kingdom*

Background: In July 2009, due to supply problems the dose of fabrazyme (r) given to patients with Fabry disease had to be reduced from 1 mg/kg to 0.5 mg/kg. In October 2009, this was further reduced to 0.3 mg/kg.
Objective: To monitor the effects of the reduction of medication on 55 adult patients (23 males, 32 females) previously treated with 1 mg/kg fabrazyme(r).
Method: Monthly questionnaires were posted out to treated patients. Pain, abdominal symptoms, bowel problems, sweating, and tiredness were specifically queried. General well-being was self-reported (scale 0–100). Urine CTH was measured monthly. General comments were requested.
Results: 45 patients returned questionnaires on at least one occasion. Well-being scores either stayed stable over time or decreased. A number of patients reported increased pain scores and return of abdominalsymptoms. Patients reported increased tiredness which had previously decreased on full-dose treatment. CTH levels remained stable in the majority of patients.
Conclusion: For some patients the reduction in dose of fabrazyme(r) has resulted in the return of pre-treatment symptoms of Fabry disease. For the majority it doesn't appear, at this time, to have had a major impact on their health status.

506-P

CLINICAL OUTCOME IN RUSSIAN PATIENTS WITH MPS I FOLLOWING BONE MARROW TRANSPLANTATION

Mikhaylova SV[1], Bologov AA[1], Skorobogatova EV[1], Balashov DN[1], Trachtman PE[1], Voskoboeva EY[2], Zakharova EY[2]
[1]*Russian Children Clinical Hospital, Moscow, Russian Federation*
[2]*Lab INH Met Dis, Res Cen Med Gen, Moscow, Russian Federation*

Mucopolysaccharidosis Type I (MPSI) is an autosomal recessive disorder caused by the deficiency of a-iduronidase, resulting in the storage of the glycosaminoglycans in different tissues. MPS I is clinically heterogeneous and there is now the enzyme replacement therapy is treatment option for patients without neurological involvement. Haematopoietic stem cell transplant (HSCT) has a positive effect on the course of the disease of affected children with Hurler syndrome (HS). We carried out HSCT in 12 children with HS over the last 7 years. As a source of HSCT used bone marrow (n=9), or umbilical cord blood (n=3). Median age at the time of HSCT was 2.25 years (range 10 months–4 years). Most patients (9 \ 11) received a transplant from an unrelated donor, 2 from healthy siblings. Survival: 1 patient died of cardiopulmonary arrest, 1 patient—of transplant-related causes following engraftment. All but 1 of the 11 patients who engrafted had complete donor engraftment. All alive patients showed improvement from the visceral organs, reducing the degree of corneal opacity, decrease joint stiffness. 5 of 8 patients (> 6 months after HSCT) have improving of the psychomotor development. Nevertheless, these patients have a moderate delay of neuropsychological development. 3 patients (5 and 6 years old) have severe delay of psychomotor development. Neurological outcome after HSCT vary widely for patients with HS and depends on age of HSCT and basic level of neurocognitive development.

508-O

ATYPICAL PRESENTATION OF ANTIQUITIN DEFICIENCY IN A FEMALE WITH NEONATAL HYPOGLYCEMIA, HYPERLACTICACIDEMIA AND INTRACTABLE MYOCLONIC EPILEPSY

Mercimek-Mahmutoglu S[1], Horvath GA[1], Coulter-Mackie M[1], Connolly M[2], Waters PJ[3], Jakobs C[4], Stockler-Ipsiroglu S[1]
[1]Div Biochemical Dis, Dep Ped, Univ BC, Vancouver, Canada
[2]Div Neurol, Dep Ped, Univ BC, Vancouver, Canada
[3]Dep Path, Univ BC, Vancouver, Canada
[4]Dep Clin Chem, Metab Unit, VU Univ Med C, Amsterdam, Netherlands

Background: Pyridoxine dependent epilepsy due to antiquitin deficiency (ATQD) is an autosomal recessive disorder characterized by neonatal encephalopathy and intractable epilepsy.
Case presentation and results: This 12-month-old girl presented with myoclonic jerks at age 4 days following hyperalertness and sleeplessness for 3 days. She had severe hypoglycaemia (0.6 mmol/l;ref range>2.4) and hyperlacticacidemia (11 mmol/l;ref range<2.2). Despite Phenobarbital, Midazolam and Phenytoin, EEG showed multiple electrographic subclinical seizures. Because of ongoing seizures, pyridoxine was given 50 mg IV and her seizures stopped next day. Her cranial MRI showed evidence of intracranial bleeding and hypoxic-ischemic encephalopathy at age 7 days with no history of birth trauma. Because of hypoglycemia and hyperlacticacidemia (resolved with 4.2 mg/kg/min glucose infusion) investigations for glycogenolysis and gluconeogenesis defects were initiated. After clinical improvement, she was discharged on Phenobarbital at age 16 days and pyridoxine was discontinued. One week later she was readmitted for increased myoclonic jerks and irritability. She was unresponsive to more than eight anti-epileptic medications for 3 weeks. Because of ongoing seizures pyridoxine was re-started after 3 weeks. She became seizure free next day. Urine alpha-amino-adipic-semialdehyde and plasma pipecolic acid levels were highly elevated. She had 2 mutations in the ALDH7A1 gene confirming ATQD. Currently she is only on pyridoxine (200 mg/kg/d) and seizure free since age of 1.5 month. Her EEG normalized at age during follow-up.
Conclusion: We presented a 12-month-old girl with ATQD and neonatal encephalopathy. ATQD should be included in the differential diagnosis of neonatal hypoglycaemia, hyperlacticacidemia and intractable myoclonic epilepsy.

509-O

CEREBRAL FOLATE TRANSPORT DEFICIENCY: A NOVEL INHERITED DISORDER OF FOLATE METABOLISM

Steinfeld R[1], Grapp M[1], Krätzner R[1], Dreha-Kulaczewski S[1], Wevers R[2], Gärtner J[1]
[1]Dept of Pediatrics, Univ of Goettingen, Goettingen, Germany
[2]Lab of Pediatrics, Univ of Nijmwegen, Nijmwegen, Netherlands

Folates are essential cofactors for important metabolic pathways such as synthesis of amino acids, DNA and lipophilic substances. The main folate compound 5-methyltetrahydofolate (5MTHF) is actively transported across the blood-cerebrospinal fluid (CSF)-barrier to supply the brain. Cellular uptake of folates is mediated by the proton-coupled intestinal transporter, the reduced folate carrier, and by two GPI-anchored receptors, folate receptor alpha (FRalpha) and beta (FRbeta). At least five distinct inherited disorders of folate transport and metabolism are presently. An inherited disorder of folate transport into brain has not been described yet.
We studied a group of pediatric patients with progressive movement disturbance, psychomotor decline and epilepsy with severely reduced folate concentrations in the CSF. Brain MRI showed profound hypomyelination and in vivo MR spectroscopy a combined loss of white matter choline and inositol. A candidate gene approach was used to identify the primary genetic defect and was confirmed by rescue of folate binding by retroviral gene transfer into patient cells.
In three patients we identified mutations in the FOLR1 gene coding for the FRalpha to cause an autosomal-recessively inherited, brain-specific folate transport defect. Folinic acid therapy could restore CSF folate concentrations, reverse white matter choline and inositol depletion and consecutively improve clinical symptoms. Mutations in the FOLR1 gene coding for FRalpha are responsible for inherited cerebral folate transport deficiency with manifestation in early childhood. Our results characterize a novel severe, but treatable neurodegenerative disorder and provide new insights into the folate metabolic pathways involved in myelin formation and human brain function.

510-P

MILD HOMOCYSTEINEMIA AND METHYLMALONIC ACIDURIA IN A CASE WITH PANCYTOPENIA DUE TO TRANSCOBALAMIN II DEFICIENCY

Merinero B[1], Lama R[2], Moráis A[2], Ruiz Sala P[1], Sanz P[1], Castro M[1], García MJ[1], Leal F[1], Pérez-Cerdá C[1], Pérez B[1], Ugarte M[1]
[1]CEDEM, CIBERER, Univ Autónoma Madrid, Madrid, Spain
[2]Unit Paediat Nutr &Metab, Hosp La Paz, Madrid, Spain

Cellular cobalamin uptake is mediated by transcobalamin II (TC II) and its plasma membrane receptor through receptor-mediated endocytosis of the TC-cobalamin complex in peripheral tissues. Inherited deficiency of TCII appears clinically and biochemically as vitamin B12 deficiency during the first year of life.
Two-month-old male, third child of consanguineous parents, was admitted to hospital with pancytopenia, failure to thrive, diarrhea and severe metabolic acidosis. Microbiological cultures, viral serologies and bone marrow biopsy were normal. Mild excretion of methylmalonic acid (419 mmol/mol creat; reference value RV <13), methylcitrate, 3-hydroxypropionate and homocystine, and abnormal plasma levels of homocysteine (26 μmol/L; RV 6.3±2.4), propionylcarnitine (3.4 μmol/L; RV <0.89) and free carnitine (18.8 μmol/L; RV 30±8) were found. Therapy with B12, carnitine and protein restriction allowed achieving complete clinical and biochemical recovery within 2 weeks. Since the patient was in good condition, oral B12 was suspended 2 years later, and he again developed pancytopenia, hyperhomocysteinemia (23 μmol/L) and excretion of methylmalonic acid (68 mmol/mol creat). All alterations were controlled with B12 therapy. Propionate uptake in cultured fibroblasts (± OHCbl in culture medium) was found in the control range. Genetic analysis of MMADHC gene was normal, ruling out a cblD defect. Sequencing of TCN2 gene revealed a novel change p.L166fs (c.497_498delTC) in homozygosity in exon 4. The child, 4 years old at present, remains asymptomatic and his mental and somatic development is optimum.
We propose that TC II deficiency should be included in the differential diagnosis of pancytopenia with mild methylmalonic aciduria and homocysteinemia.

511-O

PYRIDOXAL 5'-PHOSPHATE CONCENTRATION IN CEREBROSPINAL FLUID: FACTORS INFLUENCING CONCENTRATION

Footitt EJ[1], Heales SJ[2], Mills PB[1], Allen G[3], Oppenheim M[4], Clayton PT[1]
[1]Institute of Child Health, London, United Kingdom
[2]Great Ormond Street Hospital, London, United Kingdom
[3]Institute of Neurology, London, United Kingdom
[4]National Hospital Queen Square, London, United Kingdom

Analysis of pyridoxal 5′-phosphate (PLP) concentration in 256 samples of cerebrospinal fluid (CSF) from children with a variety of neurological symptoms showed that the variance is greater than indicated by previous studies. The age-related lower reference limit has to be set lower than previously reported to detect inborn errors of metabolism that lead to marked PLP depletion without a high false positive rate—26 nmol/L for under 30 days; 14 nmol/L for 30d to 12 mo; 11 nmol/L for 1–2 y; and 10 nmol/L for >3y. Inborn errors leading to PLP concentrations below these values included pyridoxine dependent epilepsy due to antiquitin deficiency and molybdenum cofactor deficiency which leads to the build up of sulphite, a nucleophile capable of reacting with PLP. There was no evidence that seizure per se, or the anticonvulsant drugs prescribed for patients in this study, led to significant lowering of CSF PLP. A small proportion of patients receiving L-dopa therapy were found to have a CSF PLP concentration below the appropriate reference range. This may have implications for monitoring and treatment.
A positive correlation was seen between the CSF PLP and 5-methyltetrahydrofolate (5-MTHF) concentration. Both are considered susceptible to attack by nucleophiles and oxygen-derived free radicals and CSF has relatively low concentrations of other molecules that can react with these compounds. Further studies in a wide range of neurological diseases might lead to improved understanding of pathogenesis and new possibilities for treatment.

512-P

CEREBRAL FOLATE DEFICIENCY AND DISEASES OF THE CENTRAL NERVOUS SYSTEM IN CHILDHOOD

Pérez-Dueñas B[1], Ormazábal A[2], Toma C[3], Torrico B[3], Cormand B[3], Serrano M[1], Sierra C[2], De Grandis E[4], Pineda M[1], Campistol J[1], García-Cazorla A[1], Artuch R[5]
[1]Dep Neurol Hosp Sant Joan de Déu, Barcelona, Spain
[2]Dep Biochem Hosp Sant Joan de Déu, Barcelona, Spain
[3]Dep Genet, Faculty Biol, Univ Barcelona, Barcelona, Spain
[4]Dep Child Neuropsych, Gaslini Institute, Genova, Spain
[5]CIBERER, ISCIII, Barcelona, Spain

Background: Cerebral folate deficiency is caused by a defective transport of folate across the blood-CSF barrier. The inherited defect is due to mutations in the FOLR1 gene coding for folate receptor alpha (FRα). We aimed to identify the etiology, prevalence and significance of reduced CSF 5-methyltetrahydrofolate (5MTHF) concentrations in a large population of children with neurological disorders.
Methods: In CSF samples from 134 controls (1 day–18 years; mean 3.8 years) and 584 patients (1 day–47 years; mean 4.8 years) we measured 5MTHF concentrations by reverse phase HPLC. Total folate in serum was analyzed in 318 of 584 patients by automated chemiluminescent immunoassays. In two patients with suspected cerebral folate transport defect we performed a screening of mutations of the FOLR1 gene.
Results: 71 of 584 patients (12%) showed 5MTHF deficiency. Partial deficiencies (N=63; mean 36 nmol/L; range 19–63) were associated with acquired (perinatal asphyxia, CNS infections) and genetic diseases (inborn errors of metabolism, white matter disorders, Rett syndrome and epilepsy). Severe 5MTHF deficiencies (N=8; mean 6 nmol/L, range 0.6–13) were detected in severe MTHFR deficiency, Kearns-Sayre and cerebral folate transport deficiency caused by FRα defect. A strong positive correlation was observed between CSF and plasma folate values in the whole sample.
Conclusions: Partial 5MTHF deficiency is a common secondary abnormality in several neurological diseases of childhood; many of them have been recognized for the first time in this study. In children with profound 5MTHF deficiency screening for mutations in the FOLR1 gene is advisable.

513-P

REFERENCE INTERVAL DETERMINATION OF BIOTINIDASE ACTIVITY IN HEALTHY CHILDREN AND ADULTS

Basol G[1], Barutcuoglu B[1], Koc F[2], Parildar Z[1], Habif S[1], Kurugol Z[2], Bayindir O[1]
[1]Dep Clin Biochem, Ege Univ, Izmir, Turkey
[2]Dep Soc Ped, Ege Univ, Izmir, Turkey

Background: Biotinidase deficiency is an autosomal recessively inherited disorder of biotin recycling. Early diagnosis and administration of pharmacological doses of biotin can prevent irreversible brain damage. Biotinidase deficiency is diagnosed by demonstrating deficient enzyme activity in serum. Individuals with profound biotinidase deficiency have less than 10% of mean normal serum biotinidase activity, whereas individuals with partial biotinidase deficiency have 10%-30%. The objectives of this study were to establish pediatric and adulthood reference intervals for serum biotinidase and to assess sex differences. Furthermore, the calculated mean values for each group will be used in computing the residual enzyme activities and in classification of the biotinidase deficient patients.
Methods: 241 reference individuals, including 120 children (2 months–17 years) and 121 adults (18–49 years) were enrolled to the study. The health status was confirmed by history, physical examination and a questionnaire. Serum biotinidase activity was determined quantitatively by the colorimetric method using biotinyl-p-aminobenzoic acid as substrate. The 95th percentile reference limits were determined by using the non-parametric method.
Results: The central 95% reference interval in children was 4.64–8.85 nmol/min/mL with a mean value of 6.8. Gender-based reference intervals were calculated in adults, as the mean activity was lower in women than men were (6.2 vs. 7.3).
Conclusion: Our study provided new reference intervals for biotinidase in healthy children and adults. We emphasized that a single reference interval and a single mean value could not be used for all age and gender groups, as pediatric patients require a unique medical approach.

514-P

THE ROLE OF THE INTESTINE IN HUMAN VITAMIN B6 METABOLISM

Albersen M[1], Bosma M[1], Diekman EF[1], De Ruijter J[1], Klomp LWJ[1], De Koning TJ[1], Visser WF[2], Verhoeven-Duif NM[1]
[1]Dept Metab Endocr Dis, WKZ, UMCU, Utrecht, Netherlands
[2]Netherlands Metabolomics Centre, Utrecht, Netherlands

Background: The importance of pyridoxal phosphate (the active form of vitamin B6) in brain metabolism is evident from inborn errors affecting its concentrations. Although numerous aspects of vitamin B6 metabolism are known, still many questions remain unanswered. One of these questions concerns the organ in which pyridoxal phosphate is formed from other B6 vitamers by the action of pyridox(am)ine phosphate oxidase (PNPO), since both intestine and liver have been mentioned in literature.
Objective: To gain insight into the role of the intestine in the formation of pyridoxal (phosphate) from other B6 vitamers, especially pyridoxine, with a focus on pyridox(am)ine phosphate oxidase (PNPO).
Materials and Methods: Polarized and differentiated Caco-2 cells were cultured in the presence of pyridoxine for three consecutive days. Apical and basolateral B6 vitamer concentrations were measured daily using a UPLC-MSMS method. Western Blot analysis was used to study PNPO expression in Caco-2 cells and enzyme assays were performed to quantify PNPO enzyme activity.
Results: PNPO was found to be increasingly expressed and active during Caco-2 cell differentiation. Furthermore, we demonstrated apical uptake of pyridoxine, conversion into pyridoxal and pyridoxamine and excretion of both B6 vitamers at the basolateral side of the Caco-2 cell layer.
Conclusion: Our results strongly suggest that the intestine plays an active role in vitamin B6 metabolism, not only by transporting the vitamers into the portal blood, but also by formation of the active cofactor pyridoxal phosphate.

515-P

ACUTE THIAMINE DEFICIENCY CAUSES ENERGY-DEPENDENT PROXIMAL TUBULAR DISFUNCTION AND SEVERE ELECTROLYTE IMBALANCE: AN ALTERNATIVE PATHOMECHANISM BEHIND THE "REFEEDING SYNDROME"?

Maiorana A[1], Vergine G[2], Coletti V[3], Luciani M[3], Rizzo C[1], Martinelli D[1], Emma F[2], Dionisi-Vici C[1]
[1]Div. Metabolism, Bambino Gesù Hospital, Rome, Italy
[2]Div. Nephrology, Bambino Gesù Hospital, Rome, Italy
[3]Div.Hematol-Oncol, Bambino Gesù Hospital, Rome, Italy

Refeeding syndrome (RS) is a life-threatening condition which can occurs when malnourished patients receive high carbohydrate feeding causing sudden reversal from fat to carbohydrate metabolism and increased insulin secretion. This results in a rapid fall in serum phosphate, potassium, magnesium, along with water retention and hyperglycemia. Electrolyte imbalance in RF is due to insulin action which induces a sudden shift of salt from extracellular to intracellular compartment. Acute thiamine deficiency (ATD) can cause a RS-like with refractory lactic acidosis. Non-alcoholic ATD has been observed in different conditions such as patients receiving TPN without vitamin supplement, anorexia nervosa, leukemia/cancer, and defective soy-based formula feeding. Interestingly ATD and/or sings of RF have been reported in organic acidurias and in MSUD. Two leukemia patients, both treated with TNP and one received high dose metotrexate (which competes with thiamine transport), developed neurological deterioration, refractory metabolic acidosis with massive lactic acidosis and electrolyte abnormalities characteristic of RS. Severe signs of proximal renal tubular dysfunction with increased phosphate and electrolyte loss along with an organic acid pattern typical of ACT were detected. After a single thiamine administration clinical and biochemical abnormalities suddenly recovered. Furthermore, we also observed the complete normalization of renal tubular function.
Contrary to the inter-compartimental shift observed in classical RS, we hypothesize that ATD causes electrolyte depletion through an energy dependent renal tubular dysfunction sustained by transient deficiency of thiamine dependent enzymes. Careful monitoring of thiamine intake is recommended in patients with organic aciduria and MSUD receiving prolonged period glucose infusion.

516-P

MUTATION ANALYSIS IN BIOTINIDASE GENE BY DENATURING HIGH PRESSURE LIQUID CHROMATOGRAPHY

Karaca M[1], Ozgul RK[2], Guzel A[2], Kilic M[2], Tokatli A[2], Coskun T[2], Goksun E[2], Dursun A[2], Sivri HS[2]
[1]*Faculty Sci&Arts, Dept.Bio, Aksaray Unv., Aksaray, Turkey*
[2]*Dept.Pediat.Metab.Unit.Fac.Med.Hac.Unv. Ankara, Turkey*

Biotinidase deficiency (MIM#253260) is an autosomal recessive metabolic disorder resulted from impaired function of the biotinidase (BTD) gene. In this study, we introduce the mutation patterns of biotinidase gene in 56 Turkish patients diagnosed with biotinidase deficiency. Denaturing high pressure liquid chromatography (dHPLC) was used for pre-screening of nucleotide changes, which allowed identification of 96.4% of BTD gene mutations (54 of 56 patients). Subsequently selected amplicons showing different peak on dHPLC were subjected to direct DNA sequencing. Mutational screening revealed 13 different mutations of which, 9 have already been reported and four were novel (c.205–206ins4, c.1213–1221del9, p.P178T, p.G480R). The most frequent mutation in screened cohort was c.470 G>A (p.R157H) with 35.7% frequency. Seven of the patients carrying this mutation, are homozygous, whereas 13 out of 20 carried it as compound heterozygous. Second frequent mutations are c.98–104del7ins3 and c.1330 G>C (p.D444H) and each of the mutations segregated with 21.4% of affected chromosomes in either homozygously or compound heterozygously. This study revealed that dHPLC is robust, automated, and highly sensitive mutation screening method for the molecular analysis of biotinidase gene.

517-P

TRANSCOBALAMIN II DEFICIENCY IN TWO CASES WITH A NOVEL MUTATION

Unal S[1], Ozgul RK[2], Dursun A[2], Yetgin S[1], Coskun T[2], Rupar T[3], Cetin M[1]
[1]*Dept of Ped, Hemato Uni, Hacettepe Univ, Ankara, Turkey*
[2]*Dept of Ped, Metab Uni, Hacettepe Univ, Ankara, Turkey*
[3]*Biochemical Genetics Lab, London, Ontario, Canada*

Transcobalamin II (TC II) is a primary transport protein for vitamin B12, with inherited deficiency associated with early onset macrocytic anemia, pancytopenia, failure to thrive, vomiting or diarrhea. Serum vitamin B12 levels may have normal or high despite of homocysteinemia in the disease. First patient is a 2 month-old girl with Turkish origin from Cyprus presented with petechial rash, failure to thrive and intermittent diarrhea. She was the sixth child of consanguineous parents and had two siblings died at infancy period with diarrhea, vomiting, and bleeding. Laboratory investigation revealed pancytopenia, and megaloblastic changes in erythroid and myeloid lineages in bone marrow. Serum homocysteine and serum vitamin B12 40 μmol/L and 351 pg/ml, respectively. Hydroxycobalamin (IM) was initiated and pancytopenia and diarrhea resolved. Mutation screening in TCN2 gene was performed in the patients and a large deletion was identified. The identified mutation is c.1106+1516_1222+1231del which begins 1516 bp into intron 7 and ends 1231 bp into intron 8. This 5304 nucleotides deletion includes exon 8 and results in a frame shift to produce a premature protein synthesis. Other patient is a 3 month-old boy presented with failure to thrive and poor feeding. Blood and bone marrow investigation showed pancytopenia, hypersegmentation, and megaloblastic changes. Serum homocysteine and vitamin B12 levels were 46 μmol/L and 677 pg/ml, respectively. Signs and symptoms declined after cyanocobalamine (IM) treatment. Molecular analyses revealed the same novel mutation in this patient. TCII deficiency is a potentially fatal disease but very well responsive to high dose cobalamine.

518-P

SCREENING OF ATP7B GENE MUTATIONS IN TURKISH PATIENTS WITH WILSON DISEASE BY CUSTOM DESIGNED RESEQUENCING MICROARRAYS

Yilmaz A[1], Guzel A[1], Dundar H[2], Dursun A[2], Uslu N[3], Yuce A[3], Ozgul RK[2]
[1]*Dept of Biology, Hacettepe Univ, Ankara, Turkey*
[2]*Dept of Ped, Metab Uni, Hacettepe Univ, Ankara, Turkey*
[3]*Dept of Ped, Gastro Uni, Hacettepe Univ, Ankara, Turkey*

Wilson Disease (WD) is an autosomal recessive disorder of the copper metabolism leading to the accumulation of copper in different organs and tissues. WD is caused by mutations in the ATP7B gene localized on chromosome 13q14.3 comprised of 21 exons. The gene encodes the ATP7B protein, which is essential for copper transport and the elimination of excess copper from the body. Defective ATP7B function results in pathological copper accumulation, initially in liver. In this study, 40 Turkish patients diagnosed with Wilson Disease were screened for mutations using custom designed Affymetrix resequencing microarrays and all the detected mutations were confirmed by direct sequencing. As a consequence of mutation screening study, different type of mutations in ATP7B gene was detected in 24 patients. The detected mutations include 12 missense mutations and one nonsense mutation. It was found that all the mutations detected were known mutations. Two missense nucleotide changes, Arg778Gly and His1069Gln, were found to be common mutations in patients. These mutations located in exon 8 and exon 14 of ATP7B gene and can be used for rapid mutation screening of the gene. Rest of the detected mutations, except from these two common mutations, showed heterogeneous distribution throughout the different exons of ATP7B gene.

519-P

CLINICAL OUTCOMES OF 20 PATIENTS WITH MENKES DISEASE

Kodama H[1], Fujisawa C[1], Shiga H[1], Vatanavicharn H[1], Gu YH[2], Ozawa H[3]
[1]*Teikyo Univ School of Med, Tokyo, Japan*
[2]*National Res Child Health and Develop, Tokyo, Japan*
[3]*Simada Cent Rehab Neurodevelop Interv, Tokyo, Japan*

Background: Menkes disease is an X-linked genetic disorder of copper metabolism. The current treatment is parenteral copper-histidine injections.
Objectives: To clarify clinical outcomes in patients with Menkes disease.
Patients and Methods: The clinical outcomes in 20 Japanese patients with Menkes disease (5 months to 24 years of age) were examined. Various types of mutations in ATP7A gene, including mutations of insertion/deletion, missence, nonsence and splice site, were observed in the patients.
Results: Eighteen of them had been treated with subnutanoeus injections of copper-histidine since the age of 1 month to 1 year. However, intractable convulsions were observed in 90% of the patients, and 50% of them had West syndrome. Bone fractures and pneumonia were observed in 70% and 65%, respectively. Urinary diverticula and urinary infection were observed in 80% and 55% of the patients, respectively. Two patients had been treated with copper-histidine before the age of 2 months. All patients except the patients treated before 2 months of age suffered from severe neurological disturbances. Ninth of them died at the mean age of 17 months to 9 years (mean age of 3 years and 7 months). The two patients treated early showed little improvement of the neurological disturbances, and connective tissue abnormalities were progressed.
Conclusions: The neurological disturbances improved a little bit in patients who were treated before the age of 2 months, suggesting that novel treatments should be developed for patients with Menkes disease.

520-P

THE EFFECT OF DISULFIRAM WITH MENKES DISEASE—A CASE REPORT

Takeda T[1], Fujioka H[1], Nomura S[1], Ninomiya E[1], Fujisawa C[2], Kodama H[2], Shintaku H[1]
[1]*Dep Pediatr Osaka City Univ Grad Sch Med, Osaka, Japan*
[2]*Dep Pediatr Teikyo Univ Sch of Med, Tokyo, Japan*

Background: Menkes disease (MD) is caused by defect of ATP7A, which is a transporter of copper. Due to severe copper shortage, patients of MD show disorders of central nervous system (CNS) and connective tissues. Disulfiram, an anti-alcoholic drug, was expected to improve their CNS symptoms, for it was reported to accumulate copper in neurons.
Patient: He was a 3 year-old boy. At 5 month-old, partial white hair was observed. At 7 month-old, he was admitted to our hospital due to hypotonia. Cranial MR angiography indicated meanderings in arteries. Serum copper level was 19 μg/dl and serum ceruroplasmin level was 10.3 mg/dl. We diagnosed that he was MD patient, for no increase was observed in oral copper loading test. Analysis of ATP7A gene indicated a 1359 C to T mutation in exon 4 (R409X). As intracutaneous supplementation of histidine-copper (130 μg/kg x 6 times/months) did not show any improvement of CNS symptom, we considered starting disulfiram therapy.
Results: Disulfiram was started per orally at 0.02 g/day and reached at 0.1 g/day 2 months after. The amount of copper supplementation was decreased to 100 μg/kg x 3 times/months, for serum copper level was gradually increased after the treatment. His appetite was obviously increased at 4 months after treatment. He also laughed well at 6 months after treatment. His mental improvements were disappeared when we stopped the disulfiram treatment. However they were observed again after re-starting the therapy.
Conclusion: Mental status of a MD patient at 3 year-old was improved by disulfiram and copper supplementation.

521-P

ASSOCIATION OF FOLATE CYCLE GENES POLYMORPHISMS WITH INHERITED FORMS OF PANCREATIC DEFICIENCY

Grechanina YB[1], Grechanina OY[1], Gusar VA[2], Ozerova LS[1], Vasylieva OV[1]
[1]*Dep Med.Genetetic of KhNMU, Kharkov, Ukraine*
[2]*Specialized Med Genetic Cent, Kharkov, Ukraine*

High frequency of C677T MTHFR and A66G MTRR alleles in Ukraine led us to study of their association with monogenic pathology (MP). Previous studies found that the development of chronic pancreatitis (ChP) with pancreatic deficiency (PD) can be led by cystic fibrosis (CF), some organic acidurias, disorders of fatty and sulfur-containing acids metabolism, mitochondrial syndromes. Since deficiency of folate cycle (FC) enzymes leads to decreasing of DNA methylation and losing of protection from harmful recessive mutations, it is promising to study the association of FC-genes polymorphisms and expression of point mutations that lead to PD.
Polymorphisms C677T MTHFR and A66G MTRR by allele-specific PCR were studied in 19 patients with CF and 24 patients with ChP (mitochondrial or amino acids disorders).
In 18(94.74%) patients with CF we identified polymorphisms C677T MTHFR and/or A66G MTRR in homo- or heterozygous state. Compounds of genes alleles MTHFR/MTRR is as follows: htrzg/htrzg 1(5.3%); hmzg/hmzg 0(0%); htrzg/hmzg 5(26.3%); hmzg/htrzg 2(10.5%); N/hmzg 6 (31.6%); N/htrzg 2(10.5%); hmzg/N 1(5.3%); htrzg/N 1(5.3%). All patients of another group were carriers of allele C677T MTHFR and/or A66G MTRR: hmzg/hmzg 3(12.5%); htrzg/htrzg 4(16.7%); htrzg/hmzg 5 (20.8%); hmzg/htrzg 1(4.1%); N/hmzg 3(12.5%); N/htrzg 5(20.8%); hmzg/N 1(4.1%); htrzg/N 2(8.3%). Thus 97.7% of patients with inherited forms of PD are the carriers of FC-genes polymorphisms, being more specific is A66G MTRR—79.1% than C677T MTHFR—in 60.5% cases. This should be considered during developing an individual treatment strategy and prevention of complications, as underlying MP and conditions associated with the presence of these polymorphisms.

522-P

LABORATORY DIAGNOSIS, TREATMENT, AND FOLLOW-UP OF 78 PATIENTS WITH AROMATIC L-AMINO ACID DECARBOXYLASE DEFICIENCY

Brun L[1], Ngu LH[2], Keng WT[2], Ch'ng GS[2], Choy YS[3], Hwu WL[4], Lee WT[4], Willemsen MAAP[5], Verbeek MM[5], Wassenberg T[5], Régal L[6], Orcesi S[7], Accorsi P[8], Tonduti D[7], Téstard H[9], Abdenur JE[10], Tay S[11], Allen GF[12], Heales S[12], Kern I[13], Kato K[14], Burlina A[15], Manegold C[16], Hoffmann GF[16], Blau N[1]
[1]*University Children's Hospital, Zurich, Switzerland*
[2]*Institute of Pediatrics, Kuala Lumpur, Malaysia*
[3]*Prince Court Medical Centre, Kuala Lumpur, Malaysia*
[4]*National Taiwan University Hospital, Taipei, Taiwan*
[5]*Radboud University Nijmegen Medical Cen, Nijmegen, Netherlands*
[6]*University Hospital Leuven, Leuven, Belgium*
[7]*C. Mondino Institute of Neurology Found, Pavia, Italy*
[8]*Spedali Civili, Brescia, Italy*
[9]*Centre Hospitalier Intercommunal, Annemasse, France*
[10]*CHOC Children's, Orange, CA, United States*
[11]*National University Hospital, Singapore, Singapore*
[12]*National Hospital & Inst of Neurology, London, United Kingdom*
[13]*University Children's Hospital, Geneva, Switzerland*
[14]*Yamagata University Hospital, Yamagata, Japan*
[15]*Div. Metabolic Dis, University Padova, Padua, Italy*
[16]*Univesity Children's Hospital, Heidelberg, Germany*

In the database of Pediatric Neurotransmitter Disorders (JAKE; www.biopku.org) information on treatment, clinical, biochemical and molecular findings, and the outcome of 78 patients with aromatic L-amino acid decarboxylase (AADC) deficiency was tabulated. 45 patients have been previously reported, 33 patients are described for the first time. In most of AADC-deficient patients, hypotonia (95%), oculogyric crises (86%), and developmental retardation (63%) became clinically evident during infancy or childhood. Biochemical diagnosis is based on very low homovanillic acid, 5-hydroxyindoleacidic acid, and 3-methoxy-4-hydroxyphenolglycole, and elevated 3-O-methyl-L-dopa, L-dopa, and 5-hydroxytryptophane in CSF, absent plasma AADC activity, or elevated urinary vanillactic acid. A total of 24 mutations in the DDC gene were detected in 50 patients (8 of them for the first time: p.L38P, p.I42fs, p.Y79C, p.A110Q, p.R412W, p.I433fs, p.G123R, p.E292E), with IVS6+4A>T being the most common one (AF 38%). Treatment options (B6 or PLP, dopamine agonists, and MAO-B inhibitors) are in many cases not beneficial, and prognosis is uncertain. Only 15 patients with a relative mild form improved on a combined therapy.

523-P

SEPIAPTERIN REDUCTASE DEFICIENCY CAUSED BY NEW MUTATION OF THE SPR GENE IN A 7 MONTH OLD GIRL: CASE REPORT AND REVIEW OF THE LITERATURE

Dill P[1], Koczygit-Wagner M[2], Weber P[1], Meili D[3], Rassi A[3], Thony B[3], Blau N[3]
[1]*Div Neuropediatr, Univ Children's Hosp, Basel, Switzerland*
[2]*Div Pediatrics, Univ Children's Hospital, Basel, Switzerland*
[3]*University Children's Hospital, Zurich, Switzerland*

Sepiapterin reductase deficiency (SRD) is a rare autosomal recessive disorder, caused by mutation of the SPR gene located 2p14-p12. Sepiapterin reductase (SR) catalyses the final two-step reduction in the biosynthesis of tetrahydrobiopterin (BH4). Blood phenylalanine is normal and diagnosis reveals on elevated biopterin, dihydrobioptertin and sepiapterin levels in CSF. The diagnosis can be confirmed by enzymatic fibroblast analysis and mutation analysis. So far 27 cases with 14 mutations in the SPR gene including our patient have been detected (BIODEFdb, www.biodef.org). In SRD certain symptoms appear early in infancy and childhood, such as oculogyric crises, paroxysmal stiffening, and hypotonia. Other symptoms like ataxia, dysarthria and dystonia develop later in childhood. We report a girl with SRD, born to consanguine Turkish parents, who was diagnosed at 8 months of age. Since 3 months of age she presented recurrent episodes, often after meals with paroxysmal stiffening of the body, upward gaze, and chewing movements. CSF analysis revealed a severe dopamine and serotonin deficiency and elevated biopterin and sepiapterin level, indicating SRD. This could be confirmed by enzymatic fibroblast analysis (no activity detectable) and a novel homozygous missense mutation (p.R219X) in exon 3.
Before treatment the girl showed mild motor delay and cognitive impairment. Upon therapy with levodopa/benserazide (3.2 mg/kg/d) and 5-hydroxytryptophan (3 mg/kg/d) the crises resolved completely, and both motor and cognitive skills improved. To our knowledge our patient is the only case, who not only completely recovered from motor deficits, but also shows normal cognitive development upon therapy with L-dopa and 5-hydroxytryptophan.

524-P

FUNCTIONAL ANALYSIS OF TWO POINT MUTATIONS IN THE SPR GENE IDENTIFIED IN SPANISH PATIENTS WITH DOPA-RESPONSIVE DYSTONIA

Teresa L[1], Pérez B[1], Castro M[1], Medrano C[1], Merinero B[1], Ugarte M[1], Desviat LR[1]
[1]*CEDEM, C. Biología Molecular CSIC-UAM, Madrid, Spain*

In this work we report the expression analysis of mutations c.751A>T and c.304G>T in the SPR gene, identified in Spanish Dopa-responsive dystonia patients. Mutation c.751A>T previously described in other Mediterranean patients creates a premature stop codon (p.K251X), resulting in a truncated protein with a C-terminal deletion of eleven amino acids. The novel mutation c.304G>T affects the last nucleotide of exon 1 and could potentially cause a missense change (p.G102C) or affect the splicing process. It was found to be absent in 100 control alleles screened by high resolution melting. Both changes (p.K251X and p.G102C) were functionally evaluated by expression analysis in E. coli, using the pHSR9 plasmid encoding SPR cDNA. The changes were introduced by PCR mutagenesis and SPR enzymatic activity was measured in cell extracts of induced bacteria expressing the wild-type and mutant constructs. Mutation p.K251X was functionally null (<1% activity) while p.G102C was associated with 17% residual activity. These results correlate with the observed phenotypic severity in the patients. To complete the functional analysis of the nucleotide change c.304G>T and to determine if it affects the splicing process, in silico analysis was performed. Several splice prediction programs revealed that the change caused a significant reduction in splicing score for the 5′ splice donor site of exon 1. Splicing analysis using minigenes will confirm if the change is a splicing mutation and if some normally spliced transcript with the missense change p.G102C is produced.

525-O

VALUE OF PHENYLALANINE LOADING IN PEDIATRIC PATIENTS WITH DOPA-RESPONSIVE DYSTONIA

Opladen Th[1], Okun JG[1], Burgard P[1], Blau N[2], Hoffmann GF[1]
[1]*Div Metab Dis, Univ Child Hosp, Heidelberg, Germany*
[2]*Div Clin Chem Biochem, Univ Child Hosp, Zurich, Switzerland*

Background: Phenylalanine (Phe) loading is a useful tool in the differential diagnosis of autosomal dominant dopa-responsive dystonia (DRD). Available data for valid interpretation in pediatric patients undergoing Phe loading are missing.
Objectives: To determine criteria for the diagnosis of DRD in children using standardized Phe loading.
Patients and Methods: We investigated oral loading with 100 mg Phe/kg in eight patients with confirmed DRD and 17 pediatric patients with clinically suspected but biochemically or genetically excluded DRD. Results of Phe, tyrosine (Tyr) and biopterin from plasma and dried blood spot analyses were correlated. Pediatric cut-off values were established.
Results: By using adult cut-off values and either only Phe/Tryr ratios or biopterin concentrations false positive as well as false negative results are frequent. Only the combined analysis of the Phe/Tyr ratio and the biopterin concentration is valid in children. In children with DRD dried blood Phe/Tyr ratio exceeded 4.6 (plasma Phe/Tyr ratio > 5.4) after two hours and biopterin concentration in dried blood remained below 16.2 nmol/L (plasma biopterin < 14 nmol/L) one hour after Phe loading.
Conclusions: Phe loading is a useful tool for the diagnosis of DRD in children, but specific pediatric cut-off values need to be applied. Simultaneous measurements of the Phe/Tyr ratio and biopterin in plasma or dried blood spots are essential in pediatric patients. Test duration can be reduced to only two hours.

526-P

PLASTICITY OF POSTSYNAPTIC, BUT NOT PRESYNAPTIC, GABA(B) RECEPTORS IN ALDEHYDE DEHYDROGENASE 5A1 (ALDH5A1; SUCCINIC SEMIALDEHYDE DEHYDROGENASE) DEFICIENT MICE

Vardya I[1], Drasbek KR[2], Jensen K[1], Gibson KM[3]
[1]*Dept Physiol Biophysics, Aarhus Univ, Aarhus, Denmark*
[2]*Dept Mol Biol, Aarhus Univ, Aarhus, Denmark*
[3]*Dept Biol Sci, Michigan Tech Univ, Houghton, MI, United States*

Background: Human Aldh5a1 deficiency represents a defect of gamma-aminobutyrate (GABA) degradation whose neurological phenotype includes epilepsy. Patients and mutant (MUT) mice show high extracellular GABA and the GABA(B) receptor (GABA(B)R) agonist gamma-hydroxybutyrate (GHB). MUT mice display absence seizures which progress into lethal tonic-clonic seizures by 1 month.
Methods: To test the hypothesis that GABA(B)R densensitization plays a role in epileptic mechanisms, we performed patch-clamp recordings from layer 2/3 pyramidal neurons in neocortical brain slices of wild-type (WT) and MUT mice in order to explore pre- and postsynaptic GABA(B)R function.
Results: Presynaptic GABA(B)R-mediated inhibition, estimated during wash-in of the GABA(B)R agonist baclofen, was normal in MUT mice, whereas postsynaptic baclofen-induced potassium (K+) currents were decreased. The latter was not likely related to elevated K+, GABA or GHB in slices, nor altered expression of regulators of G-protein signaling (RGS) proteins. Adenosine-induced K+ currents were likewise reduced in MUT mice, suggesting heterologous desensitization of G-protein-dependent effectors and concomitant reduction in G-protein-coupled inwardly rectifying K+ (GIRK) channel responses.
Conclusions: These data indicate that elevated GABA and GHB desensitize postsynaptic, but not presynaptic, GABA(B)Rs, and decrease GIRK channel function, thereby yielding new insight into epileptic pathophysiology of MUT mice and affected patients.

527-P

KEARNS-SAYRE SYNDROME AND CEREBRAL FOLATE DEFICIENCY

Artuch R[1], Garcia-Silva MT[2], O'Callaghan M[3], Ormazabal A[1], Blazquez A[3], Martin MA[2], Lopez-Gallardo E[4], Montoya J[4], Pineda M[1]
[1]*Hospital Sant Joan de Déu., Barcelona, Spain*
[2]*Hospital Universitario 12 de Octubre, Madrid, Spain*
[3]*CIBERER-ISCIII, Madrid, Spain*
[4]*Universidad de Zaragoza, Zaragoza, Spain*

Background: Kearns-Sayre syndrome (KSS) is a mitochondrial DNA deletion syndrome which presents severe 5-methyltetrahydrofolate (5-MTHF) deficiency in cerebrospinal fluid (CSF).
Objectives: To evaluate white matter status and analyze CSF biogenic amines in patients suffering KSS and presenting cerebral folate deficiency.
Material and Methods: Six patients with diagnosis of KSS in whom CSF analysis had been performed were included.
Results: A severe cerebral 5-MTHF deficiency was observed in 5 patients (ranging from 0.5 to 8 nmol/L), and a moderate deficiency in the other. A significant negative correlation was observed between CSF 5-MTHF and protein concentration. CSF homovanillic acid values were clearly high compared to age-matched reference values in all patients, but no correlations were found between this biogenic amine and the other variables. Regarding neuroimaging, the main feature was hyperintensity in the basal ganglia, the subcortical cerebral and cerebellar white matter, and the brainstem. Hemispheric white matter disturbances appeared to be qualitatively associated with 5-MTHF values.
Conclusions: The negative correlation between 5-MTHF and proteins in CSF supports the hypothesis of impaired transport through the choroid plexus. Interestingly, clearly high dopamine metabolites were found in our series. A relationship was observed between CSF 5-MTHF concentrations and the severity of white matter lesions.

528-O

PHARMACOLOGICAL CHAPERONES FOR THE AROMATIC AMINO ACID HYDROXYLASES

Martinez A[1], Calvo AC[1], Scherer T[2], Pey AL[1], Ying M[1], Winge I[1], McKinney J[1], Haavik J[1], Thony B[2]
[1]*Dept Biomedicine, Univ Bergen, Bergen, Norway*
[2]*Dept Pediatrics, Univ Zurich, Zurich, Switzerland*

Phenylalanine hydroxylase (PAH), tyrosine hydroxylase (TH) and the tryptophan hydroxylases (TPH1 and TPH2) are structurally and functionally related enzymes that share substrates, pterin cofactors and inhibitors. We have recently identified four compounds (I-IV) with pharmacological chaperone effect for PAH and phenylketonuria (PKU) mutants (1). We now investigated the effect of these compounds on brain TH and TPH2, comparative to hepatic PAH. As assayed by differential scanning fluorimetry purified human PAH, TH and TPH2 were differently stabilized by the compounds and only compound III (3-amino-2-benzyl-7-nitro-4-(2-quinolyl)-1,2-dihydroisoquinolin-1-one) stabilized the three enzymes. We also investigated the effect of compounds II-IV in mice upon oral loading with 5 mg/kg/day. Significant effects were obtained by treatment with compound III—which increased total TH activity in mouse brain by 100 % with no measurable effects either on TPH activity or on monoamine neurotransmitter metabolites dopamine, DOPAC, HVA, serotonin and 5-HIAA—and with compound IV—which led to 10–30% decrease of these metabolites. Our results indicate that pharmacological chaperones aiming the stabilization of one of the aromatic amino acid hydroxylases should be tested on other members of the enzyme family. Moreover, compound III stabilizes in vitro the human TH mutant pR202H, associated to autosomal recessive L-DOPA-responsive dystonia. Pharmacological chaperones thus appear as a promising approach for the treatment of disorders associated with TH misfolding, as previously revealed for PKU (1).

1. Pey AL, Ying M, Cremades N, Velazquez-Campoy A, Scherer T, Thony B, Sancho J and Martinez A (2008) J Clin Invest 118, 2858–2867.

529-P

DOPA RESPONSIVE HYPERSOMNIA IN COMBINED SEPIAPTERINE REDUCTASE (SR) AND METHYL MALONYL CoA EPIMERASE (MCEE) DEFICIENCIES

Mazzuca M[1], Christa L[2], Damaj L[3], Plouin P[4], Dauvilliers Y[5], Rabier D[2], Clot F[6], Odent S[3], Benoist Jf[7], De Lonlay P[8]
[1]*Inserm U781 – Hôp Necker Enfants-Malades, Paris, France*
[2]*Serv Bioch Métab–Hôp Necker-Univ Paris V, Paris, France*
[3]*Serv génétique clinique – CHRU, Rennes, France*
[4]*Fonction neuro explo unit - Hôp Necker, Paris, France*
[5]*Neurologie, Hôpital Gui de Chauliac, CHU, Montpellier, France*
[6]*UF Mol&Cel Neurogenet-Pitié Salpétrière, Paris, France*
[7]*Serv Bioch-Horm, Hôp Robert Debré, Paris, France*
[8]*Ref Center Metab Inherit Dis-Hôp Necker, Paris, France*

SR and MCEE deficiencies are rare autosomal recessive disorders of neurotransmitter and propionyl coA metabolism, respectively. We report a consanguineous patient with delayed psychomotor development diagnosed with permanent but moderate MMA-uria during childhood related to a homozygous nonsense mutation in the MCEE gene. Vitamin B12 and L-carnitine supplementations were inefficient. Progressive severe neurological deterioration and axial hypotonia were observed. The boy became wheelchair-bound. He was further investigated at 19 years of age because of hypersomnia and movement induced dystonia. Long duration EEG showed early onset of REM. Brain MRI and CSF Orexine level were normal. Decreased levels of HVA and HIAA, elevated level of Biopterin, normal level of Neopterin, and presence of Sepiapterin were found in CSF. Serum prolactin was increased. Sepiapterin reductase deficiency was confirmed by molecular analysis of the SR gene revealing a homoallelic nonsense mutation leading to a truncated protein. On L-dopa/carbidopa and 5-HTTP supplementations, the boy showed clinical improvement.
1°Two coincidences are facts that our patient presents two homoallelic mutations in two genes mapped in chromosome 2p13–3 and that another case report has been identified with combined SR and MCEE homozygous mutations: MCEE gene mutation was similar, but mutation in the SR gene was different. 2° although the treatment by neurotransmitter precursors is efficient, neuronal oxidative stress induced by central low BH4/BH2 ratio may justify a specific treatment by anti-oxidants in SR.

530-O

ASSOCIATION OF LOW CSF SEROTONIN AND THE SLC6A4–Gly56Ala MUTANT SEROTONIN TRANSPORTER GENE WITH ATYPICAL AUTISM

Adamsen D[1], Meili D[1], Blau N[1], Ramaekers V[2], Thony B[1]
[1]*Clin Chem and Biochem, Univ Child Hosp, Zurich, Switzerland*
[2]*Centre of Autism, Univ Hosp, Liège, Belgium*

Autism belongs to a spectrum of neurodevelopmental disorders characterized by deficits in social interaction, language and behavior. Autism spectrum disorders consist of "typical" Kanner autism, Asperger's syndrome and an "atypical" form also known as pervasive developmental disorder—not otherwise specified (PDD-NOS). The heterozygous Gly56Ala mutation in the SLC6A4 serotonin transporter (SERT, 5-HTT) gene has been shown to be associated with autism and rigid-compulsive behavior. Both the homozygous and heterozygous form of Gly56Ala results in elevated serotonin reuptake activity (Sutcliffe et al. 2005). This study describes the metabolic and underlying genetic abnormalities for atypical autism (PDD-NOS). The SLC6A4 gene was analyzed for DNA mutations as well as promoter variants, and the CSF was analyzed for monoamine neurotransmitters, including 5-hydroxytryptophan and 5-hydroxyindoleacetic acid (5HIAA). The analysis showed that the patient was carrying the L/L promoter variant, and the heterozygous Gly56Ala allele of the SLC6A4 gene, both known to result in elevated SERT activity. Moreover, the CSF revealed normal 5-hydroxytryptophan but low levels of the serotonin end-metabolite 5HIAA, suggesting down-regulation of the serotonin turnover. The patient received daily treatment with 5-hydroxytryptophan (5 mg/kg) and carbidopa (1 mg/kg) from the age of 5 years which led to clinical improvements and normalization of 5HIAA. This study, which for the first time shows an association between atypical autism, low serotonin and heterozygous Gly56Ala allele, suggests that patients with atypical autism, carrying the heterozygous Gly56Ala mutation, may respond to oral treatment with 5-hydroxytryptophan and carbidopa by clinical improvement and normalization of serotonin turnover in the brain.

531-P

THE PHENOTYPIC VARIABILITY IN BIOGENIC AMINES SYNTHESIS DEFECT

Zaman TZ[1], Einollahi NE[2]
[1]*Iranian National Research Society, Tehran, Islamic Republic of Iran*
[2]*Metab Unit, Tehran Univ, Tehran, Islamic Republic of Iran*

Background: Tetrahydrobiopterin (BH4) activates phenylalanine hydroxylase as well as tyrosine 3 hydrxyolase and tryptophan hydroxylase. BH4 deficiency causes biogenic amine synthesis defects and non-specific neurologic abnormalities as well. Elevated urine neopterine as well as hyperphenylalaninemia is most often in favor of diagnosis of 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency.
Materials and Methods: We studied 14 patients suspected of having biogenic amine synthesis defect, among 257 patients of PKU group and 534 patients with non-specific neurologic manifestations without defined diagnosis.
Results: 14 cases; (4 female, 10 male), Age: 26 month-27 yr(3 adults. Clinical findings were: psychomotor retardation in 14(14/14), convulsion in 5 (5/14), hypersalivation in 5 (5/14), distal chorea 2 (2/14), temperature disturbances in 3 (3/14) autism 1 (1/14), oculogyric crises; 1, awkward walking 4, hypertonia (3) hypotonia (1) hyper/hypo (1).High urine neopterin and urine neopterin/creatinine in 14(14/14),high plasma phenylalanine;3(3/10) and low urine VMA; 6(6/10).Treatment with levodopa-carbidopa,5 hydroxy tryptophan and BH4 was effective in those in whom treatment was started in the early stages of symptomatic disease.
Conclusion: In any undiagnosed neurologic disease we should consider biogenic amines synthesis defects and recommend urine, CSF investigations to treat as early as possible, to prevent brain damage and to improve outcome. Each new case of PKU should be tested for malignant types to be treated soon. PTPS deficiency can cause movement disorders in adults.

532-O

MOUSE MODELS FOR BH4 DEFICIENCY BY TARGETING THE 6-PYRUVOLYTETRAHYDROPTERIN SYNTHASE GENE PTS

Adamsen D[1], Scavelli R[1], Ledermann B[2], Blau N[1], Thony B[1]
[1]*Clin Chem and Biochem, Univ Child Hosp, Zurich, Switzerland*
[2]*Div Animal Facility, Uni, Zurich, Switzerland*

Tetrahydrobiopterin (BH4) deficiencies are a heterogeneous group of disorders mainly due to recessive mutations in the PTS gene, encoding 6-pyruvoyltetrahydropterin synthase (PTPS). Approximately 1/4 of patients with PTPS deficiency have a mild or peripheral phenotype with isolated hyperphenylalaninemia and lowered hepatic BH4, whereas 3/4 have a severe or central phenotype with additional depletion of BH4, catecholamine and serotonin neurotransmitters in CNS, besides systemically elevated neopterin as a precursor of BH4. The molecular mechanism for these phenotypic distinctions is unknown. Studies with mice have shown that a complete Pts knock-out (Pts-ko) exhibited a severe phenotype with perinatal lethality [1]. Here we report on a new Pts-Arg15Cys-knock-in (Pts-ki) mouse, equivalent to the human PTS-Arg16Cys with a mild peripheral phenotype. Homozygous Pts-ki mice showed no hyperphenylalaninemia or monoamine neurotransmitter deficiency, but reduced PTPS activity and elevated neopterin. Recently, we bred the Pts-allele to compound heterozygosity, leading to Pts-ki/ko animals with two different phenotypes: a severely affected group that died around 3–4 days after birth, and a second unaffected group with normal survival. At birth, all Pts-ki/ko showed reduced BH4 and PTPS activity in liver and brain, elevated neopterin, monoamine neurotransmitter deficiency in brain, and mild hyperphenylalaninemia (~0.9 mmol/l). Whereas the affected group worsened, the so called unaffected group showed reduced PTPS activity but normal levels of liver and/or brain biopterin, neopterin, monoamine neurotransmitters, and blood phenylalanine. The reason for this two phenotypes and molecular differences is unknown and currently under investigation.
[1] Elzaouk, L. et al (2003) J Biol Chem 278, 28303–11.

533-P

DOPAMINE MAY INFLUENCE BRAIN GLUTATHIONE: IMPLICATIONS FOR AROMATIC L-AMINO ACID DECARBOXYLASE DEFICIENCY AND OTHER INHERITED CONDITIONS OF DOPAMINE METABOLISM

Allen G[1], Ullah Y[2], Land J[3], Heales S[4]
[1]*UCL Institute of Neurology, London, United Kingdom*
[2]*University of Copenhagen, Copenhagen, Denmark*
[3]*Neurometabolic Unit, National Hospital, London, United Kingdom*
[4]*UCL Institute of Child Health, London, United Kingdom*

Aromatic L-amino acid decarboxylase (AADC) deficiency, a disorder of dopamine and serotonin metabolism, remains debilitating and difficult to treat for most patients. We are investigating this condition in order to improve understanding of the underlying pathogenic mechanisms. Reduced glutathione (GSH) plays a major role in cellular homeostasis as an antioxidant. We found that treatment of human neuroblastoma cells with 50 μM dopamine increased intracellular GSH 2.9-fold. 50 μML-dopa also increased intracellular GSH 2.5-fold, however inhibiting AADC, which converts L-dopa to dopamine, prevented this increase. Furthermore dopamine but not L-dopa, increased 1.5-fold GSH release from human astrocytoma cells, which do not express AADC. GSH release from astrocytes is the first stage in trafficking of GSH to neurons. This data indicates dopamine may play a role in controlling brain GSH levels and consequently antioxidant status. The inability of L-dopa to influence GSH concentrations in the absence of AADC or with AADC inhibited indicates GSH trafficking/metabolism may be compromised in AADC deficiency. Patients with other dopamine deficiency conditions such as tyrosine hydroxylase deficiency and pterin related disorders could be similarly affected, potentially leaving the central nervous system of these patients more vulnerable to oxidative stress. This work is supported by the AADC Research Trust (www.aadcresearch.org).

534-O

KINETIC ANALYSES GUIDE THE THERAPEUTIC DECISION IN A NOVEL FORM OF MODERATE AROMATIC ACID DECARBOXYLASE DEFICIENCY

Barth M[1], Chaabouni Y[2], Hubert L[3], Serre V[3], Bahi-Buisson N[4], Cadoudal M[2], Rabier D[2], Nguyen The Tich S[5], Bonneau D[1], Ribeiro M[1], Munnich A[3], de Lonlay P[6], Christa L[7]
[1]*Div Genet, Univ Medicine, Angers, France*
[2]*Div Metab, Necker–Enfants Malades Hosp, Paris, France*
[3]*Inserm U781, Paris, France*
[4]*Div Paediatric Neurology, Necker Hosp, Paris, France*
[5]*Div of Paediatric Neurology, Angers, France*
[6]*Ref center for Metab Dis, Necker Hosp, Paris, France*
[7]*Univ Paris Descartes, Paris, France*

Background: Aromatic amino acid decarboxylase (DDC) deficiency is a rare autosomal recessive disorder resulting in a combined dopamine, serotonin and catecholamines deficiency. The patients usually present severe neonatal clinical features. Here, we report an atypical clinical presentation with moderate symptoms.
Patient: This girl was born from unrelated parents. At 10 months-old, eye revulsions were noted without seizures, and feeding difficulties were attributed to gastro-oesophageal reflux. She was investigated at 7-years-old, because of orofacial dyspraxia, hypomimie, axial hypotonia and focal segmental dystonia, bilateral ptosis, without evidence for cognitive impairment.
Results: HVA (110 nM; rv: 202–596) and HIAA (<50nM; rv: 87–366) were decreased, OMD (520 nM; rv: 5–60) and HTTP (56 nM; rv: 2–16) were increased in CSF. We have diagnosed the DDC deficiency by measuring the low plasma enzyme activity: 4pmol/min/ml; rv: 16–137. Moreover, the kinetic analysis revealed a 12.5-fold increase in the apparent KM for L-Dopa (4 mM; control=0.32), with unchanged Vmax (30 pmol dopamine/min/ml; control=34), suggesting a modification in the substrate binding-site. Molecular analysis of the DDC gene revealed 2 heterozygous mutations: c.1040G>A; p.R347Q already described, and a novel mutation c.478C>T, p.R160W.
Conclusion: 1) According to the DDC 3D-model, the substitutions may destabilize the dimeric structure by loss of 2 arginines involved in intersubunit salt-bridge interactions 2) L-dopa therapy was combined to dopamine agonist and vitamin B6: the quality of life, fatigability and eye revulsions were markedly improved.

535-P

LEVO-DOPA RESPONSIVE-MILD PHENTOTYPE DUE TO A LARGE DELETION IN THE TYROSINE HYDROXYLASE GENE

Ormazabal A[1], Serrano M[1], De Castro P[2], Armstrong J[1], García-Cazorla A[1], Cormand B[3], Campistol J[1], Artuch R[1]
[1]*Hospital Sant Joan de Deu, Barcelona, Spain*
[2]*Hospital Gregorio Marañon, Madrid, Spain*
[3]*Universidad de Barcelona, Barcelona, Spain*

Objective: To report the first case with dopa responsive encephalopathy caused by a large deletion in the Tyrosine hydroxylase (TH) gene plus a mutation in TH promoter.
Case Report: A 19-month-old female was studied because of psychomotor delay of unknown aetiology.
Biochemical analysis: Biogenic amines and pterins were analyzed by HPLC with electrochemical and fluorescence detection procedures.
Genetic study: We amplified and sequenced the TH coding region, splice sites, 114 bp preceding the initiation codon and 354 bp following the stop codon of the TH gene in the patient. MLPA was performed in exons 1, 3, 4, 8, 12 and 14 of TH following the manufacturer's instructions. New primers were designed to perform long sequences from exon 11 to 14.
Results: A moderate decrease in HVA concentration (151 nmo/L: reference values: 304–658) and HVA/5-HIAA ratio (1.1; reference values: 1.5–3.5) was observed. Complete sequencing of the TH gene revealed a previously reported mutation in the promoter region (c.1–70G>A). MLPA showed one allele deletion of exon 12 but not in exon 8 and 14. Long PCR amplification showed a 716 pb deletion initiating in intron 11 and ending in exon 13 (c.1128+25del716pb; p.Asp377GlufsX39).
Conclusions: Since some dopa-responsive encephalopathic patients with biochemical evidence of TH deficiency show no mutations within the TH coding region, promoter region or splicing sites by direct sequencing, we suggest the inclusion of MLPA techniques to improve the genetic diagnosis of this disorder.

536-O

FUNCTIONAL STUDIES OF DISEASE-RELATED VARIANTS IN HUMAN TRYPTOPHAN HYDROXYLASE 1 AND 2

Winge I[1], McKinney JA[1], Halmxy A[1], Johansson S[2], Knappskog PM[2], Haavik J[1]
[1]*Dep. Biomedicine, Univ Bergen, Bergen, Norway*
[2]*Ctr Med Gen & Mol Med, Haukeland Hosp, Bergen, Norway*

Tryptophan hydroxylase (TPH) catalyzes the rate-limiting step in serotonin biosynthesis in the nervous system. There are two distinct TPH genes code for their respective enzymes, TPH1 and TPH2. Recently, several variants of human TPH1 and TPH2 have been reported to be associated with a spectrum of neuropsychiatric disorders such as unipolar major depression, bipolar disorder, suicidality, and attention-deficit/ hyperactivity disorder (ADHD). We used three different expression systems: rabbit reticulocyte lysate, Escherichia coli, and human embryonic kidney cells, to identify functional effects of all human TPH1 and TPH2 missense variants reported to date. Some of these mutations were novel, found in our own material from adult patients with ADHD. The properties of mutants affecting the regulatory domain were indistinguishable from the wild-type (WT). Moderate loss-of-function effects were observed for mutants in the catalytic and oligomerization domains which were manifested via stability and solubility effects, and in addition two variants, p.Arg145X in TPH1 and p. Arg303Trp in TPH2, were completely inactive. All variants were tested as substrates for protein kinase A and were found to have similar phosphorylation stoichiometries. A standardized assay protocol as described here for activity and solubility screening should also be useful for determining properties of other TPH variants that will be discovered in the future.

537-P

PLASMA 3-O-METHYL DOPA AS A SCREENING BIOMARKER FOR AROMATIC AMINO ACID DECARBOXYLASE (AADC) DEFICIENCY AND OTHER NEUROTRANSMITTER DISORDERS

Turner C[1], Lumsden D[2], Rahman Y[2], Mundy H[2], Champion M[2], Dalton RN[3]
[1]WellChild Lab, Evelina Children's Hosp, London, United Kingdom
[2]Dept. INH. Metab Dis, Evelina Child Hosp, London, United Kingdom
[3]Wellchild Lab, King's College London, London, United Kingdom

Background: Measurement of CSF neurotransmitters, pivotal to the original elucidation of AADC deficiency, is used in the investigation of patients with suspected disorders of biopterin, pyridoxine or pyridoxal phosphate metabolism. A plasma biomarker for such disorders could limit the reliance on lumber puncture and speed diagnosis. The original report of AADC described increased 3-O-methyl DOPA (3OMD) in CSF, plasma, and urine. Accurate measurement of normal plasma concentrations of 3OMD allowed investigation of this biomarker in disorders expected to result in increased (AADC deficiency) and decreased levels (phenylketonuria).
Method: 183 plasma samples were collected from 170 patients being investigated for neurological disease. Samples (50 μl) were analyzed by liquid chromatography stable isotope dilution electrospray MSMS on an API5000 MSMS instrument (ABSciex, Warrington). The limit of quantitation was 0.5 nmol/L.
Results: Plasma 3OMD concentrations in 165 patients without AADC or concurrent L-DOPA treatment ranged from 25–766 nmol/L (median 149 nmol/L). Results were positively skewed due to higher values in neonatal patients. In 10 patients with phenylketonuria, 3OMD ranged from 25–156 nmol/L (median 53 nmol/L). In 2 patients with confirmed AADC, plasma 3OMD concentrations were 2130 nmol/L and 10,000 nmol/L. In 2 patients receiving L-DOPA Treatment, one with tyrosine hydroxylase deficiency and the other idiopathic generalized dystonia, plasma concentrations of 6,600 nmol/L and 25,500 nmol/L were recorded. In a known AADC heterozygote the result, 182 nmol/L, was well within the putative normal range.
Conclusion: Plasma 3OMD is increased in AADC deficiency and decreased in phenylketonuria. Screening plasma 3OMD could both reduce the need for CSF neurotransmitter metabolite analysis and speed the diagnostic process.

538-P

SEROTONIN-RELATED GENES IN ANOREXIA NERVOSA: META-ANALYSES AND CZECH POPULATION STUDY

Slachtova L[1], Martaskova D[2], Kemlink D[3], Martasek P[1], Papezova H[2]
[1]Dept Pediatr, 1st Fac Med, Charles U, Prague, Czech Republic
[2]Dept. of Psychiat. 1st Fac of Med, Charl, Prague, Czech Republic
[3]Dept of Neurology, 1st Fac Med, Charles, Prague, Czech Republic

Anorexia nervosa (AN) is a serious psychiatric disease characterized by the inability to maintain normal body weight. It is characterized by restricted eating and obsessive fears of becoming overweight. The pathogenesis of AN is multifactorial with a clear genetic component. Serotonin transmission and its relationship to AN has been a topic of numerous studies. The study addressed association of the polymorphisms in the promoter of the serotonin 5-HT2A receptor (-1438 A/G) gene and promoter of serotonin transporter (5-HTT LPR, VNTR) with AN in the Czech population. We genotyped a well-defined group of 112 patients with AN (average age of 25.4 years, BMI on average 14.65). The control group consisted of 95 healthy Caucasian females (average age 26,2 years, average BMI was 20.7). The 5-HT2A receptor -1438 polymorphism analysis showed a trend for the association with OR (odds ratio) for risk allele A being in the same direction. In a meta-analysis, which includes all published results for allelic tests, the resulting P value is highly significant (0.0003, c2 statistic, 1 df). Using a quantitative association of 5-HTR2A polymorphism with BMI in the Czech sample, a borderline association (p=0.055) was observed. In 5-HTT S, L allele and in multiallelic 5-HTT VNTR polymorphism analysis, neither allelic nor quantitative association with BMI was observed. Results support previous reports of a significant role of A allele (-1438 A/G, 5-HT2A receptor) as a risk factor in AN.
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539-O

COMPARATIVE STUDY OF CHIMERIC LIVER-SPECIFIC PROMOTER EXPRESSION FROM A NON-VIRAL VECTOR FOR HEPATIC GENE THERAPY

Viecelli HM[1], Wong SP[2], Harbottle RP[2], Petrus I[3], Chuah M[3], VandenDriessche T[3], Thony B[1]
[1]Dept Pediatrics, Univ Zurich, Zurich, Switzerland
[2]Imperial College London, London, United Kingdom
[3]Vesalius Research Center, Univ Leuven, Leuven, Belgium

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. Besides efficient hepatocyte targeting and vector dosing, sustained transgene expression from a liver-specific promoter is also an important parameter for therapeutic efficacy. In this study we performed an in vivo comparison of different chimeric liver-specific promoters driving the firefly luciferase reporter transgene. Promoter strength was monitored by quantitative bioluminescence in a non-invasive imaging technology in living mice (IVIS screening). Non-viral plasmid-DNA vectors containing the luciferase reporter gene plus a downstream mammalian scaffold/matrix attachment region (S/MAR) element for episomal maintenance and/or extra-chromosomal stability were delivered to the murine liver after hydrodynamic tail vein injection (HTV). We compared longitudinal luciferase expression from the human α 1-antitrypsin (hAAT) or the human transthyretin (hTTR) promoter with a hepatocyte-specific enhancer 8 (HSE8) upstream. Our data showed that gene expression driven from the hTTR promoter with HSE8 enhancer is over 20 fold higher than from hAAT promoter over a period of several months. The efficient and sustained expression observed from this hTTR promoter and HSE8 enhancer combination in an episomal plasmid DNA-vector may result in therapeutically significant production of clinically important gene products in liver cells of mammals.

540-O

PSEUDOEXON EXCLUSION BY ANTISENSE THERAPY IN 6-PYRUVOYL-TETRAHYDROPTERIN SYNTHASE DEFICIENCY

Brasil S[1], Meili D[2], Rassi A[2], R.Desviat L[3], Pérez B[3], Ugarte M[3], Thony B[2]
[1]CEDEM CBMSO UAM FCT (SFRH/BD/45753/2008), Madrid, Spain
[2]Div Clin Chem Biochem Univ Child Hosp, Zurich, Switzerland
[3]CEDEM CBMSO Dept Biología Molec UAM-CSIC, Madrid, Spain

The number of mutations identified in deep intronic sequences that activate disease-causing pseudoexon-inclusion in mRNAs is increasing. We report the effect of cellular antisense therapy to suppress pseudoexon activation in primary dermal fibroblasts from three patients with mutations in the gene encoding the 6-pyruvoyltetrahydropterin synthase (PTPS), which leads to tetrahydrobiopterin deficiency (OMIM 261640). Patient MD130 presented an insertion of 45 nt between exon 2 and 3 (r.163_164ins45) due to an intronic deletion (g.3760_3816del57) located in the 3′splice site of an inserted antisense Alu pseudoexon. Similarly, patient MD335 had inserted the same antisense Alu sequence due to a mutation (c.164–716A>T) located in the 5′splice site of the pseudoexon. In patient MD96, a 79-nt pseudoexon between exons 1 and 2 was activated by an A>T substitution (c.84–322A>T) at the 3′ end of a LINE-2 sequence (r.83,-84ins79). Antisense morpholino oligonucleotides directed to the 3′ or 5′splice sites of the corresponding pseudoexons were designed to block intronic insertions into the mRNA of all three patients. Twenty-four hours post transfection, mRNA was isolated and transcriptional profiling analysis was performed. The cDNA pattern indicated that in all three cases a dose and sequence specific recovery of normal splicing was achieved. Furthermore, PTPS enzyme activity in all three patients' fibroblasts was recovered to normal values 2–3 days posttransfection. In addition the pterine levels after antisense transfection was within the control range, and the amount of immunodetected protein also increased. These results represent another excellent example of pseudoexon exclusion therapy using antisense morpholino oligonucleotides in inherited metabolic disease.

541-O

DIRECT INJECTIONS OF HDAd INTO THE LIVER IS AS EFFECTIVE BUT LESS TOXIC THAN INTRAVENOUS INJECTION FOR CRIGLER-NAJJAR SYNDROME GENE THERAPY

Vetrini F[1], Pastore N[2], Grove N[1], Palmer D[1], Ng P[1], Brunetti-Pierri N[3]
[1]*Dept. Mol & Hum Genet, BCM, Houston, United States*
[2]*TIGEM, Naples, Italy*
[3]*Dept. Pediatrics, Federico II University, Naples, Italy*

Background: Crigler-Najjar syndrome type I is an inborn error of bilirubin metabolism due to mutations in the uridine diphospho-glucuronosyl transferase (UGT1A1) gene. Affected patients have life-threatening increase in serum bilirubin and are often treated with liver transplantation. Life-long phenotypic elimination of hyperbilirubinemia has been achieved with a single intravenous injection of HDAd expressing UGT1A1 into Gunn rats, the animal model of Crigler-Najjar syndrome. However, this route of vector administration can activate an acute inflammatory immune response with potentially lethal consequences. To overcome this, we have investigated safety and efficacy of direct injections of HDAd into the liver parenchyma.
Methods: HDAd expressing UGT1A1 was administered at the doses of 5x10e11 vp/kg or 1x10e12 vp/kg either by intravenous or intraparenchymal injections into Gunn rats.
Results: Both doses and routes of vector administration resulted in correction of the hyperbilirubinemia in Gunn rats. At 5x10e11 vp/kg, platelets remained in the normal range for both intravenous and intraparenchymal injections. However, at 1x10e12 vp/kg, a reduction in platelets was observed for intravenous, but not intraparenchymal injections. Similar elevations in serum IL-6 were observed at 5x10e11 vp/kg for both intravenous and intraparenchymal injections. However, at 1x10e12 vp/kg greater elevation in serum IL-6 was observed for intravenous compared to intraparenchymal injections. Intravenous injection resulted in higher levels of serum LDH and reduced vector splenic uptake as compared to intraparenchymal injections.
Conclusions: Direct injections into the liver parenchyma improve the therapeutic index of HDAd and may represent a safe and efficient approach for liver-directed gene therapy of Crigler-Najjar syndrome.

542-O

COST OF DIET THERAPY IN PHENYLKETONURIA IN 10 EUROPEAN CENTERS

Lammardo AM[1], Bélanger-Quintana A[2], Dokoupil K[3], Gokmen-Ozel H[4], MacDonald A[5], Motzfeldt K[6], Nowacka M[7], Robert M[8], van Rijn M[9], Ahring K[10]
[1]*S. Paolo Hosp, Univ of Milan, Milan, Italy*
[2]*Ped Hosp Ramon y Cajal, Madrid, Spain*
[3]*Dr von Hauner Child Hosp Univ Munich, Munich, Germany*
[4]*Dept Nutr Dietetic Hacettepe Univ, Ankara, Turkey*
[5]*The Children's Hosp, Birmingham, United Kingdom*
[6]*Oslo Univ Hosp Rikshospitalet,, Oslo, Norway*
[7]*Nat Res Inst of Mother and Child, Warsaw, Poland*
[8]*Hôp Univ des Enfants Reine Fabiola, Brussels, Belgium*
[9]*Univ Med Center, Groningen, Netherlands*
[10]*Dept of PKU, Kennedy Centre, Glostrup, Denmark*

Background: The treatment of phenylketonuria (PKU) varies throughout Europe, but little is known concerning the variability of costs of medical foods (amino acid supplements) and special low-protein foods.
Objectives: We surveyed costs of these products for patients attending 10 European centers.
Methods: One centre routinely managing patients with PKU from each of 10 European countries (see author list for centers/countries) estimated the cost of these products for patients of 2, 8, 15 or 30 years of age, according to local retail costs, local or national guidelines (or, for comparison, a single guideline).
Results: Costs of protein substitutes, based on the standard age-specific amount prescribed, were variable between countries, with very different reimbursement systems operating. Mean (range) of costs for medical foods were: age 2 8613 (2968–13381); age 8 14646 (2968–25057); age 15 15812 (7687–25492); age 30 15976 (7512–31602). In general, costs of protein substitutes were highest in The Netherlands and Norway and lowest in Turkey and Poland. Medical food was the most expensive item (generally more than ten-fold more expensive than special low-protein foods).
Conclusion: Patients face significant cost/reimbursement issues with amino acid supplements and special low-protein foods across Europe. Our findings may assist patients to obtain their special foods and supplements in a more equitable manner than at present.

543-O

EFFECT OF SIMPLIFIED PKU DIET ON PHENYLALANINE LEVELS IN PATIENTS WITH HYPERPHENYLALANINAEMIA

Jacobs JP[1], Zimmermann M[2]
[1]*Div Gastr. & Nutr, Univ Child Hosp, Zurich, Switzerland*
[2]*Div Metab Dis, Univ Child Hosp, Zurich, Switzerland*

The current treatment of hyperphenylalaniaemia (HPA) is limited to a lifelong phenylalanine-restricted diet in particular in the severe form. Food must be scaled and phenylalanine calculated. This might impair quality of life significantly. We have implemented a simplified diet handling and we recommended three portions (handful) of fruits and two portions of vegetables. Low-protein foods could be eaten freely. Scaling and calculation was reduced to only few foods.
To evaluate the effect of the change, all patients diagnosed from 1997 to 2009 were reviewed retrospectively for continuous monitoring of phenylalanine levels and for diet compliance on a "classical" and on a "simplified" diet.
Totally 83 patients were followed by dietitians and entered into an anonymous registry: 7/83 patients were on "simplified" diet from birth on. 22 refused to change their diet. 54/ 83 switched from "classical" to "simplified" diet including 29 classical PKU, 14 moderate PKU, 7 mild PKU and 4 mild HPA.
For all those who switched diet, the mean phenylalanine-levels before and after change were 326 and 360 μmol/l (p=0.062). Mean phenylalanine-levels in the group of classical PKU were 372 before and 393 μmol/l after switching. In the unchanged classical diet cohort mean phenylalanine levels were 303 μmol/l and in the simplified diet from birth cohort 409 μmol/l.
Our results clearly indicate that a simplified PKU diet has no negative effect on phenylalanine control in patients with HPA independent of severity of the phenotype. Further research will be necessary to confirm these findings in other HPA populations.

544-O

CULTURALLY SENSITIVE CARE OR WHY WE NEED NEW PARADIGMS IN CHRONIC CARE MANAGEMENT?

Stockler SS[1], Ipsiroglu OI[1]
[1]*British Columbia Children's Hospital, Vancouver, Canada*

Background: "Compliance" to diet is the main determinant of PKU disease management and successful outcome. "Diet" as a medical treatment concept places considerable burden on families, as it may interfere with basic cultural practices and traditions. Thus PKU represents an exemplary disease model to study cultural aspects of "compliance" and quality of care in chronic disease management.
Aim: To explore "compliance"/adherence affecting factors using the PKU-Model and work out strategies to optimize treatment of patients.
Methods & Results: Medical analysis shows children from Turkish immigrant families in Austria to have worse control of PHE-levels and neurocognitive-outcome compared to others with PKU. Psychological/social-work analysis reveals differences in disease related knowledge, coping strategies, disease acceptance as a-matter-of-fate, and dependence on medical professionals. Medical-anthropological analysis of semi-structured interviews reveals that individual disease perception and outcome result in an accumulation of communication barriers with professionals which produce adverse interpretations of therapeutic goals from both sides.
Conclusions: Based on our analyses we developed 'culturally sensitive' patient care criteria: Exploration of individual disease perception and cultural background; use of interpreter services as a standard; respect individuals as part of their sociocultural surrounding; recognition of the mutual understanding that patients and health care providers create, about the course of care through interaction in constructing a course of treatment that recognizes contextually relevant socio-cultural information; 'empathy' as a new 'virtue' bridging cultural gaps between professionals and clients. We further suggest the implementation of 'case managers as navigators' in challenging situations.

545-O

GROWTH AND PROTEIN INTAKE IN PHENYLKETONURIA: RESULTS OF 398 TURKISH CHILDREN

Gokmen-Ozel H[1], Buyuktuncer Z[1], Koksal G[1], Kilic M[2], Dursun A[2], Kalkanoglu-Sivri S[2], Tokatli A[2], Coskun T[2]
[1]Hacettepe Univ, Dept Nutr Diet, Ankara, Turkey
[2]Hacettepe Univ, Dept Paed, Metab Unit, Ankara, Turkey

Background: The relation of growth and blood phenylalanine control and protein intake in phenylketonuria is a part of an ongoing debate. In Turkey there was no data on growth and protein intake in phenylketonuria. In this single centre, retrospective study, the aim was to evaluate z scores and their relation to total protein intake and blood phenylalanine levels.
Methods: Three hundred and ninety eight children (220 boys, 178 girls) with phenylketonuria were recruited. The median age was 4.6 years (1.7 month to 18 years). Exclusion criteria included: not on dietary treatment, pregnant, 19 years and older and lost to follow-up. Weight for height (WHZ), weight for age (WHA), height for age (HAZ) and body mass index (BMIZ) z scores were calculated using WHO Anthro programme. A-1-day dietary recalls were obtained. The latest phenylalanine levels were included. The median total protein intake was 28.1 g/day (8.6 to 68.3 g/day) and blood phenylalanine was 511 mmol/L (3.6 to 3114 mmol/L).
Results: The HAZ scores (-0.96) was significantly lower compared to WHZ (0.37), WAZ (-0.35) and BMIZ (0.33) scores (p=0.000). A significant correlation was found between total protein intake (g/day) and WHZ, WAZ, HAZ, BMIZ scores (r=0.545, p=0.000; r=0.487, p=0.000; r=0.240, p=0.000; r=0.276, p=0.000, respectively). No correlation was found between blood phenylalanine levels and WHZ, WAZ, HAZ, BMIZ scores (r=-0.31, p=0.648; r=-0.51, p=0.361; r=0.015, p=0.769; r=-0.78, p=0.122, respectively).
Conclusions: This study suggests that WHZ, WAZ, HAZ and BMIZ scores may be related to total protein intake in optimizing growth in phenylketonuria.

546-P

MONITORIZATION OF DIFFERENT ELEMENTS IN BLOOD SAMPLES FROM PATIENTS WITH INBORN ERRORS OF METABOLISM

Tondo M[1], Lambruschini N[1], Gomez-Lopez L[1], Gutierrez A[1], Moreno J[1], Garcia-Cazorla A[1], Perez-Dueñas B[1], Pineda M[1], Campistol J[1], Vilaseca MA[1], Artuch R[1]
[1]Inborn Errors of Metab Unity, Esplugues de LLobregat, Spain

Background: Patients having inborn errors of intermediary metabolism (IEM) may present element deficiencies related with dietary treatment.
Objectives: Our objective was to study several elements (Co, Cu, Zn, Se, Mn, Mo and Mg) in patients with IEM with and without dietary treatment and to compare these results with those established in a healthy paediatric population.
Material and methods: We studied 72 patients with IEM (age range 2 months- 44 years; average 11.3 years), with and without protein-restricted dietary treatment. Control values were established in 92 subjects (age range 1 day to 42 years; average 11.1 years). Dietary treatment consisted of a natural protein-restricted diet supplemented with a special formula depending on the specific metabolic defect. Samples were analyzed with an Agilent 7500ce-ICP mass spectrometer.
Results: Significant differences were observed when we compared patients under dietary treatment and control values for Se and Co ($p < 0.0001$). No differences were observed for the other elements when comparing the different groups. For Se and cobalamin, the daily intake of our patients (Se: 48 μg/day; Cobalamin: 3.5 μg/day) was slightly higher than RDAs (40 μg/day and 1.8 μg/day, respectively).
Conclusion: Overall, IEM patients under dietary treatment showed significantly lower selenium values in spite of correct supplementation, reinforcing the idea that these patients should be regularly monitored, at least for this element. The values of the other elements evaluated in IEM patients both with and without dietary treatment were similar to control values.

547-P

IMPLICATIONS FOR EXTENDED DIETARY GUIDELINES WITH NEW MEDICAL OPTIONS IN PKU

van Rijn M[1], ter Horst NM[2], de Boer F[1], Bosch AM[2], Heiner-Fokkema MR[3], Duran M[4], van Spronsen FJ[1]
[1]Beatrix Child Hosp Univ Med Center Gron., Groningen, Netherlands
[2]Dep Ped, Met Dis, Emma's Child Hosp AMC, Amsterdam, Netherlands
[3]Dep Lab Med, Univ Med Center Groningen, Groningen, Netherlands
[4]Dep Clin Chem, Met Dis, Emma's Child H, AMC, Amsterdam, Netherlands

Background: Existing guidelines for PKU were published before the approval to treat sapropterin (BH4) responsive PKU-patients ≥4 years with saproterin. BH4 effectively lowers blood phenylalanine levels in a selection of PKU-patients.
Objective: To study the need of adaptation of existing guidelines due to the introduction of BH4.
Methods: In line with recent guidelines, a 2-phase protocol for BH4 responsiveness was developed in The Netherlands. In phase 1, a 48-hours BH4 loading test (BLT) is performed. When positive in phase 2, the optimal amount of BH4, Phe tolerance and adaptation of protein supplementation is established. Finally, the contribution to individual treatment is evaluated according to the protocol criteria.
Results: In UMCG 87 PKU patients (4–44 years) were invited, 62 (≥18:26,≤18 years:36) were tested between 10-2009 and 02-2010. Results in ≤18 years were combined with 15 AMC-patients. Prescribed natural protein in the regular diets was 4.5–35 g/day (200–1600 mg Phe) in ≤18 years, and 6–40 g/day (270–1800 mg Phe), respectively. Extra Phe intake to achieve Phe >400 μmol/L to enable adequate BLT was necessary in 10 patients ≥18, and 32 ≤18 years. Patients were prescribed extra Phe as natural protein due to logistic problems with pharmaceutical Phe. Since 03-2010 till 05-2010, 36 of the 47 BH4-positive tested patients have enrolled fase 2.
Conclusion: Besides clear data on BH4-responsiveness in terms of blood Phe concentration and medication compliance, strict instruction and evaluation of dietary intake is crucial in correct judgement of effectiveness of new treatment options as BH4.
1) Blau et al. MGM 2009

548-P

BODY COMPOSITION AND MARKERS OF METABOLIC SYNDROME IN ADULTS WITH PKU

Rocha JC[1], Almeida MF[1], Soares G[1], Bastos J[2], Guimarães JT[3], Borges N[4], van Spronsen FJ[5]
[1]Centre of Medical Genetics JM - INSA, Porto, Portugal
[2]Hyg Epidem, Fac Med, Inst P Health, UP, Porto, Portugal
[3]Biochem, Fac of Medicine, UP, Sao J Hosp, Porto, Portugal
[4]Fac of Nutr and Food Sci, Univ of Porto, Porto, Portugal
[5]Beatrix Child Hosp, UMC of Groningen, Groningen, Netherlands

Background: The special diet in PKU may increase the risk of obesity. We aimed to study the prevalence of overweight, obesity and metabolic syndrome (MS) in adults with PKU, and their associations with body composition (BC) and c-reactive protein (CRP).
Methods: Twenty seven adults aged 18–38 years were studied on anthropometry, BC, blood pressure, nutritional intake and analytical determinations (including CRP as inflammation indicator, and HDL, glucose and triglycerides, as biochemical components of MS), using the National Cholesterol Education Program (NCEP) criteria. Association between BMI and quantitative variables was assessed using Pearson correlation coefficient. Quantitative variables were compared using the t-test for independent samples, or the Kruskal-Wallis test, when appropriated.
Results: Overweight and obesity were present in 44.4% of our patients. Using NCEP classification, 8 patients (29.6%) had increased waist circumference (WC) and 2 (7.4%) MS. BMI showed significant positive association with fat mass percentage (r=0.97; p<0.001 in women and r=0.96; p<0.001 in men), WC (r=0.88; p<0.001) and CRP (r=0.44; p=0.025). Patients with overweight and obesity had a higher body fat percentage (36.3 vs 21.7; p<0.001), and lower fat free mass percentage (63.7 vs 78.3; p<0.001). Medians of natural protein and protein substitute intake were not significantly different in patients with overweight/obesity compared to the others.
Conclusion: Notwithstanding the special diet with a relatively large amount of fat and carbohydrates, the prevalence of obesity is relatively low. Therefore, PKU patients should be studied more intensively to teach us how to prevent obesity and MS in the general population.

549-P

SECONDARY BIOTIN DEFICIENCY OBSERVED IN TWO JAPANESE INFANTS DUE TO CHRONIC USE OF HYPOALLERGIC INFANT FORMULA

Watanabe Y[1], Ohya T[2], Ohira T[2], Okada J[2], Fukui T[3], Watanabe T[3], Inokuchi T[1], Yoshino M[2], Matsuishi T[2]
[1]*Res Inst of Medical MS, Kurume Univ, Kurume, Japan*
[2]*Dept Pediat/Child Health. Kurume Univ, Kurume, Japan*
[3]*Sch Hum Science/Environ, Hyogo Univ, Himeji, Japan*

Background: Biotin is a water-soluble vitamin and is widely distributed in many foods. Biotin functions as a cofactor for acetyl-CoA, methylcrotonyl-CoA, propionyl-CoA, and pyruvate carboxylases, and is important in fatty acid synthesis, amino acid catabolism, and gluconeogenesis. Symptoms of biotin deficiency include dermatitis, hypoglycemia, organic acidemia, and developmental delay. Although biotin deficiency is believed to be rare, there have been several reports of symptomatic infants with secondary biotin deficiency in Japan because biotin is not supplemented in infant formula.
Objective: To report asymptomatic infants with biotin deficiency fed with infant formula without supplementation of biotin.
Case Reports: Patient 1 is an 8-month-old Japanese female, ex-33 week premature infant, with seizures and history of idiopathic gastric rupture. Hypoallergenic formula was implemented since age 20 days. Her physical exam was unremarkable. Laboratory studies including metabolic acidosis, a low free and high C3 and C5OH carnitines, and elevated urine 3-OH-propionic acid and 3-methylcrotonylglycine, prompted us to measure biotin levels (plasma: free 0.3 ng/ml ref. 0.4–1.1; urine < 2 ref: 4–25). Patient 2 is an 11-month-old, ex-33 week premature, Japanese male. He was solely fed with lactose free hypoallergenic formula until 7 months when small solid foods were started. His physical exam was unremarkable but mild metabolic acidosis was noted. Laboratory studies including plasma and urine biotin levels, splasma acylcarnitines and urine organic acids were consistent with biotin deficiency.
Conclusion: Asymptomatic infants with biotin deficiency might be not rare in Japan. Supplementation of biotin in hypoallergenic infant special formula is essential for infant's well-being.

550-P

THE EVOLUTION OF THE SSIEM-DIETITIANS GROUP (SSIEM-DG)

Link R.M.[1]
[1]*Chair of SSIEM-DG, Wiesbaden, Germany*

The SSIEM-DG is a networking group for dietitians working in the field of inherited metabolic diseases (IMD). The group was initially founded in London, 2008 as the European Metabolic Dietitians Group but this year was established as the first SSIEM working group and has representation on the SSIEM Council. The goal of SSIEM-DG is to develop IMD dietetics to a highly scientific and professional level for the benefit of all SSIEM dietitians and their countries.
The group will be co-ordinated by a committee with corresponding members from represented countries. Membership is open to all dietitian members of the SSIEM. Currently SSIEM-DG has 27 members representing 14 countries: Austria, Belgium, Denmark, England, France, Germany, Ireland, Netherlands, Norway, Poland, Portugal, Switzerland, Sweden and Turkey. Communication will be through meetings and the newly developed web page on the SSIEM website (www.ssiem.org/dg).
The objectives of the Group are to develop training standards for IMD dietitians, facilitate communication and educational courses, share knowledge, discuss and publicize best practice to ultimately improve the nutritional management and long-term outcome of IMD patients.
The group has already started evaluating the metabolic dietetic training provision of its member countries. This work was presented at the ICIEM 2009. As a consequence of the limited IMD dietetic training opportunities and wide differences in training standards, the SSIEM-DG plans to develop core IMD training standards and validate the training of IMD dietitians. SSIEM–DG aims to promote the important role of metabolic dietitians as key members of all IMD teams.

551-P

THE ANALYSIS OF DAILY NUTRITIONAL RATIO OF PKU CHILDREN

Kaluzny L[1], Drzymala-Czyz S[1], Dudek A[1], Walkowiak J[1], Cichy W[1]
[1]*Dept of Gastroent and Metab, Med Univ, Poznan, Poland*

Phenylketonuria (PKU, OMIM 261600) is the most frequent inborn error of amino acid metabolism. Diagnosis by newborn screening and implementation of low phenylalanine diet in the neonatal period and beyond have resulted in normal neurological and intellectual development in PKU patients. In this diet 60–80% of daily protein intake is from phe-free l-aminoacid mixture, 20–40% from fruits, vegetables and special manufactured low protein products. Parents of treated in our metabolic outpatients clinic patients analyzed (like as in most of European countries) phenylalanine, protein and energy intake.
The aim of the study was the evaluation of daily nutritional ratio (DNR) in PKU children in the preschool and school age. Diet of children (n-17; F=13, M=4, aged 2–9, mean 5,06) was analyzed on the basis of nutritional records, collected for 7 days. Phenylalanine, energy and protein intake in DNR was satisfactory. Diets of PKU children were unbalanced with reference to calcium, potassium and vitamin D. Zn, Cu, vitamin A and B6 intake was higher than recommended. Lipids, carbohydrates, Na, P, Mg, Fe, vitamin E, B1, B2, PP, B12, and C intake was adequate.
The result of our study suggests that children with PKU are under risk of disorders connected with unbalanced diet.

552-P

HIGH PLASMA FOLATE LEVELS IN CHILDREN WITH PKU

Lilje R[1], Almaas R[1], Bliksrud YT[2], Motzfeldt K[3], Joergensen JV[3]
[1]*Paed Div, Oslo Univ Hosp, Rikshospitalet, Oslo, Norway*
[2]*Med Biochem, Oslo Univ Hosp, Oslo, Norway*
[3]*Paed Reasearch Div, Oslo Univ Hosp, Oslo, Norway*

Background: Patients with PKU get most of their needs for protein, vitamins and minerals covered by the protein substitute. Several of the protein substitutes are very high in folic acid. Resent reports show an increased cancer risk related to high intakes of folic acid.
Objectives: Present plasma folate levels and compare this to folic acid intake from the protein substitutes in PKU children.
Patients and Methods: Fifty-five PKU patients aged 2 to 19 years (median age 11 years), all seen in our clinic the last 9 months, have been checked for plasma folate levels. Intake of folic acid was calculated from amount of protein substitute used.
Results: Mean intake of folic acid from the protein substitute was 460 μg/day (range 52-1003 μg/day). Nineteen of the patients (35 %) had an intake above upper recommended level for their age group. Forty-seven patients (85 %) had a folate level above 27 nmol/L (ref. value: 7.1–27 nmol/L). Median plasma folate level was 54 nmol/L (range 17—above 54 nmol/L). Twenty-eight patients (51 %) had a plasma level of folate above 54 nmol/L. None of the patients had plasma values below the lower reference level.
Conclusion: 35 % of the patients had a folic acid intake above the recommended upper level. 85 % of the patients had plasma folate levels above the upper reference range, none had low levels. Intakes of folic acid from protein substitutes are high, lowering the amount of folic acid in the protein substitutes should be considered.

553-P

HIGH PLASMA FOLATE LEVELS IN ADULTS WITH PKU ON PROTEIN SUBSTITUTE

Stoelen LH[1], Almaas R[1], Bliksrud YT[2], Mathisen P[3]
[1]*Paed Div, Oslo Univ Hosp Rikshospitalet, Oslo, Norway*
[2]*Med Biochem, Oslo Univ Hosp Rikshosp, Oslo, Norway*
[3]*Dep Gen Med, Oslo Univ Hosp Rikshosp, Oslo, Norway*

Background: Several protein substitutes for PKU contain high amounts of folic acid. Concern has been raised about the safety of high levels of folic acid, especially in relation to cancer risk.
Objectives: To compare intake of folic acid from protein substitutes with plasma folate levels among adult patients with PKU.
Patients and Methods: Plasma folate levels were measured in seventy adult patients with PKU at their annual assessment at our clinic from January 2008 until March 2010. Patients were asked about type and amount of protein substitute used, from which intake of folic acid was calculated.
Results: Forty-three patients were compliant with their protein substitute in either powder or liquid form. Median intake of folic acid from protein substitute was 747 µg/day (range 300–1120). Median plasma folate level was 50 nmol/L (range 21- above 54 nmol/L). Twelve patients (28%) had plasma folate levels > 54 nmol/L (reference values 7.1–27 nmol/L). Twenty-seven patients partially or non-compliant with their protein substitute or taking protein substitute tablets with little or no added folic acid had plasma folate levels with a median of 15 nmol/L (range 6.1–28). Only one patient had low plasma levels. Median plasma folate level was significantly lower in the partially or non-compliant group compared to the compliant group ($p<0.0001$).
Conclusion: Ninety-three percent of patients compliant with their protein substitute had plasma folate levels above the upper reference value. Reducing levels of folic acid in several protein substitutes should be considered.

554-P

FEEDING DIFFICULTIES IN CHILDREN WITH INBORN ERRORS OF PROTEIN & AMINO ACID METABOLISM

Evans S[1], Alroqaiba N[1], Daly A[1], Neville C[1], MacDonald A[1]
[1]*Birmingham Children's Hospital, Birmingham, United Kingdom*

Background: In children with inherited metabolic disorders (IMD) feeding difficulties are assumed to be inherent in many conditions, but there is little evidence describing their frequency.
Objective: To describe feeding patterns/difficulties among children with IMD on protein restricted diets (except phenylketonuria).
Methods: An observational, case-control study of 20 IMD children (median age: 2.7y [range: 1–6y]) compared with retrospective data on 15 healthy children (CG) aged 1–5y (median 3.0y). Carers completed a feeding assessment questionnaire, and 3 video recordings were taken of children eating at home.
Results: 50% of children were tube fed +/- oral diet. Main feeding problems in the IMD group (compared with CG) were poor appetite (55% vs 7%), limited food variety (55% vs 27%) and lengthy mealtimes (70% vs 20%). 70% of IMD children had >1 feeding problem compared with 67% (n=10) of the CG with no feeding problems. The IMD group was more likely to experience vomiting, gastrointestinal symptoms, eat fewer meals/snacks, demonstrate negative mealtime behaviours, become distracted during mealtimes and self-fed less often. While median meal duration was similar for the 2 groups (18 min IMD vs 16 min CG); the CG ate at least twice the quantity of food (3.4 mouthfuls/min vs 1.5 mouthfuls/minute). Carers were less likely to talk to IMD children (median 7x/10 min vs 17x/10 min) during mealtimes, and 83% of IMD children regularly ate alone.
Conclusions: In children with IMD on protein restrictions, feeding difficulties were common. It is important to reinforce the importance of the social aspects of feeding at all times.

555-P

DIETARY MANAGEMENT OF UREA CYCLE DISORDERS: UK PRACTICE

Adam S[1], Champion H[2], Dawson S[3], Daly A[4], Dixon M[5], Dunlop C[6], Eardley J[7], Evans S[4], Ferguson C[8], Lowry S[9], MacDonald A[4], Maritz C[10], Micciche A[11], Robertson L[12], Stafford J[5], Terry A[13], van Wyk K[7], White F[14], Wildgoose J[15]
[1]*Royal Hospital for Sick Children Glasgow, Glasgow, United Kingdom*
[2]*Addenbrookes Hospital, Cambridge, United Kingdom*
[3]*Royal Hosp for Sick Children Edinburgh, Edinburgh, United Kingdom*
[4]*Birmingham Children's Hospital, Birmingham, United Kingdom*
[5]*Great Ormond Street Hosp for Children, London, United Kingdom*
[6]*Glasgow Royal Infirmary, Glasgow, United Kingdom*
[7]*Evelina Child Hosp, Guy's & St Thomas', London, United Kingdom*
[8]*Newcastle General Hospital, Newcastle, United Kingdom*
[9]*Sheffield Children's Hospital, Sheffield, United Kingdom*
[10]*National Hosp for Neurol & Neurosurg, London, United Kingdom*
[11]*Guy's & St Thomas' Hospitals, London, United Kingdom*
[12]*University Hospitals, Birmingham, United Kingdom*
[13]*Alder Hey Children's Hospital, Liverpool, United Kingdom*
[14]*Central Manchester University Hospitals, Manchester, United Kingdom*
[15]*Bradford Teaching Hospital, Bradford, United Kingdom*

Introduction: There is no published research describing the dietary management of Urea Cycle Disorders (UCD) in the UK.
Aim: To describe dietary practices in 14 UK metabolic centers.
Methods: Cross-sectional dietary data on 165 patients (NAGS n=3; CPS n=6; OTC n=70; citrullinaemia n=38; argininosuccinic aciduria n=36; arginase deficiency n=12) on prescribed protein restricted diets, (72% [n=118] aged 0–16y; 28% [n=47] >16y) was collected by questionnaire from dietitians representing 14 metabolic centers.
Results: Prescribed mean protein intake decreased with age (0–6 m: 2 g/kg/day; 7–12 m: 1.7 g/kg/day; 1–10y: 1.3 g/kg/day; 11–16y: 0.9 g/kg/day and >16y: 0.8 g/kg/day) with little variation between disorders. Adult protein prescription ranged 0.5–1.3 g/kg/day (40–60 g daily). In the previous 2y, 30% were given Essential Amino Acid supplements (EAAs) (CPS n=2; OTC n=18; citrullinaemia n=15; argininosuccinic aciduria n=6; arginase deficiency n=9). Centers prescribed EAAs for: inadequate protein intake (n=9), poor metabolic control (n=9), low quantitative plasma amino acids (n=9), and from diagnosis in all patients (n=1) or only in severe defects (n=2). Only 4% (n=6) were given branch chain amino acid supplements. Tubes were used for feeds (26%, n=43) or medications only (3%, n=5); 77% were gastrostomies. Additional energy was provided as oral supplements (18%, n=29) and prescribable low protein foods (27%, n=44). Vitamin and mineral supplements were routinely given by 64% (n=9) of centers. No centre had formal written dietary guidelines.
Conclusions: Protein restriction is the principle dietary treatment for UCD in the UK, with EAA supplements prescribed mainly on clinical need. National dietary management guidelines, supported by all centers are required.

556-P

HOME VISITS IN PHENYLKETONURIA: A-12-MONTH LONGITIDUNAL STUDY

Gokmen-Ozel H[1], Buyuktuncer Z[1], Arpaci N[1], Kasapogullari P[1], Koksal G[1], Kalkanoglu-Sivri HS[2], Coskun T[2]
[1]*Hacettepe Univ, Dept Nutr Diet, Ankara, Turkey*
[2]*Hacettepe Univ, Dept Paed, Metab Unit, Ankara, Turkey*

Background: Some inherited metabolic disorders centers in Europe provide home visits for patients with phenylketonuria. In Turkey none of the health professionals do home visits routinely. In a-12-month, single centre, longitudinal study, the aim was to evaluate the effects of dietary education about children's blood phenylalanine (phe) levels given to the caregivers in their home environment.
Methods: Thirty-six children (15 boys, 21 girls) with phenylketonuria were recruited. The median age was 8 years (2 to 12 years). Each caregiver was visited on consecutive 3 days and given a detailed dietary education by a paediatric dietitian at baseline. Fasting morning skin puncture blood samples were collected on the Guthrie cards by the caregivers for three days during baseline and at the end of weeks 1, 4, 12, 24 and 48.
Results: The mean blood phe level obtained from the baseline was 365 mmol/L. The blood phe levels after the 1st week (mean=314 mmol/L) significantly decreased ($p<0.05$). At the end of the 4th week, the blood phe level was still lower (mean=329 mmol/L) than the baseline but the difference was not significant. At the end of the 12th, 24th and 48th weeks, the blood phe levels significantly increased ($p<0.05$) compared to baseline (means=385 mmol/L, 447 mmol/L and 486 mmol/L respectively).
Conclusions: Improvements in blood phe control can be achieved by intense and continuing education, regular and frequent blood testing, recording of food intake, and regular and frequent visits at home. Children who have poor control should be evaluated in their home environment.

557-P

A MODIFIED ATKINS DIET FOR THE TREATMENT OF CITRIN DEFICIENCY

MacDonald A[1], Daly A[1], Neville C[1], Preece MA[1], Vijay S[1], Hendriksz C[1], Chakrapani A[1]
[1]*Birmingham Children's Hospital, Birmingham, United Kingdom*

Background: Citrin deficiency (CD) presents either as neonatal intrahepatic cholestasis (NICCD) or adult onset Citrullinaemia type 2 (CTLN2). The underlying biochemical defect, the mitochondrial aspartate/glutamate carrier, is caused by a mutation in the SLC25A13 gene. In CD, high protein foods, particularly rich in aspartate (+asparagines) and arginine could potentially stimulate urea synthesis. In contrast, carbohydrate restriction may cause increased NADH production, reduce likelihood of lipid metabolism derangement and depletion of cytosolic aspartate. Aversion to high carbohydrate foods and protein-rich food preference is well-recognized. It is recommended patients consume a diet providing the following energy distribution (% of total kcal intake): protein: 17–21%; fat 40–47%; and carbohydrate 33–40%.
Case study: We describe a 6y old boy with CD, with a 2y history of frequent abdominal pain. He prefers high protein foods; avoids sugar containing foods. His carers report mood changes and abdominal pain with sweet foods. His energy distribution (% kcal) from food at aged 2, 3 and 5y was protein: 19%, 20% and 23%; fat: 42%, 43%, and 51%; and carbohydrate: 39% 37% and 26%. Normoglycaemic was documented by 24 hour continuous blood glucose monitoring. At aged 6y, a modified Atkins diet (energy distribution (% kcal): 30% protein, 65% fat and 5% carbohydrate), was commenced. His abdominal pain reduced substantially (with no hospital admissions since dietary change) with no hyperammonaemia or hypoglycaemia. Weight gain is poor.
Conclusions: The use of a modified Atkins diet is promising in CD, but warrants further study to ensure safety and establish its efficacy.

558-P

PRACTICAL EMERGENCY FEEDING MANAGEMENT IN GA1 DURING ILLNESS

MacDonald A[1], Daly A[1], Neville C[1], Vijay S[1], Hendriksz C[1], Chakrapani A[1]

Introduction: In Glutaric aciduria Type 1 (GA1), intercurrent illness increases the risk of encephalopathic crisis. Emergency management guidelines (Kolker et al 2007) recommend no natural protein, administration of maltodextrin/dextrose, lysine-free, low tryptophan amino acids (LF/LTAA) and carnitine. However, the practical administration of emergency feeds (EF) has received little attention.
Aim: To describe the use of EF in children with GA1 on LF/LTAA.
Methods: 9 children with GA1, median age 3y; range 1–11y, (3 pre-encephalopathic and 6 encephalopathic), had EF that provided 1 g/kg/day LF/LTAA, together with age-appropriate maltodextrin. The LF/LTAA was pre-measured in 5 g protein equivalent sachets (GA amino acids; Vitaflo International) together with age-appropriate, pre-measured, glucose polymer sachets (SOS; Vitaflo International). Six of 9 children had EF administered by 24 h continuous home enteral tube feeds (unless feed intolerance or pyrexia); the others drank it orally. With feed intolerance, LF/LTAA (1 g/kg/day) were given by continuous enteral tubes with concurrent IV 10% glucose.
Results: since LF/LTAA addition, EF have been used at least 45 times over 2y (usage per child 6; range 1–10). 33% (n=15) of intercurrent illnesses led to hospital admission, spread evenly between patients with and without home tube feeds. Encephalopathic deterioration was observed in one child with a second pathology associated with white matter degeneration. No others have deteriorated. The EF, using pre-measured ingredients, was easy to calculate by professionals and quick to prepare by caregivers.
Conclusions: In GA1, EF are commonly used. To aid efficacy and safety, it is essential they are easy to prepare and administer.

559-P

ERRORS IN PRESCRIPTIONS OF SPECIAL LOW PROTEIN FOODS: TIME TO REVIEW THE NATIONAL PRESCRIPTION SYSTEM

MacDonald A[1], Roberts C[2], Stringer K[2], Daly A[1], Neville C[1]
[1]*Nutricia, Trowbridge, United Kingdom*

Introduction: Systems for the supply and prescription of low protein foods differ throughout Europe. In the UK, there are approximately 120 low protein foods. Each food is individually approved and is available 'free of charge' via the UK National Health Service to many groups of people. Patients access these foods via their local, non-specialist doctor (general practitioner: GPs), who issue medical prescriptions for each food item. The prescriptions are given either to a 'high-street' pharmacist or sent to a home delivery company (HDC), who then dispense the products. A retrospective patient survey (MacDonald et al 2009) identified that patients commonly receive prescriptions for gluten-free foods instead of low protein foods.
Aim: To assess, prospectively, the frequency of written prescription errors with low protein foods.
Method: One HDC (Homeward) monitored the accuracy of all incoming prescriptions from GPs for low protein foods for one IMD treatment centre over 6 months between October 2009 and March 2010.
Results: 1471 low protein food prescriptions were issued in a 6 month period. 3% (n=45) of all prescriptions were incorrectly written for gluten-free foods instead of low protein foods, 3% (n=43) were issued for non-requested low protein products, and 9% (n=129) of prescriptions stated an incorrect product amount.
Conclusions: In the UK, prescription of low protein products via the traditional GP system is associated with regular error. It is time to reappraise and modernize the supply of low protein foods and give control of prescriptions to specialist units caring for patients with IMD.

560-P

TYROSINAEMIA TYPE 1: NATURAL PROTEIN TOLERANCE INCREASES WITH AGE

Daly A[1], Neville C[1], McKiernan P[1], MacDonald A[1]
[1]Birmingham Childrens's Hospital, Birmingham, United Kingdom

Introduction: In Tyosinaemia Type 1 (HT1), there is little information about the natural protein tolerance of patients treated with Nitisinone (Swedish Orphan) and a tyrosine/ phenylalanine-restricted diet, supplemented with protein substitute free of precursor amino acids.
Aim: To study the change in natural protein tolerance with age in well-controlled HT1 children on dietary treatment with Nitisinone.
Methods: In 11 HT1 children (median age 6y, range 2-13y), aiming to maintain plasma tyrosine between 200–400 μmol/l and plasma phenylalanine > 30 μmol/l. Natural protein was increased by 1 g/day when 6 consecutive, weekly plasma tyrosine concentrations were within target range. All patients were taking a tyrosine/phenylalanine-free protein substitute, supplemented with phenylalanine if 2 consecutive fasting concentrations were below target range.
Results: Daily natural protein tolerance increased with age in 55% (6/11) patients. The median g/day of natural protein was: 1–4y, 4 g; 5–6y, 7 g; 7y, 8 g; 8y, 11 g; 10–11y, 13 g; and 12–13y, 16 g. Temporary phenylalanine supplements were prescribed in 64% (7/11) of patients (median 200 mg/d, range 100–650); they were stopped by a median age of 2y (1 to 8y). The median protein equivalent (g/kg/day) from protein substitute declined with age from 0–3y, 2.7; 3–5y, 2.4; 5–6y, 2.2; 6–8y, 1.9; 9y, 1.8; 10y, 1.7; 11y, 1.5; 12y, 1.2; 13y, 0.8.
Conclusion: In HT1, although natural protein increases with age, there are inter-patient differences, possibly related to dietary compliance. Individual tolerance should be regularly reassessed.

561-P

GLOBAL PANDEMIC OBESITY: HOW ABOUT IN PKU?

Anakoc M[1], Kucukkasap T[2], Koksal G[2], Coskun T[2]
[1]Nutr and Diet, Univ Hacettepe, Ankara, Turkey
[2]Hacettepe University Children's Hospital, Ankara, Turkey

Background: Although in children with PKU dietary therapy began in the first days of life and continued throughout life, obesity is being a problem. The aim of this study was to evaluate the relationship between the diagnosis of the illness, birthweight, breastmilk, blood phenylalanine (PA) level, dietary compliance and obesity in school-age children with PKU.
Methods: Time of the diagnosis, birthweight, duration of breast milk, blood PA level of the last three years, body weight and height of 201 children with PKU (57.2% male, 42.8% female) between the age of 6–18 years are learned from the file records. The body weight and height of the children are evaluated in accordance with WHO 2007 standards. Median values of the last three years blood phenylalanine PA measurements have been used as metabolic indicator of dietary compliance.
Results: In 26.9 % of the children's mild obesity, 11.9 % obesity, 56.2 % underweight, 5 % very underweight, 14.4 % shortness has been determined. It hasn't been defined a relationship between the birthweight, duration of breastmilk and the obesity of children, but nearly the half of the children (40 %) who are mild obesity's birthweight is low (<2500 gr). A linear and statistically significant ($p<0.05$) relation has been indicated between the median values of the last three years blood PA measurements, age and BMI (kg/m2) of children.
Conclusion: In particular, in school age of children with PKU the anthropometric measurements such as body height and weight should be monitored in appropriate intervals.

562-P

GALACTOSEMIA TYPE II: A CASE REPORT

Bal MO[1], Monti S[1], Bettocchi I[1], Baronio F[1], Cassio A[1], Cicognani A[1]
[1]Dip Ped, Univ, Bologna, Italy

Galactosemia an inborn error due to mutations on one of three genes: GALT (most frequent and serious clinical expression), codifies for galactose 1-phosphate uridyltransferase, GALK that codifies for galactokinasi cause type 2 Galactosemia (extremely rare, results only in the formation of nuclear cataracts) and GALE that codifies for uridine diphosphate galattose 4-epimerase (two forms: a rare severe deficiency clinically resembles classical galatosemia and a more frequent partial deficiency).
Neonatal screening in Emilia Romagna is perform measuring galactose level (cut-off <15 mg/dl).
Case Report: male born from not consanguineous parents, at 36 weeks of gestational age, weight 2100 g, he presented acute respiratory insufficiency, hypoglycaemia, iperbilirubinemia in Rh-incompatibility.
Mixed feeding.
At neonatal screening galactose value >35 mg/dl.
The baby call to our Centre for the diagnostic confirmation: negative clinical evaluation.
Assessments performed:
Liver and kidney functionality and coagulation: normal;
Abdominal scan: normal; oculistic evaluation: normal;
- Qualitative and quantitative determination of urinary sugars: Positive
- Galactose 1-phosphate uridiltransferasi activity: Normal
Lactose was immediately excluded from his diet.
The molecular analysis evidenced no mutations in GALT gene and the mutation p.P28T (exon 1: c.82C>A) in omozigosis in GALK 1.
This form, if not treated, brings to cataract.
Currently the child has 12 months, an adequate length-weight development and good general clinical conditions. He continues the diet without lactose.
Conclusion: In the suspicion of galactosemia, at the moment of the diagnostic confirmation, it is absolutely important undertake a diet lacking in lactose also in presence of an asymptomatic baby.

563-P

INCREASING DIETARY PHENYLALANINE TOLERANCE WITHOUT THE AID OF NON-DIETARY TREATMENTS

Daly A[1], Neville C[1], MacDonald A[1]
[1]Birmingham Childrens's Hospital, Birmingham, United Kingdom

Introduction: The daily amount of dietary phenylalanine tolerated, although individual, is likely to increase with age and with patients with milder/moderate PKU. However, lifetime inter/intra-patient differences in phenylalanine tolerance are not well defined.
Methods: We report the change in phenylalanine tolerance in 3 diet treated patients with mild-moderate PKU, who have all maintained their annual mean plasma phenylalanine concentrations below 300 μmol/l.
Case 1: A 13y boy, with diagnostic phenylalanine concentration at 720 μmol/l. Between 1–12y of age, the median natural protein intake was 7.5 g/day (0.3 g/kg/day). Median protein equivalent intake from protein substitute (PS) was 2.2 g/kg/d. At age 13y, his natural protein intake was challenged and successfully increased to 24 g/day.
Case 2: A 14y girl, with diagnostic phenylalanine concentration at 680 μmol/l. Between 1–3y of age, the median natural protein intake was 8.5 g/day (0.4 g/kg/day). Median protein equivalent intake from PS was 2.1 g/kg/d. At age 14y, her natural protein tolerance was challenged and also successfully increased to 24 g/day.
Case 3: A 17y girl, with diagnostic phenylalanine concentration at 600 μmol/l. Between 1–16y of age, the median natural protein intake was 14 g/day (0.3 g/kg/day). Median protein equivalent from PS was 1.4 g/kg/d. At age 17y, her natural protein tolerance was increased to 25 g/day.
Conclusion: These 3 children have increased their phenylalanine intake by at least 700 mg/daily, and are likely to tolerate further dietary phenylalanine increase. It is important before using non-dietary treatments in PKU, that maximum phenylalanine tolerance is assessed and tested. All 3 children enjoy their greater dietary freedom.

564-O

DIFFICULTIES IN THE DIETETIC MANAGEMENT OF PATIENTS WITH EARLY CHILDHOOD ONSET: MULTIPLE ACYL CO-A DEHYDROGENASE DEFICIENCY (MADD)

Dalkeith T[1], Dennison B[1], Wilcken B[2], Ellaway C[1], Thompson S[1], Carpenter K[2], Bhattacharya K[1]
[1]*Gen Metab Service, Child Hosp Westmead, Sydney, Australia*
[2]*NSW Biochem Genet, Child Hosp Westmead, Sydney, Australia*

Background: MADD is caused by deficiency of the electron transfer flavoprotein ETF or ETFDH. A broad phenotypic spectrum due to impaired function of multiple dehydrogenases ensues. Dietary recommendations are to avoid prolonged fasting and maintain a fat and protein restricted diet, but our group describes some of the difficulties encountered.
Patients: 4 patients identified by the New South Wales newborn screening programme with riboflavin non-responsive MADD were prospectively treated with a low fat (<25% total energy), low protein diet. Three had symptoms of feeding difficulty, hypotonia and hypoglycaemia in the neonatal period whilst the fourth patient developed hypotonia in the first 6 months of life.
Discussion: All take 3-Hydroxybutyrate and L-Carnitine. The neonatal-onset patients were sensitive to dietary alteration, having frequent acute decompensations with one dying aged 13 months.Two had labile potassium control when unwell and had isolated low plasma histidine and intermittent grossly increased isovalerylcarnitine. One also had significant decrease in plasma glutamine when an essential amino acid supplement was used, improving with complete protein supplementation. The third neonatal onset patient was treated with MCT-rich formula after a severe decompensation aged 5 months, being more stable afterwards, contrary to other reports. The fourth child became profoundly hypotonic aged 6 months and improved upon introducing nocturnal uncooked cornstarch and increasing the 3-hydoxybutyrate dose.
Conclusion: MADD has a broad spectrum of clinical and biochemical manifestations. The ideal dietary composition is not known and may indeed differ between patients. Collaborative work and data analysis between centers may help define ideal treatments better.

565-P

TREATMENT OF ELEVATED TRIGLYCERIDES IN GLYCOGEN STORAGE DISEASE TYPE1A AND HYPERTRIGLYCERIDEMIA WITH MEDIUM CHAIN TRIGLYCERIDES SOURCES

Bernstein LE[1], Burns CE[1], Wilkinson LJ[2], Boney A[3], Balliet J[4], Van Hove J[1]
[1]*Div Metab Dis, the Children's Hospital, Aurora, United States*
[2]*The Children's Hospital, Aurora, United States*
[3]*Duke University Medical Center, Durham, United States*
[4]*The Children's Hospital Wisconsin, Milwaukee, United States*

Medium Chain Triglycerides (MCT) is a treatment for long chain fatty acid oxidation disorders (FAOD). MCT supplementation may also aid in the lowering of triglyceride levels in disorders such as Glycogen Storage Disease 1a (GSD1a) and hypertriglyceridemia. Six patients were identified with either GSD1a or hypertriglyceridemia disorders. Triglyceride levels were monitored prior to and following the introduction of MCT.
Two siblings with GSD 1a showed possible improvement in triglyceride levels with the addition of 1 sachet of MCT Procal. The older sibling had triglycerides of 1527 mg/dl decreasing to 823 mg/dl with most recent levels being 417 mg/dl. The younger had triglycerides of 861 mg/dl decreasing to 208 mg/dl with most recent levels being 266 mg/dl. Another patient with GSD1a had a triglyceride level of 923 mg/dl decreasing to 791 mg/dl. MCT Procal was increased to 2 sachets per day and levels dropped to 410 mg/dl. Most recent levels were 630 mg/dl while still on 2 sachets MCT Procal.
Two patients with hypertriglyceridemia also had improvement in triglyceride levels when a majority of long chain fat was replaced with MCT Procal, Lipistart, or Portagen, nutritionally complete powdered formulas. The first patient had an improvement of triglycerides from over 11,000 mg/dl to 184 mg/dl on MCT Procal. The second patient had an improvement from over 100,000 mg/dl to 786 mg/dl on Lipistart. The third patient had an improvement from over 34,000 mg/dl to 2139 mg/dl.
This early data shows a potentially broader treatment use for MCT in these diagnoses.

566-O

OVERWEIGHT AND OBESITY IN A POPULATION OF CHILDREN WITH PHENYLKETONURIA

Skeath R[1], Mumford N[2], Stafford J[1], Abulhoul L[2]
[1]*Dept Dietetics, Great Ormond St Hosp, London, United Kingdom*
[2]*Metab Med, Great Ormond St Hosp, London, United Kingdom*

Background: Dietary intervention in our Phenylketonuria (PKU) group clinic has generally focused on achieving a restricted phenylalanine diet. Energy intake has been assumed to be appropriate. However, diet histories suggest an excessive intake of energy-dense foods raising concerns of increased Body Mass Index (BMI). We report an analysis of BMI in our ethnically diverse PKU population.
Method: Patient height and weight are routinely measured in clinic. A cross-section of patient BMI was calculated from the most recent clinic measurements (82 boys, 81 girls). BMI was plotted on UK 90 BMI charts and patients classified as overweight or obese using International Obesity Task force definitions.
Results: BMI of 2–15 year olds with PKU were compared to the UK population (shown in brackets); in boys 24.7% are overweight (14.6%), 11.0% obese (16.8%) and in girls 20.3% are overweight (14%), 10.1% obese (15.2%). In 11–15 year old boys the incidence of overweight is 37.5% (15.7%). Ethnicity makes no difference to prevalence of overweight or obesity. There was no correlation between phenylalanine tolerance or phenylalanine control and BMI.
Conclusion: Compared to the UK population, there is a lower prevalence of obesity, but greater incidence of overweight in our children with PKU. The particularly high percentage of overweight boys in the 11–15 year age group is concerning. Healthy lifestyle and awareness of appropriate energy intake needs to be incorporated into current dietary education alongside phenylalanine restriction.

567-A

GENOME-WIDE GENOTYPING FOR THE CHARACTERIZATION OF DISEASE LOCUS IN A FAMILY WITH AN UNCHARACTERIZED NEUROMETABOLIC DISEASE

Dundar H[1], Yucel D[1], Dursun A[1], Ozgul RK[1]
[1]*Dept of Ped, Metab Uni, Hacettepe Univ, Ankara, Turkey*

In this study, we report the genetic analysis of a Turkish family with an uncharacterized neurometabolic disease with two effected children, which have a consanguineous marriage. Clinic and laboratory studies of the effected individuals suggested that the family have an uncharacterized autosomal recessively inherited neurometabolic disorder. Genome-wide genotyping was carried out with Affymetrix GeneChip 500 K NspI SNP array for genetic mapping of disease causing locus in the family members. The genome-wide haplotypes were constructed and haplotypes indicating homozygosity by descent were compared among effected individuals by VIGENOS (Visual Genome Studio Program, personnel communication) assuming an autosomal recessive model. Homozygous chromosomal segments were selected as critical intervals for the disease causing locus. Homozygosity mapping in the family displayed three large regions (spans >5 Mb) of shared homozygosity on chromosomes 2, 18, and 21 with the physical positions of 153590298–158749144, 4750897–11248372 and 15013057–20823179, respectively. The genes that responsible for the previously described clinical phenotypes in these chromosomal regions were excluded. Candidate genes that were located in these chromosomal regions will be analyzed in future studies.
The study was supported by State Planning Organization of TURKEY (Project No: DPT 2006 K120640)

568-P

MICRODELETION SYNDROMES WITH ABNORMAL BEHAVIORAL PHENOTYPES AS DIAGNOSED IN ONE CLINICAL GENETICS CENTER, PRAGUE, CZECH REPUBLIC

Baxova A[1], Mihalova R[1], Prihodova I[2], Skopova J[3], Zidkova K[1]
[1]Dept Biol Genet, 1st Fac Med, Charles U, Prague, Czech Republic
[2]Dept Neurol, 1st Fac Med, Charles Univ, Prague, Czech Republic
[3]Dept Pediatr, 1st Fac Med, Charles Univ, Prague, Czech Republic

Microdeletion syndromes are defined as a group of genetically determined diseases with characteristic clinical manifestations that lead to diagnosis. The majority of these diseases are caused by small chromosome deletions (< 5 Mb), not diagnosable by classical cytogenetic investigation. In most cases, modern cytogenetics/molecular techniques, such as FISH or MLPA, need to be performed. Therefore, diagnosis based on clinical manifestation is extremely important in such situations, where in addition to phenotypic changes, such as dysmorphisms and organ abnormalities, abnormal behavioral phenotypes are also usually present; these might help to establish a correct diagnosis. In some cases, serious psychiatric symptomatology appears during adulthood of such patients. All these facts are important for our task of addressing an individual prognosis and family planning. In the period of 2005–2009, we analyzed 180 patients. The indications for cytogenetic-molecular analysis were as follows: hypotonia after birth, mental retardation, congenital anomalies, and face dysmorphisms. Classic cytogenetic screening performed as the basic test did not show any abnormality. Using FISH and MLPA techniques, we discovered that cca 10% individuals in our cohort have cryptic chromosomal aberations. The most frequent syndrome found was Velocardiofacial syndrome (22q11 microdeletion), follow by Prader-Willi syndrome, Angelman syndrome (15q11–13 region), Williams syndrome (7q11 microdeletion), Smith-Magenis Syndrome (17p microdeletion). In rare instances Miller-Dieker syndrome (17p13), deletion 1p36, duplication 17p11.2, duplication 22q11.21, was diagnosed. Our clinical experience shows that recognition of an abnormal behavioral phenotype in childhood is very important for establishing the correct diagnosis (Grants support: IGA MZ NS10327-3/2009; MSMT - MSM0021620806 and 0021620849).

569-P

PARTIALLY FOLDED STATES OF A MISTARGETING VARIANT OF THE HUMAN ALANINE: GLYOXYLATE AMINOTRANSFERASE STABLY INTERACT WITH HSC70 CHAPERONES

Pey AL[1], Martinez A[2], Sanchez-Ruiz JM[1], Salido EC[3]
[1]Dep Phys Chem, Univ of Granada, Granada, Spain
[2]Dep Biomed, Univ of Bergen, Bergen, Norway
[3]Hosp Univ Can, La Laguna, Spain

Some mutations in the human alanine: glyoxylate aminotransferase (hAGT) associated to primary hyperoxaluria type I (PH1) cause peroxisome-to-mitochondria mistargeting, resulting in a metabolically inefficient enzyme. It is known that mistargeting requires the presence of two polimorphisms (P11L and I340M; AGT-LM) and an additional mutation (for instance, G170R; AGT-LRM), but the molecular mechanisms responsible for mistargeting are largely unknown. In this report, we have evaluated the potential role of native and partially folded states of hAGT in the mitochondrial mistargeting using recombinantly expressed AGT proteins. Our results show that the dimeric folded AGT-wt, AGT-LM and AGT-LRM variants display similar activities and overall structures, but the AGT-LRM variants show dramatic effects on kinetic stability. Under partially unfolding conditions (mild acidic pH), all AGT variants adopt a stable partially folded state, which is able to stably interact with Hsc70 chaperones in vitro. Moreover, AGT-LRM variant synthesized in a mammalian cell-free system under native conditions show enhanced ability to form stable complexes with chaperones compared to AGT-wt. Our results suggest a role of the chaperone-mediated presentation of partially folded AGT mistargeting variants to the mitochondrial import machinery.

570-P

TWO CASES OF DISCORDANT INHERITANCE FOR A HOMOZYGOUS MUTATION DUE TO UNIPARENTAL DISOMY AS REVEALED BY SNP-ARRAYS

Desviat LR[1], Pérez-Cerdá C[1], Merinero B[1], Gallego L[1], Barshop BA[2], Ugarte M[1], Pérez B[1]
[1]CEDEM, C. Biología Molecular CSIC-UAM, Madrid, Spain
[2]UCSD Dept of Pediatrics, La Jolla, California, United States

Uniparental disomy (UPD) refers to the inheritance of both homologous chromosomes or segments of a chromosome from one parent. Consequences of UPD are imprinting defects or homozygosity of a recessive mutation, among others. In this study we report two cases with a homozygous mutation in the MTRR and PCCA genes, respectively and for whom one of the parents was not a carrier of the mutation. The discordant inheritance was studied by multiplex ligation probe amplification (MLPA) and/or whole-genome SNP-array technology to investigate a possible genomic deletions or UPD. The results showed segmental UPD causing homozygosity for the pathogenic mutations. These represent the first description of UPD resulting in these inherited metabolic diseases, namely homocystinuria cblE type and propionic acidemia. Further analysis of microsatellite markers along the corresponding chromosomes was performed to confirm the segmental UPD and to determine the pattern of parental segregation of the chromosomes, in order to give clues to the mechanism leading to UPD. The results underscore the importance of performing the genetic analysis in parents of a child with an autosomal recessive disease to provide accurate genetic counseling especially in terms of recurrence risk for future pregnancies.

571-O

PROTEOMICS REVEALS NEW INSIGHTS INTO THE CAUSES OF HYPERAMMONEMIA IN METHYLMALONIC ACIDEMIA

Chandler RJ[1], Phillips D[1], Boja ES[1], Carrillo-Carrasco N[1], Caldovic L[2], Morizono H[2], Balaban RS[1], Venditti CP[1]
[1]National Institutes of Health, Bethesda, United States
[2]Children's National Medical Center, Washington DC, United States

Methylmalonic Acidemia (MMA) is an autosomal recessive disorder of metabolism caused by a deficiency in the mitochondrial enzyme methylmalonyl-CoA mutase. MMA patients can experience life-threatening metabolic instability with intermittent bouts of hyperammonemia; the underlying mechanisms of the pathology of these episodes are not well understood. Presently, the inhibition of N-acetylglutamate synthetase (NAGS) by propionyl-CoA, a metabolite elevated in MMA, is thought to indirectly decrease carbamoyl phosphate synthetase I (CPS1) activity causing hyperammonemia. To investigate possible mechanism(s) of pathology, we performed proteomic analysis of liver extracts from a mouse model of MMA using two dimensional fluorescences difference in-gel electrophoresis (DIGE) and a quantitative analysis using iTRAQ labeling and tandem mass spectrometry. Proteomic results from the liver of MMA mice revealed decreased levels of the urea cycle (UC) enzymes Cps1, ornithine transcarbamylase (Otc), argininosuccinate synthetase 1 (Ass1), argininosuccinate lyase and arginase compared to controls. The mRNA levels of Nags and Otc, but not other UC enzymes, are significantly lower in the liver of the MMA mice. MMA mice have decreased levels of plasma ornithine, but normal levels of urine orotate, plasma arginine and plasma citrulline. Limited CPS1 activity due to NAGS inhibition is consistent with low to normal urinary orotate levels. Protein immunoblots of the liver from metabolically stable MMA patients exhibited normal levels of CPS1, OTC and ASS1. Since deficiencies in the urea cycle enzymes cause hyperammonemia, we hypothesize that increased metabolic stress causes a decrease in the levels of UC enzymes, contributing to the intermittent hyperammonemia observed in MMA.

572-P

NATIVE READ-THROUGH OF A NONSENSE MUTATION IN A MAPLE SYRUP URINE DISEASE PATIENT

Fernández-Guerra P[1], Artuch R[2], Lambruschini N[2], Ugarte M[1], Rodríguez-Pombo P[1]
[1]*CEDEM, CBMSO, UAM-CSIC, CIBERER, Madrid, Spain*
[2]*Hosp. Sant Joan de Déu, CIBERER, Barcelona, Spain*

Mutations that create premature stop codons typically cause premature translation termination. The end of translation occurs when a stop codon in a gene's messenger RNA transcript enters the ribosomal A site. The efficiency of translation termination seems to be primarely determined by the stop codon and the immediate 3′ nucleotide present in the sequence. It has been proposed that the tetranucleotide UGAC acts as a mediator of the least efficient termination and the greater basal suppression of the 12 possible tetranucleotide termination signals. Nonsense suppression is a well known mechanism in yeast, but it has been scarcely documented in humans.

Regarding the importance of type and sequence's context of the nonsense mutation, we selected the change p.R324X (UGAC); borne in heterozygosis fashion by a classical maple syrup urine disease patient with a defect in the BCKDHB gene, to test the hypothesis of a possible existence of a natural read-through for this change.

The translation efficiency of the mutant allele was assessed using an in vitro transcription-translation cell-free expression analysis/system, and by western blotting analysis of HEK293T proteins after transient expression of a pReceiver vector containing the complete cDNA BCKDHB with the p.R324X mutation.

Along with the predicted truncated peptide, a full-length BCKDHB protein was detected by both approaches; quantitative analysis of the results, corrected by loading and/or basal level of expressed protein, showed a variable recovery (6- 10%) respectively.

These results highlight the possible use of read-through drugs as therapeutic agents for patients who carry out this type of mutations.

573-P

DOMINANT NEGATIVE EFFECT OF A MUTATION IN THE GLUTARYL-CoA DE-HYDROGENASE GENE ASSOCIATED WITH AN APPARENTLY DOMINANTLY INHERITED FORM OF GLUTARIC ACIDURIA TYPE I

Bross P[1], Palmfeldt J[1], Frederiksen JB[1], Hansen J[1], Nielsen MN[1], Gregersen N[1], Dunx M[2], Lund AM[2], Christensen E[2]
[1]*Res Unit f Mol Med, Aarhus Univ Hosp SKS, Aarhus, Denmark*
[2]*Dep Clin Gen, Rigshospitalet, Copenhagen, Denmark*

A patient with suspected glutaryl-CoA dehydrogenase (GCDH) deficiency, a usually autosomal recessively inherited defect of mitochondrial amino acid metabolism, was identified by elevated glutarylcarnitine (C5DC) during neonatal screening. Further biochemical analysis of blood and urine from the proband and low GCDH activity (20% of normal mean) in cultured fibroblasts were consistent with a glutaric aciduria type I phenotype with high residual enzyme activity. Subsequent genetic analysis detected a 18 bp deletion (c.553_570del18) resulting in deletion of 6 amino acids (p.Gly185_Ser190del) in one allele of the GCDH gene and no sequence changes in the other allele. Recombinant expression of the mutant variant in E. coli showed that the GCDH-(p.Gly185_Ser190del) protein was expressed; however, its ability to assemble into the active tetrameric structure was severely impaired. To investigate the hypothesis that expression of the mutant allele negatively affects a co-expressed wild type allele, we engineered a prokaryotic expression system with two plasmids carrying the two GCDH variants under control of arabinose- and IPTG-inducible promoters, respectively. Cells expressing both wild type and the GCDH-(p.Gly185_Ser190del) protein displayed increased levels of total GCDH protein compared to cells expressing wild type GCDH only, but the levels of GCDH tetramer and activity were significantly decreased. These experiments suggest that the GCDH-(p.Gly185_Ser190del) protein interferes with tetramer formation of the wild type protein and are thus consistent with the notion that the presence of the GCDH-(p.Gly185_Ser190del) protein significantly reduces the formation of active GCDH enzyme from wild type monomers thus explaining the biochemical phenotype of the heterozygous patient.

574-P

HYPOXANTHINE-GUANINE PHOSPHORIBOSYL TRANSFERASE REGULATES EARLY DEVELOPMENTAL PROGRAMMING OF DOPAMINE NEURONS: IMPLICATIONS FOR LESCH-NYHAN DISEASE PATHOGENESIS

Ceballos-Picot I[1], Mockel L[2], Potier MC[3], Dauphinot L[3], Shirley TL[4], Torero-Ibad R[5], Fuchs J[5], Jinnah HA[4]
[1]*Paris Descartes University, Paris, France*
[2]*Necker-Enfants Malades Hospital, APHP, Paris, France*
[3]*CNRS UMR 7637, Paris, France*
[4]*Emory University, Atlanta, United States*
[5]*CNRS UMR 8542, Paris, France*

Background: Hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency results in Lesch-Nyhan disease (LND), where affected individuals exhibit a characteristic neurobehavioral disorder that has been linked with dysfunction of dopaminergic pathways of the basal ganglia. Since the functions of HPRT, a housekeeping enzyme responsible for recycling purines, have no direct relationships with the dopaminergic pathways, the mechanisms whereby HPRT deficiency affect them remain unknown.

Methods: Microarray methods and quantitative PCR were applied to 10 different HPRT-deficient sublines derived from the MN9D cell line. Demonstration of a causal relationship between HPRT deficiency and gene deregulation by HPRT transfection. Validation of the results by using Fibroblasts of LND patients.

Results: The current studies demonstrate that HPRT deficiency influences early developmental processes controlling the dopaminergic phenotype, using several different cell models for HPRT deficiency. Most notable results were increases in the mRNAs for engrailed 1 and 2, transcription factors known to play a key role in the specification and survival of dopamine neurons. The increases in mRNAs were accompanied by increases in engrailed proteins, and restoration of HPRT reverted engrailed expression towards normal levels, demonstrating a functional relationship between HPRT and engrailed. Over-expression of engraileds occurred even in HPRT-deficient sublines from the SK-N-BE(2) M17 human neuroblastoma line and in primary fibroblasts from patients with LND in a manner that suggested a correlation with disease severity.

Conclusion: These results provide novel evidence that HPRT deficiency may affect dopaminergic neurons by influencing early developmental mechanisms

575-P

COMPARISON OF PROTEIN PROFILES OF CULTIVATED SKIN FIBROBLASTS FROM PATIENTS WITH GENETIC DEFECTS IN ETHE1 VERSUS HEALTHY CONTROLS

Palmfeldt J[1], Stenbroen V[1], Vang S[1], Knudsen C[1], Pavlou E[2], Baycheva M[3], Buchal G[4], Yap S[5], Augoustides-Savvopoulou P[2], Mandel H[6], Gregersen N[1]
[1]*Res Unit Mol Med, University Hospital, Aarhus, Denmark*
[2]*Dep Pediatrics, Aristotle University, Thessaloniki, Greece*
[3]*Univ Pediatric Hospital, Sofia, Bulgaria*
[4]*Dep Pediatrics, Kirchen, Germany*
[5]*Met Dis Children's Univ Hosp, Dublin, Ireland*
[6]*Meyer Children's Hosp, Haifa, Israel*

Large scale quantitative protein profiling is suitable for studying perturbations in protein amounts as a function of e.g. inborn errors, and is especially valuable when studying complex diseases with several symptoms. Defects in the gene ETHE1 causes ethylmalonic encephalopathy (EE) associated with the clinical symptoms neurodevelopmental delay, petechiae, orthostatic acrocyanosis and chronic diarrhea, as well as the biochemical symptoms; elevated C4 and C5 plasma acylcarnitine and urinary excretion of ethylmalonic acid (EMA). The disease etiology is enigmatic, but the ETHE1 protein has been found to be involved in the metabolism of sulfide. To further characterize the effects of ETHE1 deficiency we have performed large scale proteomics on mitochondrial protein extracts from several individuals. A mass spectrometry based proteomics method was applied, where the various fibroblast samples were compared using relative quantification of iTRAQ-labelled peptides. To account for variation between individuals we included six patient fibroblasts with ETHE1 gene defects, and compared with three healthy controls. More than hundred fifty mitochondrial proteins were quantified in all the samples studied. Statistical analysis of the quantitative data from patient samples versus controls points to perturbations in oxidative stress protection, metabolism of arginine and pyruvate, as well as release of an apoptosis factor from mitochondria. When performing hierarchical clustering on all the quantitative data we observed that some of the patient samples clearly cluster together whereas healthy controls were more segregated. This indicates a higher degree of common proteome fingerprint in the patient samples than in the healthy controls.

576-P

TARGETING NONSENSE MUTATIONS IN MAPLE SYRUP URINE DISEASE

Fernandez-Guerra P[1], Merinero B[1], Oyarzabal A[1], Desviat LR[1], Ugarte M[1], Rodriguez-Pombo P[1]
[1]*CEDEM, CBMSO, UAM-CSIC, CIBERER, Madrid, Spain*

Premature termination codons (PTCs) are a cause of numerous genetic disorders. Aminoglycosides were the first agents demonstrated to suppress PTCs and a novel strategy based on the restoration of full-length protein synthesis to treat genetic disorders.

Herein, we test the hypothesis that some of the primary PTCs changes detected repeatedly in the BCKDHB gene of classical MSUD patients p. E163X (UAAG), p.Q267X (UAGG), p.R285X (UGAG), and p.R324X (UGAC), could be functionally rescued by read-through agents as geneticin, gentamicin. The efficacy of these treatments was first assessed using an "in vitro" cDNA coupled transcription/translation test. A variable read-through response was observed for the different aminoglycosides assayed and PTCs. Positives recoveries were obtained for all changes from 1–4 μg/mL geneticin and 20–30 μg/mL gentamicin respectively. The maximal rescues of full length proteins ranged from 60% for p.R324X to 5% for p.E163X. Our results confirm the important role of the tetranucleotide signal in determining the efficiency of translation termination and PTC suppression. As low level of available mRNA substrate due to nonsense mediated decay may also pose a limitation to the efficacy of PTC suppression in the clinical settings, we have evaluated the consequences of these PTCs mutations on BCKDHB mRNA abundance by performing qRT-PCR of the BCKDHB transcripts in the available patient's cell strains. According to our data, only slight but significant decrease amounts of BCKDHB transcripts were obtained for all the PTC changes analysed. Together our results encourage the use of read-through drugs as a therapeutic option for this inherited metabolic disease

577-O

ABSOLUTE QUANTIFICATION OF METABOLIC ENZYMES: STOICHIOMETRY OF GLYCOLYSIS

Martens GA[1], Jiang L[2], Conolly JB[3], Geromanos SG[3], Pipeleers D[2], Vissers JPC[3], Gorus F[1]
[1]*Universitair Ziekenhuis Brussel, Brussel, Belgium*
[2]*Vrije universiteit Brussel, Brussel, Belgium*
[3]*Waters Corporation, Manchester, United Kingdom*

Validity of (ion current) label-free LC-MSE was evaluated for quantification of metabolic enzymes in liver, brain and pancreatic beta and alpha cells. With total imprecision<20%, LC-MSE confidently measured molar protein abundance differences down to 45%. Moreover, it discriminated tissue-specific isoforms and correctly measured subunit-stoichiometry of mitochondrial F0F1-ATPase and major dehydrogenase complexes (PDH, OGDH, BCKDH) with accuracy=104±25%.

Glycolytic enzymes featured among the abundant cellular proteins, particularly in neurons. Molar levels of individual enzymes varied greatly within each cell type, with >100-fold difference between most (Gapdh) and least abundant (Pfk1). Stoichiometry within the pathway, however, was comparable in all tissues, with enzymes downstream of lytic aldolase step systematically much more abundant than upstream enzymes. Calculation of theoretical flux capacity (Vmax=molar abundance x turnover number) indicated highest capacity at level of glucose-6-phosphate isomerase, pyruvate kinase and triose phosphate isomerase. Calculated capacities correlated linearly with Km (R2=0.8) for all enzymes except triose phosphate isomerase, suggesting that its abundance is excessive to justify only its catalytic role in glycolysis.

In conclusion: LC-MSE is useful for quantification of molar enzyme abundances and their stoichiometries. It reveals that enzymatic build-up of glycolysis is preserved in different cell types and shows interesting imbalances that require further study.

578-P

EXPRESSION AND STRUCTURE-BASED ANALYSIS OF CARBAMOYL PHOSPHATE SYNTHETASE I DEFICIENCY

Pekkala S[1], Martínez AI[1], Barcelona B[2], Yefimenko I[3], Finckh U[4], Rubio V[5], Cervera J[2]
[1]*Centro Investigación Príncipe Felipe, Valencia, Spain*
[2]*Cent Inv Princ Felipe & CIBERER-ISCIII, Valencia, Spain*
[3]*Instituto Biomedicina Valencia CSIC, Valencia, Spain*
[4]*MVZ Dortmund Dr. Eberhard & Partner, Dortmund, Germany*
[5]*Inst Biomedicina CSIC & CIBERER-ISCIII, Valencia, Spain*

Background: Carbamoyl-phosphate synthetase I (CPS1) deficiency (CPS1D), a recessively inherited urea cycle error due to CPS1 gene mutations, causes life-threatening hyperammonemia. The CPS1 gene consists of 38/37 exons/introns. Many of the relatively few mutations reported in CPS1D are "private" missense changes of non-ascertained disease-causing potential. Site-directed mutagenesis studies could not be used, since CPS1 expression systems were missing.

Methods: A novel baculovirus-insect cell system has been set up for expression and purification of wild-type or mutant CPS1, allowing characterization of the effects of clinical mutations on CPS1 solubility, stability, activity, and kinetic parameters for the essential CPS1 activator, N-acetyl-L-glutamate (NAG). The crystal structure of the CPS1 C-terminal domain and the localization therein of the NAG site [Pekkala et al. Biochem J 424:211–220] has been used to rationalize the effects of the mutations affecting this domain.

Results: The effects of nine clinical mutations and one polymorphism have been studied. Five mutations (p.T471N, p.Q678P, p.P774L, p.R1453Q, and p.R1453W) are first reported here, in three severe CPS1D patients. p.P774L, p.R1453Q, and p.R1453W inactivate CPS1, p.T471N and p.Y1491H greatly decrease the apparent affinity for NAG, p.Q678P hampers correct enzyme folding, and p.S123F, p.H337R, and p.P1411L modestly decrease activity. p.G1376S is confirmed a trivial polymorphism.

Conclusion: This novel CPS1 expression/purification system is proven successful for testing a mutation disease-causing role, and opens the way to the identification of CPS1D patients who might benefit from specific treatment with NAG analogues because they exhibit reduced affinity for NAG.

579-P

MOLECULAR AND STRUCTURAL ANALYSIS OF SIX NONSENSE MUTATIONS IN MUT METHYLMALONIC ACIDEMIA PATIENTS INCLUDING TWO NOVEL NONSENSE MUTATIONS

Dundar H[1], Ozgul RK[1], Unal O[1], Karaca M[2], Aydin HI[3], Tokatli A[1], Sivri HS[1], Coskun T[1], Dursun A[1]
[1]*Dept of Ped, Metab Uni, Hacettepe Univ, Ankara, Turkey*
[2]*Dept of Biology, Aksaray University, Aksaray, Turkey*
[3]*Metabolism Unit, GATA, Ankara, Turkey*

Methylmalonic acidemia (MMA) is an autosomal recessive inherited inborn error of propionate metabolism. Inadequate function of methylmalonyl-CoA mutase (MCM) causes MMA. The open reading frame of the MCM gene consists of 2.7 kb, encoding 750 amino acids. The first 32 amino acids is the N-terminal leader sequence involved in directing the precursor apoenzyme into the mitochondria where it is translocated and cleaved to form the mature subunit. The mitochondrial leader sequence is followed by the N-terminal extended segment (residues 33–87), which is involved in subunit interaction. The N-terminal $(\beta/\alpha)8$ barrel is the substrate binding domain(residues 88–422) and is attached to the C-terminal $(\beta/\alpha)5$ domain (cobalamin binding domain, residues 578–750) by a long linker region (residues 423–577).
In this study, seven different nonsense mutations were detected in eight MMA patients. Mutations include R31X, K54X, Q132X, R152X, R228X, R467X and R727X all of which result in premature protein synthesis. K54X and Q132X mutations are novel mutations, the remaining are known ones. These detected mutations were evaluated according to the effect of the mutation on the functional domains of MCM enzyme. R31X mutation is located in mitochondrial leader sequence. K54X mutation was located in N-terminal extended segment. Q132X, R152X and R228X was located in $(\beta/\alpha)8$ substrate binding domain. The R467X was located in linker-region and R727X mutation was located in $(\beta/\alpha)5$ cobalamin binding domain. These potential truncated proteins do not express enzyme activity because each one lacks a key portion of the enzyme.
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580-P

ACUTE INTERMITTENT PORPHYRIA—IMPACT OF PATHOLOGICAL MUTATIONS ON THE BIOCHEMICAL AND ENZYMATIC PROTEIN PROPERTIES

Douderova D[1], Martasek P[1]
[1]*1st Faculty of Medicine, Charles Univ, Prague, Czech Republic*

Acute intermittent porphyria (AIP) is an autosomal dominantly inherited disorder, caused by a deficiency of hydroxymethylbilane synthase (HMBS), the third enzyme in heme biosynthesis. Clinical features include autonomous, central, motor or sensory symptoms, but the most common clinical presentation is abdominal pain caused by neurovisceral crises. Diagnosis of AIP is crucial to prevent life-threatening acute attacks. HMBS is independently expressed by two isoenzymes, 42-kDa housekeeping and 40-kDa erythroid-specific. HMBS isoforms from several different species have been studied and their biochemical properties have been described. The crystallographic structures of HMBS from E. coli and human have been determined.
The aim of this study was to establish structure/function consequences of the pathological mutations we described. To establish the effects of mutations on enzyme function, biochemical characterization of the expressed normal and mutated proteins was performed. Prokaryotic expression of the HMBS mutant alleles revealed that, with the exception of one, all mutations produced little, if any, enzymatic activity. E. coli and human PBGD amino acid sequences have 35% homology and more than 70 % similarity, and considering this fact, it is possible to extrapolate structure/function relationships for human mutations leading to simple amino acid substitution based on comparative E. coli/human analyses. These analyses together with the kinetic studies of existing mutations can help predict the impact on the enzyme function in the living organism and further improve our understanding in this field.
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581-P

CHARACTERIZATION OF THE MOLECULAR BASIS OF ACUTE INTERMITTENT PORPHYRIA

Bustad Johannessen H[1], Rxnneseth E[2], Underhaug J[1], Skjfrven L[1], Toska K[2], Martinez A[1], Sandberg S[2]
[1]*Dep of Biomedicine, Univ of Bergen, Bergen, Norway*
[2]*NAPOS, Bergen Univ Hospital, Bergen, Norway*

Acute intermittent porphyria (AIP) is an inherited disease caused by reduced activity due to mutations in hydroxymethylbilane synthase (HMBS), the third enzyme of the heme synthesis pathway. The molecular basis of the disease and the consequences of the mutations are not completely understood. Valuable information about the phenotype-genotype correlations can be obtained by functional studies on the wild-type (wt) and disease causing mutants. For these studies we have initially selected pR116W, pK132N, pR167W, pR173W and pV215E, representing: i) mutations around the active site and/or interfering with binding of the cofactor dipyrromethane, expected to have catalytic effects although misfolding and destabilization cannot be ruled out (pR167W, pR173W, pV215E) and ii) mutations far from the active site and predicted to affect overall folding and flexibility (pR116W, pK132N). We have expressed recombinant human wt-HMBS and the 5 selected mutants in E. coli using pGEX expression vectors, and purified the enzymes on Glutathione-Sepharose 4B with high yields and purity for wt and most mutants. We then characterized the steady-state kinetic parameters of the mutants comparative to the wt. Moreover, the conformational stability was investigated by differential scanning calorimetry, circular dichroism and fluorescence spectroscopy. Wt-HMBS shows a high thermostability (Tm = 79°C), and some of the mutants reveal a similar conformation and stability (e.g. K132N and R167W), while R173W shows decreased thermostability (Tm ~51°C), in addition to kinetic defects. The results obtained aid to understand the pathogenic mechanisms in AIP and to select potential therapeutic strategies.

582-O

SYSTEMS ANALYSIS OF INHERITED DISEASES: STUDYING THE IMPACT OF MUTATIONS ON PROTEIN INTERACTIONS

Gersting SW[1], Lotz AS[1], Woidy M[1], Messing DD[1], Danecka MK[1], Staudigl M[1], Muntau AC[1]
[1]*Molec Pediatr, Hauner Child Hosp, LMU, Munich, Germany*

Complex associations of genotype and phenotype are increasingly appreciated for inborn errors of metabolism. This is implied by phenomena like allelic and locus heterogeneity, varying penetrance, and variable expressivity that are challenging the classic model of a mutation in one gene leading to loss of its gene product. Recent advances in proteome research revealed that missense mutations and small insertions or deletions are frequently associated with aberrant protein folding. However, this is not necessarily confined to a loss-of-protein molecular phenotype. As proteins mainly exert their function as higher order complexes, which are often connected to other functional modules, mutations can interfere with formation of these networks.
Here we present an automated, high confidence method for the detection of binary protein interactions in living cells. This informatics aided and bioluminescence resonance energy transfer based method (iBRET) shows high sensitivity (72%) and is applicable to virtually all kinds of proteins in any subcellular compartment. Designed as high-throughput technique, iBRET enables the investigation of numerous mutations in one disease gene aiming to dissect the specific molecular phenotype of individual mutations.
The technical approach presented here adds to and goes beyond strategies assessing single endpoints such as residual enzyme activity. In the era of systems biology it will allow for a comprehensive view on the impact of gene mutations on functional networks of protein-protein interactions and on the effect of pharmacological interventions affecting these interactions.

583-P

HOW DO PARENTS PRIORITISE NEEDS–THEIR OWN, THEIR PARTNER'S OR THEIR CHILDREN'S? MANAGING RELATIONSHIP NEEDS WHILE LIVING WITH CHRONIC METABOLIC ILLNESS

Pearce F[1]
[1]Genetic Health Service Royal Childs Hosp, Melbourne, Australia

The special care requirements of children who progressively become physically and intellectually disabled due to metabolic illness, can be emotionally and physically overwhelming.
All family relationships are under pressure in these circumstances. At the same time, people discover personal capabilities and strengths that would rarely be uncovered or used in more normal life situations.
Often, the sick child's primary care-giver (usually the mother) has to make choices from several competing responsibilities at the one time. How are these choices made? On what basis are they prioritized? Many relationships fail under the pressure of these circumstances, while others are awe-inspiring and grow stronger.
This presentation will include case material that demonstrates how many couples cope and how others feel forced to 'give up'. It will also incorporate some information about the role of the metabolic social worker within the multi-disciplinary team of the Metabolic Service at a major paediatric hospital.

584-P

GENE ANALYSIS OF BETA-UREIDOPROPIONASE DEFICIENCY IN 13 CHINESE PATIENTS

Ito T[1], Ichiki S[1], Nakajima Y[1], Maeda Y[2], Shen K[3], Wang X[3], Wu H[3], Zhang C[4], Sugiyama N[5], Togari H[1], Meijer J[6], van Kuilenburg ABP[6]
[1]Dept Pediatr, Nagoya City Univ, Nagoya, Japan
[2]Dept Hosp Pharm, Nagoya City Univ, Nagoya, Japan
[3]Beijing Child Hosp, Capital Medical Univ, Beijing, China
[4]MILS International, Kanazawa, Japan
[5]Dept Pediatr, Aichi-Gakuin Univ, Nagoya, Japan
[6]Lab Gene Metab Dis, AMC, Amsterdam, Netherlands

Background: beta-ureidopropionase (beta-UP) is the third enzyme of the pyrimidine degradation pathway and catalyses the conversion of N-carbamyl-beta-alanine to beta-alanine and N-carbamyl- beta-aminoisobutyric acid to beta-aminoisobutyric acid. We found 13 new Chinese cases presenting with N-carbamyl-beta-amino aciduria during selective screening for inborn errors of metabolism in patients with unspecific symptoms such as developmental delay, convulsion and hypotonia. The N-carbamyl-beta-amino aciduria in these patients suggested a deficiency of beta-UP.
Objectives: To investigate whether the N-carbamyl-beta-amino aciduria is caused by mutations in beta-UP gene (UPB1).
Material and Methods: After obtaining informed consents from the parents, patients genomic DNAs were obtained from their white blood cell or dried filter papers. Sequence analysis were performed in all exon of UPB1 gene after PCR
Results: Eleven out of 13 patients proved to be homozygous for a missense mutation c.977G>A (R326Q) in exon 9. One patient was heterozygous of this mutation. Heterologous expression of the mutant enzyme in Escherichia coli showed that the R326Q mutation resulted in a mutant beta-UP enzyme without residual activity.
Conclusion/Discussion: As seen in other pyrimidine degradation pathway defects, the symptoms of beta-UP deficiency are not specific. We need special analytical methods to detect the specific metabolites such as N-carbamyl-beta-alanine and N-carbamyl-beta-aminoisobutylic acid for the diagnosis of beta-UP deficiency. In our genetic analysis, R326Q mutation was detected at high rates. This mutation was already reported in a Japanese patient with West syndrome. Therefore, this mutation might be a common in Asian population.

585-P

INBORN ERRORS OF METABOLISM IN A TERTIARY PEDIATRIC INTENSIVE CARE UNIT

Blasco Alonso J[1], Gil R[2], Serrano J[1], Navas VM[1], Milano G[2], Yahyaoui R[3], Ortiz Pérez P[1], Sierra C[1]
[1]Ped Gastroenterol, Hosp Materno-Infantil, Málaga, Spain
[2]PICU, Hospital Materno-Infantil, Málaga, Spain
[3]Clinical Laboratory, Carlos Haya Hosp, Málaga, Spain

Introduction: Inborn errors of metabolism (IEM) are an increasingly cause of admission to pediatric intensive care units (PICU).
Methods: retrospective analysis of patients admitted to a tertiary PICU due to onset of IEM during a ten-year period (January 2001- February 2010).
Results: Twenty-four patients (14 girls, 17 Caucasian and 7 Arabs) were admitted. Median age 120 days (2 days–5 years).
Clinical onset: altered level of consciousness (13/24), convulsion (3/24), apnea (2/24) and others. Blood biochemical alterations: metabolic acidosis (10), hyperammonemia (7), coagulopathy (5), hypoglycemia (1), and electrolytes disorders (2). During admission 15 patients suffered from shock, 6 multiorganic failures, 5 severe neurologic insults, 3 coagulopathy and 3 acute liver failures. 17 aminoacid metabolism disorders (6/17 UCD–4 OTC deficiency, 2 citrullinemia, 2/17 CDG-I, 2/17 tyrosinemia, 5/17 organic acidemias–2 methyl-malonic, 1 methylmalonic aciduria and homocystinuria, 1 propionic acidemia and 1 betaketothiolase deficiency, 1/17 non-ketotic hyperglycinemia and 1/17 cystinosis), 4/24 mitochondrial diseases, 1 glycogenosis, 1 fatty-acid oxidation disorder and 1 hemochromatosis. Most patients required agressive support, including mechanical ventilation (20/24) and extrarenal removal therapy (7/24 peritoneal dialysis (PD), 6/24 continuous veno-venous hemofiltration (CVVHF)), as well as vasoactive drugs (15/24). Specific medical treatment and specific diet were needed in all cases. In terms of morbidity and mortality, 12/24 died, 5 suffer from severe neurologic sequelae, 1 liver failure (transplantation) and 1 chronic respiratory failure.
Conclusion: Acute decompesation in a IEM is a metabolic emergency which should be diagnosed and treated urgently. Newborn screening could have diagnosed 11 patients before onset.

586-P

ASSOCIATION OF POLYNEUROPATHY, MENTAL RETARDATION, SENSORINEURAL HEARING LOSS, 6th NERVE PALSY, CONVULSIONS, AND ORAL DYSKINESIA; A PROPABLE NEW NEUROMETABOLIC DISORDER

Dursun A[1], Yalnizoglu D[2], H Dundar[1], Erdem S[3], Akarsu AN[4], RK Ozgul[1]
[1]Dept of Ped, Metab Uni, Hacettepe Univ, Ankara, Turkey
[2]Dept of Childhood Neurol, Hacettepe Univ, Ankara, Turkey
[3]Dept of Neurology, Hacettepe Univ, Ankara, Turkey
[4]Dept of Med Genet, Hacettepe Univ, Ankara, Turkey

We present four siblings from an inbred family with a history of three affected children died between 5–13 years of age and two unaffected sisters. The clinical picture consisted of polyneuropathy, mental retardation, sensorineural hearing loss, 6th nerve palsy, febrile convulsions, and oral dyskinesia. The early developmental milestones were near normal. In general, a febrile illness with febrile seizures preceeded the onset of neurological deterioration. Charcot-Marie-Tooth disease was excluded by clinical and laboratory findings. Metabolic work up including Tandem MS, urine organic acid analysis, peroxisomal profile, CDG screening, and amino acid analysis were normal. Evoked potential studies showed abnormal VEP, BAEP results and normal ERG in all patients, EEG features were unremarkable. EMG was compatible with sensoryneural polyneuropathy in three cases; peripheral nerve biopsy of an affected sibling showed demyelinating axonal polyneuropathy. We conclude that this clinical picture may represent a novel disease with autosomal recessive inheritance. Homozygosity mapping of the disease locus in the family is under investigation.
The study is supported by TUBITAK (108 S259).

587-P

FOLLOW UP OF METABOLIC DISORDERS DIAGNOSED BY THE SERVICE OF INFORMATION ON INBORN ERRORS OF METABOLISM (SIEM)

Nalin T[1], Herber S[2], de Barba ML[2], Netto CBO[2], Sanseverino MT[2], Refosco L[2], Rafaelli C[2], Giugliani R[2], Souza CFM[2]
[1]*Pos Prog Med: Med Sciences, UFRGS, Porto Alegre, Brazil*
[2]*Med Genetics Serv, HCPA, Porto Alegre, Brazil*

Inborn Errors of Metabolism (IEM) are serious diseases that especially affect children and newborns. Accurate diagnosis and promptly treatment are important for prognosis of these patients. SIEM is a toll free service in Brazil that has the objective to supply support to health professionals. From October 2001 to April 2010 the SIEM had 1684 registers, from that, 1237 had been followed up and concluded. Of these, 161 (14,5%) had been diagnosed as having some IEM. Organic acidemias and amino acid disorders were the most frequent, both with 32 patients (19,8%) each. In 65% of the cases, patients presented symptoms within the first year. Consanguinity was present in 21% of the cases. In our sample, 17% patients died, showing the high mortality rate of these diseases. The data obtained confirm the severity of the metabolic disease and the need for a more precise and earlier diagnosis, in order to improve survival rate and help to choose the best therapeutic strategy. The SIEM assist professionals in a more effective diagnosis and a better predictor for the best quality of life of patients with some IEM, including those that are far from of reference centers. (PROREXT-UFRGS/ comidaMed./ Fundacão Médica do RGS).

588-P

SURVEY OF IEM DIAGNOSED BY THE BRAZILIAN INFORMATION SERVICE FOR INBORN ERRORS OF METABOLISM (SIEM)

Souza CFM[1], Herber S[2], Nalin T[1], De Barba ML[1], Netto CBO[1], Sanseverino MT[1], Raffaeli C[1], Giugliani R[1]
[1]*Medical Genetics Service, HCPA, Porto Alegre, Brazil*
[2]*UFRGS, Postgraduation, Brazil*

The SIEM is a pioneer toll free service in Brazil and South America with a team specialized in IEM to help health professionals to diagnose, manage and treat suspected patient. Since the IEM are frequent pathologies, but often poorly recognized by physicians in Brazil, fast and efficient diagnosis and management are crucial for the patient's better prognosis and health. Between October 2001 and May 2010, 1700 cases were registered. Of the total 1700 registries, 1122 (66%) had their investigation for IEM concluded, and 163 (14.5%) of these cases confirmed diagnosis of IEM, 432 (38.5%) were non-metabolic cases, 281 (25%) were considered inconclusive and 244 (22%) had their follow-up lost. Consanguinity was present in 21% of the metabolic cases and 10% in the non-metabolic. Recurrence was reported in 23% of metabolic cases and 15% in non-metabolic. The mortality rate in the metabolic cases was 17% and 2,7% in the non-metabolic. We believe the SIEM is an extremely important source of information about IEM in a country were such group of disorders is often unrecognized, aiding different medical specialists to a better and more efficient diagnosis and treatment, avoiding burden to such patients. (PROREXT-UFRGS/ comidaMed./ Fundacão Médica do RGS).

589-O

DIAGNOSIS OF PORPHYRIAS: THE MAYO CLINIC BIOCHEMICAL GENETICS LABORATORY FOUR-YEAR EXPERIENCE

Tortorelli S[1], Hofherr SE[1], Kloke KM[1], Raymond KM[1]
[1]*Mayo Clinic, Rochester, MN, United States*

Objective: Few laboratories perform specialized biochemical analyses for diagnosing porphyrias. Our laboratory provides a wide range of biochemical and enzymatic analyses. Here we report our experience for 4 year period.
Methods: A total of 43,720 tests for porphyrias were performed between January 2006 and December 2009, and yielded positive diagnoses for 1,195 patients. The total number of positive patients is based exclusively on the number of patients and not the total number of positive tests. Patients were classified with individual porphyrias by the characteristic biochemical pattern identified by studies in various matrices, such as erythrocytes, plasma, stool, urine, and/or by the defective enzyme (PBG deaminase, URO III synthase, URO decarboxylase).
Results: 1,195 patients (2.7%) were diagnosed with porphyrias out of 43,720 porphyrin tests. The biochemical diagnoses were: Acute porphyrias 164 (AIP 62, HCP 13, VP 13, NSAP 76), CEP 12, PCT 890, EPP 130. The 76 diagnoses of non-specific acute porphyria were not differentiated due to a lack of additional necessary testing. In two cases, two different disorders coexist in a single patient (dual porphyria).
Conclusions: Although porphyrias are considered rare disorders, they accounted for 15% of all diagnoses made by our laboratory during the 2006–2009 time period. 74% of all positive porphyria cases were porphyria cutanea tarda (PCT), with 22 type II PCT cases confirmed with erythrocyte enzymatic deficiency by our assay. 49% of the 130 cases of EPP (the second most frequent diagnosis) were in patients below the age of 18, suggesting an improved awareness of this disease.

590-P

N-ACETYLASPARTYLGLUTAMATE IN HYPOMYELINATING DISORDERS

Wamelink MMC[1], Jakobs C[1], Holwerda U[1], Struys EA[1], Sistermans EA[2], Verheijen FW[3], van der Knaap MS[4], Wolf NI[4]
[1]*Dept of Clinical Chemistry, VUMC, Amsterdam, Netherlands*
[2]*Dept Clinical Genetics, VUMC, Amsterdam, Netherlands*
[3]*Dept Clinical Genetics, Erasmus MC, Rotterdam, Netherlands*
[4]*Dept Peadiatric Neurology, VUMC, Amsterdam, Netherlands*

Introduction: Hypomyelinating disorders are a heterogeneous group of white matter disorders characterised by severe cerebral myelin deficit. In only half of these patients, a definitive diagnosis is possible. The dipeptide N-acetylaspartylglutamate (NAAG) has been shown to be elevated in some hypomyelinating disorders.
Method: We measured CSF NAAG by LC-MS/MS in a group of 21 children with hypomyelination, both with and without definitive diagnosis. As controls we used 54 children without hypomyelinisation.
Results: Normal range in controls was 0–12 μmol/l. NAAG was strongly elevated in a child with Pelizaeus-Merzbacher-like disease (PMLD) due to GJC2 mutation and in a child with Salla disease. In two children with Pelizaeus-Merzbacher disease (PMD), caused by PLP1 duplication, NAAG was normal to slightly elevated. In four children without a definitive diagnosis, NAAG was strongly elevated in two children while in the other two children it was only mildly elevated. CSF NAAG levels were normal in 4H syndrome (hypomyelination, hypodontia, hypogonadotropic hypogonadism), hypomyelination with congenital cataract and hypomyelination with atrophy of the basal ganglia and cerebellum.
Discussion: This study demonstrates that NAAG is not a universal marker for hypomyelination. Its elevation in CSF is confined to certain disorders including PMD, PMLD and Salla disease, but is also found in some children without definitive diagnosis. The mechanism of NAAG elevation is still not understood, but its CSF levels may help in differentiating hypomyelinating disorders of unknown origin.

591-P

NOVEL MUTATION IN GENE FOR UROPORPHYRINOGEN DECARBOXYLASE IN EGYPTIAN PATIENTS WITH PORPHYRIA CUTANEA TARDA

Farrag Mohamed Sameh[1], Douderova Dana[1], Martasek Pavel[1], Weshahy Hany[2]
[1]*Dept Pediatrics, 1st Fac Med, Charles Univ, Prague, Czech Republic*
[2]*Dept Derma, Fac Med, Cairo Univ, Cairo, Egypt*

Porphyria cutanea tarda (PCT) is caused by the deficiency of uroporphyrinogen decarboxylase (UROD). Familial PCT is an autosomal dominant disorder characterized by light-sensitive dermatitis and excretion of large amounts of uroporphyrin III in urine.

The human UROD gene is located on Chromosome1 and contains 10 exons. The cDNA encodes a protein containing 387 amino acids. The UROD gene codes for uroporphyrinogen decarboxylase which transforms uroporphyrinogen III to coproporphyrinogen III. Approximately 50 mutations that cause PCT have been identified in the UROD gene reducing the activity of uroporphyrinogen decarboxylase by 50 percent. As a result, porphyrins build up in the body, particularly in the liver. This build up, in combination with non-genetic factors cause PCT.

In this study, molecular analyses of the UROD gene was done for an Egyptian family with PCT.The analyses revealed a new unpublished homozygous mutation in 2 patients (brother & sister) while their mother was a heterozygous carrier. T→A transition at position 163 in exon 3 was identified (c.163 T>A), that leads to the substitution of phenylalanine to isoleucine at the codon 55 (F55I). We describe the first UROD mutation identified in the Egyptian population.

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592-P

INBORN ERRORS OF METABOLISM IN NEONATAL AND PEDIATRIC INTENSIVE CARE UNIT: FIVE YEAR EXPIRIENCE

Djordjevic M[1], Sarajlija A[1], Martic Nikitovic J[2], Kecman B[1], Martic J[3], Jankovic B[2]
[1]*Metab Div, Moth and Child Health Inst, Belgrade, Serbia and Montenegro*
[2]*NICU, Moth and Child Health Inst, Belgrade, Serbia and Montenegro*
[3]*PICU, Moth and Child Health Inst, Belgrade, Serbia and Montenegro*

Background: Reports of inborn errors of metabolism (IEM) epidemiology in pediatric and neonatal intensive care units (ICU) are scarce. A number of IEM present as severe and life-threatening disorders that significantly contribute to mortality in these age groups.

Objectives: To investigate clinical features, treatment strategies and outcomes of IEM patients admitted to pediatric and neonatal ICU.

Materials and Methods: Medical records of all patients diagnosed with IEM that were admitted in Mother and Child Health Care Institute of Serbia between January 2005 and December 2009. Descriptive statistical methods were used for retrospective analysis.

Results: During study period, pediatric and neonatal ICUs had a total of 4013 admissions. Forty four admissions counted for IEM (1,1%). Eight patients were newborns and 36 older children. Main indications for admission were somnolence (29,5%), respiratory insufficiency (27,2%) and severe metabolic acidosis (22,8%). Diagnosis of IEM was known at the time of admission in 20/44 patients. Most frequent IEMs were maple syrup urine disease and citrullinemia (2 patients each) among newborn patients and mitochondrial disorders in older children (9). Mechanical ventilation was required in 22 patients (5 newborns) and extracorporeal removal therapy in 8 (5 newborns). Median length of stay in ICUs was 5 days (range 1–46 days). Overall mortality was 31.8% (14/44).

Conclusion: Although relatively rare, IEM constitute significant and complex problem in neonatal and pediatric ICUs. High mortality rate despite aggressive support implies need for further improvement of treatment strategies.

593-P

HARDEROPORPHYRIA PHENOTYPE DUE TO A HOMOZYGOUS H237R MISSENSE MUTATION

Kasapkara CS[1], Hasanoglu A[1] , Ezgu FS[1], Okur I[1], Tumer L[1], Cakmak A[2], Balwani M[3], Nazarenko I[3], Clavero S[3], Yu C[3], Bishop DF[3], Desnick RJ[3]
[1]*Dept Ped Metab & Nutri, Gazi Univ Hosp, Ankara, Turkey*
[2]*Dept Peds, Med School of Harran Univ, Sanliurfa, Turkey*
[3]*Dept Genet/Genom Sci, Mt Sinai Sch Med, New York, NY, United States*

Hereditary coproporphyria (HCP) is an autosomal dominant acute hepatic porphyria due to coproporphyrinogen oxidase (CPO) deficiency, resulting in the accumulation of coproporphyrin III. The CPO enzyme normally catalyzes the step-wise oxidative decarboxylation of the heme precursor, coproporphyrinogen III (COPRO'gen III), to protoporphyrinogen IX via a tricarboxylic intermediate, harderoporphyrinogen. To date, only a few homozygous HCP patients have been described; most have had harderoporphyria, a rare variant due to mutations in CPO enzyme residues 400–405. Patients with harderoporphyria present with neonatal hyperbilirubinemia, hemolytic anemia, hepatosplenomegaly, and skin lesions when exposed to sunlight, and may have acute neurovisceral attacks after puberty. Patients with homozygous HCP have acute neurologic attacks and may have skin lesions. Here, we describe a 4-month old male, the product of a consanguineous union, who presented with neonatal hyperbilirubinemia treated with phototherapy, hemolytic anemia, hepatosplenomegaly, and skin lesions when exposed to sunlight. He was homoallelic for CPO mutation H327R (c.980A>G), and had massively increased urinary uroporphyrinogen I and III (9250 and 2910 μM, respectively) and coproporphyrinogen I and III (895 and 19,400 μM, respectively). (Normal levels for these porphyrin isomers: trace to < 82 μM.) The patient expired at 4 months of age, and fecal porphyrins were not studied. Both parents and a brother were H327R heterozygotes who had HCP. Of note, H327 is known to interact with W399 in the CPO active site, presumably accounting for the harderoporphyria phenotype. Further studies to express the mutation will substantiate its involvement in the conversion of harderoporphyrinogen to its dicarboxylic intermediate.

594-P

QUANTITATIVE IN VIVO 1H-MAGNETIC RESONANCE SPECTROSCOPY OF THE BRAIN IN CHILDREN AT THE HOSPITAL FOR SICK CHILDREN

Al-Hertani W[1], Mason E[1], Schmitt B[2], Blaser S[3], Branson H[3], Schulze A[1]
[1]*Dept of Metab Genet, Hosp Sick Children, Toronto, Canada*
[2]*German Cancer Research Center, Heidelberg, Germany*
[3]*Dept of Diag Imag, Hosp Sick Children, Toronto, Canada*

MRS is one of the few techniques that can be used for the in vivo assessment of metabolic processes in the brain. We analyzed brain proton MRS data from 902 children, to formulate age dependent reference ranges of metabolites from both the basal ganglia (BG) and the 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 scanners. Our patient population was referred for: isolated abnormal neurological symptoms or signs (AN, N=440), isolated developmental delay (DD, 77), AN and DD (185), isolated dysmorphisms or structural malformations (DY, 36), DD and DY (47), DD, DY and AN (20), AN and DY (25), a known single gene/chromosomal disorder (24), and a known metabolic disorder (48). The patients were divided into 3 age groups (0–12 months, 12–48 months and 48 months-18 years). In the BG at 144 ms, we observed an age dependant increase for all of NAA, tCr, and choline metabolites. However, in the PVW at 33 ms, measurements showed an age dependant increase for NAA, but a decrease for choline, while tCr was highest in the 12–48 months group and lowest in the 0–12 months group. To our knowledge, this is the largest study of its kind to embark on measuring age dependant ranges for in vivo concentrations of brain metabolites in children.

595-P

THE EFFECT OF EARLY DIAGNOSIS AND TREATMENT IN INFANTS WITH INBORN METABOLIC DISEASES

Zaman TZ[1], Nosrati AN[2], Moradian RM[1]
[1]*Iranian National Research Society, Tehran, Islamic Republic of Iran*
[2]*Metab Unit, Tehran Univ, Tehran, Islamic Republic of Iran*

Background: Newborn screening using tandem mass spectrometry (MS/MS) is an extremely sensitive method of identifying a variety of inborn errors of metabolism mainly aminoacidopathies, organic acidemias and fatty acid oxidation defects. Early diagnosis and treatment is very effective in most of these disorders.
Methods: We conducted a case series retrospective study in 30 high risk neonates: i) 17 neonates with one or more affected siblings with mental retardation, severe motor deficit, convulsion and microcephaly, including 7 cases that had prenatal diagnosis and treatment. ii) 13 cases with non-specific sign and symptoms: Convulsions, 8 cases (26.7%); coma, 6 (20 %); poor feeding; 5 (16.7%); vomiting, 5 (16.7%); hepatomegaly, prolonged hyperbilirubinemia, and respiratory distress in the neonatal period, each one (6.6%).
Results: The results of MS/MS were obtained as follows; Organic acidemia: 70%; MMA,6; MMA +respiratory chain defect, 2; propionic academia,3; lactic acidosis, 5; fatty acid oxidation defect, 4; LCAD, 1; 3HMG , 1; glutaric acidemia II, 2; respiratory chain disease with tubulopathy, 1. Carbohydrate disorders: 2 (galactosemia, 1); aminoacidopathies, 3 (PKU, 2; hepato-renal tyrosinemia, 1); urea cycle defect, 4. Five year follow up care with early specific treatment was surprising with regard to growth and development: 70% very well, 23.3% well, and only 2 cases without significant improvement (fulminant neonatal cases).
Conclusion: Early diagnosis and treatment is effective in most inborn errors of metabolism.

596-O

CHARACTERIZATION OF THE LOCOREGIONAL BRAIN ENERGY PROFILE IN WILD-TYPE MICE AND IDENTIFICATION OF AN ENERGY DEFICIT IN A NEURODEGENERATIVE MODEL

Mochel F[1], Durant B[2], Schiffmann R[2], Durr A[1]
[1]*Dpt Genet, La Salpêtrière hosp, Paris, France*
[2]*Instit Metab Dis, Baylor Res Instit, Dallas, United States*

Background: Cerebral energy deficit plays an important role in various neurometabolic and neurogenetic diseases, and can be the target of promising therapeutic approaches.
Methods: To reliably characterize the locoregional and temporal brain energy profile in wild-type (WT) mice of different body weights, we used a microwave fixation system that instantly inactivates brain enzymes and preserves in vivo concentrations of adenine nucleotides. High energy phosphates were measured by HPLC in striatum, hippocampus and frontal cortex. We validated our system in an acute model of energy deficit using 3-nitroproprionic acid (3NP) and we determined the brain energy profile of a transgenic model of Huntington disease (HD), an autosomal dominant neurodegenerative disease.
Results: We established for the first time the locoregional levels of AMP, ADP, ATP, creatine and phosphocreatine in brains of WT mice. In the 3NP model, a significant decrease of ATP was observed in the striatum associated with a significant decrease of phosphocreatine. In the HD model, we found a significant decrease of ATP in the striatum, hippocampus and frontal cortex. Unlike previous studies, we also found a significant increase of creatine and phosphocreatine in HD mice, preceding the decrease of ATP at a presymptomatic age, and suggesting compensatory mechanisms for the chronic brain energy deficit in HD.
Conclusion: Our system can be used to characterize the brain energy profile of many neurological disease models for which an energy deficit is suspected.

597-P

REFERENCE VALUES OF EXPANDED NEONATAL SCREENING WITH A MS/MS NON-DERIVATIZED METHOD IN SOUTHERN SPAIN

Yahyaoui R[1], Rueda I[1], Dayaldasani A[1], Olea M[1], Serrano J[2], Gonzalo M[3], Sierra C[2], Pérez V[1]
[1]*Clinical Laboratory, Carlos Haya Hosp, Málaga, Spain*
[2]*Dep Pediatrics, Carlos Haya Hosp, Málaga, Spain*
[3]*Dep Endocrinology, Carlos Haya Hosp, Málaga, Spain*

Background: The implementation of MS/MS in a newborn screening laboratory requires the establishment of appropriate reference intervals, based on the method used and the population under study. Recently, we have introduced in our laboratory an expanded newborn screening in Oriental Andalucia (45000 samples/year) with a commercial kit that does not require prior derivatization of the samples and permits the analysis of 12 aminoacids and 30 acylcarnitines.
Objectives: The establishment of global reference values (p1/p99.5) for aminoacids and acylcarnitines in the first 3348 samples analyzed in our laboratory, using the reagent kit MassChrom. (Chromsystems, Germany), and stratified by weight and feeding type.
Methods: From each dried blood card, one 3.2-mm diameter disc was punched into 96-well microplates, and 100 μL of internal standard was added to each well. Elution was carried out by gentle rotation during 20 min, and then transferred to fresh 96-well microtiter plates, this working solution was ready for injection. We loaded 20μL aliquots of working solution into an API3200-triple quadrupole mass spectrometer (ABSciex). Analysis was performed in the multiple-reaction–monitoring (MRM) mode. Precision and accuracy were monitored in each analytical run.
Results: The global reference values obtained for aminoacids (p99.5,μmol/L) were: Ala523.82, Arg27.64, Asp558.19, Cit32.37, Glu769.16, Gly730.01, Leu/Ile279.24, Met26.88, Tyr219.21, Phe104.85, Orn367.91 y Val192.14.For 13 acylcarnitines (p99.5/p1,μmol/L) were: C050.20/7.71, C248.82, C34.09, C40.61, C50.46, C5DC0.37, C60.08, C80.10, C100.17, C120.18, C140.33, C164.76/0.38, C181.19/1.19.
Discussion: Every screening laboratory should determine its own cutoff values in a pilot study.These cutoff values are for guidance purposes only, and may vary depending on the patient groups and the MS/MS system used.

598-P

TREATABLE METABOLIC DISORDERS CAUSING INTELLECTUAL DISABILITY: A SYSTEMATIC LITERATURE REVIEW

van Karnebeek CD[1], Leenders AG[2], Stockler-Ipsiroglu S[1]
[1]*Div Metab Dis, BC Child Hospital/UBC, Vancouver, Canada*
[2]*Emma Child Hosp/AMC, Amsterdam, Netherlands*

Background: Intellectual disability / Developmental delay (ID/DD) affects 2.5% of children worldwide. Studies reporting the yield of diagnostic work-up in individuals with ID/DD have focused primarily on the frequency of causal conditions. However, the aim of every clinician is not to miss the small but increasing subset of treatable inborn errors of metabolism (IEMs) causing ID/DD.
Aim & Methods: To provide an overview of the currently treatable IEMs causing ID/DD, we have performed a systematic literature review. All steps were performed by 2 independent reviewers with regular consensus meetings regarding formulation of definitions, search (strategy) in Pubmed (1965–2010) & Scriver's 'Metabolic and Molecular Bases of Inherited Disease', selection & categorization of treatable IEMs.
Results: Inherent to rare diseases, available evidence for effect of therapy is scarce and often of limited quality. A total of 55 'treatable IEMs' causing ID/DD were identified; Levels of evidence included A: Standard treatment; B possible effect on ID/DD; C: experimental. Therapeutic modalities include diet, co-factor/vitamin supplements, substrate inhibition, enzyme replacement, stem cell transplant. Outcome (defined as effect on IQ, developmental test scores, behavior, epilepsy, neuro-imaging) varied from improvement to halting or slowing neurocognitive regression.
Applications: Our review data establish the basis for: 1) evidence-based guidelines to aid the clinician in recognition of treatable conditions causing ID/DD (which will be presented in an interactive fashion) 2) an ongoing High-Throughput Sequencing study to screen simultaneously for a subset of these IEMs in large groups of individuals with ID/DD.

599-P

INCIDENCE OF INOSINE TRIPHOSPHATE PYROPHOSPHOHYDROLASE (ITPA) DEFICIENCY IN PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES

Zamzami M[1], Catley L[1], Marinaki A[2], Bowling F[1], Duley J[1]
[1]*The University of Queensland, Brisbane, Australia*
[2]*St Thomas' Hospital, London, United Kingdom*

Mutations affecting genes which produce nucleotide imbalances may represent mutational risk factors for the development of haematological and other malignancies, e.g. myelodysplasias (MDS). We tested the novel idea that low ITPase activity in ITPA carriers may be associated with late onset haematological malignancies. We hypothesised that accumulation of rogue nucleotides such as deoxy-ITP may be mutagenic.

Aim: The study assessed the incidence of ITPA 94C>A and IVS2+21A>C polymorphisms associated with ITPase deficiency among stable untreated patients with chronic lymphocytic leukaemia (CLL), acute myeloid leukaemia (AML) and MDS.

Methods: 22 MDS, 28 CLL and 18 AML patients were screened for ITPA SNPs. Standard PCR primers were used to amplify exons 2,3 and 4 (Sumi et al, 2002). Thermocycler conditions were 95:Cx5min, 35 cycles (94:Cx20s, 56:Cx20s, 72:Cx30s), ending 72:Cx10min, followed by sequencing.

Results and Discussion: For Caucasian populations, expected frequencies of 94C>A and IVS2+21A>C are 6% and 13% respectively. In our study, the frequency of 94C>A with MDS was 11.4% (p=0.134) and IVS2+21A>C 20.5% (p=0.141). For CLL, these frequencies were 7.1% (p=0.719) and 12.5% (p=0.911), and for AML 2.8% (p=0.416) and 8.3% (p=0.405) respectively. Silent ITPA mutations were normal frequencies for all diseases. The ITPA 94C>A and IVS2+21A>C frequencies were therefore not statistically different from normal for the hematological malignancies, but were close to significance (e.g. 1 more carrier required) for MDS. The international prognostic scoring system (IPSS) values were not available for this patient base and further studies of ITPA effects on prognosis may be productive.

600-P

A METABOLIC SCREEN

Turner C[1], Dalton RN[2]
[1]*WellChild Lab, Evelina Children's Hosp, London, United Kingdom*
[2]*Wellchild Lab, King's College London, London, United Kingdom*

Background: The continued elucidation of new inborn errors of metabolism (IEM) provides both clinical and analytical challenges. The increased number of potential tests available and the serendipity involved in many clinical diagnoses presents the clinician with both intellectual and financial choices, while the laboratory is faced with the logistical problem of providing an increasing range of analyses in a clinically relevant time frame. Recent increases in the sensitivity of electrospray mass spectrometry-mass spectrometry (MSMS) instruments have made it possible to measure normal whole blood/plasma concentrations of diagnostic metabolites, e.g. orotic acid, previously only measured in urine. In addition, the flexibility of the instrumentation challenges the conventional class compound analytical approach.

Methods & Results: We have developed an analytical system, based on an AB SCIEX API5000 platform, that combines "routine" and "esoteric" metabolites in a rapid, <30 min, and highly cost effective Metabolic Screen. A single sample preparation with multiple injections, chromatography, and positive and negative ion acquisitions allows the inclusion of the majority of diagnoses achieved with conventional amino acid, acylcarnitine, purine, pyrimidine, and organic acid analyses. The "esoteric" analytes include creatine, guanidinoacetate, ureidopropionate, galactose-1-phosphate, sialic acid, and 3-O-methyl-DOPA. Stable isotope internal standards are included for most analytes. Difficult but critical analyses are performed at the same time but on separate samples and include total homocysteine, succinylacetone, and biotinidase activity. Diagnostic protein analysis is being evaluated.

Conclusion: We regard this as the first step in an innovative metabolomic and proteomic approach to developing a rapid and cost effective Metabolic Screen.

601-P

ADULT INBORN ERRORS OF METABOLISM: THE ITALIAN EXPERIENCE

Burlina AP[1], Cazzorla C[2], Manara R[3], Bombardi R[1], Turinese E[1], Zanco C[2], Bordugo A[2], Burlina AB[2]
[1]*Neurological Unit, San Bassiano Hosp, Bassano del Grappa, Italy*
[2]*Inherit Metab Dis Unit, Univ Hosp, Padova, Italy*
[3]*Neuroradiology Unit, Univ Hosp, Padova, Italy*

In 2001 we set up an outpatient clinic for adult patients with inborn errors of metabolism (IEM). At that time, this was the first Italian experience in the field. Due to the highly frequent and severe neurological complications, our primary clinical interest was the neurological follow-up of adult patients with IEM. Nowadays, more than 100 adult patients (age range: 16–71 years) are regularly followed-up. According to disease category our cohort of patients can classified as follows: PKU & BH4 18%, Lysosomal disorders 17%, Organic acids 17%, Homocysteine 16%, Urea cycle disorders 11%, Carbohydrates 9%, Peroxisomal disorders 6 %, Amino acids 5%, Wilson disease 4%, Lipids, sterols, lipoproteins 3%, Fatty acid oxidation 1%, Neurotransmitters 1%, Porphyria 1% (we have only few patients with mitochondrial disorders because in Italy there are specialized centers for mitochondrial diseases). Almost 30% of our adult patients were diagnosed in the adulthood, mainly in the groups of Lysosomal disorders, Homocysteine, and Urea cycle disorders. Diagnosis in adult age is difficult due to poor awareness of IEM among general practioners and adult specialists. Despite the present distant geographical locations, there is a strict collaboration between the pediatric team and the neurological one. The neuropsychologist, the neuroradiologist, and the dietician are working in both teams and there is one reference specialized biochemical laboratory for all patients. In our experience, the adult neurologist represents the best bridging specialist for the clinical transition from the metabolic pediatric setting to the adult medical care.

602-P

LONG TERM FOLLOW UP OF CHILDREN IDENTIFIED THROUGH EXPANDED NEWBORN SCREENING: CLINICAL AND PUBLIC HEALTH ASPECTS

Botto LD[1], Feuchtbaum L[2], Dowray S[3], Noble Piper K[4], Romitti PA[5], Wang Y[6], Palmer M[7], Olney RS[8], Hinton C[8]
[1]*Div Medical Genetics, Univ of Utah, Salt Lake City, United States*
[2]*Genet Dis Screen Prg California Dpt Hlth, California, United States*
[3]*Public Health Foundation Enterprises, California, United States*
[4]*Cntr Cong Inherit Dis, Iowa Dpt Pub Hlth, Iowa, United States*
[5]*Dpt Epidemiology, Univ Iowa, Iowa, United States*
[6]*Cong Malf Registry, NY State Dpt Hlth, Albany, United States*
[7]*Utah Dpt Health, Utah, United States*
[8]*Nat Cntr Birth Def Dev Disab, CDC, Atlanta GA, United States*

Background: Expanded newborn screening is a reality in many countries, but population-based data on outcomes and public health benefits are scarce. We are pilot-testing a population-based monitoring system to track and evaluate trends, outcomes, and service utilization using as a basis existing public health programs (newborn screening and birth defect monitoring programs).
Methods: In four US states with expanded newborn screening (California, Iowa, New York, Utah) we used multiple modalities of ascertainment, including active case finding, record linkages, and direct input from metabolic centers to identify affected newborns and characterize their outcomes. The system tracks diagnosis, treatment, complications, morbidity, mortality, and use of services (outpatient and inpatient). Data are pooled centrally for ongoing monitoring.
Results: Among a birth cohort of 1.1 million births, 238 babies (1 in 4,800 births) with one of 19 conditions identified through tandem MS screening were followed through age two years. Examples include GA-1 (9 cases), 3-MCC (55 cases), MCAD (61 cases), and VLCAD (17 cases). By their first birthday, one fourth of children had an emergency room visit; one third had a hospitalization (nearly all precautionary); one child died; and 1% of children with fatty acid oxidation disorders did not gain skills, vs.16% with organic acidemias or amino acid disorders.
Conclusions: Longitudinal population-based monitoring of occurrence and outcomes can be enhanced by integrating existing public health programs, including birth defect surveillance systems, and by tracking data that can be used not only clinically but also for public health evaluation.

603-P

USING HIGH RESOLUTION MELTING FOR MUTATION SCANNING ON PAH, GALT, GCDH, AND CBS GENES

De Lucca M[1], Albanez S[1], Arias I[1], Casique L[1], Florez I[1], Rodríguez T[1], Araujo Y[1]
[1]*Instituto de Estudios Avanzados-IDEA, Caracas, Venezuela*

Background: Molecular diagnosis of inborn errors from metabolism has been made using different time-consuming techniques which make diagnosis these pathologies a laborious process. Furthermore, it is known that each of these diseases can be caused by hundreds of different mutations and mutation spectrum depend on genetic background of populations. Here we evaluated a simple and sensitive diagnosis screening method using high resolution melting (HRM) with direct sequencing confirmation for detecting sequence variations in PAH, GALT, GCDH, and CBS genes.
Methods: The samples used had been previously analyzed by single-strand conformation polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE), restriction analysis, and sequencing in our laboratory. HRM was used for mutation scanning of selected samples derived from phenylketonuria, galactosemia, glutaric aciduria type 1 and homocystinuria patients with a known genotype. HRM was standardized for each of those genes with the same primers used in the methods named above and the addition of LCGreen plus melting dye. Amplicons were between 129 and 378pb long.
Results: All analyzed samples with mutant genotypes were unambiguously distinguished from wild-type samples. However, in some homozygote patients was necessary to create heteroduplex, mixing the patient samples with a normal DNA. Application of this approach has allowed us to analyze about nine patients and eighteen different mutations has so far been detected.
Conclusions: HRM is a rapid, sensitive, economical, and specific close-tube screening technology that can be used in diagnostic laboratories to facilitate whole gene mutation screening, including unknown mutations.

604-P

REFERENCE INTERVALS FOR AMINOACIDS AND ORGANIC ACIDS OBTAINED BY UNSUPERVISED MULTIVARIATE ANALYSES

Ottolenghi C[1], Nedorezov T[2], Chadefaux-Vekemans B[1], Ricquier D[3], Barouki R[1], de Lonlay P[4], Rabier D[3]
[1]*INSERM U747, Metab B, Necker H, Descartes U, Paris, France*
[2]*Delmarva Foundation, Columbia (MD), United States*
[3]*Metab Bioch, Necker H, AP-HP, Descartes U, Paris, France*
[4]*INSERM U781, Div Pediatr, Necker Hosp, Paris, France*

Background: Reference intervals for metabolic markers require criteria to unambiguously define the reference population from which normative values are derived. In practice, selection of bona fide reference populations may be hampered by incomplete knowledge of the many possible confounding factors (e.g., diet and therapy). Here, we devised an "omics"-like strategy to select reference individuals in a completely agnostic fashion.
Methods: The analysis included all the patients investigated in a French National Center of Reference for Metabolic Diseases (Necker Hospital) over a period of 3 years. Reference individuals were identified by statistical exclusion criteria involving iterative unsupervised multivariate analyses of aminoacid and organic acid profiles regressed on age. All external information, including available knowledge on disease status, was disregarded. Statistical analyses employed public domain software.
Results: Overall, reference intervals were consistent with previous studies, but discrepancies were differentially distributed among metabolites suggesting bias. Unbiased estimation was instrumental to extract statistical signatures of diagnostic relevance. The approach enabled us to cope effectively with the heterogeneity of metabolic profiles that are associated with rare diseases (e.g., deficiency for dihydrolipoamide dehydrogenase, E3). Similarly, we generated reference intervals for patients' follow-up markers. The latter deviated from the reference intervals of the unselected population in a disease- and age-dependent fashion.
Conclusions: Defining reference intervals by purely statistical criteria may facilitate diagnosis in the presence of biochemical heterogeneity and may help to improve follow-up of patients.

605-P

LITIGATION IN HEALTH: THE EXAMPLE OF LARONIDASE FOR THE TREATMENT OF MPSI IN BRAZIL

Boy R[1], Krug B[2], Picon C[3], Santana-da-Silva L[4], Steiner C[5], Acosta A[6], Ribeiro E[7], Marcial F[8], Braz A[9], Leivas P[10], Braz M[9], Schwartz I[2]
[1]*Ped Dept, UERJ, Rio de Janeiro, Brazil*
[2]*Medic Genet Serv, HCPA, Porto ALegre, Brazil*
[3]*Int Med, HCPA, UFRGS, Porto Alegre, Brazil*
[4]*Biol Sci Inst, UFPA, Pará, Brazil*
[5]*Med Gen Dept, UNICAMP, Campinas, Brazil*
[6]*Ped Dep, UFBA,, Salvador, Brazil*
[7]*Albert Sabin Child Hosp, Fortaleza, Brazil*
[8]*Med Genet Mol Biol U, Cuiabá, Brazil*
[9]*DepSocSci, FIOCRUZ, Rio de Janeiro, Brazil*
[10]*Law School, Porto Alegre, Brazil*

MPSI is a lysosomal disease whose treatment involves laronidase, a high-cost drug, approved by some regulatory agencies including the Brazilian one. Since it is not covered by the Specialized Component of Pharmaceutical Assistance of the Ministry of Health, the access to it is mostly guaranteed through lawsuits and little is known about them. Our aims are to characterize judicial requests of laronidase, in two states of Brasil- RJ and RS, as well as the related decisions.
An observational, cross-sectional, and retrospective study was developed and approved by ethical committees. Data were collected from January 2008 to August 2008-lawsuits proposed until December 2007.An instrument for analysis was elaborated. Ten lawsuits were analyzed: RS(07),RJ (03).Profile of the plaintiffs: male/female (04/06) under 18 (08/10); all had only one plaintiff and were instituted by private counselors. All medical prescriptions were provided by clinical geneticists of the public health services. In all cases there was preliminary injunction granting a mandate. Main arguments for granting: the right to health, avoiding progress of the disease and suffering of the plaintiff. Defendants' main answers: impact on the public budget; participation in Research Protocols. Medical expertise advice (02/10) ratified the need of the medications.
Funding of orphan drugs represents a strong effort especially for poor countries. The Brazilian Constitution reinforces the right to treatment with high-cost drugs, through the definition of a rational evidence-based policy. Mechanisms of sustainability for the treatment of orphan diseases, such as MPS I, ought to be encouraged based on the analyses of efficacy and safety on the long term and through the institution of clinical guidelines.

606-P

THE COMPULSORY MILITARY DUTY IN TURKEY PROVIDES AN OPPORTUNITY TO DETECT INHERITED METABOLIC DISEASES IN MALE ADULTS

Kurt I[1], Tapan S[1], Caglar YE[1], Ozturk O[1], Sertoglu E[1], Erbil MK[1]
[1]*Dept Med Biochem, Gulhane School of Med, Ankara, Turkey*

Background: Although characteristic features of the most inherited metabolic diseases (IMDs) appear until late childhood, some IMDs are hard to diagnose due to late onset and/or well tolerated signs and symptoms and/or, misdiagnosis or inattention owing numerous reasons.
Objective: To determine the usefulness of compulsory military duty for diagnosing IMDs in adult males.
Methods: This was a retrospective review of the IMDs diagnosed according to the results of the analysis performed in Biochemical Genetic Laboratory in the soldiers consigned to Gulhane Military Medical Academy either during military service or at pre-recruitment evaluations to their official examination for military service done within last 13 years.
Results: During the period 1997 to 2010, such 49 patients who were missed from medical care until then, and have been diagnosed to have IMDs. The most frequent IMDs diagnosed were porphyrias(n=28), metabolic myopathies(n=8) and disorders of carbohydrate metabolism (n=9). The diagnostic distribution of the porphyria cases were as follows: erythropoietic protoporphyria (n=10), porphyria cutanea tarda (n=3), variegate porphyria (n=10), congenital erythropoietic porphyria (n=3), acute intermittent porphyria (n=2) Consists of other inherited rare metabolic diseases were Wilson disease, Dubin-Johnson syndrome, Fabry disease and Pompe disease.
Conclusion: Compulsory military duty is a favorable chance means to detect some patients with inherited metabolic disease mainly porphyria and metabolic myopathy who were not diagnosed previously.

607-P

EVOLUTIONARY STUDY OF FOUR PROTEINS FUNDAMENTAL FOR THE MYELIN METABOLISM

Barrera LA[1], Echeverri OY[1], Montaño AM[2]
[1]*I.E.I.M. Universidad Javeriana, Bogota, Colombia*
[2]*St Louis University, St Louis M.O., United States*

Galactocerebrosidase (GALC) and Arylsulfatase A (ARSA) are two lysosomal enzymes important for myelin degradation and turnover in eukaryotic organisms. Both enzymes use saposins A and B respectively as activators. From the evolutionary standpoint, the four proteins belong to different families: saposins belong to a highly conserved family whose origin and evolutionary mechanisms are well studied. GALC, which deficiency causes Krabbe disease, has not been widely studied. ARSA, which deficiency causes Metachromatic Leukodystrophy, is highly conserved through evolution and it is present only in eukaryotes. We propose that the emergence of ARSA and GALC is evolutionarily linked to the specialization of the nervous system in higher organisms and that saposins already present before the emergence of ARSA end GALC, play a role in the process. Our aim is to elucidate the evolutionary relationship between the two enzymes and their activators, in myelin metabolism. We used Ensembl and NCBI databases to select amino acid and nucleotide sequences of 25 different eukaryotic species from jawed fishes to higher mammals. We have established the ideal alignment to assess evolutionary distances, age, and selective pressures for the two enzymes studied. Our preliminary results show that the emergence of ARSA in eukaryote evolution has not undergone any major evolutionary event. In Contrast GALC shows a significant conformational change in fishes and amphibians lines. Saposins, although present already in evolution, specialized to participate in myelin turnover. The results of this work will give insights on the interaction of these proteins in the evolution of the nervous system.

608-P

ESTABLISHMENT OF REFERENCE INTERVALS FOR FREE CARNITINE AND ACYLCARNITINES FROM BIRTH TO ADULTHOOD: A POSTERIORI SAMPLING APPROACH

Basol G[1], Barutcuoglu B[1], Bozdemir AE[1], Habif S[1], Kabaroglu C[1], Coker M[2], Parildar Z[1], Bayindir O[1]
[1]*Dep Clin Biochem, Ege Univ, Izmir, Turkey*
[2]*Dep Ped Endo and Metab, Ege Univ, Izmir, Turkey*

Background: Analysis of the free carnitine and acylcarnitine profiles by tandem mass spectrometry has improved the detection of inherited errors of metabolism in newborns. Age- and gender-specific reference intervals are an important prerequisite for interpreting carnitine measurements in children. The objective of this study was to determine the reference intervals for whole blood free carnitine and acylcarnitines from birth to 18 years.
Methods: Test results obtained from 2820 individuals of an outpatient clinic pediatric population were included into the analysis. Reference individuals were selected using a posteriori sampling technique, the process in which exclusion and partition of reference individuals occurs after specimen collection and analysis. The health status of children was determined from the medical records and laboratory results. Individuals were divided into subgroups by age ranging from 3–7 days, 8–30 days, 1–24 months and 2–18 years, respectively. Free carnitine and acylcarnitines from dried blood specimens were analyzed by tandem mass spectrometer.
Results: The final sample size for reference data creation was 2554. For each age group 2.5–97.5 percentiles were calculated by using the non-parametric method. Free carnitine levels were significantly lower in newborns than in older children ($P < 0.05$) whereas the several acylcarnitine levels were significantly higher in the newborns than in groups of older children.
Conclusion: A posteriori sampling approach may be useful for the determination of reference intervals in select situations including pediatric population where collection of sufficient numbers of reference samples may be difficult.

609-P

EXPANDED NEONATAL SCREENING OF INHERITED METABOLIC DISORDERS BY TANDEM MASS SPECTROMETRY IN THE CZECH REPUBLIC: RESULTS OF 6 MONTHS PERIOD IN ONE CENTER

Bártl J[1], Chrastina P[2], Hornik P[2], Krouská L[2], Pinkasová R[2], Hladíková J[2], Koubíková H[2], Ko-ich V[2], ťastná S[2]
[1]*Inst Inher Metab Dis, Gen Facul Hosp, Prague, Czech Republic*
[2]*Inst Inher Metab Dis, Gen Facul Hosp, Prague, Czech Republic*

Objectives: To evaluate the performance of the screening program for 10 inherited metabolic diseases (IMD) mandated by the Ministry of Health of the Czech Republic since October 1, 2009 using criteria recommended by the international project on neonatal screening- Region4Genetics (R4G). According to R4G the 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 by help of Chromsystem kit into methanol with deuterium-labeled internal standards, derivatized by butylation and analyzed by MS/MS profiling of selected amino acids and acylcarnitines on API 2000 or API 3200.
Results: Between 1st October 2009 and 31st March 2010 we have analyzed samples from 40 951 newborns from various regions of the Czech Republic. We have detected 8 neonates with subsequently confirmed IMD, i.e. 7 patients with PKU/HPA and 1 patient with MCAD deficiency, thus, the calculated incidence of PKU and MCAD deficiency was 1:5,850 and 1:41,000, respectively. The detection rate for 10 IMDs in the first half year of expanded screening was 1:5,119. The total false positive rate was 0.13% with much higher frequency of false positivity (0.8%) among neonates with low birth weight (< 2500 g).
Conclusions: The first six 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.

610-P

DEVELOPMENT OF VISUAL COUNSELING AIDS FOR INHERITED METABOLIC DISORDERS: NEWBORN SCREENING THROUGH DIAGNOSIS

Bernstein LE[1], Wright E[1], Long C[2], Rice C[1]
[1]*Div Metab Dis, the Children's Hospital, Aurora, United States*
[2]*Children's Memorial Hospital, Chicago, United States*

Newborn screening of inherited metabolic disorders possess many challenges; particularly regards to effective communication and education of parents of infants with abnormal newborn screening results. Parental understanding needs to occur quickly in order for parents to grasp the complexity of the disorder so that treatment and home management are effective. Teaching aids for metabolic disorders have been limited to complex diagrams that physicians have been trained on. Inherited Metabolic Disorders: Visual Aids (IMD:VA) book was developed by a team of genetic counselors, dietitians, and biochemical geneticists from the Inherited Metabolic Diseases Clinic at The Children's Hospital in Colorado. The primary goal was to create visual aids that aid in parental understanding of basic concepts of biochemistry and multiple metabolic disorders. The book is comprised of two sections: general metabolic and specific biochemical pathways. The book depends on provider expertise for successful explanation with a final goal of improved treatment and management of the metabolic disorder. The book consists of visual aids covering basic concepts of metabolism, newborn screening, and multiple biochemical pathways. The visuals aids were created with both patients and providers in mind. IMD: VA is currently being distributed throughout the metabolic community. The IMD: VA has been translated into Dutch and is now available online www.metabolicteachingaid.com
A future survey will be conducted with the goal to evaluate the effectiveness in terms of parental understanding and ease to the provider. The results of the survey will enable providers to introduce new concepts to be added to the IMD: VA.

611-P

ASSESSMENT OF THE EFFECTIVENESS OF METABOLIC UNIVERSITY: AN ENTRY LEVEL PROGRAM FOR REGISTERED DIETITIANS, NURSES, GENETIC COUNSELORS AND PHYSICIANS

Bernstein LE[1], Freehauf C[1], Thomas JA[1], Yannicelli S[2]
[1]*Nutricia NA, Rockville, United States*

Metabolic University is an interactive program designed to educate clinicians within the field of inborn errors of metabolism (IEM). With expanded newborn screening and other advances the need for healthcare professionals trained in IEM has become paramount. Diagnosis and management of patients with IEM requires an interdisciplinary approach including: dietitians, nurses, genetic counselors and physicians. Metabolic University is targeted to professionals newly entering the field. The training program was designed to provide information on natural history, biochemistry, genetics and pathophysiology of common metabolic disorders. Goals are to enhance knowledge and critical thinking skills. Education methods implemented include interactive lectures, group teaching and observations. One hundred and thirty one registered dietitians, nurses, geneticist and physicians participated over six sessions of the program. Participant knowledge was measured by a pre-test and a post-test. The program itself is evaluated by participants using questionnaires that addressed program content, presentation and knowledge gained. Participants had a mean 26% statistically significant increase in correct answers from the pre-test to the post-test ($p<0.001$) which indicated knowledge gained regarding treatment of patients with IEM. Participant comments regarding the clarity, organization, expertise and enthusiasm of the speakers were largely positive. Overall, participants rated Metabolic University a 9.78 on a Likert scale of 1 to 10. The Metabolic University unique education approach has been a successful venue in training clinicians treating patients with IEM. A follow up survey will be published looking at long term benefits.

612-P

REMOTE TRAINING PROGRAMME IN INBORN ERRORS OF METABOLISM (IEM) FOR PEDIATRICIANS LOCATED FAR FROM HIGH COMPLEXITY HOSPITALS IN ARGENTINA

Bay L[1], Eiroa H[1], De Pinho S[1]
[1]*Div Metab Dis, Hosp Garrahan, Buenos Aires, Argentina*

Background: Argentina has 40 million inhabitants living in 23 provinces along 2780400 km2. There are few diagnostic and treatment centers for patients with IEM, so physicians have to consult, send laboratory samples or their patients to these centers. Screening for 3 IEM is performed. Most Pediatricians are not able to suspect IEM and lack information about emergency treatments and on follow up of cases.
Objective: consider an IEM remote consultation and training program, to promote clinical suspicion, early detection and monitoring guidelines for there.
Material: The Hospital has a Distance Communication Office (DCO), where pediatricians can inquire by fax about cases that can´t be solved locally. The number of inquiries about IEM increased from 2008 to 2010. In 2008 the Hospital started a free one year online course with an on line forum plus three-face meetings. One pediatrician by province is selected to participate. They can learn and discuss aspects of the laboratory and clinical cases and be trained about newborn screening, early clinical suspicion, emergency treatment and monitoring children with IEM.
Results: The CDO shows that since the beginning of the course the number and quality of consultations about IEM improved. Since the beginning of training program 50 pediatricians in different provinces can suspect and follow locally IEM patients who were initially diagnosed and treated at the Hospital, with permanent contact with DCO.
Conclusions: Due to Argentina´s Health System characteristics the DCO is very useful. Pediatricians distance training helped raising IEM suspicion and facilitated monitoring patients living far from the referred centers.

613-A

TANDEM MASS SPECTROMETRY FOR INBORN ERRORS OF METABOLISM SCREENING IN HIGH RISK BRAZILIAN PATIENTS

Oliveira AC[1], Miragaia AS[1], D'Almeida V[1], Micheletti C[2], Mendes CSC[1], Holanda M[1], Fonseca AA[3], Gomes LNLF[3], Fonseca JHR[3], Vieira Neto E[3], Martins AM[1]
[1]*CREIM, Dep. Ped. - UNIFESP, São Paulo, Brazil*
[2]*Dep. Ped. - UNIFESP, São Paulo, Brazil*
[3]*DLE - Diag. Lab. Especializados, Rio de Janeiro, Brazil*

Background: Mass spectrometry has been widely used for the screening of inherited metabolic diseases, and many neonatal screening programs have demonstrated the efficacy of the methodology. As well, the use of dried blood spots on filter paper (DBS) enables safe transportation through long distances. Material and methods: The use of tandem mass spectrometry for the diagnosis of inborn errors of metabolism (IEM) in high risk Brazilian patients was evaluated in a collaboration between CREIM (Centro de Referência em Erros Inatos do Metabolismo)—UNIFESP, a national reference center for the diagnosis and treatment of IEM, IGEIM (Instituto de Genética e Erros Inatos de Metabolismo) a non-governmental institution which supports investigation and treatment for IEM and, DLE (Diagnósticos Laboratoriais Especializados), a private clinical laboratory which was the only one in Brazil performing these assays.
Results: One hundred and forty nine DBS samples from 124 patients were analyzed between February/2007 and February/2010.Thirty two of these analyses from twenty one patients were considered positive for the diagnosis of an IEM, resulting in an IEM prevalence of 17% in the studied population: 15 organic acidurias, 2 aminoacidopaties, 2 urea cycle defects, 1 beta-oxidation defect and 1 ketolysis defect.
Conclusions: Mass spectrometry in an important tool in IEM investigation allowing a significant rate of diagnosis which, in turn, provides possibilities of treatment for these patients.
Financial support: CNPq, AFIP and IGEIM.

614-A

ACCREDITATION OF THE METABOLIC LABORATORY UNDER THE EN ISO 15189

Stastna S[1], Maskova V[1], Kostalova E[1], Chrastina P[1]
[1]*Inst Inherit Metab Dis, Gen Univ Hosp, Prague, Czech Republic*

Metabolic laboratory of the Institute of Inherited Metabolic Disorders of General University Hospital and 1st Medical Faculty of Charles University in Prague is preparing for accreditation by Czech Institute for Accreditation under EN ISO 15189:2007 with deadline XII/2010.
Preparation for the Accreditation is a part of the project Metabolic Diagnostic Centre.
European standard EN ISO 15189 Medical laboratories—Particular requirements for quality and competence specifies requirements for quality and competence particular to medical laboratories. It is used by medical laboratories in developing their quality management systems and by accreditation institutions in confirming the competence of medical laboratories.
The Standard includes mainly:
1. Management requirements—organization and management, quality management system, document control, review contracts, examination of referral laboratories, external services and supplies, advisory services, resolution of complaints, identification and control of nonconformities and corrective and prevention action, continual improvement, quality and technical records, internal audits and management review;
2. Technical requirements—staff, accommodation and environmental conditions, laboratory equipment, pre-examination procedures, examination procedures, assuring quality of examination procedures, post-examination procedures and reporting of results.
The project Metabolic Diagnostic Centre is aimed at increasing of effectiveness of laboratory and clinical diagnostics of inherited metabolic disorders in our Institute in children from the Czech Republic thanks to new top analytical devices, extended diagnostic procedures, accreditation of the Metabolic laboratory and publications dedicated to Czech doctors of all specializations.
The Project is supported by Norway Grants and General University Hospital in Prague and realized in years 2008–2011.

615-P

PITT-HOPKINS SYNDROME AND ERYTHROPOIETIC PROTOPORPHYRIA IN A PATIENT WITH 18Q21 DELETION DIAGNOSED BY SNP ARRAY

Tsiakas K[1], Grosse R[2], Uyanik G[3], Fuchs S[3], Santer R[1]
[1]*Dep Ped, Univ Med Cen, Hamburg-Eppendorf, Hamburg, Germany*
[2]*Ped Hem, Univ Med Cen, Hamburg-Eppendorf, Hamburg, Germany*
[3]*Hum Gen, Univ Med Cen, Hamburg-Eppendorf, Hamburg, Germany*

Haploinsufficiency of TCF4 on chromosome 18q21.2 or heterozygosity for point mutations within this gene encoding transcription factor 4 were recently identified as the underlying cause of Pitt–Hopkins syndrome (PTHS), an autism spectrum disorder with dysmorphic features and breathing abnormalities. Erythropoietic protoporphyria (EPP) typically follows an autosomal (pseudo)dominant trait; it is caused by mutations of the ferrochelatase gene (FECH) on chromosome 18q21.3. Clinical expression of the disease with photosensitivity and liver complications results from coinheritance of the common hypomorphic allele IVS3 48C trans to a deleterious FECH mutation. We present a patient with PTHS and EPP as a contiguous gene syndrome caused by a large deletion in 18q21.2-q21.32.
Case Report: Clinical characteristics of the 13-years-old female patient, born to consanguineous Turkish parents, were dysmorphic features, severe muscular hypotonia, apnea episodes, hyperventilation, severe psychomotor and mental retardation, dystonia, lack of speech development, lack of walking ability, short stature and glaucoma. Slight photosensitivity was noticed after direct sun exposition; no hepatic manifestations till now. EPP was confirmed biochemically by increased erythrocyte and fecal protophorphyrin. SNP array revealed a 5.4 Mbp deletion in 18q21.2-q21.32 affecting both the TCF4 and FECH locus. The second allele harbored the low expression FECH polymorphism IVS3–48C.
Discussion: Coexistence of PTHS und EPP has only once been published in the literature in a patient with an 18q21 deletion long ago before the genetic background of these two entities was fully understood. To our knowledge, this is the first patient with PTHS and EPP, who is completely characterized on the genetic level.

616-P

INFANTILE SCA7 IS ASSOCIATED WITH SEVERE RENAL DISEASE

Maertens P[1], Manci E[1], Chen TJ[1]
[1]*University of South Alabama, Mobile, United States*

Background: Renal disease has only been previously reported in one infant with large CAG repeats in the SCA7 gene. The infantile phenotype differs significantly from adult and juvenile onset phenotype.
Methods: Clinical history and evaluation was obtained in all affected family members of a large family recently diagnosed with SCA7. Urine samples were obtained in all family members. Renal pathology was obtained in patients with renal disease.
Results: Patients with juvenile and adults onset SCA7 failed to demonstrate signs and symptoms of nephrotic syndrome. Their urine was negative for proteins. Two infants with large CAG repeats (>110) had signs and symptoms of nephritic syndrome. There was a massive proteinuria. Kidney was ectopic in one infant. Renal pathology showed collapsing glomerulopathy and enlarged proximal tubules with increased granularity or mild vacuolization. In both infants visual symptoms were not present early on. Developmental regression preceded dysphagia and tongue atrophy with fasciculation
Conclusions: Renal disease with collapsing glomerulopathy is due to large CAG repeats in the SCA7. Renal disease is only seen in patients with infantile onset. Such patients present with ataxia and tongue fasciculations.

617-P

PRORESSIVE SPASTIC PARAPLEGIA IN A FEMALE WITH DUPLICATION OF THE PLP-1 GENE

Maertens P[1], Dees D[1]
[1]*University of South Alabama, Mobile, United States*

Background: Pelizaeus-Merzbacher disease (PMD) is X-linked and females are usually asymptomatic. Spastic paraplegia has rarely been reported in females affected by this condition.
Case report: We experienced a sixteen year old female who was born healthy and had normal early development. She failed the first grade, but remains in regular classes. When she was 11 year-old, she was first noticed to have increasing foot drop when walking and slumping into a chair when sitting. Clumsiness and frequent falls got worse at age twelve. The examination shows a spastic gait, weak lower extremities and no atrophy. Sensory examination is normal. Extensive work up was done.
Results: Electromyography and nerve conduction studies were normal. The child had a negative workup for hereditary spastic paraplegia and genetic testing for Charcot-Marie-Tooth. MRI of brain showed a thin corpus callosum as would be expected in a male PMD patient. MRI of the spine excluded syringomyelia and tethered cord. FISH analysis showed a duplication of the PLP-1 gene consistent with the diagnosis of a carrier state of the Pelizaeus-Merzbacher gene.
Conclusion: Duplication of the PLP-1 gene rarely can be expressed as a spastic paraplegia PMD phenotype in females. A similar situation is seen in X-linked adrenoleukodystrophy, a disease that affects in addition the peripheral nerves, where females rarely present with a myeloneurophty phenotype.

618-P

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY BASED ASSAY OF ADENYLOSUCCINATE LYASE ACTIVITY IN ERYTHROCYTES AND DRIED BLOOD SPOTS

Bierau J[1], Pooters INA[1], Visser D[1], Bakker JA[1]
[1]*Dept Clin Gen, Lab Biochem Gen, MUMC, Maastricht, Netherlands*

Background: Adenylosuccinate lyase defiency is a disorder characterized by a broad clinical spectrum. The laboratory diagnosis is made by detection of succinylpurines in urine, but is severely hampered by the lack of availability of the index metabolites succinylamino-imidazole carboxamide riboside (SAICAR) and Succinyl-Adenosine (SAdo). Alternatively, patients can be identified by enzyme activity assays. A simple HPLC-based ADSL activity assay was developed and tested in erythrocytes and dried blood spots (DBS).
Methods: ADSL activity was measured in erythrocyte lysates and DBS eluates using S-AMP as substrate. Reaction mixtures were analysed using isocratic ion-pairing reversed-phase HPLC with UV-detection. Reference values in erythrocyte lysates were established.
Results: The intra- and inter assay variation in erythrocytes were 2 % and 8 %, respectively. Enzyme activity in erythrocyte lysates was stable upon freeze-thaw cycles. ADSL activity in DBS was highly unstable, 10% residual activity remained one day after spotting when compared to lysates, which declined rapidly to very low activities after 16 days in control samples.
Conclusion: A rapid and reproducible assay to assess ADSL activity in erythrocytes was developed. DBS was not a reliable medium for diagnostic services. We advocate enzyme activity measurement in erythrocytes in case of suspicion for ADSL deficiency.

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