001-P

METAGENE.DE – RAMEDIS.DE: ONLINE DISEASES AND PATIENT DATABASE FOR GENETIC METABOLIC DISORDERS

Mischke U, Scheible D, Frauendienst-Egger G, Trefz F
Childrens Hospital Reutlingen, Steinenbergstrasse 31, D-72764 Reutlingen, Germany

There is a rapid increase in numbers and complexity of genetic metabolic disorders. Databases on the internet contribute to faster diagnoses and treatments of these disorders. As result on our approach to give diagnostic support in IEMs [1] we developed the reference book METAGENE (diseases) and the electronic publishing tool RAMEDIS (patient data). So far almost 400 diseases and 700 patients with 87 different diagnoses have been implemented in a standardised terminology. METAGENE provides information regarding to clinical symptoms, general and special laboratory findings, and gives citations and links (OMIM, PubMed, Expasy). RAMEDIS provides longitudinal information about clinical, biochemical and molecular genetic findings in patients with IEM. In contrast to printed publications RAMEDIS offers the opportunity for long term follow up of (treated) patients. RAMEDIS can also be used for scientific evaluation, a basic analysing tool is implemented. Confidentiality is guaranteed by anonymous data processing. In addition informed consent was obtained from patients or their parents. The rights of the data remain with the author. In future RAMEDIS will offer the possibility to extract patients data for a continous update of disease knowledge.

[1] U Mischke, G Frauendienst-Egger, P Matthis, P Gao, FK Trefz. KBS-DIAMET: Database and expert system for diagnosis and treatment of patients with inborn errors of metabolism

002-P

METABOLIC INFORMATION HELPLINE: A TOOL FOR THE INVESTIGATION AND MANAGEMENT OF CHILDREN WITH ACUTE METABOLIC DISORDERS IN BRAZIL

C Souza, S Brustolin, L Refosco, R Pires, R Giugliani
Medical Genetics Service, Hospital de Clínicas de Porto Alegre. Porto Alegre RS – Brazil (cfsouza@hcpa.ufrgs.br)

Brazil is a large country with few diagnostic laboratories and metabolic treatment centres, and transportation may be difficult. The area of genetic metabolic disorders is still a young field in the country and most paediatricians are not familiar with the management of these diseases. To help to address these issues, a metabolic information helpline (Service of Information on Inborn Errors of Metabolism, SIEM) was set up in Brazil in October 2001. SIEM has a tool-free telephone number (0800-5102858) which can be accessed from any area of the country by physicians who are dealing with an acutely-ill child with suspected or already diagnosed metabolic disorder. The service provides information on emergency management measures, tests for follow-up and diagnosis. Until March 2004, the total number of cases is 359, 50% asking help for the diagnosis, 42% of the consultants are paediatricians and neurologists, the origin of the request 41% from southeast Brazilian; 63.7% because the child has symptoms and in these cases 45% of the symptoms begin between 28 days and first year of life; 11% of the request are because altered neonatal screening tests. We have obtained definitive diagnosis for 37 cases (10.3%) and 68% are still under investigations. In countries like Brazil, it is expected that the program would increase the identification of metabolic diseases, improve the management and increase the awareness about this group of disorders.

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003-P

EARLY SPONTANEOUS MOTOR ACTIVITY AS PREDICTOR OF LATER OUTCOME IN NEONATES WITH NEONATAL PRESENTATION OF INBORN ERRORS OF METABOLISM: A PILOT STUDY

AF Bos, JF Eizenga, GPA Smit, RI Soorani, KA Bergman, BJ Wijnberg, FJ van Spronsen
Beatrix Children's Hospital, University Hospital of Groningen, The Netherlands

Objective: The assessment of spontaneous motor activity, from video, is a sensitive method to determine the integrity of the central nervous system in neonates and young infants. Predicting later outcome in neonates presenting with severe inborn errors of metabolism (IEM) is very difficult. We studied the course of spontaneous motor activity, in particular the quality of general movements (GMs), and questioned its predictive value for later outcome. **Methods**: In 5 infants, the quality of GMs was assessed from serial videotape recordings up to 5 months. Four had a neonatal presentation of severe IEM: a still undefined gluconeogenese defect (GGD), propionic acidaemia (PPA), arginosuccinate synthetase (AS) and arginosuccinate lyase (AL) deficiency. One neonate was known with AS prenatally. Outcome at the age of at least 18 months was determined by neurological examination; additionally a developmental test (Bayley) was performed. **Results**: All infants had initially abnormal GMs: hypokinesia, followed by a poor repertoire of GMs. In 2, (both AS) the GMs normalised, in one during the first week (antenatal AS) and one during the 3rd month. In one (AL) the quality of GMs normalised at 3 months, albeit with a reduced repertoire. In 2 the quality of GMs remained abnormal (PPA) or severely abnormal (GGD) during the entire study period. The more severe or longer lasting abnormalities of GMs occurred, the more neurological outcomes and scores on developmental tests were abnormal. **Conclusions**: The number of patients is still low, but the data show that assessment of spontaneous motor activity might help in predicting later outcome in infants with IEM.

004-P

LONG-TERM OUTCOME OF UREA CYCLE DEFECTS AND ORGANIC ACIDURIAS

Deodato F, Caviglia S, Bartuli A, Sabetta G, Dionisi-Vici C
Metabolic Unit and Psychology Unit, Bambino Gesù Hospital, IRCCS, Rome, Italy

We retrospectively analysed 60 patients 36 UCDs (3 CPS, 6 OTC male, 13 OTC females, 4 AS, 5 AL, 5 HHH) and 24 OAs (12 PA, 8 mutMMA, 2 HMG, 1 IVA, 1 KT). All were treated with low protein diet and, according to their disease, with recommended supplements. Pts were divided in 2 groups. A) neonatal onset, 29 patients (14 UCDs, 15 OAs), B) later onset, 31 patients (22 UCDs, 9 OAs). At long term follow-up, they were classified as normal (IQ/DQ > 79), mildly (IQ50-79/DQ60-74), and severely-retarded (IQ < 49/DQ < 59). **Group A**: 7 patients died neonatally and 7 between 1.5–15 yrs (overall mortality rate of 48%). At short-term follow-up (2 yrs) a normal/borderline development was recorded in 68% of cases, however at longer term evaluation 66% became mentally retarded (MR). Among the 15 long-term survivors,MR was severe in 9, mild in 4, with only 2 patients presenting normal cognitive development. **Group B**: the mortality rate was 10% (3 OTC female), and among the 26 long-term survivors 65% shows normal IQ. Neonatal onset patients have higher mortality compared to those with a later onset. Although the short-term evaluation of neonatal-onset patients was satisfactory in most of the survivors, their long term developmental outcome was very poor. At follow-up, a significant difference in the number and severity of life-threatening relapses of metabolic decompensation was observed between the two groups of patients. Since most of neonatal cases show an early favourable course, followed by progressive deterioration, the option for early liver transplantation should be carefully considered in these patients.

005-P

COMBINATION OF TANDEM MASS SPECTROMETRY AND LYSOSOMAL ENZYMES ANALYSIS – EFFECTIVE TOOL FOR SELECTIVE SCREENING FOR IEM IN NEUROLOGICAL CLINIC

Baydakova GV, Boukina AM, Boukina TM, Shechter OV, Michaylova SV, I'lina ES, Zakharova EY
Research Centre for Medical Genetics RAMS, Moscow, Russia

Most of inborn errors of metabolism (IEM) manifest in early years with different neurological symptoms. Not infrequently, IEM fall outside current standard diagnostic tools. For more effective detection of IEM we include in programme of selective screening electrospray tandem mass spectrometry (to diagnose defects of fatty acid oxidation, aminoacidopathies and organic acidurias) and tests for the enzymatic detection of lysosomal storage disorders. For this study patients were selected from neurological department of Russian Child Hospital. In our study clinical findings of patients were characterized by failure to thrive, psychomotor retardation, muscle hypotonia, drug-resistant epileptic seizures, episodic lethargy and coma, hepatosplenomegaly. The biochemical investigation was completed in 217 patients and an IEM were confirmed in 9.7% (21 cases). In 5.5% (12 cases) aminoacidopathies/organic acidurias were detected. Lysosomal storage disorders were detected in 4.2% (9 cases). The disorders most frequently identified were GM1 gangliosidosis ($n = 4$), biotinidase deficiency ($n = 3$), neuronal ceroid lipophyscinosis type 2 ($n = 3$). **Conclusion**: Combination of electrospray tandem mass spectrometry and tests for the enzymatic detection of lysosomal storage disorders provides an effective method for IEM detection in patients of neurological clinics.

006-P

SERUM S-100B LEVELS IN X-ALD AND GAUCHER DISEASE

H Michelakakis[1], C Kariyannis[2], M Moraitou[1], E Dimitriou[1], J Sarafidou[1], I Papassotiriou[2]
[1]Institute of Child Health, 11527 Athens, Greece, [2]Department of Clinical Biochemistry, 'Aghia Sophia Children's Hospital', 11527 Athens, Greece

S-100B is a member of a multigenic family of calcium-modulated proteins synthesized in astroglial cells in all parts of the central nervous system. Serum concentrations of S-100B have been used as markers of brain damage. In the present study serum S-100B levels were measured (immunoluminometric assay, Sangtec 100) in patients with X-ALD ($n = 16$; 10 childhood, 4 adult, 2 asymptomatic) and Gaucher disease ($n = 22$; 19 type I, 3 type II). Overall, no statistically significant differences (Mann-Whitney test) were observed between either the X-ALD (range 0.05–0.46, median 0.13; 25th-75th percentile 0.07–0.18 μmg/l; $p = 0.191$) or Gaucher type I patients (range 0.03 0.45, median 0.07, 25th-75th percentile 0.05–0.13 μg/L; $p = 0.095$) and healthy controls of similar age (range 0.05–0.15, median 0.10, 25th–75th percentiles 0.08–0.12 μg/L, $n = 22$). The serum S-100B levels of the type II Gaucher patients were also within the normal for their age limits (patients: 0.2, 0.22, 0.65; control range 0.44–2.55, median 0.95, 25th–75th percentile 0.44–2,55 μg/L, $n = 44$). Furthermore, within the X-ALD group, lack of clinical symptoms and/or MRI findings was not associated with lower values. Our results indicate that serum S-100B levels cannot be used as a marker of brain damage in X-ALD and Gaucher disease.

007-A

FREQUENCY OF DIFFERENT TYPES OF LEUKODYSTROPHIES IN RUSSIA

Mikhaylova SV[1], Ilina ES[1], Pilia SV[1], Akhunov VS[2], Boukina TM[2], Shechter OV[2], Zakharova EY[1], Petrukhin AS[3]
[1]*Russian Clinical Child Hospital, Moscow, Russia;* [2]*Research Centre for Medical Genetics RAMS, Moscow, Russia;* [3]*Russian Medical University, Moscow, Russia*

The leukodystrophies (LD) are heterogeneous group of degenerative diseases that involve primarily the white matter of the brain and sometimes also the peripheral nerves. During the period 1988–2003 over 230 patients with clinical findings and brain magnetic resonance imaging proven white matter involvement were investigated in our neurological department. In 69 cases primary LD was confirmed using of enzymatic and metabolite analysis. Within this group metachromatic leukodystrophy (MLD) and X-linked adrenoleukodystrophy (XALD) are the most frequent forms. MLD was representing 42% and XALD 39% of all diagnosed primary LD. Krabbe disease (14%) and Canavan disease (4%) were also detected. Eighteen cases may be classified as secondary changes in myelin development (organic acidurias and mitochondrial disease, GM1-gangliosidosis). And a significant proportion of inherited leukodystrophies/leukoencephalopathies has not been linked with special metabolic deficiency. Our results demonstrates that MLD and XALD are the most frequent forms of leukodystrophies with known metabolic origin in Russian patients.

008-A

A COMPUTER-BASED SOLUTION FOR SCREENING OF INHERITED METABOLIC DISEASES

M Pinheiro[1], JL Oliveira[1], MAS Santos[1], H Rocha[2], ML Cardoso[2], L Vilarinho[2]
[1]*University of Aveiro, IEETA, Portugal;* [2]*Instituto de Genética Médica, Portugal*

In the last few years the advances in tandem mass spectrometer (MS/MS) technology had led to its introduction in neonatal screening laboratories. Significant challenges still remain since it implies a significant financial effort, a change in the way of thinking the diagnosis of IEM and the need to manage and process a massive amount of data.

The introduction of MS/MS (two API2000 from AppliedBiosystems) in the Portuguese national neonatal screening laboratory (with over 110 000 samples/year) has led to the development of a new application that can help technicians handle the large amount of data involved (with more than 80 parameters/sample) and can assist in the implementation of a reliable quality control procedure.

The application consists of an information system supported by mathematical and statistical tools that automate the evaluation data procedure. The incorporated statistical tools allow a daily update of the marker cut-offs through the use of percentile scores of valid historical data. Mathematical expressions, for future use, can also be created from the markers used in the system. The layout of the operator interface is constructed to familiar paradigms allowing for increased user friendliness.

The system also allows an effective control of the intensities of the internal standards and control values as well as it performs control charts for the markers.

009-P

OUR EXPERIENCE WITH SCREENING BY TANDEM MASS SPECTROMETRY

Chrastina P, Stastna S, Myskova H, Kosarova M, Kostalova E, Elleder M, Zeman J
Institute of Inherited Metabolic Disorders, General Faculty Hospital and 1st Faculty of Medicine of Charles University, Prague, Czech Republic

With the aim to prepare conditions for national-wide neonatal screening of inherited metabolic disorders (IMDs) in Czech Republic by tandem mass spectrometry (MS/MS), we analysed retrospectively 1130 samples from 118 patients with IMDs and 13 315 samples from patients suspected for IMDs. We characterized reference range for concentration of amino acids and acylcarnitines in blood spot and pathological levels for IMDs occurred in our region. We prospectively analysed 40 393 samples from neonates born between 2002 and 2003.

Results: In the *selective* screening we detected 14 patients with IMDs (5 × MCAD deficiency, 3 × PKU/HPA, 2 × multiple acyl-CoA dehydrogenase deficiency, 1 × tyrosinemia type I, 1 × VLCAD deficiency, 1 × methylmalonic acidaemia, 1 × deficiency of transport of acylcarnitines). Profile of acylcarnitines was normal in some patients with SCAD, CPT II and LCHAD deficiency in well metabolically compensated states.

In the *neonatal* screening we detected 7 patients with IMDs (4 × PKU/HPA, 1 × LCHAD deficiency, 1 × MCAD deficiency and 1 × methylmalonic acidaemia, frequency of IMDs 1:5771).

Conclusions: We verified that we are now able to detect 19 IMDs. Some patients with defect in beta oxidation of fatty acids can escape the detection in the screening. We are prepared to start the national-wide neonatal screening program for IMDs by MS/MS in Czech Republic.

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010-P

ORGANIC ACIDURIAS: MASS SCREENING, MANAGEMENT AND FOLLOW-UP IN THE PROVINCE OF QUEBEC

Auray-Blais C, Giguère R, Lemieux B
Newborn Mass Urinary Screening Program, Department Medical Genetics, Centre hospitalier universitaire de Sherbrooke (CHUS), Sherbrooke, Quebec, Canada

Screening for aminoacidurias (AA) and methylmalonicacidurias (MMA) was implemented in 1973 in the Province of Quebec as part of a voluntary neonatal urine screening program. Since then, more than 2 136 879 babies have been screened for MMA. A thin layer chromatographic (TLC) technique with sequential use of different sprays on the same plate permits the detection of abnormalities in AA as well as MMA in urine filter paper specimens of 21-day-old babies. Since October 2000, we have devised an add-on TLC technique to screen for 10 other organic acidurias using bromocresol green reagent. More than 209 054 babies have been screened with this add-on technique. Two cases of methylcrotonylglycinuria and one case of glutaric aciduria type 1 were detected. MMA cases detected by screening were classified in 3 groups : mild excretor (250–500 μmoles/mmole creatinine), potential high-risk excretor (500–1000) and definite high-risk excretor (> 1000). Fifty-six cases were detected, with an incidence of 1:38 000. In all positive cases (excreting more than 250 μmoles/mmole creat), the parents are phoned directly (without contacting the child's physician). Explanations regarding the urine results and the follow-up procedure are then given to them. Afterwards, they select one of four regional centres where diagnosis, counselling, management and follow-up are done. The incidence of other confirmed organic acidurias cases is 1: 69 000. At the time of referral, direct contact with the parents allows a more rapid clinical intervention and at the same time lowers the anxiety on their part.

011-P

EXPANDED NEWBORN SCREENING FOR INBORN ERRORS OF METABOLISM BY TANDEM MASS SPECTROMETRY IN AUSTRIA

Möslinger D, Mühl A, Mercimek-Mahmutoglu S, Konstantopoulou V, Stöckler-Ipsiroglu S, Bodamer OA
Austrian Newborn Screening Program, University Children's Hospital Vienna, Austria

Objective: Expanded newborn screening for inborn errors of metabolism (IEM) using tandem mass-spectrometry was started in Austria two years ago. To date more than 158 000 infants were screened for a total of 18 disorders including aminoacidopathies, organoacidopathies and fatty acid oxidation defects (FOD).

Results: Total recall was 0.54% (n = 866) with a diagnostic yield of 0.04% (n = 42) and a false positive rate of 0.5% (n = 823). Diagnoses included: HPA/PKU (n = 19), FOD (n = 14), propionic acidaemia (n = 2), MMA (n = 1), GA I (n =1), 3-methylcrotonyl carboxylase (3-MCC) deficiency (n = 5), 3-methyl glutaconyl-CoA hydratase deficiency and MSUD (n = 1). Family investigations identified 3 asymptomatic siblings and 6 mothers with 3-MCC deficiency. Four children with IEM (LCHAD, cblC, tyrosinemia) were not identified through our program due to diagnostic markers below the respective cut-off values.

Conclusions: Expanded newborn screening programs allow reliable early diagnosis of many different IEM with the possibility of early therapeutic intervention and subsequent reduction of mortality and morbidity.

012-P

COMBINED MS/MS AND DNA-BASED NEWBORN SCREENING IN TWO CANADIAN PROVINCES

DC Lehotay[3], C Rockman-Greenberg[1], JR Thompson[2], F Cantlon[2], L Seargeant[1]
[1]*University of Manitoba,* [2]*Cadham Provincial Laboratory, Winnipeg, and* [3]*Provincial Laboratory, Regina, Canada*

Routine MS/MS based newborn screening was introduced in Saskatchewan (SK) in 2000 and targeted DNA-based neonatal screening for HHH disease, over-represented in a First Nations community in northern SK, is being introduced. In Manitoba (MB), a neighbouring province with a similar population base (1 million), in addition to 'traditional' newborn screening, supplemental DNA-based neonatal screening for glutaric acidaemia type 1 (GA1) of all First Nations babies of Oji-Cree descent began in 1998 and supplemental DNA-based neonatal screening for carnitine palmitoyl transferase-1 (CPT1) deficiency in all Hutterite babies began in 2002. DNA-based newborn screening was adopted because of the high carrier frequency ($\sim$1 in 15 for each of these diseases), and because MS/MS screening does not always detect these diseases at birth. Of $\sim$2670 Oji-Cree newborns screened, 8 babies affected with the GCDH IVS-$1^{+5\ g>t}$ mutation causing GA1 have been identified. Of $\sim$500 Hutterite newborns screened for the CPT1 G710E causative of CPT1 deficiency, one affected baby has been diagnosed. Only half of all GA1 cases had elevated glutarylcarnitine, and acylcarnitines in the newborn affected with CPT1 deficiency were normal. Detection of inborn errors of metabolism in MB and SK was most effective with a combination of MS/MS and targeted DNA-based neonatal screening. A prior understanding of the spectrum, frequency, aetiology and biochemical phenotype of metabolic disorders in the catchment area is essential for the design of an effective newborn screening program. In our experience MS-MS and DNA-based newborn screening are mutually complementary.

013-P

MATERNAL DISORDERS DETECTED BY CORD BLOOD SCREENING

Patterson A[1], Till J[2], Parke D[2], Fleming G[2], Olpin SE[3], Henderson MH[1], Besley GTN[2], Walter JH[2]
[1]Department of Clinical Chemistry, St James's Hospital, Leeds, [2]Willink Unit, Manchester Children's Hospital, Manchester [3]Department of Clinical Chemistry, Sheffield Children's Hospital, UK

Cord blood samples from centres with a significant number of consanguineous families were screened by TMS for acylcarnitines and amino acids. We have now screened approximately 10 000 deliveries. In two, abnormalities in cord blood acylcarnitines have led to the identification of inborn errors in the mothers, whereas their infants were not affected. One infant had a low free carnitine in cord blood of 1.5 μmol/L. Subsequent studies at 4 days gave values of 2.5 μmol/L in both mother and infant. Fatty acid oxidation flux using tritiated myristate, palmitate and oleate in fibroblasts was normal in the infant but reduced in the mother to 25–46% of simultaneous controls. Pre-incubation of cultured fibroblasts with 0.5 mmol/L L-carnitine normalised fatty acid oxidation flux. Fibroblast carnitine transport with 5 μmol/L carnitine was $<1\%$ of controls confirming a maternal OCTN2 defect. The other abnormality was in an infant with a raised C5-OH acylcarnitine in cord blood of 8.6 μmol/L (normal <1.0); at 6 days it was 4.8 μmol/L and by 36 days, 2.5 μmol/L. The baby was well and there was no abnormality on organic acid analysis. Mother was also well but her blood C5-OH was high at 21.4 μmol/L, indicting that she probably had 3-methylcrotonyl-CoA carboxylase deficiency. No confirmatory studies were done on the mother but subsequent family studies revealed a cousin with a confirmed enzyme deficiency.

Abnormal acylcarnitine in cord blood may reflect metabolic disorders in the mother rather than infant.

014-P

UNDISCLOSED TRANSFUSION: A RISK FOR EXPANDED WHOLE POPULATION NEWBORN SCREENING

M Downing, AJ Matthews, JR Bonham, JC Allen, S Ellin, M Maloney, NJ Manning, SE Olpin, G Race
Regional Newborn Screening Service, Childrens Hospital, Sheffield, UK

In March 2004 we added sickle cell disease (SCD) and MCADD to our newborn screening programme which screens 55 000 babies per year for PKU, hypothyroidism and cystic fibrosis. Samples are taken on day 6; 91% of babies are at home, 9% are in hospital and 0.7% have had a transfusion. To minimize the possibility of a false negative result due to the transfusion we have a policy of recall for metabolite screening where samples are taken <3 days post transfusion (PT) (0.05%). So far, SCD screening has revealed 8/7605 (0.11%) samples with significant Hb A > Hb F where no transfusion was recorded on the Guthrie card (though only one of these samples was found to be taken <3 days PT). With our combined incidence of screened disorders, excluding SCD, being 1:1150, this incidence of non-disclosed transfusions presents a significant risk of an invalid result (similarly reported by AP Reynolds et al., 2002). This risk will clearly be much greater for services offering expanded MSMS screening. These findings highlight the need for a PT sample policy, a 2003 survey by the UKNSLN revealed 35% of UK laboratories had no such policy. It has resulted in improvement of our own service for all disorders by alerting us to the need for recall even when we are not notified of transfusions and prompted us to enhance our education programme to promote pre-transfusion sample collection and accurate recording of details on the Guthrie card. It also highlights the need for screening to be provided as an integrated service.

015-P

TANDEM MASS SPECTROMETRY (MS/MS) SCREENING WAS NOT A DIAGNOSTIC TOOL IN A PATIENT WITH SHORT CHAIN ACYL-CoA DEHYDROGENASE (SCAD) DEFICIENCY

Kostalova E, Stastna S, Kosarova M, Chrastina P
Institute of Inherited Metabolic Disorders, General Faculty Hospital and 1st Medical Faculty of Charles University, Prague, Czech Republic

Objective: To evaluate whether measurement of acylcarnitine concentrations and diagnostic ratios using MS/MS is a powerful diagnostic tool in a patient with SCAD deficiency with EMA-uria, confirmed on molecular genetic level.

Methods: Acylcarnitines extracted from dried blood spots into a metanol solution with deuterium-labeled internal standards and derivatized were analysed using MS/MS. The patient's samples for selective screening were taking in the age of 4 months to 14 months. One sample was collected when the boy suffered from encephalopathy. Newborn blood spot was analysed too.

Results: Nine acylcarnitine profiles were determined (8 profiles from selective screening and 1 profile from newborn screening). Butyrylcarnitine concentrations and C4/C2 and C4/C3 ratios were under cut-offs in all 8/8 selective screening and 1/1 newborn screening.

Conclusions: MS/MS newborn and selective screening was not reliable marker for diagnosis of SCAD deficiency in our patient. Acylcarnitine analysis by MS/MS may not reveal all patients with SCAD deficiency. Normal acylcarnitine concentrations and diagnostic ratios in newborn and/or selective screening do not exclude SCAD deficiency.

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016-P

MEDIUM-CHAIN ACYL-CoA DEHYDROGENASE DEFICIENCY FOUND THROUGH NEWBORN SCREENING BY TANDEM MASS SPECTROMETRY IN JAPAN

G Tajima, N Sakura, H Ono, M Kobayashi, I Hata[1], Y Shigematsu[1]
Department of Pediatrics, Hiroshima University Graduate School of Biomedical Sciences, Hiroshima, Japan; [1]Department of Pediatrics, University of Fukui, Fukui, Japan

Introduction: Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency was believed to be an extremely rare inherited disorder in Japan, and had not been reported in the neonatal period among Japanese before our study. We show that the introduction of tandem mass spectrometry (MS-MS) in newborn screening led to the detection of three patients with MCAD deficiency in Hiroshima area.

Methods: Diagnosis of MCAD deficiency in newborns with elevated C8-acylcarnitine levels in dried blood spots was confirmed by the enzyme assay using lymphocytes. Mutation in the gene encoding MCAD was identified by direct sequencing of PCR products.

Results: In the last five years, 88 886 out of 139 317 newborns in Hiroshima area were eligible for this MS-MS screening. Three patients with MCAD deficiency were diagnosed by the enzyme assay among five suspected cases. The MCAD activity in the three patients was 2.8%, 13.8%, and 10.9% of the mean in normal subjects, respectively. The sites of mutation identified in MCAD gene were heterogenous; a novel mutation (del449-452) was found in one allele of two patients.

Conclusion: These results indicate that the application of MS-MS to newborn screening is essential to find MCAD deficiency in Japan. It is important to establish rapid and simple tests of enzyme assay and gene analysis of MCAD for early confirmation of this disorder.

017-P

GC/MS METHOD STILL HAS A SPACE FOR NEONATAL METABOLIC SCREENING

K Tashiro, I Yoshida, T Inokuchi, K Aoki, M Inaba, A Fumimori, K Matsumoto, C Hara, M Tanaka

Research Institute of Medical Mass Spectrometry, Kurume University School of Medicine, Kurume, Japan

We evaluated effectiveness of the GC/MS method for newborn screening for inherited metabolic disorders. A total of 48 614 urine samples from healthy newborns including premature babies were screened during the 8 years study period (1996–2004). This screening method is based on the urease treatment on neonatal urine (Shoemaker et al., 1991). The samples were analyzed by automated GC/MS. Thirty-three patients were discovered including 8 patients with methylmalonic acidaemia, 2 patients with propionic acidaemia, 2 patients with glycerol kinase deficiency, 1 patient with citrullinemia, 1 patient with ornithine transcarbamylase deficiency, 1 patient with maple syrup urine disease, 1 patient with dihydropyrimidinase deficiency, 1 patient with dihydropyrimidine dehydrogenase deficiency, 2 patients with 3-ureidopropionase deficiency, 12 patients with 2-ketoadipic aciduria, 2 patients with neuroblastoma. The incidence of methylmalonic acidaemia (1/6077) is more than 10 times higher compared to the incidence by MS/MS in Japan (Shigematsu et al., 2004). We believe that GC/MS method still has a good space for neonatal metabolic screening, especially for methylmalonic acidaemia.

018-P

OROTIC ACID AND URACIL ARE NOT GOOD DIAGNOSTIC MARKERS IN NEONATAL SCREENING FOR INHERITED METABOLIC DISEASE

M Inaba, I Yoshida, T Inokuchi, K Aoki, K Tashiro, A Fumimori, K Matsumoto, C Hara, M Tanaka

Research Institute of Medical Mass Spectrometry, Kurume University School of Medicine, Kurume, Japan

The aim of this study is to evaluate the usefulness of orotic acid and uracil as diagnostic marker for inherited metabolic diseases.

From January, 1996 to March, 2004, the urine samples of 48 614 newborns were screened. Orotic acid was high in 30 newborns and uracil was high in 41 newborns. Both orotic acid and uracil were elevated in 28 newborns. Among 99 (30+41+28) newborns, only 6 newborns were diagnosed as inherited metabolic diseases. Six newborns are citrullinemia type 1 (n = 1), ornithine transcarbamylase deficiency (n = 1), dihydropyrimidinase deficiency (n = 1), dihydropyrimidine dehydrogenase deficiency (n = 1), 3-ureidopropionase deficiency (n = 2). The remaining 93 newborns may include transient abnormalities, persistent orotic aciduria, persistent uraciluria and other unknown disorders.

In conclusion orotic acid and uracil are not good diagnostic markers for the diagnosis of inherited metabolic diseases in specificity.

019-A

CAN WE SCREEN FOR GALACTOSEMIA BASED ON AMINO ACIDS DATA FROM MS/MS?

H Rocha, ML Cardoso, L Vilarinho
Instituto de Genetica Médica Jacinto de Magalhães Praça Pedro Nunes 88, 4050-466 Porto Portugal

Classical galactosemia is an inherited disorder of galactose metabolism caused by deficiency of the enzyme galactose-1-phosphate uridil-transferase, and is included in many neonatal screening programs. The methodologies used are total galactose measurement, Beutler test or MS/MS for galactose-1-phosphate, on blood spots.

We retrospectively analysed blood spot samples (obtained 4–7 days postpartum) for amino acids and acylcarnitines, of eight classical galactosemic patients and verify, as expected, that they all present marked signs of hepatic dysfunction. The analysis by MS/MS showed a significant increase of phenylalanine and tyrosine as well as an abnormal unspecific acylcarnitine pattern. For long it is known the increase of these amino acids in galactosemias and we hypothesise about the use of this information to select suspected samples, for specific galactosemia test, in newborn expanded screening. All the eight samples with proven classical galactosemia revealed an increase of ((Phe+Tyr)/(Leu+Ile)), in comparison to normal controls. Based on these findings, we propose that Beutler test and/or galactose-1-phosphate measurement should be performed only in the samples that reveal an increase of this parameter. This approach, although not specific, seems quite sensitive to be assayed. More studies are needed to validate this approach and to define false negative rates. The advantages of this strategy are reduction of costs and increase labour productivity.

020-P

MOLECULAR BASIS OF VLCAD DEFICIENCY IN NEWBORNS DETECTED BY EXPANDED NEONATAL SCREENING IN GERMANY

Schymik I, Mueller M, Wendel U, Mayatepek E, Spiekerkoetter U
Department of General Pediatrics, University Children's Hospital, Düsseldorf, Germany

Background: Patients with deficiency of the very long-chain acyl-CoA dehydrogenase (VLCAD) present with heterogeneous clinical phenotypes. With expanded neonatal screening using tandem mass spectrometry, asymptomatic newborns with biochemical evidence of VLCAD deficiency can be identified.

Methods: To confirm disease, we sequenced all 20 exons of the VLCAD gene in these newborns (n = 6).

Results: On 11 of 12 alleles, we delineated mutations. Two patients were homozygous, three compound heterozygous, in one patient only one mutation was found. 8/11 mutations were single missense mutations, in 1/11 a frameshift due to an insertion of one base pair occurred, in 2/11 a homozygous splice site mutation was found. 9 different mutations were found, 4 of them were already known from literature and occurred in clinically symptomatic patients. The V243A mutation that has frequently been delineated in patients detected by newborn screening in earlier studies, was present only on one allele.

Conclusion: There is remarkable molecular heterogeneity in asymptomatic patients with VLCAD deficiency identified by newborn screening. The occurrence of mainly missense mutations suggests milder clinical phenotypes in the majority of these newborns.

021-P

DEVELOPMENT OF POPULATION SCREENING FOR WILSON DISEASE

Hahn SH, Hartman SJ, Magera MJ, Kroll C, Mensink KA, Jacobson RM, Dobrowolski S[1], Winters JL, Ferber MJ, Dawson DB, O'Brien JF, Matern D, Rinaldo P
Department of Laboratory Medicine and Pathology, Pediatric and Adolescent Medicine, Mayo Clinic College of Medicine, Rochester, MN, USA;[1]Idaho Technology, Inc, Salt Lake City, Utah, USA

Wilson's disease (WD) is an autosomal recessive disorder of copper metabolism, resulting in hepatic cirrhosis and neuronal degeneration. WD is treatable and if an early diagnosis is made, serious symptoms can be avoided. Recent reports of pilot studies in Japan and Korea using an ELISA method to measure ceruloplasmin in blood spots suggest that population screening for WD is feasible. We developed the similar in-house sandwich ELISA assay using two specific anti-ceruloplasmin antibodies and purified ceruloplasmin for standards. Intra-assay precision showed acceptable CV's within 20% and the recovery for various concentrations averaged 90% of the expected values. This assay is currently being evaluated in an IRB approved pilot study at Mayo Clinic. At least 4000 children between 3 months and 18 years of age will be enrolled by March, 2005. Abnormal results (cut off: <15 mg/dl) are followed up by 2nd tier molecular genetic analysis of the ATP7B gene using a LightCycler assay for the frequent mutation, H1069Q and a conformation sensitive gel electrophoresis (CSGE) screening of 26 amplicons of the gene's 21 exons and 5′ UTR region, followed by gene sequencing if necessary. Twenty-five candidate polymorphisms in 15 exons of the gene were characterized by performing CSGE on 96 anonymous normal controls, which is critical in interpreting the CSGE patterns and sequences in patient samples.

022-P

INFLUENCE OF HEMATOCRIT AND LOCATION OF PUNCH WITHIN DRIED BLOOD SPOTS ON AMINO ACID AND ACYLCARNITINE LEVELS MEASURED BY TANDEM MASS SPECTROMETRY

M Holub[1], A Mühl[1], G Heinze[2], B Hagerty[1], OA Bodamer[1]
[1]Departments of Pediatrics and [2]Medical Computer Sciences, University of Vienna, Austria

Objective: Expanded newborn screening programs rely on the analysis of amino acids (AA) and acylcarnitine profiles (AC) in dried blood for the diagnosis of fatty acid oxidation defects, organic acidaemias and amino acidopathies. Neonates show considerable inter-individual variability of haematocrit levels which has an effect on blood viscosity.

Methods: Blood samples with defined haematocrit levels (20, 30, 40, 50, 60%) were produced by diluting full blood cells with plasma from a single healthy donor. Forty dried blood spots were generated for each haematocrit level and a central as well as a peripheral 3 mm spot was punched and analysed for AA and AC respectively, by ms/ms.

Results: Levels of most AA and guanidinoacetate increased significantly with increasing haematocrit ($p<0.001$), while the effect of haematocrit on asparagine and tyrosine was less pronounced. Total AC, free carnitine, some long chain AC as well as some short and medium chain AC depended significantly on haematocrit levels ($p<0{,}001$), while other AC-species (C5DC, C6, C8, C10, C12, C14, C14-OH, C16-OH, C18-OH, C18:1-OH, C18:2-OH) did not. In samples with low haematocrit levels of most AA and free carnitine were higher in the peripheral than in the central areas.

Conclusions: Both haematocrit and location of punched spots have a significant (and sometimes additive) effect on levels of AA and AC measured by ms/ms in blood dried on filter cards. Theoretically, diagnoses may be missed depending on haematocrit and location of the punch.

023-P

DIAGNOSIS OF METHYLMALONIC ACIDEMIAS FROM DRY BLOOD SPOTS BY HPLC AND INTRAMOLECULAR EXCIMER FLUORESCENCE

Al-Dirbashi OY[1], Jacob M[1], Al-Badaoui F[1], Al-Hassnan ZN[2], Rashed MS[1,2]
[1]Departments of Genetics and [2]Medical Genetics, King Faisal Specialist Hospital and Research Center, Department of Genetics, PO Box 3354, Riyadh 11211, Saudi Arabia

We describe a novel HPLC method with intramolecular excimer fluorescence (IEF) detection for the diagnosis and confirmation of methylmalonic acidaemia (MMA) in dried blood spots (DBSs) of newborns and patients. MMA was extracted from DBSs, converted into fluorescence derivative by reaction with 4-(1-pyrene) butyric hydrazide, and the structure was confirmed by MS/MS analysis. Using acetonitrile/water (2:1; *v/v*) as a mobile phase, the derivative of MMA and internal standard (IS) were separated by reversed phase HPLC with retention times of 4.5 min for the IS and 5.5 min for MMA. The derivatives were stable for at least 24 hours (4°C, dark). Excellent linearity covering two orders of magnitude, sensitivity, and reproducibility were obtained. Utilization of IEF resulted in a great selectivity with no fluorescence from monocarboxylic acids and significantly enhanced the signal-to-noise ratio compared with monomer fluorescence. We carried out a blinded study using DBSs from known MMA and propionic acidaemia (PA) cases ($n = 25$, each) randomized with control DBSs (total $n = 100$) from our archives (1995 to present). The derivative was detected only in MMA cases ($n = 25$), including DBSs collected on the first day of life. The method is of great value as a second-tier testing when the MS/MS analysis of newborn screening DBSs shows abnormal propionylcarnitine, a marker for both MMA and PA. With further validation, the method may eliminate the need for urinary organic acid analysis for diagnosis of MMA.

024-P

POOR SPECIFICITY OF PROPIONYLCARNITINE IN CORD BLOOD SCREENING FOR PROPIONIC AND METHYLMALONIC ACIDAEMIA

Till J[1], Patterson A[2], Parke D[1], Fleming G[1], Henderson MH[2], Besley GTN[1], Walter JH[1]
[1]Willink Unit, Manchester Children's Hospital, Manchester; [2]Department of Clinical Chemistry, St James's Hospital, Leeds, UK

The outcome for propionic and methylmalonic acidaemia may be improved if diagnosis and treatment can be made before the onset of severe neonatal disease. In order to determine the feasibility of earlier screening for disorders of intermediary metabolism we have undertaken TMS analysis on cord blood samples collected at delivery from 4 major maternity units with populations at increased risk from these disorders. We report here our experience with the analysis of propionylcarnitine (C3). 7681 cord blood samples from separate pregnancies were collected. Of these 5841 were analysed by TMS following derivatisation and the remainder by TMS using underivatised samples. Using the derivatised method the median value for C3 was 2.21μmol/L with 95% of samples within the range of 0.85–4.70 μmol/L. For the underivatised analyses the median was 2.33 μmol/L with 95% of samples within the range 0.93 to 5.13 μmol/L. In 52 samples the C3 level was > 6 μmol/L; 6 were >8 μmol/L. In all these infants the C3 in the newborn screening sample, collected between 6 and 10 days of age, had all fallen within our normal range. One sample from a sibling of a known patient with propionic acidaemia had a cord blood C3 of 7.6 μmol/L. The infant was therefore initially thought to be affected. However C3 in the subsequent screening sample was 3.5 μmol/L and urine organic acid analysis by GCMS was normal.

We conclude that a high C3 in cord blood may not be sufficiently specific to be reliably used to screen for propionic or methylmalonic acidaemia.

025-O

MUTATIONS IN IBD (ACAD8) MAY EXPLAIN ELEVATED C4-CARNITINE DETECTED BY MS/MS SCREENING IN NEWBORNS

Bischoff C[1], Christensen E[2], Simonsen H[3], Lund A[2], Young SP[4], Koeberl DD[4], Millington DS[4], Roe C[5], Roe D[5], Pedersen CB[1], Knudsen I[1], Gregersen N[1], Andresen BS[1,6]
[1]Research Unit for Molecular Medicine, Århus University Hospital, Denmark, [2]Rigshospitalet, Copenhagen, Denmark, [3]Statens Serum Institut, Copenhagen, Denmark, [4]Duke University, North Carolina, USA, [5]Baylor College, Texas, USA, [6]Department of Human Genetics, Århus University, Denmark

MS/MS-based newborn screening for SCAD deficiency identified a Danish girl with elevated C4-carnitine, but analysis of her SCAD gene did not identify any mutations. However, GC-MS analysis of urine showed elevated levels of isobutyryl-glycine - a marker for isobutyryl-CoA dehydrogenase (IBD) deficiency. Mutation analysis of genomic DNA identified two mutations in the IBD (ACAD8) gene, and family analysis confirmed that the girl is compound heterozygous for 409G>A (G137R) and 958G>A (A320T). Analysis of 400 control individuals showed that none of these mutations are polymorphic in the Danish population, though a single individual was heterozygous for 958G>A. Despite no treatment the girl has been asymptomatic since birth.

Additionally, we have analyzed three newborns identified with elevated C4-carnitine in USA. All had mutations in both IBD alleles indicating IBD deficiency. 5 different mutations were identified, indicating that the mutation spectrum in IBD deficiency is wide with no prevalent mutations.

We conclude that newborns with IBD deficiency are identified by MS/MS-based newborn screening, and that this enzyme defect may be more prevalent than initially believed. The possible clinical implications of this enzyme defect is not known and careful follow up is necessary.

026-P

HIGH DIAGNOSTIC SENSITIVITY FOR ISOVALERIC ACIDAEMIA IN NEWBORN SCREENING DURING THE FIRST FIFTEEN HOURS OF LIFE

[1]R Fingerhut, [2]EM Maier, [2]W Röschinger, [1]B Olgemöller, [2]AC Muntau
[1]Laboratory Becker, Olgemöller and Colleagues, Munich, Germany; [2]Dr von Hauner Children's Hospital, Department of Clinical Chemistry and Biochemical Genetics, Ludwig-Maximilians-University, Munich, Germany

Isovaleric acidaemia (IVA) is caused by a defect in leucine metabolism due to a deficiency of isovaleryl-CoA dehydrogenase, a mitochondrial flavoprotein. The first clinical signs are poor feeding, drowsiness and, when untreated, progressive coma. The age of onset ranges from a few hours after birth up to several weeks. The accumulation of isovaleryl-carnitine allows the detection of IVA by tandem mass-spectrometry based neonatal screening. Data on the time course of isovaleryl-carnitine concentrations in patients with IVA after birth are not yet available.

We report about twins born at risk for IVA because of a positive family history. Isovaleryl-carnitine was determined in dried blood spots at various time points (cord blood, 3–15 hours of age). Isovaleryl-carnitine was significantly elevated in cord blood of both twins (15.4 and 13.8 μmol/L; reference range <0.3 μmol/L). After birth the twins were put on a glucose infusion. Although they did not receive any natural protein during the first fifteen hours of life, isovaleryl-carnitine levels were persistently elevated (10.0–15.4 μmol/L). The patients were put on a low protein diet. At the age of now ten months both children show a normal psychomotor development.

Our observation of early elevation of isovaleryl-carnitine concentrations has a major impact on the interpretation of acylcarnitine profiles in neonatal screening and points to the validity of results obtained from early collected samples.

027-P

A COMMON MUTATION IS ASSOCIATED WITH A NOVEL PHENOTYPE AMONG PATIENTS WITH ISOVALERIC ACIDAEMIA (IVA) DIAGNOSED BY NEWBORN SCREENING (NBS)

Ensenauer RE[1], Vockley J[2], Burton BK[3], Willard JM[1], Sass JO[4], Santer R[5], Marquardt I[6], Koch HG[7], Rinaldo P[1], Matern D[1]
[1]Mayo Clinic College of Medicine, Rochester, MN, [2]University of Pittsburgh Medical Center, PA, [3]Children's Memorial Hospital, Chicago, IL, USA; University Children's Hospitals [4]Freiburg, [5]Hamburg, [7]Münster and [6]Children's Hospital, Oldenburg, Germany

Objective: IVA manifests as either acute neonatal encephalopathy or recurrent crises with developmental delays. We report the occurrence of a common mutation among patients diagnosed by NBS that is associated with a novel, mild phenotype of IVA. **Methods**: Eighteen newborns were identified with C5 acylcarnitine (C5AC) elevations by NBS. IVA was confirmed following biochemical and molecular genetic testing, including organic acid analysis, acylcarnitine analysis, in vitro studies of fibroblast or lymphoblast cultures, and *IVD* gene sequencing. **Results**: Sixteen mutations, including 12 novel alleles, were identified. A 932C>T (A282V) mutation was found in 17 of 36 (47%) mutant alleles, including 4 homozygous cases. All were asymptomatic. This allele was not found by PCR and restriction enzyme analysis in 100 subjects with normal NBS results. Biochemical studies of family members identified 5 affected but asymptomatic siblings in 5 unrelated families. NBS blood spot C5AC and urine isovalerylglycine appeared to be of prognostic value in patients. **Conclusions**: These data demonstrate the frequent occurrence of a mild, potentially asymptomatic phenotype of IVA that is diagnosed through expanded NBS. Genotyping of newly identified cases is therefore required to recognize patients with a favorable prognosis.

028-P

SUCCINYLACETONE DETERMINATION IN DRIED URINE FILTER PAPER BY TANDEM MASS SPECTROMETRY FOR NEWBORN SCREENING OF TYROSINEMIA I

Castiñeiras DE, Cocho JA, Rebollido M, Boveda MD, Alonso JR, Couce ML, Fraga JM
Unidad Metabolopatías, Hospital Clínico Universitario, 15706 Santiago de Compostela, Spain (pdneonat@usc.es)

Tyrosinemia I can be detected in newborn screening programs by tandem mass spectrometry (MS/MS) but with risk of false negative results in some cases. Succinylacetone (SCA) in urine, measured by gas chromatography-mass spectrometry, is the metabolite used for diagnosis confirmation.

Our Newborn Screening Program includes dried urine samples and we developed a MS/MS method for SCA quantitation in the newborn samples (3 days). We extract the urine samples, $\frac{1}{4}$ inch discs, and after butylation they are directly measured in positive mode with an API 2000 (Applied Biosystems) instrument. The MRM experiment includes also the creatinine measurement in the extract. We make a calibration curve until 100 μmol/L for SCA and 400 μmol/L for creatinine. The detection limit of SCA is 2.4 μmol/L and the quantitation limit is 12.6 μmol/L. Our reference range for SCA is 95.7 ± 59.6 (16.8–208.2) μmol/mmol creatinine.

We detected a case of Tyrosinemia I with a SCA level of 512.4 μmol/mmol creatinine and borderline tyrosine level in the dried blood spot, both at the fourth day of life.

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029-P

SUCCINYLACETONE ANALYSIS IN BLOOD SPOTS BY TANDEM MASS SPECTROMETRY

Matern D, Magera MJ, Gunawardena N, Mitchell G[1], Hahn SH, Rinaldo P
Mayo Clinic College of Medicine, Rochester, MN, USA; [1]Hôpital Sainte-Justine, Montreal, Canada

Background: Tyrosinemia type I (Tyr I; fumarylacetoacetase deficiency) is a severe disorder causing early death if left untreated. Over the last decade, NTBC treatment became available which is particularly effective when initiated in newborns. While tyrosine can be determined in dried blood spots (DBS), it is not a specific marker for Tyr I and most often associated with benign transient tyrosinemia of the newborn. Succinylacetone (SUAC) is a specific marker for Tyr I but not detectable by routine newborn screening. We developed a new assay that determines SUAC in DBS by liquid-chromatography tandem mass spectrometry (LC-MS/MS).

Methods: DBS of 7 Tyr I patients were analyzed before and after initiation of NTBC treatment. SUAC is extracted from each 3/16″ DBS into an aqueous solution containing deuterium labeled SUAC as internal standard (IS; gift from SI Goodman, Denver). SUAC and IS are oximated, then extracted, butylated, and analyzed by positive electrospray LC-MS/MS using a C18 LC column. Quantitation is from SUAC spiked calibrator DBS over the range 0–200 μmol/L using selected reaction monitoring of transitions *m/z* 212 to 156 and *m/z* 214 to 140 for SUAC and IS respectively. Analysis time is 5 min.

Results: SUAC was elevated in all pre-NTBC samples (37–150 μmol/L; median: 60 μmol/L) compared to the post-NTBC samples and 100 controls (< 5 μmol/L).

Conclusion: This new LC-MS/MS based method for the determination of SUAC in DBS will significantly reduce the number of false positive results in newborn screening for this severe disease. In addition, it can be used for the laboratory follow up of patients treated for Tyr I.

030-P

FALSE POSITIVE NEWBORN SCREENING FOR BIOTINIDASE DEFICIENCY CAUSED BY HYPERCHYLOMICRONEMIA

R Santer[1,2], N Lorenzen[2], Z Lukacs[1]
Department of Pediatrics, University Children's Hospitals [1]Hamburg and [2]Kiel, Germany

Objective: Alcohol contamination and high storage temperature of Guthrie testcards are known causes for false positive results of the neonatal screening test for biotinidase deficiency. Here, we report a case of lipoprotein lipase (LPL) deficiency detected by a false positive neonatal screening result for biotinidase deficiency.

Methods: Fluorometric biotinidase assay using biotinyl-6-amidochinolin as a substrate.

Case Report: A neonate of non-consanguinous Turkish parents had a normal neonatal screening test on day 2. A routinely performed repeat test on day 11, and further tests on days 19 and 26 showed a severely decreased biotinidase activity. On day 26, lipemic plasma with a triglyceride concentration >25 000 mg/dl and hyperchylomicronemia on lipid electrophoresis were found. Apart from mildly impaired skin perfusion (*Cutis marmorata*), the clinical status was entirely normal. Molecular genetic testing revealed compound heterozygosity for two mutations of the *LPL* gene (G159E/I194T). On a fat-free diet (later switched to an MCT-based formula), plasma triglycerides normalized and repeated determinations of biotinidase activity together with analyses of organic acids and acyl carnitines excluded biotinidase deficiency.

Conclusions: The fluorometric test for biotinidase deficiency is not reliable in hyperlipidemic states; parameters of lipid metabolism should be determined in cases with a positive neonatal screening for biotinidase deficiency.

031-P

PKU TREATMENT BEYOND NATIONAL GUIDELINES

J Jansma[1], M v Rijn[1], A Brinksma[1], FC v Wijmen[2], LJM Hollands[2], HD Bakker[3], NM ter Horst[3], AC Douwes[4], H Termeulen[4], RCA Sengers[5], G Boers[5], H Zweers[5], M Rubio[6], L vd Ploeg[6], JP Sels[6], TJ de Koning[7], HW de Valk[7], E Carbasius[7], A vd Herberg[8], JBC de Klerk[9], FJ v Spronsen[1]
[1]Univ Hospital Groningen, [2]University Maastricht, [3]Academic Medical Centre Amsterdam, [4]VU Medical Centre Amsterdam, [5]University Medical Centre Nijmegen, [6]Academic Hospital Maastricht, [7]University Medical Centre Utrecht, [8]University Medical Centre Leiden, [9]Sophia Children's Hospital Rotterdam, The Netherlands

A previous Dutch study on PKU showed differences in phenyalanine (Phe) level between the 8 Dutch university clinics. The study showed that not all differences could be explained by variation in age, nationality or social economic status. The present study investigated differences in the treatment centres. **Method**: In a qualitative study 17 paediatricians, adult physicians and dieticians were interviewed about the PKU guidelines of their centre, and their interpretation of Phe values. ***Results***: Only 3 centres have a multidisciplinary PKU team as proposed in the Dutch guidelines, blood sampling and out-patients visits are often less frequent than the Dutch guidelines advice. Paediatricians, adult physicians and dieticians react in different ways on casuistry questions. Only 14% of the questions was answered unanimously. **Discussion**: Much time is spend to design (inter)national guidelines. Differences in interpretation of the guidelines, however, cause differences in treatment. In order to minimize these differences, knowledge should be more frequently exchanged between the paediatricians, adult physicians and dieticians within the centres and between the centres.

032-A

ARE ENGLISH RECOMMENDATIONS FOR PKU-ADOLESCENTS BETTER THAN GERMAN?*

M Bik-Multanowski
Chair of Pediatrics, Jagiellonian University Krakow, Poland

Prefrontal brain cortex dysfunction manifesting as worsening of reaction time and of short-time memory could occur in case of dietary relaxation in adolescents with phenylketonuria (PKU). On the other side many adolescents do not develop any pathological symptoms despite liberal diet. Precise dietary recommendations for teenagers are still lacking. English centers recommend more restrictive, German centers more liberal diet. The aim of the study was to compare reaction time and short-time memory efficacy in PKU-adolescents following more restrictive (English) and more liberal (German) dietary recommendations. **Methods**: 12 persons with early treated PKU and normal mental development aged 12–21 years participated in the study. Computerized simple reaction time test, choice reaction time test and a test assessing short-time memory were used. Participants were tested after 1 month of following English dietary recommendations and subsequently after 1 month of following German ones. **Results**: No statistically significant differences in reaction time and short-time memory were detected between results achieved in both phases of the study. In 2 participants, simple reaction time prolonged and short-time memory worsened in the dietary relaxation phase. These patients, however, did not manage to control their diet sufficiently and blood phenylalanine concentration exceeded in them the recommended range. **Conclusion**: No superiority of the English or German dietary recommendations was shown for early treated, normal mentally developed PKU-adolescents.

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033-P

A TWO-YEAR AUDIT OF THE HYPERPHENYLALANINAEMIA/PKU SPECTRUM IN VICTORIA

A Boneh, D Francis, M Humphrey, H Upton, K Gibson, H Peters
Metabolic Service, Genetic Health Services Victoria, Royal Children's Hospital, Melbourne, Australia

Following reports of tetrahydrobiopterin (BH_4) responsive PKU, we have done a BH_4 load in all newborns with PKU detected by newborn screening in Victoria. Between December 2001 and December 2003 we identified 17 newborn babies with high levels of phenylalanine (Phe) by newborn screening using MSMS (total samples:130 352). Dihydrobiopterin reductase deficiency was excluded in all babies. Urinary biopterin concentration was normal in all but one baby, subsequently diagnosed as having pyruvoyl tetrahydro-pterin synthase deficiency. Five babies had persistent Phe levels of 200–300, and do not receive any dietary or pharmaceutical therapy. BH_4 load (20 mg/kg, 6R-5,6,7,8-tetrahydro-L-biopetrin dehydrochloride; Schricks Laboratories, Switzerland) was done in 10 babies (one baby with a Phe level of 2600 μmol/L was started on diet without prior load). Two babies had a significant response to BH_4 (>35% decrease in Phe level). Due to high Phe levels in one baby at 11months of age, protein restriction (to 1.2 g/kg/day) and introduction of Phe-free formula, in addition ot BH_4 treatment, were necessary. The other baby maintains optimal Phe levels on ~10 mg/kg/day of BH_4 at 5 months of age. Mutation analysis in these babies is underway. Of the 9 babies who are on a full PKU diet, 3 have high Phe tolerance (Phe intake >40 mg/kg/day). The detection of BH_4-reponsive PKU patients offers them a different quality of life and may enable near normal lifestyle in adolescence.

034-P

TURKISH DELIGHT: A TASTY TREATMENT ALTERNATIVE FOR PKU DURING PREGNANCY

HS Kalkanoğlu, A Dursun, A Tokatlı, G Köksal, F Kutluay, T Coşkun
Hacettepe University Faculty of Medicine, Department of Pediatrics, Section of Nutrition and Metabolism, Ankara, Turkey

The child of a phenylketonuric woman is exposed during pregnancy to a high risk of growth retardation and malformation. If a strict low protein diet is followed before conception and throughout gestation the risks of abnormalities are not higher than in the normal population. Pregnant women with PKU have problems during pregnancy with inappropriate weight gain, hyperemesis, and poor dietary patterns. We followed 22-year-old woman with PKU during her pregnancy. The treatment had started 3 months before conception and continued until delivery. In the 13 weeks of pregnancy she started vomiting and phenylalanine levels arised 850 mmol/L and she stopped gain weight. We thought that the limitation of natural products caused low energy intake and protein catabolism increased phenylalanine level. Turkish delight was added on her diet without limitation. As soon as she started eating Turkish delight her phenylalanine level immediately decreased and she put adequate weight gain according to her gestational weeks. Turkish delight is basic energy supply composed simply by water, sugar, and cornstarch. It does not contain phenylalanine and gives high energy (240 kkcal/100 g) and good taste. It is known that the adequate intake of energy is important in treatment of PKU and we suggest that Turkish delight is one of the most tasteful, cheap and easy supply to all individuals affected with PKU or any other metabolic diseases which protein restriction is essential.

035-P

MATERNAL PHENYLKETONURIA: THE FRENCH SURVEY

F Feillet, V Abadie, J Berthelot, N Maurin, H Ogier, M Vidailhet, JP Farriaux, L de Parscau and the PKU workgroup of the AFDPHE
Department of Pediatrics, Nancy, France (AFDPHE: Association Française pour le Dépistage et la Prévention des Handicaps de l'Enfant, France)

We report the French experience in pregnancies with maternal phenylketonuria (PKU). In 2001, a questionnaire was sent to each referring PKU specialist in the 20 centres of each region of France, collecting reports on 135 pregnancies in 79 women born between 1958 and 1980. Forty-two women were informed of the risks of untreated pregnancy, while 26 were not informed (no data for 11). A strict diet was achieved in 83% of informed and in 16% of uninformed mothers prior to conception. Healthy offspring were observed in 43% of the 135 pregnancies, spontaneous abortions in 10.4%, elective abortions in 4.4%, therapeutic abortions in 12.6%, and embryopathies (EP) in 21.5%. In 8.1% of cases, the outcomes are unknown. The proportion of healthy children increased over time and reached 80% of the pregnancies of informed females. There were 7 heart defects, all in cases of EP, but though microcephaly and intrauterine growth retardation (IUGR) were almost constant in EP, we also found 9 healthy children with IUGR. A continuum between EP and healthy children is suggested. The anthropometric data of the mothers showed that their body mass index (BMI) distribution was shifted to the left compared to women of the general population.

Conclusion: the information and the preconception diet are effective for avoiding EP in maternal PKU. Nutritional parameters can influence foetal growth and the nutritional state must be closely monitored throughout pregnancies of PKU women.

036-P

BONE METABOLISM IN RELATION TO DIETARY TREATMENT IN PHENYLKETONURIC (PKU) CHILDREN AGED 6–8 YEARS

Lange A[1], Starostecka E[1], Heleniak G[2], Lukamowicz J[3], Kudra A[3]
[1]*Metabolic Department,* [2]*Department of Dietetics,* [3]*Biochemical Laboratory, Polish Mother's Health Memorial Institute, Lodz, Poland*

Treatment of PKU is based on phenylalanine restricted diet combined with the phenyl-free substitutes of protein. The diet is deficient in natural source of calcium and other nutritional components necessary for normal bone structure. The aim of the study was the evaluation of the skeletal status in PKU children.

The prospective, intervention, open study was conducted among 20 PKU children aged 6–8 years with good metabolic control. They were divided into 2 groups: I (investigated, $n = 10$) treated both with Milupa PKU 2 mix and PKU 2 (3:1), II (control, $n = 10$) who received PKU 2 for 6 months. Nutritional status was analysed using anthropometric parameters. Mean dietary intake of chosen nutrients was assessed. Calcium-phosphorus metabolism indices, bone turnover markers activity: serum bone alkaline phosphatase, osteocalcin (OC), terminal telopeptide of collagen type I (crosslaps), bone mineral density (DEXA) were performed initially and after six-month intervention period. Initial lower mean values of Z-score for total body (–0.397) and for spine (–0.973) have improved significantly in investigated group ($p < 0.05$). Calcium phosphorus metabolism evaluation revealed no disturbances in both groups. Bone markers activity (OC and crosslaps) increased in investigated group compared to control after 6 months but significant change was found in OC concentration ($p < 005$).

Conclusions: (1) Diet supplemented with PKU 2 mix formula had positive influence on bone metabolism; (2) this was accompanied with higher bone accretion marker (OC concentration)

037-P

CLINICAL AND NEUROPHYSIOLOGICAL STUDY OF TREMOR IN PATIENTS WITH PHENYLKETONURIA

Pérez-Dueñas B[1], Fernández E[1], Conill J[1], Vilaseca MA[2], Artuch R[2], Valls J[3], Campistol J[1]
[1]Neurology and [2]Biochemistry Departments Hospital Sant Joan de Déu; [3]Neurology Department Hospital Clínic, University of Barcelona, Spain

Background: Tremor of unknown origin is detected in 10–30% of early-treated phenylketonuric patients. **Objective**: To investigate the characteristics of tremor and its correlation to other clinical and biochemical variables in our cohort of phenylketonuric patients. **Methods**: Observational study of tremor in 55 phenylketonuric patients (mean age 18.9 years, 32 females; 37 early-treated) by means of a neurological examination, the application of the WHIGET Tremor Rating Scale and a neurophysiological study (accelerometer, BYOPAC System MP100WSW, analysis of tremor frequency with fast Fourier transformation). Age at diet onset, IQ test results, concomitant plasma phenylalanine levels and index of dietary control were studied. **Results**: No patient had a positive family history of tremor. Postural hand tremor was recorded in 16 patients (mean frequency 10.6 Hz, range 7.5–14 Hz); tremor was more frequent among late-treated patients (61%) compared to early-treated patients (32%) ($p = 0.043$). Moreover, ratings of 1 for postural and/or kinetic tremor were observed in all early-treated patients while 4 late-treated patients received ratings of 2–3, two of them recognizing disability for daily living activities. A later age at onset of phenylalanine-restricted diet ($p = 0.004$) and higher phenylalanine values in the first 6 years of life ($p = 0.041$) were detected in patients with tremor when compared to patients without tremor. **Conclusion**: A higher prevalence of tremor in phenylketonuric patients compared to previously reported series was observed. Tremor was more common and also more severe among late-treated patients.

038-P

BRANCHED CHAIN AMINO ACIDS AS A PARAMETER FOR CATABOLISM IN PKU

S Illsinger, U Meyer, T Lücke, B Vaske[1], AM Das
Department of Paediatrics and [1]Biometric Institute Medical School Hannover, Germany

Introduction: Elevated branched chain amino acids (BCAA) can reflect catabolism. The aim of this study was to establish an association between caloric intake and nutritional status on one hand and BCAA and Phe concentrations in plasma on the other hand in PKU patients free of infection

Methods: 242 plasma AA profiles from 70 PKU patients were measured. Nine patients had elevated Phe and BCAA levels. Dietary records were obtained; low caloric intake was compensated by giving Duocal® or ANAMIX® without modifying protein and Phe intake. Control of AA profiles was done after 2 (and 4) wks. Additionally, we investigated 47 plasma AA profiles from 26 liver transplanted patients with increased carbohydrate and caloric intake (~ 16 g/kg per day; $\geqslant 120$ kcal/kg per day) as an example for anabolism.

Results: 29 PKU patients had elevated BCAA levels as a sign for catabolism associated with normal Tyr levels, no ketonuria. 55% had normal Phe, 45% had Phe concentrations above the therapeutic level. Phe and Isoleu concentrations decreased significantly ($p = 0.04$) after dietary intervention. Leu, Val and Tyr levels fell slightly but not significantly There was negative correlation of initial Phe levels on one hand and protein as well as caloric intake ($p = 0.004$; $p = 0.08$) on the other hand. BCAA concentrations of all liver transplanted patients were in the lower range of normal.

Conclusion: In treated PKU patients (latent) catabolism indicated by elevated BCAA levels is common and can be overcome by increased caloric intake.

039-P

TRANSIENT GLUTAMIC ACIDEMIA IN PHENYLKETONURIC INFANT

Behúlová D[1], Šaligová J[2], Potočňáková L[2], Škodová J[1], Ponec J[1], Bzdúch V[3]
[1]*Departments of Clinical Biochemistry and* [3]*Pediatrics, Comenius University Children's Hospital, Bratislava;* [2]*Department of Pediatrics, Šafárik University Children's Hospital, Koice, Slovakia*

Glutamic acidaemia was described in only two patients (Mon Hum Gen 1978;9:75–9; Eur J Pediatr 1996;155:308–10). We report an infant who showed a six-fold normal glutamate in plasma. The girl was admitted to the hospital at the age of 20 days with hyperphenylalaninemia detected in the neonatal screening programme. Further examinations disclosed unconjugated hyperbilirubinemia, marked elevation of γ-glutamyltransferase, mild hyperammonemia and metabolic acidosis. Screening for another metabolic disorder revealed normal results. However, plasma amino acid analysis showed, beside high phenylalanine (Phe), marked elevation of glutamic acid (Glu) and borderline-low glutamine (Gln). The girl was treated with a low-phenylalanine diet and with ursodeoxycholic acid because of suspected cholestasis. After 5 weeks, biochemical findings returned to normal and required Phe levels were maintained. The girl was released as a case of classical PKU. Despite effective PKU treatment and good clinical condition, elevated levels of Glu and reduced concentrations of Gln lasted for months. Results were confirmed by two laboratories. A decomposition of Gln to Glu as a cause of the amino acid pattern in the samples was excluded. In our opinion, the underlying defect of metabolism of Glu and Gln remains unexplained.

Age (months)	1.0	1.1	4	6	9	15	18.5	Reference range
Glu (μmol/L)	490	289	484	378	122	113	81	11–79
Gln (μmol/L)	486	495	171	170	467	384	478	475–746

040-A

COINCIDENCE OF TWO RARE DISEASES: PKU AND PORPHYRIA IN A HUNGARIAN FAMILY

[1]Schuler Á, [1]Somogyi Cs, [2]Tasnádi Gy, [1]Kiss E, [1]Tőrös I, [1]Milánkovics I, [1]Király Gy, [3]Szendei Gy
[1]*Buda Children's Hospital, Metabolic Screening Center, Budapest;* [2]*MÁV Hospital, Porphyria Center, Budapest;* [3]*1st Department of Gynecology, Semmelweiss University, Budapest*

The original aim of our study was to examine the outcome of hyperphenylalaninaemic (HPA) patients with wide phenylalanine (Phe) tolerance and their family members by laboratory, psychological methods. Phe loading test (100 mg/kg Phe; SHS) was performed and Phe and tyrosine levels were measured at 0 and 60 min of the loading spectrofluorometrically. Phe^2/tyr ratios (P^2/T) were calculated at the 60th min (norm: $\leqslant 36$). At one of the families we found that besides of our HPA patient (P^2/T: 447) her aunt had a much higher ratio (P^2/T: 434) then the other heterozygotes (P^2/T: 36–93). We tried to find an explanation when we realised that at some members of this family acut intermittent porphyria (AIP) was already enzymatically diagnosed at an other hospital as a dominant inherited defect of porphobilinogen deminase enzyme (PBGd). The residual activity of PBGd was normal (156 nmol/gHbh), while the other women family members suffered of AIP (PBGd: 40–72 nmol/gHbh). This may be a sign of a common cross-point in the phenylalanine and porphyrine metabolic pathways at the level of maleylacetate–fumarylacetoacetate and succinylacetoacetate–succinylacetone.

Conclusion: The phenylalanine loading test may be not only a useful indicator of the heterozygote status of PAH defect, but at higher values of P^2/T then it could be expected at PAH heterozygotes, we have to search for asymptomatic porphyria checking the PBGd enzyme activity.

041-P

THE INFLUENCE OF TRACE ELEMENTS AND THYROID HORMONE LEVELS ON BODY MASS INDEX AND LINEAR GROWTH IN PHENYLKETONURIA

SC Wong[2], E Smith[1], JA Skiming[2], DM Isherwood[1], DC Davidson[2]
Department of Biochemistry[1] and Metabolic Medicine[2], Alder Hey Children's Hospital, Liverpool, UK

Aim: To examine the influence of trace elements, thyroid hormone levels and B_{12} levels on growth in PKU.

Methods: We performed a retrospective case note analysis of 41 PKU patients examining height and BMI SDS in relation to trace elements, thyroid function test and B_{12} levels.

Results: There was a total of 41 patients: 22 (M), 19 (F). There were 9 overweight and 3 obese patients (29.3% of patients). BMI SDS showed positive correlation with plasma TSH ($r = 0.32$, $p = 0.04$). No significant correlation was identified between BMI SDS with total T4 ($r = 0.07$, $p = 0.67$), total T3 ($r = 0.17$, $p = 0.31$), selenium ($r = 0.11$, $p = 0.62$), zinc ($r = -0.04$, $p = 0.79$), iron ($r = -0.12$, $p = 0.49$), copper ($r = 0.04$, $p = 0.81$), manganese ($r = -0.11$, $p = 0.49$) and B_{12} levels ($r = -0.11$, $p = 0.53$). Linear growth was not influenced by trace element and thyroid hormone levels. All patients had normal thyroid function tests during time of examination.

Conclusion: Mild short term trace element deficits in PKU does not cause growth impairment. BMI did not show any significant correlation with trace elements, total T4 and T3. We surmise that the relationship of BMI with plasma TSH is more likely to be a reflection of thyroid hormone changes associated with increasing weight.

042-P

INFLUENCE OF DIFFERENT KINDS OF PROTEIN INTAKE ON LENGTH AND HEAD CIRCUMFERENCE GROWTH IN DUTCH PKU INFANTS

M Hoeksma[1], M van Rijn[1], V Fidler[2], HD Bakker[3], AC Douwes[4], JBC de Klerk[5], Rubio E[6], T de Koning[7], RCA Sengers[8], PH Verkerk[9], PJJ Sauer[1], FJ van Spronsen[1]
[1]University Hospital Groningen, [2]Department of Statistics, [3]Acadamic Medical Center, [4]VU University Medical Center, [5]Erasmus Medical Center, [6]Academic Hospital Maastricht, [7]University Medical Center Utrecht, [8]University Medical Center Nijmegen, [9]TNO Leiden, The Netherlands

Introduction: Dutch PKU children were found to be shorter than their healthy peers [1]. A higher protein intake has been suggested to optimize growth in PKU patients.2 As possible explanatory facors for retricted growth, different kinds of protein intake (total, natural, proein substitute) were studied.

Methods: Length and head circumference growth and dietary data were analyzed retropectively in 139 Dutch PKU patients born between 1974 and 1996 (0–3 years of age). Multivariate regression anayes conrolled for severity of PKU and caloric intake during the first year of life.

Results: Neither proein nor energy intake correlated with length growth. Head circumference growth correlated with natural ($p = 0.032$) and total protein intake ($p = 0.035$). Multivariate regresion analysis showed that the effect of total protein intake was explained by natural protein inake.

Conclusion: These results suggest that the composition rather than an increase of toal protein inake has significant effect on growth velocity, where head circumference seems to be afec-ted earier than length. Further research on the optimal composition of the protein substitute is needed.

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[2] Acosta PB. JPGN 1998;27:287–91

043-A

RELATIONSHIP BETWEEN INTELLECTUAL PERFORMANCES, GENOTYPE AND METABOLIC CONTROL IN A COHORT OF PHENYLKETONURIC PATIENTS LIVING IN BELGIUM

C Carlier[1], C De Laet[1], M Robert[1], G Prové[1], J Parma[2], Ph Goyens[1]
[1]Metabolic Unit, University Children's Hospital Queen Fabiola, [2]Genetic Laboratory – Free University of Brussels, Belgium

The aim of this study was to evaluate the influence of genotype and of the quality of metabolic control on intellectual performances in a cohort of patients with phenylketonuria (PKU), living in Belgium.

Method: 48 PKU patients were studied. Intellectual performances at preschool age were evaluated by the Termann–Merill test; the Weschsler's scales were used in older subjects. The quality of metabolic control was evaluated by computing the mean blood phenylalanine concentration per period of six months. Mutations in the phenylalanine hydroxylase gene were identified by DHPLC analysis and/or direct sequencing by the ABI Big Dye Terminator Cycle Sequencing kit. Genotypes were classified according to Guldberg's classification (Am J Hum Genet 1998; 63:71–9).

Results: We observed a significant correlation ($p < 0.01$) between the final IQ and the quality of the metabolic control. When the metabolic control is good, no relation is found between IQ and genotype. In untreated patients or when metabolic control is inadequate and, patients with more severe genotypes tend to have a lower IQ ($p = 0.055$).

Conclusions: The most important determinant of intellectual performances is the quality of the metabolic control. The genotype, in this group of patients, seems to be a determinant of intellectual performances only when the patient is not treated or in case of inadequate metabolic control.

044-P

MOLECULAR BASIS OF PHENYLKETONURIA AND A CORRELATION BETWEEN GENOTYPE AND BIOCHEMICAL PHENOTYPE IN NORTHERN CHINESE POPULATION

Weimin Yu, Haiyan Liang
China-Japan Friendship Hospital, Beijing 100029, China

Objective: To determine the molecular basis of phenylketonuria (PKU) and related hyperphenylketonuria (HPA) and to explore correlation between phenylalanine hydroxylase (PAH) genotype and biochemical phenotype.

Methods: Fifty-three patients were identified through neonatal screening program. PAH genotypes were determined by using PCR/SSCP or DHPLC and DNA sequencing. The phenotypic severity of patients with PKU and HPA was based on pretreatment serum phenylalanine (Phe) levels during the neonatal period.

Results: Thirteen mutations were detected. Four novel mutations were identified in Chinese PKU population as R176X, E280K, L367R and S349A. Twenty-six of the fifty-three non-related patients examined in this study were completely genotyped. Strong correlation was observed between the level of PAH activity predicted from the genotype, when known from previous in vitro expression studies of the mutant proteins, and pretreatment serum Phe levels ($r = 0.696$, $p = 0.009$).

Conclusions: These results reveal a significant correlation between PAH genotype and biochemical phenotype, which further demonstrate the clinical utility of genotype analysis in the treatment of patients with PKU and HPA.

045-P

ABNORMALITIES DETECTED IN BRAIN OF TREATED ADULT PKU PATIENTS WITH T2- AND PROTON DENSITY-MAPPING

X-Q Ding[1], B Kohlschütter[2], O Wittkugel[1], A Cohen[1], H Zeumer[1], K Ullrich[3]
[1]Department of Neuroradiology, [2]Department of Internal Medicine, [3]Department of Pediatrics, University Hospital Hamburg-Eppendorf, Hamburg, Germany

Purpose of study: To try to understand the pathomechanisms leading to neurological dysfunction in treated PKU patients.

Methods: Four treated patients (aged 17–31 years) with different therapy compliance underwent MR examinations. T2 and proton density maps were constructed from the MRI data acquired using a triple echo sequence. The T2 relaxation times (t2) and proton density were then measured in 11 regions of brain gray matter (GM) and white matter (WM), and compared to those of 4 healthy adult control persons with comparable age.

Results: In affected WM a prolonged t2 was found as expected [1]. Surprisingly, all patients demonstrated a reduction of t2 in unaffected WM and GM. Proton density in both GM and WM was increased. Results did not correlate with dietary compliance.

Discussion and conclusion: The observation of reduced t2 values and increased proton density, found independently of dietary compliance, probably reflects morphological changes in neuronal structure and enhanced cortical cell density similar to those reported for untreated PKU patients [2]. These data indicate that structural changes of brain tissue, which are not visible on conventional MRI, may even exist in treated PKU patients.

[1] Ullrich K. Acta Paediatr Suppl. 1994;407:78–82
[2] Bauman ML et al. Acta Neuropathol (Berl). 1982;58:55–63

046-O

WHITE MATTER (WM) ALTERATIONS AND BRAIN PHE IN PKU ASSESSED BY 3 TESLA (T) MAGNETIC RESONANCE IMAGING (MRI) AND PROTON MAGNETIC RESONANCE SPECTROSCOPY (^{1}H-MRS)

V Leuzzi[1], T Scarabino[2], M Tosetti[3], M Burroni[4], F Carnevale[5], Cl Carducci[1], Ca Carducci[1], I Antonozzi[1]
[1]University of Roma 'La Sapienza; [2]'Casa Sollievo della Sofferenza' Scientific Institute – San Giovanni Rotondo; [3]Scientific Institute 'Stella Maris' – Pisa; [4]Child Neurology and Psychiatry Unity – Fano; [5]Paediatric Hospital Giovanni XXIII, Bari, Italy

WM abnormalities on brain MRI are a common sign in PKU subjects. Clinical correlates, neuropathology, and pathogenesis of these alterations are not known. **Aims**: The aims of the present study were: (a) to evaluate the characteristics of WM alteration as assessed by a 3 T MRI technology in PKU patients; (b) to study the correlation between the severity of WM alterations and the concurrent level of brain Phe (*in vivo* 3 T H-MRS). **Methods**: 32 PKU patients, 17 males and 15 females, aged 7 to 34 years (mean 18.9, SD 6.0), early (21) and late (11) detected, were enrolled for the study. WM involvement was assessed on axial FLAIR (fluid attenuated inversion recovery) T1 weighted images and coronal FSE T2 weighted images and sagittal FSPGR T1 weighted images. Alterations of WM were analyzed both qualitatively, with reference to signal intensity in the pulse sequences used, and quantitatively with reference to their anatomical location and extension. Single-voxel ^{1}H MRS was performed using a short TE PRESS sequence (TE = 35 ms). Voxel volume was 8 cc and was placed in the periventricular white matter. Amplitudes of each metabolite, including PHE at 7.36 ppm, were analyzed. **Results**: (a) WM alterations was found in 93.7% of all PKU patients (30 out of 32); (b) supratentorial WM was selectively affected; (c) there was no significant difference in the severity of WM involvement between early and late detected patients; c) no correlation was found between the degree of WM involvement and the concurrent concentration of brain Phe.

047-P

THE PHARMACOKINETICS OF LARGE NEUTRAL AMINO ACIDS (LNAA) IN PKU

V Leuzzi, Cl Carducci, S De Leo, W D'Auria[1], I Antonozzi
University of Rome 'La Sapienza'; [1]Dietetic Metabolic Food, Milan, Italy

Pietz et al. (J Clin Invest. 1999;103:1169–78) proved the efficacy of large neutral amino acids (LNAA) administration in reducing brain Phe concentration in PKU patients. The aims of the present work were: (a) to study the absorption and the blood kinetics of each LNAA; (b) to test different mixtures of LNAA with prompt or retarded release, in order to establish the best combination compatible with a rapid and protracted rise of blood LNAA concentrations. 10 PKU subjects were enrolled for the study (4 females and 6 males; age range: 17 to 30). A single oral loading of 50 mg/kg of Iso, Leu, Val, His, Tyr, Trp, and Met was administered after an overnight fast; blood samples were collected before the challenge and each hour for the following 6 hour period. Different mixtures of LNAA were used as the rapidity of amino acids release was concerned: 100% prompt release (P), 100% retarded release (R), 50%P–50%R, 70%P–30%R. Results: a) although the identical dosage, the increase of blood concentrations of each LNAA was very variable, ranging from 150% (Tyr) to 700% (Met); b) 100%R and 50%P–50%R mixtures resulted in a late and slight increase of blood LNAA, while 100%P and 70%P–30%R mixtures determined a rapid and marked increase of LNAA, which in the case of the 70%P–30%R one lasted over 5 hours after the loading.

048-P

IRON OVERLOAD AMELIORATES PHENYLKETONURIA PHENOTYPE

WT Scouten[1,2], KM Camp[1,2], Y Torosyan[2], GL Francis[1,2]
[1]Departments of Pediatrics, Walter Reed Army Medical Center, Washington, DC 20307; [2]Uniformed Services University of the Health Sciences, Bethesda, MD 20814, USA

Phenylketonuria (PKU; MIM# 261600) most commonly results from mutations in the phenylalanine hydroxylase gene (PAH; EC 1.14.16.1), which reduce conversion of phenylalanine (Phe) to tyrosine. Iron deficiency further decreases the residual activity of PAH in patients with PKU. We evaluated a 22-year-old Caucasian male with the co-morbid occurrence of hereditary hemochromatosis due to homozygous C282Y mutation in the HFE gene (MIM# +235200) and PKU due to IVS10nt3c→t and S349P mutations. At initial exam the patient was on an unrestricted diet with an estimated intake of 3.75 g Phe from 75 g protein on 24-hour dietary recall. The plasma phenylalanine and ferritin levels at that time were 243 μmol/L (PKU therapeutic range 120-600 μmol/L) and 911 ng/mL (normal 22–322 ng/mL), respectively. Treatment of hemochromatosis by therapeutic phlebotomy resulted in a progressive decline of serum ferritin to 24 ng/mL and concurrent increase in the plasma Phe concentration to 1014 μmol/L. The mechanism by which iron overload improves the metabolism of Phe is not yet known. Three-dimensional protein modeling of PAH using this patient's mutations showed reduced molecular volume and surface area, and conformational changes that suggest decreased protein stability, and modification of the iron-binding domain. Increased iron availability may favor iron incorporation into mutant enzyme. Alternatively, iron may modulate the activity of the post-translational protein quality control system, increasing the amount and residual activity of mutant protein.

049-A

THE RELATIONSHIP OF SELENIUM LEVELS WITH THYROID HORMONE IN PHENYLKETONURIA

SC Wong[2], E Smith[1], JA Skiming[2], DM Isherwood[1], DC Davidson[2]
Department of Biochemistry[1] and Metabolic Medicine[2], Alder Hey Children's Hospital, Liverpool, UK

Aim: To study the effect of selenium status in PKU on thyroid hormone levels and glutathione peroxidase (GP) activity.

Methods: We performed a retrospective analysis of thyroid function tests and GP activity in patients with PKU in relation to the selenium status of the patient. Patients were classified as selenium sufficient and selenium deficient based on age dependent ranges.

Results: There were 41 patients: 27 were selenium sufficient and 14 were selenium deficient. There was no difference between the groups for mean age and sex distribution. Serum phenylalanine (SPA) at diagnosis, 1 year and at time of blood test were no different. The number of portions prescribed at 1 year and at time of blood test did not differ between the groups. Selenium deficient patient showed lower GP activity ($p = 0.004$), higher total T4 ($p = 0.04$) and higher TBG ($p = 0.04$) compared with selenium sufficient patients. All our patients were clinically euthyroid.

Conclusion: Selenium deficiency in PKU show mild alterations of thyroid function within normal ranges. There was no difference in clinical characteristics of selenium deficient PKU patients compared with those with normal selenium levels. We conclude that mild selenium deficiency in PKU does not cause overt thyroid dysfunction.

050-P

6R-BH_4/PHE VS 6R-BH_4 LOADING TEST IN PAH DEFICIENT PATIENTS

V Leuzzi, Cl Carducci, P Montieri, C Olita, Ca Carducci, M Ferrazzi, I Antonozzi
University of Rome 'La Sapienza', Italy

BH_4-responsiveness is a common tract of phenylalanine hydroxylase (PAH) deficient patients. In order to ascertain BH_4-responsiveness single (BH_4) or combined (Phe plus BH_4) loading procedures have been proposed. We report on the results of both 6R-BH_4 (20 mg/kg) and 6R-BH_4 plus Phe (100 mg/kg) loading tests in 6 PAH deficient patients, 2 females and 4 males, aged 4 months to 8 years. Blood Phe after a overnight fast or 3 hours after Phe loading were taken into account as basal values for the interpretation of BH_4 and BH_4/Phe loading test, respectively. A 30% decline of blood Phe was the threshold chosen to denote BH_4-responsiveness. Blood Phe was evaluated 4, 8, and 12 hours after each challenge. The decline of Phe was 9 to 77% after BH_4 loading, and 31 to 81% after Phe/BH_4 loading. In 5 out of 6 subjects the combined loading resulted in a higher percentage of blood Phe decline. 2 out of 6 patients were BH_4-responsive according to the result of combined loading but non-responsive at the single challenge. In one of these subjects, the treatment with 6R-BH_4 (5 to 15 mg/kg/day) for 6–8 months failed to determine a relevant decrease of blood Phe. These preliminary data suggest a higher reliability of BH_4 loading test when compared with the combined Phe/BH_4 challenge.

051-P

BH_4-RESPONSIVENESS IN PAH-DEFICIENT HYPERPHENYLALANINEMIC INFANTS IN ITALY

Fiori L, Fiege B, Casero D, Rossi S, Giovannini M
Department of Paediatrics, San Paolo Hospital, University of Milan, Italy

Introduction: Hyperphenylalaninemia (HPA) is due to deficiency of phenylalanine-hydroxylase (PAH) or of tetrahydrobiopterin (BH_4). Recently BH_4 has been inidicated as a valid alternative treatment in BH_4-responsive PAH-deficient patients on diet. **Aim of the study**: To verify the incidence of BH_4 responsiveness among patients born from 2000 until 2003 in Lombardy (Italy) detected at neonatal screening and to evaluate the genotype-phenotype correlation in BH_4-responsiveness. **Patients and methods**: All patients born in Lombardy between January 2000 and Septtember 2003 and affected by HPA (79 subjects) were investigated for BH_4 responsiveness. BH_4 deficiency was excluded in all patients. The BH_4 loading test was performed according to a standardized protocol; 6R-tetrahydrobiopterin (20 mg/kg), was administered to patients and plasma samples were collected at time 0, 4, 8, and 24 h. In patients with basal plasma phe levels lower than 360 μmol/L, a combined phe and BH_4 loading test was performed: 100 mg phe/kg body weight were administered orally 3 hours before the BH_4 load. **Results**: The incidence of BH_4-responsiveness is 77.2%. The most of responsive patients are affected by HPA, a smaller percentage by mild and moderate phenylketonuria (PKU). No responsiveness was found among patients affected by classical PKU. **Conclusions**: BH_4-responsiveness seems to be an important feature even in some patients affected by mild and moderate PKU, who undergo a dietary treatment. The mechanisms underlying BH_4 responsiveness have to be further elucidated. Pharmacological security and high costs of BH_4 therapy are limiting the introduction of pharmacological therapy in PAH deficient hyperphenylalaninemia.

052-P

MAXIMUM LEVELS OF PHENYLALANINE AT DIAGNOSIS AND THE RESPONSE TO THE BH_4 IN THE HYPERPHENYLALANINEMIA

Baldellou A[1], Campos C[1], Navarro H[1], Ruiz-EcharriMP[1], Salazar I[1], Ugarte M[2], Pérez B[2], Desviat L[2]
[1]*Hospital Miguel Servet, Zaragoza,* [2]*Universidad Autónoma de Madrid, Spain*

Intoduction: Some patients with hyperphenylalaninemia (HPA), principally those with mild or moderate forms can respond with a wide variability to the administration of high dose of tetrahydrobiopterin (BH_4). Responsiveness seems to depend on mutation in the phenylalanine hidroxylase (PAH) gene, but there is not always a definite correlation between mutations and the response to BH_4. **Methodology**: We have made the combined loading Phenylalanine (100 mg/k) and 6R-BH_4 (20 mg/k) test in 16 patients with PKU and with mild or moderate HPA in order to know their therapeutic possibilities, and the response has been considered positive when Phe decreased more than 30% (from the maximum level after Phe-loading) 8 hours after the administration of BH_4. **Results**: In spite of their mutations, the response to the test has been positive in all the patients (7) who had the maximum level of Phenylalanine less than 800 nmol/ml at diagnosis. Six of them are in treatment with BH_4 with doses between 7 and 10 mg/k/day and in all the cases has been possible to increase the daily phenylalaine intake. One of the patients is now under free diet and two of them are very close to get it. None of the patients with more than 800 nmol/ml of the Phe at diagnosis had a positive response to the BH_4 administration. **Conclusion**: The maximum level of Phe at diagnosis of the patients with hyperphenylalaninemia due to PAH deficiency can be a good predictor of the response to BH_4.

053-P

GLOBAL COGNITIVE PERFORMANCE IN PKU PATIENTS TREATED WITH BH_4

Campistol J, Gassió R, Fusté E, Lambruschini N, Vilaseca MA
PKU Follow-up Unit, Hospital Universitari Sant Joan de Déu, Barcelona, Spain

Objective: Evaluation of global cognitive performance in 8 PKU patients treated for 6 months with BH_4 and progressive Phe-free diet. **Patients**: 8 patients (6 girls and 2 boys) with a mean age of 5 years 5 mmonths (1 year 4 months–12 years 8 months). Seven patients with mild PKU (tolerance: 400–600 mg Phe/day) and one with moderate PKU (tolerance: 350–400 mg Phe/day). **Methods**: Patients younger than 3 years ($n = 4$) were evaluated by the Brunet-Lezine test and patients older than 6 years by K-ABC and WISC-R scales. Questions were made to their families about eventual hyperkinesia and presence of other behavioural associated problems. **Results**: Before treatment, patients younger than 3 years of age showed a mean Development Quotient of 104 (100–106) and mean global cognitive performance in the patients older than 6 years was 108 (96–118). During BH_4 treatment mean plasma Phe levels was 281 μmol/L (172–358). None of the patients presented hyperkinesia. Previous neurocognitive evaluations were available in 5 patients, and in none of them a progressive cognitive worsening during the 6 months of therapy was observed. The patients older than 6 years were also evaluated for attention and other executive abilities and none of them had an alteration of these intellectual functions. **Conclusions**: (a) In our treated patients neurocognitive evaluation remained within the normal range, none of them presenting problems related to hyperkinesia or behavioural troubles. (b) A longer follow-up and a larger group of patients seem necessary to be sure for the absence of alterations in other cognitive functions.

054-P

TREATMENT OF ADULTS WITH HYPERPHENYLALANINEMIA WITH TETRAHYDROBIOPTERIN (BH_4)

[1]R Moats, [2]K Moseley, [2]S Yano, [1]M Nelson, [1]S Bluml, [2]R Koch
[1]Department of Radiology and [2]Department of Medical Genetics at Childrens Hospital Los Angeles, CA, USA

Treatment of persons with various levels of hyperphenylalaninemia (HPA) traditionally have been exclusively treated with the phenylalanine (Phe) restricted diet monitored by blood Phe levels. A recent National Institute of Health Consensus Conference Statement has recommended blood Phe levels of 120–360 μmol/L during infancy and early childhood and 120-600 μmol/L for adolescence and less than 900 μmol/L for adulthood. The recent recognition that many patients are not following the diet and have blood Phe levels far above the recommendations have prompted some clinicians to seek alternative therapies.

In specific cases of HPA BH_4 may be a possible solution. In a group of 8 HPA individuals whose blood Phe ranged from 360–900 μmol/L we measured plasma amino acids along with brain Phe using Magnetic Resonance Spectroscopy (MRS). Four were supplemented with BH_4. The brain Phe levels of all individuals in the group ranged from 0.18 mmol/L to 0.3 mmol/L. The brain levels did not change significantly since these low levels are difficult to measure and a larger number of subjects may be needed to show significance. However, in the BH_4 supplemented group the blood Phe levels were decreased. We believe that supplementation with BH_4 is a viable alternative for those individuals who respond and may be useful in maternal PKU.

055-P

MULTIPLE DOSE STUDY OF TETRAHYDROBIOPTERIN (BH_4) IN PHENYLKETONURIA

R Matalon[1], R Koch[2], K Michals-Matalon[3], K Moseley[2], D Rassin[1], S Surendran[1], S Tyring[1], A Dorenbaum[4]

[1]Department of Pediatrics, University of Texas Medical Branch, Galveston TX; [2]Children's Hospital Los Angeles, CA; [3]University of Houston, Houston, TX; [4]BioMarin Pharmaceutical, Inc. Novato CA, USA

Several studies have shown that patients with phenylketonuria (PKU), especially those with mild mutations, respond with decreased blood phenylalanine (Phe) concentrations following oral administration of BH_4. The purpose of this study is to determine changes in blood Phe after ascending single doses of BH_4 with 10, 20 and 30–40 mg/kg (Part 1) and to evaluate multiple daily doses, for 7 days each, of 10 and 20 mg/kg BH_4 (Part 2) in 20 patients with PKU. We have completed Part 1 in 9 patients with PKU (6 adults and 3 children <12 years of age). All patients were genotyped and blood Phe and tyrosine concentrations were measured before and 24 h after BH_4 dosing. There was a one-week wash out period between the ascending doses of BH_4. The mean blood Phe concentration at baseline was 790 μmol/L (range 318–1191 μmol/L). Blood Phe concentration declined after BH_4 dosing in 5 of 9 subjects at 10 mg/kg and 20 mg/kg and 5 of 8 subjects at 40 mg/kg BH_4. The mean blood Phe decline in responders was 22.5% at 10 mg/kg BH_4, 40.6% at 20 mg/kg and 33.9% at 30–40 mg/kg. Of the 8 subjects scheduled to receive 40 mg/kg BH_4, two were given 30 mg/kg. One patient considered a non-responder at 10 and 20 mg/kg BH_4 had a significant response to 40 mg BH_4 (62% decline in blood Phe). The responses observed placed the blood Phe level below the recommended NIH Consensus treatment threshold for age in 6 of 9 subjects. The results in Part 1 of this study may help define an appropriate dose for BH_4 sensitivity testing in PKU patients. Long-term dosing is needed in order to define the appropriate BH_4 dose for treatment of PKU patients.

056-P

TETRAHYDROBIOPTERIN-RESPONSIVE PHENYLALANINE HYDROXYLASE DEFICIENCY IN SPAIN

LR Desviat[1], B Pérez[1], A Bèlanger-Quintana[2], M Castro[1], C Aguado[1], A Sánchez[1], MJ García[1], M Martínez-Pardo[2], M Ugarte[1]

[1]Centro de Biología Molecular 'Severo Ochoa' CSIC-UAM; [2]Unidad de Enfermedades Metabólicas, Servicio de Pediatría, Hospital Ramón y Cajal, Madrid, Spain

Tetrahydrobiopterin (BH_4) responsiveness in patients with mutations in the phenylalanine hydroxylase (PAH) gene is a novel subtype of hyperphenylalaninemia characterized by a positive BH_4 loading test. The exact underlying mechanism is yet unknown and in each population the prevalence of this phenotype and the associated genotypes may vary. In this work we describe the results of a pilot study performed with 31 Spanish PAH-deficient patients subjected to a BH_4 loading test. Overall, 11/30 (37%) showed a positive response with a 30% decrease in blood Phe levels 8 hours after the BH_4 challenge, and 3 additional patients, considered slow responders, showed this decrease only after 12–16 hours. We report for the first time a patient homozygous for a splicing mutation with a slow response, suggesting an effect of BH_4 supplementation on PAH gene expression. Most of the responsive patients belong to the mild hyperphenylalaninemia (MHP) or mild phenylketonuria (PKU) phenotypic groups. In MHP patients we report for the first time the results of parallel single Phe doses confirming the utility of these analyses for a better evaluation of the response. Genotype analysis confirms the involvement in the response of specific mutations (D415N, S87R, R176L, E390G, A309V) present in hemizygous patients, and provide relevant information for the discussion of the potential mechanisms underlying BH_4 responsiveness.

057-P

SUCCESSFUL TREATMENT WITH BH_4 MONOTHERAPY OF TEN PATIENTS WITH MILD/MODERATE PKU

Vilaseca MA, Lambruschini N, Mas A, Ormazábal A, Gómez L, Gutiérrez A, Artuch R, Pérez-Dueñas B, Campistol J
PKU Follow-up Unit, Hospital Sant Joan de Déu, Barcelona, Spain

Aim: To evaluate the increase in Phe tolerance and the micronutrient status after six-month follow-up of BH_4 monotherapy in ten patients with mild/moderate PKU. **Patients**: Eight patients with mild PKU (tolerance: 400–600 mg Phe/day) and two with moderate PKU (tolerance: 350–400 mg Phe/day). Phe (mean $\pm$ SD) at diagnosis was: 895 $\pm$ 230 μmol/L. All of them were on Phe-restricted diet supplemented with special formula. Selection of patients: the BH_4 response to the 24-h-lasting combined Phe/BH_4 loading test was from 45% to 94%. Genotypes: IVS10/D415N, R408W/E390G, Y168H/V388M, Y414C/?, R241H/?, V388M/R241H, IVS10/E178G, S349P/E390G, R408W/P275S. **Methods**: Amino acids, urine pterins, oligoelements (Se, Zn), and vitamins (E, A, folate, B_{12} and B_6) were determined. **Results**: Phe levels were: 265 $\pm$ 82 μmol/L (137–418) when treatment was started with 5 mg BH_4/kg/day (Schircks Laboratorios, Jona, Schwitzerland). Phe-restricted diet was simultaneously liberalised (1000–1200 mg Phe/day) and formula was gradually reduced until complete removal (51 $\pm$ 40 g/day→0). Tolerance increased from 356 $\pm$ 172 μmol/L (201–600) to 1626 $\pm$ 188 μmol/L in the patients ($p = 0.007$). Phe (295 $\pm$ 96 μmol/L), Tyr, oligoelements and vitamins were not significantly different. Only the percentage of biopterin in urine was significantly higher ($p = 0.028$). No secondary effects were detected in any patient. **Conclusion**: BH_4 treatment in 10 patients with mild/moderate PKU allowed a significant increase in Phe tolerance while maintaining plasma Phe levels within the save range. The successful long-term BH_4 treatment confirmed the good response achieved in the Phe/BH_4 loading test.

058-O

PHENYLALANINE HYDROXYLATION IN PATIENTS WITH CLASSIC PKU IN RESPONSE TO TETRAHYDROBIOPTERIN

Bodamer OA, Holub M, Mühl A, Hagerty B, Tuschl K
Department of General Paediatrics, University Children's Hospital Vienna, Austria

A significant number of patients mild PKU and a limited number of patients with classic PKU have been shown to be responsive to tetrahydrobiopterin (BH_4) resulting in decreased plasma phenylalanine levels and increased dietary phenylalanine tolerance. A stable isotope method employing ring D_5 phenylalanine, ring D_2 and D_4 tyrosine was used to measure phenylalanine and tyrosine flux and to calculate whole body phenylalanine hydroxylation (PAHtion) rates before and fifteen hours after an oral BH_4 load (20 mg/kg) in three adult subjects with classic PKU and three healthy adults. Dietary phenylalanine intake was kept unchanged. Plasma phenylalanine levels decreased in all PKU patients: 532, 990 and 1120 μmol/L before versus 426, 819 and 924 μmol/L after BH_4 load while there was no change in the control group. Phenylalanine hydroxylation rates remained unchanged in both groups: 14.2, 9.9, 13.0 versus 15.0, 10.9 and 11.4 μmol/kg/h in subjects with PKU and 13.7, 13.5, 8.3 versus 13.8, 14.6, 15.8 μmol/kg/h in controls, before and after BH_4 load. Although there is no change in PAHtion following a BH_4 load plasma phenylalanine levels decreased substantially in subjects with classic PKU. This may be due to increased protein synthesis and/or phenylalanine breakdown by yet unknown mechanisms. Interestingly absolute PAHtion was similar between PKU and controls but when corrected for plasma phenylalanine levels PAHtion was 5–10 fold lower in PKU. It is feasible to employ a stable isotope method to measure PAH activities in response to a BH_4 load.

059-P

THE METABOLIC AND MOLECULAR BASES OF BH_4-RESPONSIVE PHENYLALANINE HYDROXYLASE (PAH) DEFICIENCY

N Blau[1], H Erlandsen[2]
[1]*Division of Clinical Chemistry and Biochemistry, University Children's Hospital, Zurich, Switzerland;*
[2]*Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden*

About two thirds of all mild phenylketonuria (PKU) patients are tetrahydrobiopterin (BH_4)-responsive and thus can be potentially treated with BH_4 instead of a low-phenylalanine diet. Although, there has been an increase in the amount of information relating to the diagnosis and treatment of this new variant of PKU, very little is know about the mechanisms of BH_4-responsiveness. A total of 75 mutations (BH_4-responsive and non-responsive), most of them in the compound heterozygous state, were described in 121 patients and about 50% of them were detected in more than one allele. The R408W mutation is the most common one (25 alleles), followed by Y414C (23 alleles), A403V and R241C (14 alleles each), A300S and E390G (8 alleles each), IVS12nt+1g>a (7 alleles), R413P (6 alleles) and I65T, R68S, and R158Q (5 alleles each). The complete list of mutations is available from the BIOPKU database (www.bh4.org).

Based on the present knowledge of the regulative properties of the cofactor BH_4 and substrate phenylalanine, the following mechanisms have been postulated as possible causes for BH_4-responsiveness: (1) *Km* mutants of the PAH enzyme; (2) stabilization of PAH by the chaperon-like activity of BH_4; (3) BH_4- driven change in regulation of BH_4 biosynthesis; (4) induction/up-regulation of PAH enzyme expression by BH_4; and (5) PAH mRNA stabilization.

060-P

LIPOPHILIC ANTIOXIDANTS IN PKU PATIENTS ON BH_4 THERAPY

J Pineda, A Ormazabal, R Artuch, B Pérez-Dueñas, L Gómez, N Lambruschini, MA Vilaseca, J Campistol
Hospital Sant Joan de Déu, Department Clinical Chemistry, Pediatrics and Neurology, Barcelona, Spain

Aim: Our aim was to evaluate the lipophilic antioxidants retinol, tocopherol and coenzyme Q_{10} in PKU patients on BH_4 treatment. **Patients**: Nine BH_4-responsive PKU patients (age range 1–12 years) analysed in basal conditions and after 3 months of BH_4 therapy (5 mg/kg/day). **Methods**: Plasma phenylalanine, retinol and tocopherol were determined by reverse phase HPLC with ultraviolet detection and plasma Q_{10} by HPLC with electrochemical detection. **Results**: Plasma retinol and tocopherol values were within the reference ranges before BH_4 therapy and were not modified after the start of the treatment. Plasma Q_{10} concentrations were decreased in 4 out of 9 PKU patients in basal conditions (0.27–0.88; average: 0.53 μmol/L. Reference values: 0.41–1.01 (0.68). After 3 months of BH_4 therapy, Q_{10} values significantly increased compared with basal conditions (0.42–1.04, average 0.67 μmol/L: Wilcoxon test: $p = 0.020$). Furthermore, all PKU patients showed plasma Q_{10} values within our reference range. A significantly negative correlation was observed between plasma phenylalanine and Q_{10} concentrations (Spearman test: r=-0.753: $p = 0.019$).No correlation was observed between both parameters after 3 months of BH_4 therapy. **Conclusions**: Q_{10} deficiency in PKU was corrected after 3 months of BH_4 therapy. This normalisation of plasma Q_{10} values may be a consequence of an increment of dietary Q_{10} intake by the diet relaxation after BH_4 therapy. However, the negative association observed between phenylalanine and Q_{10} prior to BH_4 treatment suggests that high phenylalanine values might also be responsible of decreased Q_{10} concentrations in PKU.

061-P

PLATELET SEROTONIN CONCENTRATION IN PKU PATIENTS UNDER BH_4 THERAPY

A Ormazábal, R Artuch, J Pineda, B Pérez-Dueñas, L Gómez, N Lambruschini, J Campistol, MA Vilaseca
Biochemistry, Pediatrics and Neurology, Hospital Sant Joan de Déu, Barcelona, Spain

Aim: Our aim was to evaluate serotonin and tryptophan in PKU patients under BH_4 treatment.
Patients: 12 BH_4-responsive PKU patients (age range 1–12 years) analysed in basal conditions and after 6 months of BH_4 therapy (5 mg/kg/day). Results were compared with 12 age-matched healthy controls.
Methods: Platelet serotonin and serum tryptophan concentrations were determined by reverse phase HPLC with fluorescence detection. Plasma Phe was analysed by HPLC with ultraviolet detection.
Results: Platelet serotonin concentrations were significantly decreased in PKU patients before BH_4 treatment (range 1.20–2.89; median 1.99 nmol/10^9 platelets) when compared with the control group (1.77–4.46; 2.78) (Mann–WhitneyU test: $p = 0.019$), while serum tryptophan values were not different. Platelet serotonin concentrations significantly increased during BH_4 treatment in 8 out of 12 PKU patients who showed a good metabolic control (Phe: 169–445; 334 μmol/L): serotonin values were 1.2–2.89 (1.84) pre-treatment vs 1.9–4.31 (2.82) post-BH_4 treatment. No changes were observed in platelet serotonin values in 4 PKU patients who showed high Phe values the day of the analysis (445–718 μmol/L). No differences were observed in serum tryptophan concentrations before and after BH_4 treatment.
Conclusions: Platelet serotonin concentrations may be decreased in PKU patients. BH_4 treatment caused a significantly increment in serotonin values, especially in those PKU patients with a good metabolic control. This increment might occur also in CNS and this might improve neuropsychological functions in these patients.

062-P

PHENYLALANINE HYDROXYLASE AND THE S-OXIDATION OF L-METHIONINE IN THE RAT

GB Steventon[1], AH Goreish[1], S Bednar[2], H Jones[3], SC Mitchell[3]
[1]*Department of Pharmacy, King's College London, UK;* [2]*Institute of Pharmaceutical Chemistry, University of Vienna, Austria;* [3]*School of Biomedical Sciences, Imperial College London, UK*

Phenylalanine hydroxylase (PAH) has been investigated experimentally with respect to phenylketonuria for decades. Reports in the literature that PAH has a wider substrate specificity than Phe upon activation by post-translational modfication appear not to have been followed up. We have investigated this phenomenon in the Wistar rat using Met as a substrate for activated PAH in the cytosolic faction. Substrate and inhibitor investigation revealed that Met was a substrate for activated PAH activity *in vitro*. The apparent K_m (Phe and Met) were 0.26 ± 0.08 and 7.75 ± 3.01 mmol/L. The apparent V_{max} (Phe and Met) were 13.72 ± 4.74 and 63.88 ± 6.81 nmoles product formed min^{-1} mg^{-1}. The large aromatic amino acid hydroxylase monoclonal antibody and the Fe^{3+} chelator, deferoxamine, completely inhibited both Phe C-oxidation and Met S-oxidation to their respective metabolites. Analysis of the Dixon plots revealed that both Phe and Met competitively inhibited each other's oxidation. Correlation studies showed that the rate of production of Tyr was positively correlated to the production of both Met S-oxides in 20 female Wistar rat hepatic cytosolic fractions. These results strongly support the hypothesis that PAH was the enzyme responsible for Met S-oxidation in the rat.

063-P

CHEMICAL CHAPERONES ENHANCED THE STABILITY AND FOLDING OF V388M MUTANT PHENYLALANINE HYDROXYLASE

C Nascimento, A Conceição, P Leandro, I Tavares de Almeida
Unidade Biologia Molecular e Biopatologia Experimental, Faculdade de Farmácia, Universidade de Lisboa, Portugal

The cytosolic enzyme phenylalanine hydroxylase (PAH) is essential for the hydroxylation of Phe to Tyr. A PAH deficient activity causes phenylketonuria (PKU). When produced in the presence of natural osmolytes, glycerol and trimethylamine N-oxide (TMAO), an increase in some PAH mutant forms activity was observed. We aimed to understand the molecular mechanism underlying the observed stabilizing effect. The V388M mutant protein was overexpressed in *E.coli* in the presence and absence of glycerol or TMAO. To monitor conformational changes possibly related with enzyme activity modulation, the purified protein (IMAC) was subjected to size exclusion chromatography, thermal stability assays, urea denaturation curves and fluorescence quenching studies. The V388M protein synthesized in the presence of natural osmolytes presented: (1) an increased enzymatic activity ($\approx$2 fold); (2) an altered oligomeric profile; (3) a Tm change (43°C to 52°C); (4) a shift toward higher urea concentrations in the denaturation midpoint; and (5) an alteration in the accessibility of the Trp residues to quenchers. The obtained results proved that chemical chaperones promote an enhanced folding of the newly synthesized mutant enzyme thus contributing to its stability. Understanding the molecular mechanism of chemical chaperone action, could lead to the design of more potent stabilizing agents, opening new perspectives to improve PKU treatment.

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064-P

A STUDY OF ILLEGITIMATE TRANSCRIPTS OF PHENYLALANINE HYDROXYLASE GENE

C Carducci, C Artiola, L Ellul, M Pierella, T Giovanniello, V Leuzzi, I Antonozzi
Università degli studi di Roma, 'La Sapienza', Italy

PKU is a well studied disease at the genetic level; nevertheless relevant inconsistencies are found in genotype/phenotype relationships, and few studies have been performed on the PAH transcripts. We studied the effect of PAH gene mutations in 11 PKU patients and 5 controls, evaluating PAH ectopic transcripts by using RT-PCR and sequencing analysis of RNA extracted from patients' lymphoblastoid cell lines. Three different splicing mutations analyzed (IVS10-11G>A; IVS8-7A>G, and IVS4nt+5g>t) resulted in 3 different effects on the transcripts, i.e. in frame insertion, complete exon skipping, and aberrant splicing with coexisting aberrant transcripts, respectively. Four nonsense mutations were studied (F55fs, R111X, R261X, R176X); three of them (F55fs, R261X, R176X) did not show detectable transcripts, probably because of the nonsense mediated decay (NMD) of the molecule. The missense mutations (L48S, P261Q, R158Q and P281L) did not cause further effects on the transcripts, also if a partial exon skipping, involving exons far from the mutation site was observed in alleles carrying L48S and P261Q. Nevertheless, the same effect was also observed in the controls. Our data suggest that transcript analysis can give complementary information to in vitro residual enzyme activity and structural studies. Furthermore, the observation of exon skipping in controls as well as in PKU subjects suggests that PAH gene can undergo the so called 'spontaneous or constitutive skipping' and recommends caution in the interpretation of exon deletion where a strictly correlated genomic mutation was not identified.

065-P

PHENYLALANINE INHIBITS SYNAPTOGENESIS IN MURINE PRIMARY NEURONAL CULTURES

F Hörster[1], MA Schwab[1], S Sauer[1], J Pietz[2], GF Hoffmann[1], JG Okun[1], S Kins[3], S Kölker[1]
[1]Department of General Pediatrics, Division of Metabolic and Endocrine Diseases, and [2]Department of Pediatric Neurology, University Children's Hospital Heidelberg, Germany; [3]Centre for Molecular Biology, Heidelberg, Germany

Classical phenylketonuria (PKU) is neuropathologically characterized by reduced dendritic arborization, loss of synapses and neurodegeneration. We investigated whether increased concentrations of phenylalanine cause reduced synaptic density, altered dendritic branching and neurodegeneration. For this purpose we treated primary cortical neurons cultured for 21 days *in vitro* in the absence or presence of 5 mmol/L phenylalanine in a medium containing all essential amino acids. Immunocytochemistry at 12 and 21 DIV revealed no changes of dendritic morphology, or neuronal viability but a significant reduction of synaptogenesis about 20% in Phe-treated cultures compared with non-treated sister cultures. Since MRS showed subtle signs of pertubation of cerebral energy metabolism, we investigated whether phenylalanine inhibited respiratory chain complexes I-V. I*n vitro* analysis revealed no inhibitory effect of phenylalanine on complex I-V, but an inhibition of pyruvate kinase a key enzyme of glycolysis, catalysing the formation of pyruvate. Thus, we asume that elevated concentrations of phenylalanine might be responsible for impaired cerebral glucose metabolism and loss of synapses, which stresses the importance of all medical treatment aiming at the reduction of elevated phenylalanine levels in plasma.

066-O

PHENYLALANINE INHIBITS PROLIFERATION OF HUMAN NEUROBLASTOMA CELLS WHICH EXPRESS PPARγ RECEPTORS

Z Lukacs, R Santer, K Ullrich, U Schumacher[1]
Department of Pediatrics and [1]Anatomy II, University Hospital-Hamburg-Eppendorf, Germany

The ultimate causes for intellectual impairment in PKU patients are still not fully understood. Morphologically, the number, length, and degree of arborization of the dendritic processes as well as synaptic spines were diminished, while cell packing density is increased with individual neurons being smaller than usual (Baumann and Kemper, Acta Neuropathol. 1982). Recently, peroxisome proliferator-activated receptors (PPARs) have been discovered to govern cell proliferation and differentiation. We and others were able to demonstrate that treatment of human cancer cells with PPARγ agonists, including phenylacetate, induced apoptosis and reduced cell growth which might explain the morphological results obtained earlier (Katoh et al. Cancer Letters, in press; Han et al. Cancer Research, 2001). As phenylalanine is the relevant neurotoxin in PKU, we tested elevated concentrations of this amino acid on human neuroblastoma cell lines which were proven to express PPARγ receptors. Indeed, three human neuroblastoma cell lines (LAN5, SHSY5Y and SKNSH) repeatedly showed reduced cell counts after 5 days of growth in phenylalanine-rich media (2.4 mmol/L) which resulted in decreases ranging from 37% to 70% compared to controls. Preliminary data indicate that the reduced number of cells in these cultures is caused by increased apoptosis. Thus, for the first time, we were able to show that phenylalanine affects cell proliferation using human neuronal cell lines.

067-P

ASSESSMENT OF RESPIRATORY CHAIN ENZYME ACTIVITIES IN A MOUSE MODEL FOR PHENYLKETONURIA

Z Lukacs, R Matalon[1], S Surendran[1], K Ullrich
Department of Pediatrics, University Hospital-Hamburg-Eppendorf, Germany; [1]University of Texas Medical Branch, Children's Hospital, Galveston TX, USA

Recently, it was found that rats, which have been artificially exposed to increased phenylalanine concentrations showed a reduction in complex I+III activity (Rech et al. Neurochem Res. 2002). In addition, *in vivo* ^{31}P MR spectroscopy of human PKU patients was indicative of an impaired cerebral energy status when phenylalanine concentrations were increased during a phenylalanine load (Pietz et al. Pediatr Res. 2003). Therefore, we have evaluated activities of complex I to V of the respiratory chain in brains of ENU2 mice (n = 12), a model for PKU, vs. age-matched normal control mice (n = 12; BTBR background). Plasma phenylalanine concentrations exceeded 20 mg/dl in all PKU mice tested. It was shown before, that brain phenylalanine concentrations in this model reflect blood/brain-phenylalanine-ratios known from PKU patients (Sarkissian et al, Anal Biochem 2000). In the brains of the mice activities for complex I ranged from 99 to 365 nmol/min/mg, for complex II ranged from 15 to 52 nmol/min/mg, for complex II+III were between 29 and 146 nmol/min/mg, for complex IV varied from 311 to 1250 nmol/min/mg and complex V showed values between 238 and 736 nmol/min/mg. No significant differences to control mice were observed. Thus, it appears that the reduced energy status is not caused by functionally-impaired respiratory chain enzymes but may result from direct or indirect inhibition of energy production by phenylalanine.

068-P

SECOND SUCCESSFUL PREGNANCY IN A WOMEN WITH 6-PYRUVOYL TETRAHYDROPTERIN SYNTHASE (PTPS) DEFICIENCY – IS THE MOTHER'S TREATMENT SAFE TO THE FETUS?

M Gizewska[1], G Hnatyszyn[2], B Nestorowicz[3], L Cyrylowski[4], M Walczak[1]
[1]II Department of Children's Diseases, [2]Department of Neonatology, [3]Department of Maternal and Fetal Medicine, Department of Radiology, [4]Pomeranian Medical University, Szczecin, Poland

PTPS deficiency is the most common defect of tetrahydrobiopterin synthesis, however pregnancy of a women with this defect has been so far only once reported (Gizewska et al. 2001). The same mother with late diagnosed PTPS deficiency (N72L/N36K genotype at PTS locus) became pregnant 3.3 years after the first delivery. Her older daughter, although born prematurely with right-sided closed schisencephaly (incidental event?), after being intensively rehabilitated due to mild from of hemiplegic cerebral palsy, is healthy with DQ = 120. During the second pregnancy mother's treatment with BH_4, L-Dopa and 5-hydroxytryptophan was continued. The average Phe level was 112 μmol/L, with incidental rise up to 505 μmol/L in 19 week of pregnancy (transient overconsumption of protein). The course of pregnancy was complicated by the mother's severe nicotinism which led to increased feto-maternal flow (the fetus's hypotrophy was observed from week 29) and imminent premature delivery. The boy was born in 37 week of pregnancy (Apg. 9/9 points), with body weight 2200 g and moderate symptoms of IUGR. On brain MRI examination no pathological changes (especially no schisencephaly) were found. At the age of 6 months the patient's physical and psychological development is normal; however he has already been hospitalized 4 times due to recurrent respiratory tract infections. It seems that treatment of mothers with PTPS deficiency is safe for the fetus, yet further studies and longer observations are needed.

069-P

LONG-TERM OUTCOME OF 30 PATIENTS WITH 6-PYRUVOYL-TETRAHYDROPTERIN SYNTHASE DEFICIENCY

Lin Wang[1], Wei-min Yur[1], Chun He[1], Shu Shen[1], Tze-Tze Liu[2], Kwang-Jen Hsiao[2]
[1]China-Japan Friendship Hospital, Beijing 100029, China; [2]National Yang-Ming University, Taiwan

Objective: To evaluate the long-term outcome of patients with 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency. **Methods**: Patients were identified as tetrahydrobiopterin (BH_4) deficiency based on the results of urinary pterin analysis, detection of dihydropteridine reductase (DHPR) activity in blood from a total of 550 patients with hyperphenylalaninemia (HPA), and then BH_4 loading tests in suspected patients with abnormal pterin profiles at our outpatient clinic since 1992. BH_4 deficient Patients were treated with BH_4, levodopa and 5-hydroxytryptophan (5-HTP). Development and intelligence quotient (DQ/IQ) and magnetic resonance imaging (MRI) of the brain were followed up. **Results**: A total of 30 cases were diagnosed as BH_4 deficiency, all of them were revealed as PTPS deficiency. They were diagnosed at the age of 2.5–27 months and the follow-up duration was 36–118 months. The average full-scale DQ/IQ at diagnosis and after treatment of at least 3 years were 53 ± 16, 78 ± 15 respectively. The improvement of abnormalities in the white matter was also seen on the MRI of the brain after treatment. A significant negative correlation was observed between the level of the DQ/IQ and the age of treatment commenced (= –0.751, $p < 0.001$). **Conclusions**: Long-term follow up demonstrates that outcome of patients with PTPS deficiency benefits from treatment as early as possible with three drugs combined.

070-P

DIAGNOSIS OF SEVERE PTPS DEFICIENCY IN A 28-YEAR-OLD LAWYER WITH NORMAL IQ

L Fiori, N Blau[1], A Zenga, E Riva M Giovannini
Pediatric Department, San Paolo, Hospital, University of Milan, Italy, [1]Division of Chemistry and Biochemistry, University Children's Hospital, Zurich, Switzerland

MM was screened for PKU at birth and a diagnosis of classic PKU (plasma phenylalanine (Phe) 1200 μmol/L) was made. A low Phe diet was introduced with good control and progressive tolerance increase so that free diet was introduced at age 3 years. The patient refers a normal life (sport at agonistic levels), with good metabolic control, until the age of 14 years, when he had his first panic attack (followed by 8 attacks in 2 years) that occurred before a sport competition. He started treatment with alprazolam and consulted many psychologists until the age of 27 when he asked for a consult in our Metabolic Department He referred he got his degree at university despite many episodes of dystonic movements related to stress induced panic attacks. MR of the brain (age 26 years) was normal. At admission in our Department he presented with normal IQ (103 WISH Scale), frequent episodes characterized by flexor spasm (hands), involuntary movements of arms, tongue protrusion, hypersalivation. Plasma Phe concentration was 381 μmol/L. Prolactin level was 75 ng/ml (nv 3–20) height Neopterins. CSF neurotransmitters and pterins showed a pattern suggestive for severe PTPS deficiency (HIAA 8 nmol/L, HVA 25 nmol/L, 3OMD 10 nmol/L, Neo 54 nmol/L, Bio 3.6 nmol/L). Enzyme assay on red blood cells was performed and therapy with neurotransmitters and BH_4 was started in April 2004. This is the first case of severe PTPS deficiency diagnosed in adult life in a patient with normal IQ and dystonic movements, with onset of clinical symptoms after school age.

071-P

PKU AT HOME: AN OBSERVATIONAL STUDY

MacDonald A, Daly A, Davies P, Asplin D, Hall SK, Chakrapani A
Birmingham Children's Hospital, Steelhouse Lane, Birmingham, B4 6NH, UK

Introduction: In children with PKU, dietary success may be dependent on parental ability and discipline to continually apply and supervise the low phenylalanine (Phe) diet.

Aims: (1) to compare blood phe concentrations with maternal knowledge of diet and demographic background, and (2) to investigate maternal application of diet.

Methods: In this observational study, all children with PKU (n = 46; median age 6 years) aged 1–10 years attending Birmingham Children's Hospital were recruited. The researcher, by home visiting, reviewed demographics, the maternal knowledge of PKU and their ability to allocate and calculate phe exchanges. Median plasma phe concentrations over the previous 12 months were estimated.

Results: 24% of subjects had an annual median plasma phe above their recommended range. Plasma phe control was worse in children from parents without educational qualifications (maternal $p<0.001$; paternal $p<0.005$), but not related to other social factors. Good maternal knowledge about diet and PKU correlated with exchanges for up to 50% of the time and 17% never weighed any exchanges. Only 40% of pre-weighed phe exchanges were correctly estimated by observation, and 25% were underestimated.

Conclusions: Parental education and knowledge of PKU were important factors associated with acceptable plasma Phe control. Weighing Phe exchanges was not well-adhered to and non weighed methods of gauging dietary phenylalanine portions need exploring.

072-P

SELF MANAGEMENT VERSUS PROFESSIONAL STEERED TREATED PHENYLKETONURIA

B Dorgelo, M van Rijn, P Modderman, DJ Reijngoud, FJ van Spronsen
Beatrix Children's Hospital, University Hospital of Groningen, The Netherlands

Introduction: To further improve metabolic control in PKU more frequent measurements of the Phe concentration are needed and better compliance is warranted. To increase the frequency of blood sampling and improve compliance, a system of self-management was introduced in our hospital in January 2000. Patients themselves performed bloodsampling at home and changed their diet accordingly, without interference from professionals. A previous study showed a small increase of Phe concentrations. The percentage percentage of samples within range remained comparable. The previous study, however, reported on a period of only 6 months of experience [1]. **Objective:** to study the effects of self-management during a longstanding period of time. **Methods:** We compared 3 years of the traditional, professional steered situation with 3 years of self-management in 48 patients divided in 4 age groups (1–4, 5–10, 11–15, >15 years). **Results:** The frequency of blood sampling doubled. During 2000, Phe values increased, but decreased afterwards (mean Phe in 2000: 455 μmol/L, thereafter, 414 μmol/L, $p = 0.056$). Compared with 1999, there still is an increase probably just due to the yearly increase seen over years. When splitted up, results showed an increase in aged 11–15 yrs ($p = 0.000$), and a decrease in >15 yrs ($p = 0.008$), and no change in 1–4 yrs and 5–10 yrs. A slight increase was seen in the percentage of samples within range as recommended by the British Medical Research Council for PKU. **Conclusions**:We conclude that self-management in PKU can be introduced and increases independence and responsibility in PKU patients especially in older patients.

[1] Bekhof. Eur J Pediatr. accepted

073-P

PKU: WHAT IS THE EFFECT OF CHANGES IN THE PHE-INTAKE?

M v Rijn, P Modderman, DJ Reijngoud, PJJ Sauer, FJ v Spronsen
Beatrix Children's Hospital, University Hospital of Groningen, The Netherlands
E-mail: g.van.rijn@fd.azg.nl

Objective: Treatment of PKU is based on a diet for life. The more strict approach of the treatment in adulthood asks improved compliance and more knowledge about the effects of changes in intake of Phe (natural protein) on the blood Phe concentration. However especially in adult patients little is known on this issue. **Methods:** We studied the effect of changes in the amount of Phe in 4 adult patients. The actual Phe tolerance of the patients was 450, 550, 550 and 2000 mg. Phe concentrations were measured each morning during 5 periods of 1 week. The intervention in the intake of Phe was calculated in percentages of the daily individual tolerance. Period 1: no change in intake; period 2 and 3: Phe loading (100% extra) on day 3 as capsules with free L-Phe. When Phe values after loading did not increase to > 600 μmol/L loading was repeated in period 4 and 5 with 200% extra. When Phe exceeded > 600 μmol/L, the loading with 100% extra was repeated in combination with 2 days reduction of 50%. Energy and total protein-intake were kept as usual for the patient. **Results:** Mean rise in Phe concentration after loading with 100% was 42% (median 34%) and 52% (median 35%) after 200%. In only 1 patient results in period 2 and 3 were >600 μmol/L. Reduction before or after loading resulted in Phe <600 μmol/L also in this patient. Loading with 300% resulted in Phe >600 μmol/L during 0–4 days after loading. **Conclusion:** The results suggest that for these patients it is safe to take incidentally 100% or even 200% extra above their normal tolerance of Phe, when they are in good metabolic control.

074-P

THE COURSE OF THE TOLERANCE OF PHENYLALANINE IN EARLY TREATED DUTCH PKU PATIENTS

B Dorgelo[1] M van Rijn[1], T de Koning[2], JBC de Klerk[3], A Bosch[4], E Rubio[5], RCA Sengers[6], AC Douwes[7], FJ van Spronsen[1]
[1]University Hospital Groningen, [2]University Medical Centre Utrecht, [3]Sophia Children's Hospital Rotterdam, [4]Acadamic Medical Centre Amsterdam, [5]Academic Hospital Maastricht, [6]University Medical Centre Nijmegen, [7]VU Medical Centre Amsterdam, The Netherlands

Introduction: the dietary tolerance of phenylalanine (Phe) at 5 yrs of age is often used to define clinical severity of phenylalanine hydroxylase (PAH) deficiency. In 1980 Güttler classified 3 subgroups using this method. However, little is known about the course of the Phe tolerance until adolescence and the prediction rate of the tolerance at ages <5 yrs. A Phe tolerance with a high predictive value at an earlier age might be of advantage.

Objective: to assess the course of the Phe tolerance and the prediction rate of an early tolerance.

Methods:We studied the Phe tolerance at 8 moments (1, 6, 12, 24, 36, 60, 120 and 180 months) in 213 early and continuously treated Dutch PKU patients.

Results: We found that during the studied age period the tolerance of Phe decreased logarithmically to a mean of ±12 mg/kg/day at 15 years of age. The correlation of the tolerance at 3 and 5 yrs with the tolerance at 10 years of age was comparable, $r^2 = 0.526$ ($p = 0.000$) and $r^2 = 0.437$ ($p = 0.000$), respectively.

Conclusions: It was concluded that the tolerance at 3 rather than at 5 yrs of age might be a valuable discriminating method of the clinical severity of PAH deficiency.

075-P

COMPARISON OF PROTEIN INTAKE AND GROWTH IN DUTCH AND USA PKU PATIENTS 0–3 YEARS

M Hoeksma[1], M van Rijn[1], V Fidler[2], R Koch[3], K Moseley[3], HD Bakker[4], AC Douwes[5], JBC de Klerk[6], T de Koning[7], RCA Sengers[8], E Rubio[9], PH Verkerk[10], PJJ Sauer[1], FJ van Spronsen[1]

[1]University Hospital Groningen, [2]Department of Statistics, [4]Academic Medical Centre, [5]VU University Medical Centre, [6]Erasmus Medical Centre, [7]University Medical Centre Utrecht, [8]University Medical Centre Nijmegen, [9]Acadamic Hospital Mastricht, [10]TNO Leiden, The Netherlands, [3]Children's Hospital, Los Angeles, USA

Introduction: PKU infants in the USA seemed to attain more favorable growth than in the Netherlands with higher total protein intakes [1,2]. This study tests the hypothesis that a higher intake of proein affects length and head circumference growth.

Methods: In a retropective study growth and dietaty data of 70 USA and 28 Dutch infants, born between 1974 and 1996 with comparable severity of PKU based on tolerance at 3 years of age were compared.

Results: Growth parameters were similar in the Dutch and USA study group. Total proein intake of USA children was 141% of the intake of the Dutch group, the intake of protein from a chemically manufactured source was alost twice as high as the intake of the Dutch infants (181%). Intake of natural protein was (not statiscally) higher in Dutch infants.

Conclusion: From this study, it can not be concluded that a higher protein intake is important for optimal length and head circumference growth. However, larger studies are necessary to investigate the effect of energy and different sources of protein.

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[2] Costa PB. JPGN 1998;27:287–91

076-P

EXPERIENCE WITH THREE NEW PRODUCTS FOR THE DIETARY MANAGEMENT OF PHENYLKETONURIA PATIENTS AGED 1–14 YEARS

De Laet C[1], Goyens Ph[1], Robert M[1], Beernaert S[2], Desloovere A[2], Eyskens F[2]

[1]Queen Fabiola University Children's Hospital, Brussels; [2]Koningin Paola Children's Hospital, Antwerp, Belgium

According to the nutritional recommendations for healthy individuals it was necessary to create three new products out of PKU2 for the treatment of phenylketonuria to ensure an optimal nutrient supply with respect to different age groups: PKU2 mix, a phenylalanine-free cow's milk substitute, and PKU2 A and PKU2 B, amino acid mixtures, for children aged 1–8 years and 9–14 years, respectively.

Patients and methods: 18 children aged 1–14 years were enrolled in an open prospective study of dietary management using PKU2 mix, PKU2 A and PKU2 B for a total duration of 12 weeks, regularly evaluated by defined efficacy and safety parameters. Statistical evaluation of the data was performed by the Wilcoxon signed-rank test and Pearson correlation coefficient.

Results: normal weight, length and body mass and haematological/biochemical parameters were maintained. Plasma concentrations of zinc and magnesium, which were low in several patients from the start, did not change using the new formulas. The mean blood Phe level was within therapeutic range; the blood tyr however increased significantly in all patients. The latter could be due to improved palatability and acceptance, resulting in a better compliance and spreading the intake of the formulas over at least 3 portions per day.

Conclusion: Milupa PKU2 mix, PKU2 A and PKU2 B formulas meet all the requirements for dietary treatment of PKU patients aged 1–14 years.

077-P

CHANGE IN RECOMMENDATIONS FOR MICRONUTRIENTS IN PKU

JB Hennermann, A v Arnim-Baas, C Gebauer, C Wiegert, E Mönch
Otto Heubner Centre for Pediatric and Adolescent Medicine, Charité Berlin, Germany

In 2000, new recommendations for the application of micronutrients were established by the German, Austrian, Swiss dietary organization (DACH). Also phenylalanine (Phe) restricted diet has changed within the last years due to new low protein dietary products.

We examined whether a new Phe free amino acid mixture (AAM) (P-AM 1, 2, 3; SHS Heilbronn) with a micronutrient content adapted to the DACH recommendations had a positive effect on the micronutrient status in PKU patients. 47 PKU patients were included in the study: eight aged 0–3 years (I), 22 aged 4–13 years (II) and 17 older than 13 years (III). Micronutrient serum concentrations and dietary protocols were analyzed before starting the new AAM and 3, 6 and 12 months after. Before changing AAM selenium concentrations were decreased in 35 children (median 22 µg/L in I, 29 µg/L in II, 48 µg/L in III). An increase of vitamin B_6 was detected in 35 children (median 31 µg/L in I, 25 µg/L in II, 37 µg/L in III), of B_{12} in 11 children and of molybdenum in 38 children (median 1.6 µg/L in I, 2.2 µg/L in II, 1.6 µg/L in III). After changing AAM serum concentrations of vitamin B_6 (median 18 µg/L in I, 16 µg/L in II, 25 µg/L in III) and B_{12} decreased in all examined patients, whereas selenium increased (median 24 µg/L in I, 45 µg/L in II, 54 µg/L in III). Molybdenum decreased only in II and III.

Before changing AAM our patients showed vitamin B_6 and molybdenum increase and selenium decrease that could have resulted in clinical symptoms. After changing AAM the results improved. These data show that the nutritional status of PKU patients must be analyzed regularly and the AAM should be updated according to the DACH recommendations.

078-P

FACTORS AFFECTING NEONATAL OUTCOME IN MATERNAL PHENYLKETONURIA (PKU)

SC Wong[3], PJ Rutherford[2], JA Skiming[3], DM Isherwood[1], DC Davidson[3]
Department of Biochemistry[1], Dietetics[2] and Metabolic Medicine[3], Alder Hey Children's Hospital, Liverpool, UK

Aim: To study neonatal outcome in maternal PKU in relation to phenylalanine control, tyrosine levels, protein utilisation and maternal weight gain in the 3 trimesters of pregnancies.

Methods: We conducted a retrospective case note analysis of 30 neonatal outcome of maternal PKU (1980–2003). Factors studied include gestational age at commencement of diet, gestational age phenylalanine <0.6 mmol/L, phenylalanine levels, tyrosine levels, total protein intake and weight gain in the 3 trimesters.

Results: Factors associated with congenital malformation were later age of commencement of diet and later reduction of phenylalanine <0.6 ($p<0.04$), higher phenylalanine levels in 2nd and 3rd trimester ($p<0.03$). Mothers with microcephalic infants had lower protein intake in the 1st trimester ($p = 0.001$) and lower tyrosine levels in the 3rd trimester ($p = 0.007$). Maternal weight gain in the 3rd trimester ($r = 0.48$, $p = 0.009$) and protein intake in the 3rd trimester ($r = 0.37$, $p = 0.04$) correlated with birth weight SDS.

Conclusions: Poor control of phenylalanine levels appear to have the greatest effect on congenital malformation in maternal PKU. The important finding of our study is that different factors in the 3 trimesters of pregnancies in maternal PKU may have an influence on differing aspects of neonatal outcome. This highlights the importance of close follow-up of maternal PKU even up till the 3rd trimester and that factors other than maternal phenylalanine levels can affect outcome.

079-P

INFLUENCE OF PKU MUTATION ON PHENYLALANINE (PHE) TOLERANCE DURING PREGNANCY AMONG DANISH AND NORWEGIAN MATERNAL PKU (MPKU) PATIENTS

KK Ahring
The John F. Kennedy Institute, Denmark and Kristina Motzfeldt, Department of Pediatrics, Rikshospitalet University Hospital, Norway

Introduction: Type of PKU mutation has a major influence on the individual ability to metabolize Phe. Patients with classical mutations metabolize 12–20 mg Phe/kilo (kg) lean body mass (LBM), with moderate mutations 20-25 mg Phe/kg LBM and with mild mutations up to 35 mg Phe/kilo LBM. This amount of Phe intake allows a patient with PKU to achieve control within the recommended range. However, most MPKU women only tolerate a smaller amount of Phe pre pregnancy in order to keep the Phe level 120–300 μmol/L. Despite type of mutation, it seems like MPKU women have very individual tolerance of Phe intake during the last trimester. **Data**: Data was collected from women in Denmark and Norway with MPKU. Twenty-three women with MPKU were compared for mutation, mean Phe blood level pre pregnancy and at 0 and 9 months pregnancy. Also, ability to metabolize Phe/kg bodyweight was compared at 0 and 9 months of pregnancy. Seventeen of the individuals had classical PKU, 5 mild PKU and 1 suspected to have mild PKU (only one known mutation). **Results**: Mean intake for a MPKU woman with a classical phenotype: 7.4 mg Phe/kg/day (range 4.2–10.7) at first week of pregnancy and 11.4 at the end of pregnancy (range 5.6–16.6). Mean intake for MPKU woman with a mild phenotype: 7.7 mg Phe/kg/day (range 3.6–11.8) at first week of pregnancy and 13 at the end of pregnancy (range 8.8–22.9). Mean blood level for a MPKU woman with a classical phenotype: 384 μmol/L (range 175–434). Mean blood level for a MPKU woman with a mild phenotype: 249 μmol/L (range 193–301). **Conclusion**: There is no direct correlation between mutation and Phe intake in these individuals.

080-P

IMPROVED PHENYLALANINE COUNTING FOR PKU DIETS

Sweeney AL[1], Coxon R[2], Fletcher JM[3]
Departments of Nutrition and Dietetics[1], Psychological Medicine[2] and Chemical Pathology[3], Women's and Children's Hospital Adelaide Australia

Objective: To compare the 15 mg phenylalanine (Phe) exchange system with a protein exchange system for the dietary management of PKU in childhood and adolescence.

Methods: Eighteen patients were initially randomised to either continue counting their current Phe exchange system (control group) or change to counting protein exchanges (study group). A new protein counting diet chart was developed. Food diaries and an in-house questionnaire, developed to assess attitudes to PKU and its management, were assessed at baseline and 6 months. Regular filter paper blood spots analysed by tandem mass spectrometry were used to evaluate metabolic control. As interim data analysis after 6 months for the study group confirmed no significant deterioration in the Phe levels (ICIEM 2003), the control group were changed to protein counting.

Results: For the most recent 6 month period, Phe levels were comparable to their pre-study levels (mean Phe pre 366 μmol/L ± 169 (n = 18), mean Phe post 388 μmol/L ± 160 (n = 17). All reported easier dietary management with fewer exchanges to count, more 'free' foods. Parents report being able to reduce cooking with part meals for PKU children now shared with the family and that most children have become more involved in their diets by being able to read directly from food labels.

Conclusion: Protein exchanges are a safe method of measuring Phe intake in the dietary management of PKU. Counting protein is now standard dietary management of patients at WCH with PKU and some participants have now counting protein for 2 years with good metabolic control.

081-P

A YOUNG CHILD WITH A COMBINATION OF PHENYLKETONURIA AND DIABETES TYPE 1: A CHALLENGE FOR THE DIETICIAN

E van der Louw, J Olieman, T Lappenschaar, JB C de Klerk, GJ Bruining, A van der Ploeg
Erasmus Medical Centre Sophia Children's Hospital, Rotterdam, The Netherlands
e.vanderlouw@erasmusmc.nl

A Moroccan girl (40 weeks of gestation, birth weight 3.4 kg) from consanguineous parents was detected in neonatal screening with high levels of phenylalanine (Phe) (1497 μmol/L). A protein free diet was started and amino acids and Phe were introduced gradually. The child was discharged from hospital with Phe levels below 200 μmol/L, on a 200 mg Phe/day restricted diet. The diet contained a Phe free formula containing amino acids (PhAAM). Normal growth (P-50 for weight/height/head circumference) and development was achieved. A few days before her first birthday the child presented severe diabetic keto acidosis, for which she required intensive care for a week. At that time Phe levels were very high. A diagnose type 1 diabetes was made. Insulin therapy, multiple daily injections (MDI), was started. The concurrence of these metabolic diseases is exceptional. No data on this particular combination were found in PUBmed. A variation of factors can influence the Phe levels, blood glucose and Hba1c%. In this case different insulin schedules gave no improvement. At age of 3 years CSII pump treatment was started. Acceptable Phe levels (240–480 μmol) could only be achieved on a very strict diet. An amount of 200–220 mg Phe/day was tolerated (normal Phe restriction is 250–300 mg/day). Most protein free products used in the protein restricted diet contain large amounts of carbohydrates and must be taken over the day for good blood glucose levels. This case report shows that combination of 2 strict diets is possible. It is a great challenge for the dietician to coach the parents and make the dietary life of the child possible.

082-P

A NEW NOVEL PROTEIN SUBSTITUE FOR TYROSINAEMIA

Daly A, MacDonald A, McKiernan P, Preece MA, Asplin D, Chakrapani A
Birmingham Childrens Hospital, Steelhouse Lane, Birmingham, B4 6NH, UK

Newer, novel protein substitutes (PS), originally designed for PKU, are being adapted for other non-PKU amino acid disorders. It is important their use is evaluated as natural protein tolerance and intake of PS may vary in other disorders.

Aim: To evaluate the efficacy and acceptability of a tyrosine (Tyr) and phenylalanine (Phe) free PS gel (Tyr Gel; Vitaflo; 42 g/100 g protein equivalent) for children aged 1–10 years with tyrosinaemia. It contains added carbohydrate, vitamins and minerals.

Methods: Eight children with tyrosinaemia types 1 (n = 3), 2 (n = 1), 3 (n = 3) and undefined (n = 1), aged 1 to 10 years, were recruited when taking X Phen Tyr Maxamaid (SHS). Natural protein intake ranged from 3–40 g/daily (median 11), and one subject took PS only. Median intake of protein equivalent from PS was 1.4 g/kg/day (range 0.5–3.4). The new PS was taken for 8 weeks, and dietary intake, nutritional biochemistry, plasma Tyr and Phe were assessed at weeks 0 and 8.

Results: The median daily weight of PS reduced by 35 g/daily from 115 g (range 90–175) to 80 g (range 60–100). Plasma Tyr/Phe levels were unchanged, all subjects preferred it, and parents found it convenient. Dietary intake of calcium, zinc and and iron reduced on the new PS, but all nutrients still met UK (1991) dietary reference values, and nutritional biochemistry was satisfactory.

Conclusions: Tyr Gel is safe, popular and efficacious in tyrosinaemia but longer-term studies of its effect on nutritional biochemistry are warranted. It contains lower concentrations of some micro-nutrients than traditional products, which may be an issue with smaller dosages of PS.

083-A

IS MEDICAL FORMULA FOR STABLE ADULT UCD PRACTICAL?

Sirrs S, Verduyn I, Dixon C, Abraham J, O'Riley M, Bosdet T, Howson A
Vancouver General Hospital's Adult Metabolic Diseases Clinic, Vancouver, Canada

Background: Although UCD adults tend to be more stable than children, lifelong dietary protein restriction is still required with standards suggesting 50% of dietary protein from medical formula. Only 1/6 of our adult patients who presented as a neonate uses medical formula. The other 5 patients who presented as adults have both taste intolerance for medical formula which prevents its use and an aversion to increasing and selecting good quality dietary protein. Even without medical formula, our adults have withstood surgical stresses and infections. But low protein quality diets cause low nutritional markers. **Purpose**: We present the results of improved nutritional parameters by adding medical formula to maintenance j-tube feeds for a 46-year-old woman with presumed OTC and the effects this change had on NH3. **Methods**: Three year mean values for BCAA, glutamine, NH3, prealbumin and hemoglobin were calculated pre-treatment. Maintenance j-feeds were gradually modified by adding a total of 9 extra grams of protein/day. Total calories stayed the same. **Results**: Weight was maintained. Nutritional parameters (μmol/L) improved: glutamine 793 ± 17.2 to 683 ± 31.9, valine 148 ± 3.5 to 169 ± 8.2, isoleucine 42 ± 1.5 to 49 ± 2.8, leucine 79 ± 2.2 to 89 ± 5.5; prealbumin 159 ± 5.0 to 169 mg/L and hemoglobin 128 ± 1.4 to 130 g/L. Mean NH3 was slightly increased (40 ± 2.6 to 46 ± 3.4, p = NS). **Conclusion**: Nine extra grams of medical formula protein via j-tube improved nutritional parameters and glutamine. Given the relative stability of many adult UCD patients, and the absence of palatable medical formula for adults, the recommendations to use medical formula to provide 50% of dietary protein may need to be reanalyzed. Finding ways to include, or reduce aversions to, quality dietary protein may be better.

084-P

PROBLEMS WITH STANDARDISING ARGININE SUPPLEMENTATION DOSAGE IN ARGININOSUCCINIC ACIDURIA

S Alger[1], MA Preece[1], A Chakrapani[1], A MacDonald[2]
[1]*West Midlands Regional Laboratory for Inherited Metabolic Disorders, Clinical Chemistry Department;* [2]*Dietetic Department, Birmingham Children's Hospital, Birmingham, UK*

Argininosuccinic aciduria is a urea cycle disorder caused by a deficiency of the enzyme argininosuccinate lyase (AL). Supplementation with arginine (an essential amino acid in this disorder) promotes the synthesis of argininosuccinate which is excreted in the urine thus promoting removal of waste nitrogen. A review of the prescribed arginine dose for our eight patients with this disorder revealed wide variation ranging from 100 to 500 mg/kg/day. Plasma ammonia and glutamine were generally lower in those patients regularly receiving 250 to 500 mg/kg/day. The dosage recommended in 'Medicines for Children' (RCPCH Publications) is 300 to 700 mg/kg/day. All eight patients were changed to a standard arginine dose of 500 mg/kg/day. Over a two year period good correlation between increased plasma arginine and lowered ammonia and glutamine was observed. Compliance was a major issue in one patient. Target plasma arginine levels recommended in 'Medicines for Children' are 50–200 μmol/L. In our patients arginine was frequently increased to above 200 μmol/L and varied widely (38–643). Although evidence for toxicity is not strong, high plasma concentrations of arginine in arginase deficiency are thought to contribute to neurological damage. In our patients, the recommended arginine dose frequently produced high random plasma arginine concentrations and peak concentrations may be even higher. The possibility that this could cause neurotoxicity should be considered.

085-P

DIETARY THERAPY IN PROPIONIC ACIDEMIA: RECOMMENDED VERSUS REAL PROTEIN SUPPLY

Scholl-Bürgi S, Grissenauer G, Fendl A, Baumgartner S, Konstantopoulou V, Skladal D
University Children's Hospital, Innsbruck, Austria

Introduction: Dietary therapy of propionic acidaemia consists of a defined protein supply and a supplementation of amino acids, minerals, trace elements and vitamins. In long term treatment it often remains unclear whether the recommendations for protein supply are fulfilled. **Aim of the study**: To compare protein recommendation with supply. **Patients**: Seven patients with propionic acidaemia (3 girls, 4 boys from 4 families) were included in a prospective study. Diagnosis was confirmed molecularly and enzymatically. For every patient food intake was protocolled over 5 days (3 days by children and parents, 2 days via a telefone interview by a dietitian) and repeated after six months. All patients had a protein supply recommendation of 1.2–1.5 g/kg bw/day, six received an additional amino acid supplementation. All patients received vitamins, minerals, trace elements and carnitine. **Results**: In 8 out of 14 dietary protocols there was a lower supply of natural protein than recommended (median: 0.2; range: 0.1–0.7 g/kg bw/day), in 4 a higher protein supply than recommended (median: 0.3; *r*: 0.2–0.7 g/kg bw/day), in 2 recommendations were met. Protein made 8.1% (*r*: 6.4–10.8%) of energy intake. Supplements were taken as recommended (*r*: 0.1–0.7 g/kg bw/day). In addition, a strong selection of foods consumed could be observed. **Discussion**: Surprisingly, the protein supply in over half of the protocols was lower than recommended. A possible explanation could be the fear of metabolic derangements on the parents' side or a failure to adjust the children's eating habits to increased body wait when they have grown.

086-P

UNCOOKED CORNSTARCH IN TREATMENT OF LONG CHAIN FATTY ACID OXIDATION DISORDERS

MA Preece[1], A Chakrapani[2], A Daly[3], A MacDonald[3]
[1]Clinical Chemistry; [2]Clinical IMD, [3]Dietetic Departments, Birmingham Children's Hospital, Birmingham, UK

Uncooked cornstarch (UCCS) is a complex carbohydrate which, when given orally, is slowly digested and absorbed acting as a slow release supply of glucose over a 6–8 hour period. Its use has been proposed in LCFAOD to prevent hypoglycaemia and to suppress lipolysis. We have studied the effect of a dose of UCCS (2 g/kg) in 6 patients (3 LCHADD, 1 VLCADD, 1 CPT2 and 1 carnitine translocase, age 1–7 years) over a period of 6–7 hours. All 6 patients remained normoglycaemic. Free fatty acids were suppressed for 4 hours but then increased rapidly in 4 patients. Clinically, UCCS has improved metabolic stability and eased management in 3 patients.

Table. Plasma free fatty acid concentrations (μmol/L) after a 2 g/kg dose of UCCS

Patient no		Pre	1 h	2 h	3 h	4 h	5 h	6 h	7 h
1	LCHADD	284	<100	<100	141	486	682	972	
2	LCHADD	256	160	214	445	446	493	609	
3	LCHADD	720	<100	<100	100	101	962	1508	1737
4	CPT2	1255	174	NA	<100	536	1719	1923	
5	VLCAD	223	169	<100	242	211	512	763	1142
6	Translocase	181	136	103	129	120	203	406	

Conclusion: Regular UCCS helps maintain metabolic stability in LCFAOD patients but fasting tolerance remains limited. Individual treatment regimens should be based on UCCS loading tests.

087-P

EFFECT OF KETOGENIC DIET IN REFRACTORY EPILEPSY

ThAM van den Hurk, EC Carbasius Weber, TJ de Koning, NM de Roos, G Visser, O van Nieuwenhuizen

Departments of Dietetics, Neurology, Metabolic Diseases, University Medical Centre Utrecht, The Netherlands

Introduction The Ketogenic diet is a high fat (70–90 en%), low carbohydrate (14–19 en%) and low protein (6–10 en%) diet that has been shown to reduce the number and severity of seizures in some children resistant to anticonvulsant therapy.

Aim To prospectively evaluate the effect of diet intervention in children with medically refractory epilepsy.

Method Between 1998 and 2003, 54 patients (27♂, 27♀), age 0.5–19.0 years were treated with the ketogenic diet. Of the 54 patients, nine had a metabolic disease while in 45 patients no cause for the epilepsy was found. The effect of the diet was evaluated several times a year.

Results Five patients completed the diet within 24–42 months and 17 patients are still on the diet (range 8–57 months) and are doing well (decrease in number and severity of seizures). The diet was discontinued by 32 patients because: the effect was absent or marginal ($n = 16$), patients experienced side effects such as vomiting, constipation, diarrhoea ($n = 3$), or a combination of both ($n = 4$). Four patients died; 3 stopped the diet because of psychosocial problems and 2 because of other medical conditions.

Conclusion Our study shows that in 40% of medically refractory epilepsy the ketogenic diet, when strictly followed for at least 2 years, can decrease the number of seizures and increase the wellbeing of pediatric patients.

088-P

ENDOCRINE RESPONSES OF DIETARY AMINO ACIDS: INSULIN, GHRELIN, AND LEPTIN RESPONSES TO A SINGLE DOSE OF ESSENTIAL AMINO ACIDS

I Knerr[1], H-G Topf[1], M Gröschl[1], R Link[2], W Rascher[1], M Rauh[1]

[1] *University Children's Hospital, Erlangen-Nuremberg, 2SHS Company, Heilbronn, Germany*

Insulin, ghrelin and leptin are among the endocrine mediators of dietary ingestion, so we tested their response following the intake of essential amino acids (AA) in healthy volunteers. 12 fasted volunteers (age 18-40 years, body mass index 18.0–23.5 kg/m^2) were included, 7 consumed a dietary AA mixture (E-AM®, 0.35 g AA/kg body weight), 5 served as fasting controls. Serum AA, glucose, albumin, urea and hormones were measured at 0, 15, 30 min and thereafter in 30-min intervals up to 5 hours. Glucose, albumin and urea remained constant, peak AA concentrations were achieved at 30 min for Met, at 60 min for all other AA. The insulin peak (533% compared to basal level, $p < 0.01$) at 30 was earlier than the peaks of all AA except Met. Ghrelin showed a continuous rise towards the end of the experiment leading to a 3-fold increase of initial concentrations in the study group ($p < 0.001$), signmificantly higher than in the controls ($p < 0.05$). Both groups exhibited constant leptin concentrations.

An oral low-dose AA bolus causes not only considerable hyperaminoacidaemia and hyperinsulinemia but is also accompanied by an increased ghrelin secretion in fasted humans compared to controls, but is not a sufficient stimulus to induce satiety. Leptin is not a key mediator in this setting. Hyperinsulinemia in combination with hyperaminoacidaemia stimulates AA oxidation especially in the absence of non-protein energy, therefore reducing the efficiency of AA supplementation. This has to be considered in the dietary treatment using AA mixtures.

089-P

NOVEL DIAGNOSTIC PARAMETERS FOR AAD DEFICIENCY IN GENERAL METABOLIC URINE SCREENING

NGGM Abeling[1], JE Abdenur[2], L Jorge[3], N Chamoles[3]
[1]Laboratory of Genetic Metabolic Diseases, Department of Clinical Chemistry, Academic Medical Centre, University of Amsterdam, The Netherlands, [2]Division of Metabolism, PSF Children's Hospital of Orange County, Orange, USA, [3]Foundation for the Study of Neurometabolic Disease, Buenos Aires, Argentina

Introduction: Aromatic L-amino acid decarboxylase (AADC) deficiency in most cases is a treatable defect in the biosynthesis of the neurotransmitters dopamine and serotonin. Until now the only way to detect this disorder in general metabolic screening was to detect vanillactic acid (VLA) in GC-MS analysis of organic acids. Because of the sometimes only small increase of VLA, and/or insufficient analytical sensitivity AADC deficiency is probably often missed. **Cases**: The first case was a boy with hypotonia, hypoglycemia and metabolic acidosis detected at 13 days of age, by organic acids (OA) analysis of urine. The second case(s) were two brothers, both with dystonia, and oculogyric crises in one, detected at ages of 6 and 10 years of age, respectively, in whom the diagnosis had been missed in OA, but eventually established by the finding of elevated L-DOPA and dopamine in urine.

Methods: GC-MS of organic acids after ethoximation, HPLC-ECD of L-DOPA and dopamine.

Results: In the first case the urinary OA profile not only showed elevated VLA, but also vanilpyruvic acid, *N*-acetylvanilalanine and *N*-acetyltyrosine. The brothers appeared to have hyperdopaminuria in addition to clearly elevated L-DOPA.

Conclusion: The cases we present clearly demonstrate the additonal value of the newly discovered diagnostic parameters, providing new chances for detection of AADC deficiency.

090-A

QUANTITATIVE PLASMA AMINO ACID ANALYSIS IN THAI INFANTS WITH IEM USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

P Wasant, P Ratanarak, N Wattanawicharn, S Liammongkolkul
Division of Medical Genetics, Department of Pediatrics, Siriraj Hospital Faculty of Medicine, Mahidol University, Bangkok 10700, Thailand

The disorders of amino acid (AA) metabolism are usually diagnosed by quantitative analysis of amino acids by amino acid analyzer or high performance liquid chromatography (HPLC) which was established in developed countries since 1970s. Patients with amino acid disorders usually presented with metabolic acidosis, respiratory distress/tachypnea, lethargy, vomiting, coma, seizures, hypo/hypertonia, poor feedings, growth failure and delayed development/mental retardation. Confirmation of the diagnosis of amino acid disorders is quite difficult in developing countries due to lack of expert laboratories and only a handful of interested and experienced scientists available.

Collaboration with expert scientists at Chulabhorn Research Institute in Bangkok (1999–2000) has helped us establish the genetic metabolic center where we can perform analysis of AA and identify several disorders since July 2001. During the past 3 years we have analysed 422 samples from 282 patients and identified 14 new cases. These are: 1 case of PKU, 7 cases of MSUD, 4 cases of urea cycle defects (ALD, ASD) and 2 cases of NKH. The development of quantitative analysis of AA has led to prompt and proper treatment and better outcome in our patients.

091-A

CORRELATION OF LEU/ILE LEVELS USING DRIED BLOOD SPOT THIN LAYER CHROMATOGRAPHY (TLC) AND PLASMA HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) AMONG FILIPINO MSUD PATIENTS

Lee JY, Demata MA, Dela Cruz KT, Cavan CC, Chiong MD, Padilla CD
Institute of Human Genetics, National Institutes of Health, Philippines

Management of patients with maple syrup urine disease (MSUD) includes low protein diet supplemented with special formulas and constant monitoring of branched chain amino acids. The gold standard for monitoring is plasma amino acid analysis using HPLC. In a developing country like the Philippines, however, the cost of this test is prohibitive to majority of patients. Dried blood spot leu/ile level by TLC is often used to diagnose and monitor these patients. This study was done to determine the correlation of leu/ile levels using the two analysis (TLC vs HPLC using Shimadzu Class VP series). Paired samples (dried blood spot and plasma) of twelve MSUD patients were collected. There were 7 male and 4 female with age range from 2 months to 5 years. Majority had the classical type of MSUD and diet was between 0.6 g/kg/day to 1 g/kg/day of natural protein. Results showed a significant linear correlation (Spearman correlation = 0.800) between the two methods ($p < 0.05$). A dried blood spot leu/ile level by TLC is an alternative method that can be used in the diagnosis and monitoring of MSUD patients especially in a developing country.

092-P

THE USE OF BREATH TESTS TO MONITOR LIVER DYSFUNCTION IN HEREDITARY TYROSINAEMIA TYPE 1

D Rigante, A Stabile, A Gasbarrini[1], EC Nista[1], A Armuzzi[1], AI Cazzato[1], M Santoro[1]
M Candelli[1]
Department of Pediatric Sciences, [1]Department of Internal Medicine, Università Cattolica Sacro Cuore, Rome, Italy

Hereditary tyrosinemia type 1 (HT-1) is a fatal liver disease resulting from early cirrhosis and risk of hepatocellular carcinoma during childhood. The use of breath test (BT) with stable isotopes such as ^{13}C has been considered to be safe and accurate for the indirect estimation of liver function evaluating the ^{13}C-enrichment of expired CO_2 due to the metabolic breakdown of an orally administered ^{13}C-labeled substrate. Several ^{13}C-labeled substrates have been used such as ^{13}C-aminopyrine (^{13}C-*A*), for the evaluation of microsomial cytochrome P450 enzymatic system or ^{13}C-α-ketoisocaproic acid (^{13}C-*KICA*) for the evaluation of mithocondrial oxidative capacity. We have assessed the reliability of ^{13}C-*A*BT and of ^{13}C-*KICA*BT in a 7-year-old female child (weight 22 kg; height 112 cm) with HT-1, with Child-Pugh A liver cirrhosis, confirmed by biopsy, under treatment with nitisinone, after an overnight fast and while resting: breath samples for ^{13}C-*A*BT were obtained prior to and every 15 min for 90 min after the ingestion of 2 mg/kg body weight of ^{13}C-*A*; three months later breath samples for ^{13}C-*KICA*BT were obtained prior to and every 15 min for 90 min after the ingestion of 20 mg/kg L-leucine followed by 1 mg/kg ^{13}C-*KICA*. For each BT $^{13}CO_2$ enrichment was analyzed by isotope ratio mass dpectrometry and the fraction of the administered dose exhaled as CO_2 was calculated from the increment in the $^{13}C/^{12}C$ ratio. The cumulative % of ^{13}C-dose exhaled at 90 min was 9.02 for *A*BT (normal if compared with healthy controls) and 12.85 for *KICA*BT (reduced if compared with healthy controls). These BTs represent useful means in assessing damage entity in chronic liver diseases, but further studies are needed to establish whether ^{13}C-*KICA*BT might identify mitochondrial impairment in children with HT-1.

093-P

RENAL FUNCTION IN TYROSINEMIA TYPE I AFTER LIVER TRANSPLANTATION

LJWM Pierik[1], CMA Bijleveld[2], KML van Dael[1], FJ van Spronsen[3]
[1]Department Pediatric Nephrology, [2]Department Pediatric Gastroenterology, [3]Department Metabolic Diseases, Beatrix Children's Hospital, University Hospital Groningen, The Netherlands

NTBC is the first choice in the treatment of tyrosinemia type I (TT-I). Before introduction of NTBC, liver transplantation (LT) was the only possible treatment. LT remains still necessary in case of hepatocellular carcinoma. After LT renal function may still be at risk.We studied 9 patients with TT-I after LT (between 1988 and 1996, only 1 patient treated with NTBC before LT). An evaluation was made of the most recent glomerular and tubular function and SA excretion in urine.

Age (years)	17	12	15	16	13	11	11	10	16
Age at OLT (years)	3	1.5	4	5	5.5	0.7	1	0.5	10
Creatininclearance (ml/min/1.73 m^2)	152	83	39	89	100	141	96	95	61
TRp < 80%/Ca-excretion > 4 mg/kg/day	−/−	+/−	+/−	−/−	−/−	+/+	−/−	+/	−/−
Hypokalemia/metabolic acidosis	−/−	−/−	−/+	−/−	−/−	+/+	−/−	−/?	−/−
Glucosuria/tubular proteinuria	−/−	−/−	−/−	−/−	−/−	+/+	+/−	−/−	+/−
SA excretion (mg/mmol creatinin)	0.1	?	1.9	2.7	2.6	5.1	1.1	0.5	0.1

We found progressive tubular rather than glomerular dysfunction. This deterioration of tubular function is most probably caused by SA and not by cyclosporin. We therefore stress the importance of tubular follow-up after LT in patients with TT-I. Because the tubular problems seem to be progressive, NTBC treatment might be a future option to prevent further tubular problems.

094-P

TYROSINEMIA TYPE I TREATED BY NTBC: CAN HEPATOCELLULAR CARCINOMA (HCC) DEVELOP AT ANY TIME?

CJL Koelink[1], G Visser[2], TJ de Koning[2], A van der Ploeg[3], JBC de Klerk[3], BT van Maldegem[4], FA Wijburg[4], CMA Bijleveld[1], FJ v Spronsen[1]
[1]University Hospital of Groningen, [2]University Medical Centre Utrecht, [3]Erasmus University Centre, [4]Academic Medical Centre of Amsterdam, The Netherlands

Introduction: HCC is known to develop in tyrosinemia type I notwithstanding NTBC especially when NTBC started after 2 years of age. **Objective:** to present 3 cases with HCC in whom NTBC treatment was started < 2 (2), and just > 2 (1) years, and to point at the abnormal course of AFP. **Case 1**: AFP 8440 (normal < 20) μg/L. Start NTBC at 27 months, NTBC remained adequate. AFP stable at 25 μg/L till $5\frac{1}{2}$ years later. AFP rose to 372 μg/L in a few months. HCC was found with MRI rather than ultrasound. HCC was removed and some 6 months later, liver transplantation (OLT) was performed. **Case 2**: Second of a twin (first treated by OLT almost directly after diagnosis). AFP 9840 μg/L. Start NTBC at 15 months. NTBC for 11 years. AFP only once 10 μg/L, remained stable at ± 30 μg/L for years but rose to 4035 μg/L in a few months. HCC was removed and he is now going for OLT. **Case 3**: Start NTBC at 14 days, AFP 1 160 000 μg/L. Slow decrease to 120 000 μg/L but rose again within 3 months up to 175 530 μg/L. HCC was treated with chemotherapy, and living related OLT was performed. **Conclusions**: NTBC may not be able to prevent development of HCC in all patients. A very high initial AFP, a slow decrease, and not reaching normal values may be predictors of HCC development in further life. It may be important to start with NTBC early after birth in all patients, necessitating neonatal screening, although even this may not prevent HCC in all patients.

095-P

EVALUATION OF DICHLOROACETATE AS TREATMENT FOR HEREDITARY TYROSINEMIA IN A MOUSE MODEL

C Langlois, R Jorquera, M Finegold, RM Tanguay
Laboratory of Cellular and Developmental Genetics, CREFSIP, Department of Medicine, Pav. Marchand, Laval University, Quebec, Canada, G1K 7P4

Hereditary tyrosinemia type I (HTI) is caused by deficiency in fumarylacetoacetate hydrolase (FAH). Accumulation of toxic metabolites causes liver and kidney damage. Blockage of the pathway with 2-(2-nitro-4-trifluoro-methylbenzyol)-1,3 cyclohexanedione (NTBC) ameliorates acute symptoms, but some HTI patients do not respond to this therapy. The search for alternative treatments remains important. We evaluated whether inhibition of maleylacetoacetate isomerase (MAAI) with dichloroacetate (DCA) could replace NTBC in FAH knockout mice. Adult male were withdrawn from NTBC and given DCA (0.8 g/kg/day) orally for 5 weeks, while fed a standard diet or a tyrosine-restricted diet. NTBC-OFF mice lost weight (100% to 70%) when fed a regular diet, but gained weight (100% to 118%) when fed a tyrosine-restricted diet. Similar body weight curves were found in DCA-treated mice. Histology revealed that DCA did not ameliorate or worsen hepatic and renal damage caused by NTBC withdrawal, regardless of diet. NTBC-OFF mice showed MAAI protein levels corresponding to 60% of NTBC-treated or untreated wild-type mice; DCA decreased these levels to 20%. Curiously, NTBC-treated and NTBC-OFF mice had similar low hepatic MAAI activity levels corresponding to 10–20% of untreated wild-type mice; DCA decreased these levels to 5%. Thus, the virtual lack of effect of DCA treatment in $FAH^{-/-}$ mice could be ascribed to residual MAAI activity, which seems sufficient to maintain open the tyrosine catabolic pathway, allowing toxic metabolite accumulation.

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096-P

GLUTATHIONE MONOETHYL ESTER TREATMENT RESCUES TYROSINEMIC MICE FROM NEONATAL DEATH AND ALLOWS SURVIVAL DURING EARLY INFANCY

R Jorquera[1], C Langlois[1], M Finegold[1], WJ Rhead[2], RM Tanguay[1]
[1]*Laboratory of Cellular and Developmental Genetics, CREFSIP, Department Medicine, Pav. Marchand, Universite Laval, Quebec, Canada, G1K 7P4;* [2]*Medical College of Wisconsin, Milwaukee, WI 53201, USA*

Hereditary tyrosinemia type I (HTI) is caused by deficiency in fumarylacetoacetate hydrolase (FAH). The drug 2-(2-nitro-4-trifluoro-methylbenzyol)-1,3 cyclohexanedione (NTBC) ameliorates acute symptoms, but does not prevent development of liver cancer. Alternative treatments are cogently needed. We tested whether GSH-MEE could replace NTBC in FAH knockout mice. GSH-MEE (10 mmol/kg/day) was given orally to pregnant/nursing females. All $FAH^{-/-}$ pups survived the first 24 h of life and grew normally until postnatal day 10 (P10); thereafter, they showed failure to thrive, lethargy and died around P17. Glycosuria and hyperaminoaciduria were found at P6 and hypoglycemia at P17. Histology revealed no damage at P2, mild damage at P6, but severe acute liver damage and marked proximal tubular dilatation in kidneys at P17. Some NTBC-treated pups showed diffuse tubule dilatation at P2 and P17 and single cell necrosis and nuclear anisocytosis in liver at P17. Oxidative stress-inducible protein levels increased with age in GSH-MEE- and NTBC-treated pups; α-fetoprotein decreased with age in both groups, was still present at P17, but no longer detected in untreated wild-type pups at P17. Thus, GSH-MEE successfully replace NTBC at least during the first 48 h after birth; thereafter, a HTI phenotype develops with age. However, even NTBC does not completely abolish some manifestations of this phenotype before weaning.

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097-P

MICE WITH HEREDITARY TYROSINEMIA TYPE I ACQUIRE RESISTANCE TO CELL DEATH STIMULI IN BOTH LIVER AND KIDNEYS

SMM Jacobs, MC Luijerink, HEM Malingre, EACM van Beurden, DHA van Beurden, LWJ Klomp, R Berger, IET van den Berg
University Medical Centre, Utrecht, The Netherlands

Hereditary tyrosinemia type I (HT1) caused by deficiency of the enzyme fumarylacetoacetate hydrolase, is associated with severe liver and kidney damage, which can be largely prevented by treatment with 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC). However, patients with HT1 have developed hepatocellular carcinoma despite NTBC-treatment. The HT1 mouse model was used to gain more insight into the pathogenesis of liver and kidney disease. In $Fah^{-/-}$ mice, protected from liver and kidney injury by NTBC, a single administration of 800 mg/kg of the tyrosine metabolite homogentisic acid (HGA) caused rapid cell death in liver as well as in kidneys, which is mainly caspase-3 independent. In contrast, mice with pre-existing liver and kidney damage, induced by withdrawal of NTBC, survived this otherwise lethal dose of HGA and acquired resistance to this caspase-3-independent form of cell death. Liver and kidneys of these mice revealed limited characteristics of apoptosis and a small increase in expression of cleaved caspase 3. It is already known that patients with HT1 are at risk for developing hepatocellular carcinoma, even under NTBC treatment, which may be related to the observed resistance to cell death of HT1-affected hepatocytes. It cannot be excluded that the susceptibility of the HT1-affected kidneys to develop resistance to cell death stimuli will have harmful effects when HT1 patients become older, now that NTBC treatment and liver transplantation have greatly improved their life expectancies. Close monitoring of the kidneys of HT1 affected individuals is therefore strongly recommended.

098-P

ER STRESS SIGNALING IN MAMMALIAN CELLS TREATED WITH FUMARYLACETOACETATE, THE ACCUMULATING METABOLITE IN THE LIVER OF TYROSINEMIA TYPE I PATIENTS

RM Tanguay, A Bergeron, R Jorquera
Laboratory of Cellular and Developmental Genetics, CREFSIP, Department of Medicine, Pav. Marchand, Laval University, Ste-Foy, Québec, Canada G1K 7P4

Hereditary tyrosinemia type I (HTI) is the most severe disease of the tyrosine degradation pathway. HTI is caused by a deficiency in fumarylacetoacetate hydrolase (FAH), the enzyme responsible for the hydrolysis of fumarylacetoacetate (FAA). As a result, there is an accumulation of FAA which was shown to display mutagenic, cytostatic and apoptogenic activities and to cause chromosomal instability.

Here, we demonstrate that FAA also causes a cellular insult leading to ER (endoplasmic reticulum) stress signaling and apoptosis. Treatment of V79 chinese hamster lung cells with an apoptogenic dose of FAA (100 μmol/L) causes an early induction of the ER resident chaperone BiP and a simultaneous phosphorylation of the eIF-2α translation initiation factor. FAA treatment also causes a subsequent induction of CHOP, a transcription factor that potentiates apoptosis. Finally, FAA treatment leads to apoptotic cell death as evidenced by late activation of caspase-12 (an ER resident caspase cleaved in response to ER stress). In summary, the present data show that FAA treatment of cells causes an ER stress followed by apoptosis through an early activation of BiP and eIF-2α phosphorylation, followed by CHOP and caspase-12 late activation.

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099-P

NO EVIDENCE OF CHIMERISM IN THE MOSAIC EXPRESSION OF FUMARYLACETOACETATE HYDROLASE IN LIVERS OF TYROSINEMIA 1 PATIENTS

A Bergeron[1], P Russo[2], J Morissette[3], RM Tanguay[1]
[1]Laboratory of Cell and Developmental Genetics, CREFSIP, Department of Medicine, Pav. Marchand, Laval University, Ste-Foy, Québec, Canada G1K 7P4, [2]Department of Pathology, Children's Hospital, Philadelphia, PA 19104, USA, [3]CHUQ, Pav. CHUL, Ste-Foy, Québec, Canada G1V 4G2

Hereditary tyrosinemia type I (HTI) is a recessively inherited disease caused by a deficiency of fumarylacetoacetate hydrolase (FAH), the last enzyme of the tyrosine catabolic pathway. A mosaic pattern of FAH expression is observed in the livers of ~85% of studied patients and it was shown to result from the correction of the mutation in one of the *FAH* alleles. Bilateral cell trafficking can occur between mother and fetus and such an event could be responsible for the chimerism observed in some diseases. It has also been reported that the liver repopulation observed in a HTI murine model by transplantation of bone-marrow derived cells was due to cell fusion. These observations led us to test the possibility that the transfer of nucleated maternal cells in the fetal circulation could be responsible for the mosaic liver expression of FAH in HTI patients. We used polymorphic markers of short (C-A)n DNA repeats to compare DNA from corrected liver sections of five HTI patients to DNA from their parent's blood. The genotyping results show that one maternal allele is absent in DNA isolated from FAH expressing liver nodules of each proband for at least one marker. Our findings suggest that the corrected liver nodules in HTI patients are not of maternal origin and do not support cell trafficking as mechanism of reversion.

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100-P

TYROSINAEMIA TYPE I – DE NOVO MUTATION IN LIVER TISSUE SUPPRESSING AN INBORN SPLICING DEFECT

Bliksrud YT[1], Brodtkorb E[1], Andresen PA[2],van den Berg IET[3], Kvittingen EA[1]
[1]Institute of Clinical biochemistry/Department of Clinical Chemistry, [2]Department of Pathology, University of Oslo, The National Hospital, Norway, [3]Department of Metabolic and Endocrine Diseases, University Medical Centre Utrecht, The Netherlands

Many patients with tyrosinaemia type 1 have a mosaic pattern of fumarylacetoacetase immunopositive or negative nodules in liver tissue. This phenomenon has been explained by a spontaneous reversion of the mutation in one allele to a normal genotype, but only a few nodules have been examined. We now report a Norwegian patient, compound heterozygous for the mutations IVS12g^{+5}→a and G^{1009}→A, with liver mosaicism, but with an immunopositive nodule in which both primary mutations were intact. In the immunopositive hepatocytes of this nodule genetic analyses showed a new mutation C^{1061}→A 6 bp upstream of the primary mutation IVS12g^{+5}→a in the fumarylacetoacetase gene. The splicing defect caused by the primary mutation is most likely suppressed by the new mutation due to improvement of the splicing site. In the same liver we demonstrate another nodule of regenerating immunopositive tissue due to reversion of one of the primary mutations to a normal genotype. Together with the original cells this makes a triple mosaicism of hepatocytes with 1, 2 or 3 point mutations in the fumarylacetoacetase gene.

101-P

TAT GENE ANALYSIS IN 3 KINDREDS WITH OCULOCUTANEOUS TYROSINEMIA II: IDENTIFICATION OF A NOVEL 5′ EXONIC SPLICE SITE TRANSVERSION CAUSING COMPLETE EXON 11 SKIPPING

SH Korman[1], G Meydan[1], BS Andresen[4], PP Madsen[4], A Raas-Rothschild[2], A Zlotogorski[3], A Gutman[1], M Zeigler[2]

[1]Departments of Clinical Biochemistry, [2]Genetics and [3]Dermatology, Hebrew University–Hadassah Medical School, Jerusalem, Israel; [4]Research Unit for Molecular Medicine, Aarhus University Hospital, Aarhus, Denmark

Deficiency of the hepatic cytosolic enzyme tyrosine aminotransferase (TAT) causes marked hypertyrosinemia leading to painful palmoplantar hyperkeratoses and pseudodendritic keratitis, with variable mental retardation (Richner–Hanhart syndrome). Parents may therefore seek prenatal diagnosis but this can only be performed by *TAT* gene mutation analysis. To this end, we sought the molecular basis of tyrosinemia II in 3 consanguineous Palestinian kindreds. In 2 kindreds with 7 patients, the only potential abnormality identified after sequencing all 12 exons and exon/intron boundaries was homozygosity for a silent, single nucleotide substitution 1224G>T (T408T) at the last base of exon 11. This was predicted to affect the 5′ donor splice site of exon 11 and result in missplicing. However, as TAT is expressed only in liver tissue, patient mRNA was not available for splicing analysis. Transfection experiments were therefore performed using wild type and 1224G>T mutant minigene constructs, demonstrating that 1224G>T results in complete exon 11 skipping. Homozygosity for a 1249C>T (R417X) exon 12 nonsense mutation (previously reported in an Italian patient) was identified in the two patients from the third kindred, enabling successful prenatal diagnosis of an unaffected fetus in chorionic villous tissue from a subsequent pregnancy.

102-P

PRENATAL DIAGNOSIS OF UREA CYCLE DISORDERS BY MOLECULAR GENETIC MEANS

J Häberle, E Schmidt, S Pauli, C Berning, T Rummel, C Werner, Koch HG

Universitätsklinikum, Klinik für Kinder- und Jugendmedizin, Münster, Germany

Aim of the study was to facilitate and standardise the current approaches to prenatal diagnosis in urea cycle disorders by the use of molecular genetics.

Deceased index patients suffering from a urea cycle disorder were investigated for mutations of the biochemically most likely affected gene. Parental DNA was studied for obligate carrier status, if no index patients material was available. Fetal cells of 21 pregnancies, either chorionic villi or amniotic fluid cells, were used for direct sequence analysis of the respective mutations. 15 families were investigated of which two were affected by N-acetylglutamate synthase deficiency, six by carbamylphosphate synthetase 1 deficiency, one by ornithine transcarbamylase deficiency, three by argininosuccinate synthetase deficiency, two by argininosuccinate lyase deficiency, and one by arginase deficiency. Molecular genetic analysis allowed the determination of the fetal status in all cases. Besides 16 known mutations we detected the mutation c.544delC of the N-acetylglutamate synthase gene, the missense mutation c.721G>A (E241K) of the argininosuccinate lyase gene, and the double mutated allele comprising the known mutation c.703G>A (G235R) and the insertion c.712ins[GGACC]$_2$ (254X) of the arginase 1 gene.

Prenatal diagnosis in all urea cycle disorders by the use of direct genetic analysis of either chorionic villi or amniotic fluid cells is feasible, fast, and specific, and can be regarded as the method of choice in the future.

103-P

MUTATION ANALYSIS AND *IN VITRO* EXPRESSION OF THE HUMAN N-ACETYLGLUTAMATE SYNTHASE GENE

E Schmidt, JM Nuoffer[1], J Häberle, S Pauli, B Wermuth[1], HG Koch
Klinik für Kinder- und Jugendmedizin, Münster, Germany, [1]Inselspital Bern, Switzerland

The mitochondrial enzyme N-acetylglutamate synthase (NAGS) is expressed in liver and small intestine producing N-acetylglutamate (NAG), which serves as an activator of CPS1. NAGS activity is enhanced by L-arginine. Autosomal recessively inherited NAGS deficiency (NAGSD) leads to neonatal or late onset hyperammonemia.

Twelve families affected by NAGSD (10 neonatal onset, 2 late onset) were analyzed by PCR and direct DNA sequencing. 5 different missense mutations were functionally analyzed by expression in NAGS deficient *E. coli* NK5992. Moreover, we expressed the wildtype NAGS pre-protein, the mature NAGS protein as well as a highly conserved NAGS core protein.

We identified 12 private mutations: 8 missense, 1 deletion, 1 insertion, 1 nonsense, and 1 splice site. NAGS activity was detected in all three expressed wildtype proteins as well as an increased activity after stimulation by L-arginine. There was no difference in activity levels of mature and core protein. Expression of 5 different missense-mutations showed a strong decrease in enzyme activity. Stimulation by L-arginine was not possible.

In conclusion, we report 12 mutations of the NAGS gene and a prokaryotic and eukaryotic *in vitro* expression system, which allows functionally analyze alterations of the NAGS gene. The mutations expressed here could be verified as disease causing.

104-P

N-ACETYLGLUTAMATE SYNTHASE (NAGS) DEFICIENCY WITH NEONATAL ONSET SUCCESSFULLY TREATED WITH CARBAGLU®

Nordenström A[1], Alm J[1], von Döbeln U[1], Häberle J[2]
Karolinska University Hospital, Stockholm, Sweden; [2]Universitätsklinikum, Münster, Germany

Urea cycle defects are a heterogeneous group of disorders with varying age of onset and severity. Until now only supportive therapy has been available and the outcome is often fatal for the severe forms. Since early 2003 Carbaglu® is registered in Europe for the treatment of NAGS deficiency. We report of a child with severe neonatal hyperammonemia due to NAGS deficiency successfully treated with Carbaglu®

The boy was born full term as the first child to healthy, related parents, bw 3050 g. At $2\frac{1}{2}$ days the child's general condition deteriorated rapidly. He became comatose and had convulsions. Laboratory work up showed a P-NH3 of 926 µmol/L. The boy was transferred to intensive care, required mechanical ventilation and treatment with i.v. sodium-benzoate, arginine and glucose. Carbaglu® 100 mg/kg × day was given orally and hemofiltration was started. Within 2 days P-NH3 was <30 µmol/L. U-orotic acid was normal. At 6 days of age the boy was taken off the ventilator, protein intake was gradually increased and he started to breastfeed.

Genetic analysis showed that the patient was homozygous and the parents both heterozygous for the novel mutation R414P in exon 7 of the NAGS gene. Carbaglu® therapy has been maintained at 50 mg/kg × day with normal P-NH3 levels. At 5 months of age the boy is breastfed without protein restriction and, thus far, has a normal development.

This case emphasizes the importance to include Carbaglu® early in the treatment of all patients with hyperammonemia.

105-P

SUCCESSFUL TREATMENT OF CARBAMOYLPHOSPHATE SYNTHETASE I DEFICIENCY WITH CARBAMOYLGLUTAMATE

Rabier D[1], Touati G[2], Aboulabdeh A[3], Mention K[2], Valayannopoulos V[2], De Lonlay P[2], Saudubray JM[2]

[1]Laboratoire Biochimie, [2]Service Maladies Métaboliques, Hopital Necker, Paris, [3]Service Pédiatrie, Hopital Meaux, France

MA is a 6 year old boy born from consanguineous parents. On day 20 of life he was hospitalized for vomiting, failure to thrive, mild hypotonia and hepatomegaly. At this time biochemical investigations showed hyperammonemia (392 μmol/L; $n<40$), elevated plasma glutamine, alanine and lysine with normal citrulline. Cow milk intolerance was suspected. After three months with an artificial milk, his liver was still enlarged, plasma glutamine, alanine and lysine were also elevated. At 6 months he was investigated for his hyperammonemia: ammonia ranged from 90 to 196 μmol/L when fed 23 g protein/day, from 104 to 191 μmol/L with 15 g protein/day and from 33 to 70 μmol/L with 8 g protein/day. Urinary orotic acid was normal. N-acetylglutamate synthase or carbamoylphosphate synthetase I (CPSI) deficiency was suspected. Enzyme assay on liver biopsy showed a 30% residual activity for CPSI (OTC activity was normal) with an abnormal response to variable concentrations of N-acetylglutamate, suggesting an abnormal affinity of CPSI for its activator. Because of this finding carbamaoylglutamate treatment (Carbaglu®) (175 mg/kg bw/day) was tried while his plasma ammonia was always elevated (73 to 122 μmol/L) despite a protein intake of 12 g/day and a treatment with benzoate (218 mg/kg bw/day). After 24 h of treatment ammonia decreased dramatically (28 to 50 μmol/L) and remained normal afterwards. Plasma glutamine and lysine decreased under the lower limit of normal values.

106-P

OCT PARTIAL DEFICIT: REVEALING CLINICAL SIGNS AND BIOCHEMICAL TOOLS OF DIAGNOSIS

R Garnotel[1], N Bednarek[2], B Leroux[2], P Sabouraud[2], P Gillery[1], P Morville[2]

[1]Paediatric Biochemical Unit, Hôpital Maison Blanche; [2]Paediatric Unit, Hôpital Alix de Champagne, 49 rue Cognacq-Jay, 51092 Reims cedex

Introduction: Partial deficit in ornithine carbamyl transferase (OCT) constitutes a pathology essentially met in the girl with late revealing clinical manifestations. From four observations the various clinical manifestations and the biological tools to lead to the diagnosis are described.

Method: From four observations of patients hospitalised between 2002 and 2004, revealing clinical signs, age of the diagnosis, biologic parameters, and personal and familial past history, sex, evolution and treatment are evaluated.

Results: First symptoms were mainly psychiatric-like. The age of the first manifestations extends from 4 to 16 years. In the past history of patients, school difficulties and migraines were notified. Sex repartition is 3 females and one male. In acute period, the amino acids chromatography exhibited a homocitrulline level reduction and/or a glutamine and alanine level increase. Ammonia levels range between +15 to +400% of the physiological values, the orotic acid value between +20 to +50% where it was measured out in acute phase. The diagnosis was confirmed by the allopurinol test, which showed a strong oroticuria (10 to 20 times physiological concentrations), 6 hours after allopurinol ingestion.

Conclusion: OCT partial deficit must be considered in any acute psychiatric sign, in school delay and past history of headaches in the girl but also in the boy. The allopurinol test is a valuable tool when biological examinations could not be made at the time of the acute phase.

107-A

LATE DIAGNOSIS OF OTC DEFICIENCY: DIFFERENT CLINICAL AND BIOCHEMICAL PHENOTYPES IN TWO RELATED WOMEN

I Redonnet-Vernhet[1], C Marchal[2], C Acquaviva[3], S Mesli[1]
[1]Laboratoire de Biochimie, [2]Service de Neurologie, Hôpital Pellegrin, Bordeaux, France; [3]Service de Biochimie Génétique, Hôpital Robert Debré, Paris, France

Ornithine transcarbamylase (OTC) deficiency, an X-linked disorder, is the most common of the inherited urea cycle defects. OTC deficiency exhibits partial dominant expression in women with 15 to 20% of carrier females becoming symptomatic because of skewed lyonisation. We describe two women in the same family, carrying the same genotype and with different phenotypes. Patient 1, a 44-year-old woman, was hospitalized, presenting with confusion and aggressiveness after a family protein-rich meal. She was treated with valproate and developed a neuropsychiatric aggravation. Since she was eleven she has had a long history of recurrent vomiting, frequent headaches and behavioral changes. Biological diagnosis of OTC deficiency was based on the elevated plasma ammonia and glutamine, decreased citrulline, increased orotate excretion and decreased OTC activity in jejunal biopsy. Patient 2, the daughter of patient 1, 17-year-old, has always been asymptomatic. The different biological tests didn't show any abnormality. In particular, the allopurinol test was normal. Both patients were shown to be heterozygous for the same nucleotide substitution T638A (Met213Lys), with change in a highly conserved amino acid localized in the ornithine binding domain. In conclusion, (1) in OTC heterozygous women, the same mutation can lead to early manifestations in childhood or can be completely asymptomatic, (2) a normal allopurinol test cannot exclude the diagnosis of asymptomatic OTC carrier, (3) the diagnosis of urea cycle defects should be considered in adult patients with unexplained neurobehavioral changes.

108-P

NEUROMORPHOLOGIC AND NEUROMETABOLIC CHANGES IN NEONATAL CITRULLINEMIA

Scholl-Bürgi S, Gotwald T[1], Felber S[1], Baumgartner S, Haberlandt E, Skladal D
University Children's Hospital and [1]Department of Radiology II, Innsbruck, Austria

Introduction: In neonatal citrullinemia citrulline concentrations in various body fluids are massively elevated. **Case report**: Diagnosis of neonatal citrullinemia on 2nd day of life (max. ammonia-concentration 500 µmol/L). On day 25 acute metabolic decompensation with hyperammonemia (800 µmol/L), seizures and somnolence (due to non-compliance to dietary therapy). At age of 3 years performance of MRI and MRS for further diagnostic workup of the cerebral movement disorder. CSF- and plasma-citrulline-concentrations and -ratio were determined and compared with controls (21 patients, age: median: 4.7 years, range: 0.02–17.2 years, CSF-protein-concentration normal). **Results**: MRI on a 1,5 T system showed symmetric parenchymal lesions, involving the putamina, caudate nuclei and pyramidal tract. Using proton MRS (TE 30 ms amd TE 270 ms) there were elevated resonances in the lipid range, but no peak that could specifially be asigned to citrulline. Citrulline-concentrations in plasma 2569 µmol/L (controls (mean $\pm$SD): 34$\pm$9 µmol/L) and CSF 649 µmol/L (c: 2.2$\pm$0.9 µmol/L) were massively elevated, as was CSF/plasma-ratio for citrulline 0.2525 (c: 0.0661$\pm$0.0274). **Discussion**: We demonstrate that besides elevation in plasma and CSF the CSF/plasma-ratio for citrulline is elevated indicating that citrulline may accumulate in CSF. In addition the brain didn't show elevated metabolites that could be asigned to citrulline observed in *in-vivo*-spectroscopy.

109-P

HYPERORNITHINEMIA, HYPERAMMONEMIA, HOMOCITRULLINURIA (HHH) PRESENTING WITH FULMINANT LIVER FAILURE

G Parenti[1], S Fecarotta[1], P Vajro[1], A Zuppaldi[1], D Capalbo[1], M Internicola[1], A Correra[2], MT Carbone[2], G Andria[1]

Departments of Pediatrics, [1]Federico II University and [2]Ospedale SS. Annunziata, Naples, Italy

The HHH syndrome is a rare autosomal recessive disorder of urea cycle, caused by a defect in ornithine transport across mitochondrial inner membrane. The classical presentation of the syndrome is characterized by protein-rich food aversion, vomiting and lethargy. Neurological signs may also include seizures, coma and mental retardation. Moderate liver involvement has been occasionally described. We report a 3-year-old Italian patient, with HHH syndrome, who presented with neurological deterioration after an intercurrent infection. Hyperammonemia (308 μg/dl), coagulopathy (PT 36%) and moderate hypertransaminasemia (AST 79 UI/L, ALT 224 UI/L) were detected on hospital admission. Severe hepatocellular necrosis with hypertransaminasemia (AST 20 000 UI/L, ALT 18 400 UI/L) and coagulopathy (PT $<5\%$) rapidly developed within two days, prompting evaluation for liver transplantation (OLT). The diagnosis of HHH syndrome was based on the presence of hyperornithinemia (852–68 μmol/L), hyperammonemia, homocitrullinuria (34–6.4 μmol/mmol creatinine). Protein-restricted diet and arginine supplementation were immediately started with a rapid improvement of the patient's neurological conditions and normalization of liver function tests and blood ammonia. The diagnosis of HHH syndrome should be considered in patients with fulminant hepatitis-like presentations. Early identification and treatment of these patients can be life-saving and avoid OLT.

110-P

HYPERLYSINEMIA TYPE I: EFFECTS ON UREA-CYCLE AND METHYLATION REACTIONS

RJ Slingerland[1,2], JPN Ruiter[2], N Blau[3], MR Baumgartner[3], T Coşkun[4], BT Poll-The[2], RJA Wanders[2], M Duran[2]

[1]Department of Clinical Chemistry, Isala Klinieken, Zwolle, The Netherlands, [2]Academic Medical Center, Departments of Pediatrics/Emma Children's Hospital and Clinical Chemistry, Amsterdam, The Netherlands, [3]Kinderspital University of Zurich, Switzerland, [4]Hacettepe University, Ankara, Turkey

Hyperlysinemia type 1 results from mutations in the alpha-aminoadipic semialdehyde synthase (AASS) gene, usually affecting both the lysine: 2-oxoglutarate reductase and the saccharopine dehydrogenase activities. We diagnosed four patients from three consanguineous families. All patients have psychomotor retardation; hypotonia (3/4) and convulsions (3/4). The two patients having neonatal seizures also had microcephaly, plasma lysine varied from 602–2029 μmol/L (controls: <180), whereas ornithine was low (10–20 μmol/L) and arginine was normal. Both enzyme activities of the AASS-synthase reactions were found to be defective. Urinary aminoacids showed increased concentrations of homocitrulline and homoarginine, products of urea-cycle enzyme mediated reactions. We found no evidence of homoargininosuccinic acid.

Elevated lysine inhibits arginase; together with a low ornithine this may result in increased production of guanidinoacetic acid. This was confirmed by urine analysis. The secondary effects of hyperlysinemia type I on methylation reactions may be responsible for the clinical symptoms. We consider this condition to be severe and propose to include hyperlysinemia type 1 in neonatal screening programs.

111-O

COGNITIVE OUTCOME IN MAPLE SYRUP URINE DISEASES (MSUD)

B Hoffmann, C Helbling, U Wendel
Children's University Hospital, Heinrich-Heine-University, Düsseldorf, Germany

Background: MSUD is an inborn error of metabolism affecting the catabolism of branched chain amino acids (BCAA). Patients with classic MSUD develop severe encephalopathy and coma within the first 10 days of life due to very high concentrations of the leucine/2-oxoisocaproic acid pair. Rapid lowering of the toxic substances and lifelong maintaining of plasma BCAA at near to normal levels aim to minimize/prevent brain dysfunction. We evaluated the cognitive outcome of patients with classic MSUD related to their longitudinal plasma leucine concentrations. **Methods**: 24 patients with classic MSUD retrospectively were evaluated regarding plasma leucine levels and IQ. Median plasma leucine levels were calculated from cumulative data over six years for each patient individually. Aged six years, IQ-measurements were performed. **Results**: Using median plasma leucine levels over six years as criteria, patients were assorted to either one of three groups by cluster analysis. Cluster I (n = 8) showed median plasma leucine levels not exceeding 1.5-fold of normal. Cluster II (n = 13) showed moderately elevated levels (up to 3-fold of normal), whereas plasma leucine levels of cluster III (n = 3) reached values above 4.5-fold of normal. IQ-scores at the age of six years significantly differed between the three clusters ($p<0.05$; χ^2-test). Patients in cluster I had a median IQ of 102 (86–108) whereas patients in cluster II had a median IQ of 76 (57–99). Patients in cluster III showed a median IQ of 57 (50–64). **Conclusions**: Continuous exposure to elevated plasma leucine concentrations has a major impact on cognitive capacity of patients with MSUD. Furthermore, our data suggest a direct relation between the duration as well as the extent of elevated plasma leucine levels in the neonatal period and long term cognitive outcome represented by IQ-levels. Thus, rapid lowering of plasma leucine and longitudinally maintaining at near to normal levels may prevent loss of cognitive capacity.

112-A

MANAGEMENT OF A CHILD WITH MSUD BY PERCUTANEOUS ENDOSCOPIC GASTROSTOMY. A TWO YEAR FOLLOW-UP

Bzdúch V, Fabriciova K, Bibza J[1], Behúlová D[2]
First Department of Pediatrics, Department of Pediatric Surgery[1] and Clinical Biochemistry[2], Comenius University Children's Hospital, Bratislava, Slovakia

Long-term metabolic control have an important influence on the neurological outcome in children with MSUD. We used percutaneous endoscopic gastrostomy (PEG) in a girl with neonatal MSUD as a radical solve of her metabolic problems, caused by inadequate energetic and nutritional intake. First problems with refusal of feed and metabolic decompensation started at the age of 5 months during intercurrent infections (otitis media, gastroenteritis) and dentition, treated by increased parenteral intake of energy and nasogastric tube feeding. From 10 months of life, problems with with refusal of feed and continuous parenteral feeding required establishment of central venous catheter. Intolerance of nasogastric tube feeding and septic complication of central venous catheter increased our decision for aggressive management with PEG. Placement was without complication. After education of mother in preparing of suited food to gastrostomy, child was two days after insertion discharged from hospital. During the next two years, rate of hospitalizations significantly decreased and energetic deficit during mild infections was compensated by nocturnal feeding to PEG by enteral pump. In our opinion this is the first described practical use of PEG in a MSUD child. Our experience showed, that this relative new method is an easy and safe procedure, which can improve the quality of life of these patients, mainly during the first few years, when inadequate oral food intake frequently occur.

113-P

MAPLE SYRUP URINE DISEASE – ADOLESCENT GROWTH SPURT

ER Naughten[1], A Clark[1], M Kellett[1], A O'Shea[2], PD Mayne[2]
[1]*The National Centre of Inherited Metabolic Disorders and* [2]*Department of Clinical Chemistry, Children's University Hospital, Temple Street, Dublin, Ireland*

Thirteen cases of classical maple syrup urine disease (MSUD, McKusick 248600) have been diagnosed by newborn screening from 8 unrelated kinships in Ireland since 1972 (incidence 1 in 125 000). One additional case was not detected by screening. Twelve are alive and well (median age 17 yrs, range 3–26.5); one has a mild hemiplegia, 2 are clumsy and 2 have mild speech impairment. Routine care consists of weekly measurement of plasma leucine levels with adjustment of diet by telephone, aiming to maintain plasma leucine levels below 400 μmol/L. The growth spurt over short periods of time in two healthy adolescent boys with MSUD is presented.

Case no. 1 was a 13 year old boy who normally required about 6 g of natural protein per day to maintain plasma leucine levels below 400 μmol/L when not ill. Over a 4 week period during which his weight increased by 6.1 kg and height by 2.3 cm, natural protein intake was increased by 22 g per day and synthetic protein intake by 9.5 g per day as the plasma leucine level kept falling.

Case no. 2 was a 15 year old boy whose natural protein intake was increased by 39 g per day over a 3 week period during which time his body weight increased by 7 kg and height by one cm. Synthetic protein was increased by 28 g per day to 142 g over the same period for similar reasons.

These two cases highlight the requirement for frequent biochemical monitoring prior to and during the onset of the adolescent growth spurt in order to optimise growth and prevent decompensation in patients with classical MSUD.

114-P

LIVER TRANSPLANTATION IN MAPLE SYRUP URINE DISEASE (MSUD): SUCCESSFUL OUTCOME IN 6 PATIENTS

A Bélanger-Quintana[1], MJ García[2], C Diaz[3], L Hierro[3], P Sanz[2], C Camarena[3], A de la Vega[3], E Frauca[3], M Ugarte[2], P Jara[3], M Martinez-Pardo[1]
[1]*Unidad de Enfermedades Metabólicas, Servicio de Pediatria, Hospital Ramón y Cajal;* [2]*Centro de Diagnostico de Enfermedades Moleculares (CEDEM), UAM-Cantoblanco;* [3]*Servicio de Hepatología Pediátrica, Hospital Infantil de La Paz, Madrid, Spain*

We present the successful clinical, biochemical and nutritional evolution of 6 patients with the severe phenotype of MSUD, who after a diet very restrictive in branched-chain aminoacids since diagnosis, underwent liver trasplantation between 12 and 27 months of age. Follow-up has been from 2 to 10 years. Post liver transplantation and **on a normal diet**, these patients present:

1. Plasma levels (media ± SD in mol/L): leucine dropped from 703 ± 476 to 207 ± 34 and isoleucine from 261 ± 127 to 127 ± 45; valine raised from 178 ± 127 to 349 ± 45; alloisoleucine fell from 261 ± 127 to undetectable levels, and only has been transiently detected (maximum 21 μmol/L) in a patient with hepatic damage (rejection with activation E. Barr hepatitis)

2. The neurological development of these patients has been satisfactory and improving in the motor, social and intellectual areas. Their cerebral MRI, which had white matter abnormalities, have been normalized. Two of them had an episode of seizures secondary to cyclosporine levels.

3. Graft complications: a patient needed a second liver transplantation because of hepatic artery problems; three of them had viral hepatitis (E. Barr and CMV); one patient had a transitory lymphoprolyferative disorder.

4. The quality of life of patients and their families has improved in spite of complications.

115-P

INITIAL TREATMENT OF CLASSICAL MAPLE SYRUP URINE DISEASE WITHOUT EXTRACORPORAL DETOXIFICATION

B Schwahn[1], I Marquardt[2], K Bagner[1], M Grotzke[1], U Wendel[1]
[1]*Clinic for General Pediatrics, University Children's Hospital Düsseldorf,* [2]*Elisabeth-Hospital, Oldenburg, Germany*

Extracorporal detoxification procedures usually are advised for the reversal of acute metabolic decompensation of symptomatic newborns with the classical type of maple syrup urine disease (MSUD). We identified two patients with classical MSUD by expanded newborn screening. Due to early diagnosis and availability of adequate formula, devoid of branched chain amino acids (BCAA), we were able to manage their initial metabolic decompensation by conservative means. Treatment of both patients started at day 6 of life. Both showed somnolence and inappetence, but no further encephalopathic symptoms. Patient 1 had BCAA plasma concentrations [μmol/L] of leucine 2191, valine 803, isoleucine 580; patient 2 of leucine 1748, valine 508, isoleucine 257. Patients were tube-fed with BCAA-free formula supplemented with maltodextrine and, later, L-valine and L-isoleucine. Patient 1 was additionally treated with insulin and glucose intravenously. After three days BCAA plasma concentrations had normalized and L-leucine was introduced into the diet by natural proteins. We conclude that the oral intake of BCAA-free formula with adequate calorie supply and supplementation with L-valine and L-isoleucine is sufficient to stimulate protein synthesis and to clear the body from accumulating BCAA. Newborn screening for MSUD allows for early intervention and seems to obviate the need for extracorporal detoxification. We propose a dietary treatment protocol for the acute management of neonatal decompensation in MSUD.

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116-P

COMPLETE REVERSAL OF DYSMYELINATION IN A PATIENT WITH MAPLE SYRUP URINE DISEASE FOLLOWING IMPROVEMENT OF BIOCHEMICAL CONTROL

E Simon, S Schoenberger, B Schwahn, U Wendel
Children's Hospital, Heinrich-Heine University, D-40225 Duesseldorf, Germany

Background: Morphological alterations with signs of dysmyelination in MRI of the brain have been previously described in adolescent MSUD patients as a consequence of chronically elevated concentrations of branched-chain amino acids (BCAA) in plasma. We recently demonstrated a correlation between the extent of brain abnormalities and the median BCAA concentrations over 6-36 months prior to the MRI scans. So far it is not known whether the structural defects of myelin in MSUD are permanent or reversible with stricter metabolic control.

Methods: A Turkish girl suffering from classic MSUD underwent cerebral MRI at the age of 9 and 12 years. Median BCAA plasma concentrations were calculated for periods of 36, 24, 12 and 6 months prior to each MRI examination.

Results: At the age of 9 years the MRI showed extensive areas of increased signal in T2-weighted images, especially in deep white matter of the cerebral hemispheres and the basal ganglia. The median plasma total BCAA concentrations during the 36 to 6-months periods prior to the MRI scan were 1313, 1590, 1800, 1824 μmol/L respectively. 3 years later the BCAA concentrations had decreased due to stricter metabolic control (median plasma BCAA concentrations were 1082, 890, 870, 744 μmol/L, respectively) while the MRI scan at this time showed no signs of dysmyelination.

Conclusion: This case demonstrates the possibility of a complete resolution of cerebral MRI alterations in MSUD after long term improvement of metabolic control.

117-P

MRI IN ACUTE INTERMITTENT MAPLE SYRUP URINE DISEASE

Di Rocco M, Biancheri R[1], Rossi A[2], Allegri AEM, Vecchi V[3], Tortori-Donati P[2]
Second Unit of Pediatrics, Istituto G. Gaslini, Genoa, [1]Neuromuscular Disease Unit, University of Genoa, Istituto G. Gaslini, Genoa, [2]Department of Pediatric Neuroradiology, Istituto G. Gaslini, Genoa, [3]Pediatric Unit Hospital of Rimini, Italy

Maple syrup urine disease (MSUD) is characterized by reversible brain edema during acute metabolic decompensation. MR diffusion-weighted imaging (DWI) is an useful technique for assessing the tissue water diffusivity, differentiating between vasogenic and cytotoxic edema.

An 18-month-old girl presented with a three-day lasting episode of ataxia and lethargy. Following spontaneous recovery, neurological examination, MRI, aminoacids and organic acids were normal. Five years later, during the second episode of acute decompensation, MRI showed mild hyperintensity of the medulla and dentate nuclei and DWI demonstrated high signal in the same areas. Branched chain ketoaciduria and increased plasma branched chain aminoacids were depicted, allowing the diagnosis of MSUD. After recovery clinical, neuroradiological and biochemical examinations became normal again.

Between attacks, patients with acute intermittent MSUD are entirely normal. High signal abnormalities shown by DWI during acute episode are related to cytotoxic or intramyelinic edema due to impaired energy production secondary to branched chain aminoacids accumulation. These changes are quite specific and must lead to promptly perform biochemical investigations that are diagnostic only analyzing biological material collected during acute metabolic decompensation.

118-A

EFFECT OF THE BRANCHED CHAIN ALPHA-KETO ACIDS ACCUMULATING IN MAPLE SYRUP URINE DISEASE ON GLUTAMATE UPTAKE INTO SLICES OF CEREBRAL CORTEX OF RATS

Funchal C, Rosa AM, Wofchuk S, Wajner M, Pessoa-Pureur R
Universidade Federal do Rio Grande do Sul, Instituto de Ciências Básicas da Saúde, Departamento de Bioquímica, Porto Alegre, RS, Brasil

Severe neurological symptoms, cerebral edema and atrophy are common features of the inherited metabolic disorder maple syrup urine disease (MSUD). However, the pathomechanisms involved in the neuropathology of this disease are not well established. In the present study we investigated the effect of the branched chain alpha-keto acids (BCKA) α-ketoisocaproic (KIC), α-keto-β-methylvaleric (KMV) and α-ketoisovaleric (KIV) acids on the in vitro uptake of [^{3}H]glutamate by cerebral cortical slices from rats aged 9, 21 and 60 days of life. We initially observed that KIC inhibited this uptake by tissue slices at all ages studied, whereas KMV and KIV produced the same effect only in cortical slices of 21- and day-old rats. Kinetic assays showed that KIC significantly inhibited glutamate uptake in the presence of high glutamate concentrations (50 mmol/L and greater). We also verified that the reduction of glutamate uptake was not due to cellular death, as evidenced by tetrazolium salt and lactate dehydrogenase viability tests of cortical slices in the presence of the BCKA. It is therefore presumed that the reduced glutamate uptake caused by the BCKA accumulating in MSUD may lead to her extracellular glutamate levels and potentially to excitotoxicity, which may contribute to the neurological dysfunction of MSUD affected individuals.

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118a-A

BRANCHED CHAIN ALPHA-KETO ACIDS ACCUMULATING IN MAPLE SYRUP URINE DISEASE INDUCE MORPHOLOGICAL ALTERATIONS AND CYTOSKELETON REORGANIZATION IN ASTROCYTES

Funchal C, Gottfried C, Almeida LMV, Santos AQ, Wajner M, Pessoa-Pureur R
Universidade Federal do Rio Grande do Sul, Instituto de Ciências Básicas da Saúde, Departamento de Bioquímica, Porto Alegre, RS, Brasil

In this study we investigated the effects of the branched chain keto acids (BCKA) α-ketoisocaproic (KIC), α-ketoisovaleric (KIV) and α-ketomethylvaleric (KMV), which accumulate in maple syrup urine disease (MSUD), on astrocyte morphology and cytoskeleton reorganization. Cultured astrocytes from cerebral cortex of neonatal rats were exposed to various concentrations of the BCKA and cell morphology was studied. We observed that these cells changed their usual polygonal morphology when exposed to BCKA, leading to the appearance of fusiform or process-bearing cells. Furthermore, longer exposures to the BCKA elicited cell death at all concentrations studied, attaining massive death at the highest concentrations. Immunocytochemistry with anti-actin or anti-GFAP antibodies revealed that the BCKA induced reorganization of actin and GFAP cytoskeleton. In addition, creatine kinase activity was inhibited by KIC and KIV and this inhibition was prevented by creatine, indicating that these keto acids compromise brain energy metabolism. Considering that astroglial cells are critical to brain development and functioning, it is conceivable that alterations of the actin network by BCKA may have important implications in astrocytic function and possibly in the pathogenesis of the neurological dysfunction and brain damage of MSUD patients.

119-P

MOLECULAR ANALYSIS OF MSUD IN SPAIN

P Rodríguez-Pombo, R Navarrete, B Merinero, C Pérez-Cerdá, M Ugarte
Centro de Biología Molecular SO, Universidad Autónoma de Madrid, Spain

The aim of this work has been to perform the molecular analysis of 19 fibroblast cell strains from maple syrup urine disease (MSUD) Spanish patients, with diverse clinical and biochemical phenotypes. Mutations in the genes which encoded for the catalytic components unique for the branched-chain α-keto-acid dehydrogenase complex: E1α (BCKDHA), E1β (BCKDHB), or E2 (DBT) result in MSUD referred as molecular phenotype Ia, Ib and II, respectively. To identify the gene locus for mutant alleles, all cell lines were tested by somatic cell complementation using E1α, E1β and E2 tester cells. Dihydrolipoamide dehydrogenase (E3) activity was determined to discard the MSUD molecular phenotype III. The frequency found for the affected gene was 50% for E1β, 21% for E1α and 26% for E2. Up to now, we have characterized the 75% of the alleles in study, with 21 different alterations identified, 13 of which are new. The novel mutations were A171V, R252C, D302A, R346H, R314X and c.117delC in E1α, R168H, G172W and c.348delA in E1β, and G132R, A450D, R462P and c.754_760del in E2. Only the previously described changes, E1β-c.595_596delAG and the B1-responsive related E2-F276C appear on 4/18 and 3/10 characterized alleles, respectively. The pathogenic effect of the identified mutations is supported by the impact on the crystal structure of the E1 protein and because most of these allelic variants are located in residues and even in regions highly conserved of the corresponding proteins. This work represents the largest study to date on the genotype for Spanish MSUD patients. The diversity of the molecular spectrum characterized strengthens the clinical complexity of the disease.

120-P

ADVERSE EFFECTS OF L-SERINE THERAPY AND SUBSEQUENT WEST SYNDROME ON LOWERING THE DOSE IN A PATIENT WITH 3-PHOSPHOGLYCERATE DEHYDROGENASE DEFICIENCY

TJ de Koning[1], KPJ Braun[1], T Veen[2], G Visser[1], M de Sain[1], IET van den Berg[1], L Dorland[1], LWJ Klomp[1], R Berger[1]
University Children's Hospital Utrecht[1] and MESOS Medical Center Utrecht[2], The Netherlands

3-Phosphoglycerate dehydrogenase deficiency was diagnosed in a two months old boy who presented with intrauterine growth retardation, congenital microcephaly and hypertonia. CSF serine was 5 and 7 μmol/L (normal 52 ± 12) and 3-PGDH activity in fibroblasts 13 mol/min/mg prot (day control 74). He was treated with L-serine orally 500 mg/kg/day in 6 doses. One week later symptoms occurred of malaise, vomiting, nystagmus, acoustic startles and myoclonias in upper and lower extremities. EEG showed abnormalities compatible with cortical myoclonias. Although L-serine in CSF was 26 μmol/L (low normal range) with glycine being 8 μmol/L (normal 7 ± 2), we decided to lower the dose of L-serine to 400 mg/kg/day. Upon lowering the dose the symptoms disappeared with the myoclonias persisting for some time. During L-serine therapy he showed a catch-up in growth and head circumference. However, at 7 months his head circumference had stopped growing, flexion spasms occurred and his EEG showed hypsarrhythmia. CSF serine and glycine were below the normal range (serine 21 μmol/L, glycine 2 μmol/L). Despite increasing L-serine to 600 mg/kg/day seizures persisted and vigabatrine was added. Seizures and hypsarrhythmia hypsaritmia stopped after two weeks. During follow-up his psychomotor development is poor. This is the first report of adverse events of L-serine on the recommended dose, but also clearly demonstrates that 400 mg/kg/day resulting in (low) normal CSF serine might not be enough to prevent seizures.

121-P

RAPIDLY PROGRESSIVE INFANTILE LEUKOENCEPHALOPATHY ASSOCIATED WITH NONKETOTIC HYPERGLYCINEMIA AND PULMONARY HYPERTENSION

Del Toro M, Macaya A, Moreno, Raspall M, Vazquez E, Ortega A, Arranz A, Riudor E, Roig M
Hospital Vall d'Hebron, Barcelona, Spain

In 1992 we described three siblings with a similar phenotype: (a) onset in the first months of life; (b) pulmonary hypertension; (c) progressive neurological deterioration with hypotonia, pyramidal signs and loss of cognitive functions; (d) elevated glycine in blood, urine and CSF and (e) death before the age of 2 years. In 2002 we identified two unrelated patients with the same phenotype and we reported the bioquemichal data. We wish to report in detail their clinical and neuroradiological phenotype as well as the neuropathological findings of one of them.

Both patients were followed for primary pulmonary hypertension since the first months of life. They presented a subacute neurological deterioration following vaccination, without seizures o EEG abnormalities. Cranial MRI showed bilateral cystic white matter leukodystrophy with thalamic involvement, extending from occipital to frontal lobes. MRS detected the presence of glycine in CNS. Glycine was elevated in plasma, urine and CSF samples. Absent glycine cleavage enzyme and protein P activities were found in hepatic tissue. Neuropathological findings depicted a cavitated leukoclastic encephalopathy involving both cerebral hemispheres with preservation of the U fibres and vacuolated demyelination involving corpus callosum, cerebellar peduncles, medial lemniscus and pyramidal tracts. No involvement of cortical grey matter was detected.

We believe that all these patients belong to a new phenotype of glycine encephalopathy with some similarities with the severe form of 'vanishing white matter' disease.

122-P

ATYPICAL NON-KETOTIC HYPERGLYCINEMIA: 3 CASES WITH GLDC MUTATIONS

A Dinopoulos[1], S Kure[2], G Chuck[1], A Ichinohe[2], K Kojima[2], DL Gilbert[1], Y Matsubara[2], T DeGrauw[1]
[1]CCHMC-Neurology Cincinnati, [2]Tohoku University, Medical Genetics, Japan

We report three unrelated adult with mental retardation and atypical NKH. They presented with hypotonia and global developmental delay during infancy and behavioral problems (outbursts of aggressiveness and intractable ADHD) during childhood and adolescence. The diagnosis was based initially on increased glycine levels in serum and CSF and increased CSF/plasma glycine ratio. Two were homozygous for missense mutation (A389V) and the third was homozygous for mutation (R739H) of the human P protein gene (GLDC). Both mutations reduce the glycine decarboxylase activity to 6–8% of normal (wild type) when expressed in COS7 cells. A total of 200 control subjects were screened negative for the presence of A389V or R739H mutations. A review of the literature of atypical NKH cases revealed two forms: 'infantile' and 'later onset.' Mental retardation and behavioral abnormalities are prevalent in both forms although the phenotype in later onset form is more heterogeneous. Patients with the 'infantile' form tend to have higher CSF/Plasma glycine ratio. The hyperglycinemia in the 'infantile' form, similar to the 'classic' one, is caused by a defective Glycine Cleavage System (GCS), whereas the cause of hyperglycinemia in 'later onset' is unknown. The above mutations are uncommon in the general population, but the prevalence in populations with mild, non-specific mental retardation and behavior problems is unknown. Thus, the true incidence of atypical NKH is unknown. It is likely underdiagnosed. Since all patients have increased serum and urine glycine, screening patients with mild mental retardation and particularly with behavioral problems, may be useful.

123-P

PERSISTENT XANTHURENIC ACIDURIA PROBABLY DUE TO A KYNURENINASE DEFICIENCY IN A HEALTHY SOMALIAN BOY

M Christensen, M Dunø, A Lund, E Christensen, F Skovby
Department of Clinical Genetics, Rigshospitalet, Copenhagen, Denmark

Xanthurenic aciduria is a rare disorder in the pathway of tryptophan metabolism possibly due to deficient activity of the pyridoxal-5′-phosphate dependent enzyme, kynureninase. The enzyme is ubiquitous but mostly expressed in the liver, placenta and lung. Kynureninase catalyzes the conversion of kynurenine and 3-hydroxykynurenine to anthranillic and 3-hydroxyanthranillic acids, respectively, the later being essential for biosynthesis of nicotinic acid. We report here a case where a healthy Somalian boy, excretes abnormal quantities of 3-hydroxykynurenine, xanthurenic and kynurenic acids but no detectable anthranilline or 3-hydroxyanthranilline, suggesting a kynureninase deficiency. Massive excretion of xanthurenic acid was detected incidentally during analysis of urinary organic acids. Elevated concentrations of xanthurenic acid has been detected in all urine samples analysed so far with concentrations ranging from 0.1 to 0.5 mmol/mmol creatinine and increasing to 1 mmol/mmol creatinine during a tryptophan loading test. The boy is now 5 years old and presently without symptoms suggesting that the biochemical aberration may be without clinical significance.

Up to now no pathogenic mutations have been reported in the few patients with presumed kynureninase deficiency. The gene for kynureninase is localised to chromosome 2q22 and consists of 14 exons. We have synthesized cDNA from normal individuals and the patient and data from this work will be presented.

124-A

INHIBITION OF PYRUVATE KINASE ACTIVITY BY CYSTINE IN BRAIN CORTEX OF RATS

CMD Wannmacher, LR Feksa, A Cornelio, J Giacomazzi, D Parissoto, CS Dutra-Filho, ATS Wyse, M Wajner
Departamento de Bioquímica, ICBS, UFRGS, Porto Alegre, RS, Brazil

Cystinosis is a metabolic disturb associated with excessive lysosomal cystine accumulation secondary to defective cystine efflux. Patients affected by this disease develop a variable degree of symptoms depending on the involved tissues. Accumulation of cystine in the brain may lead to severe neurological symptoms. However, the mechanisms by which cystine is neurotoxic are not fully understood. Considering that pyruvate kinase (PK) is a thiolic enzyme crucial for the glycolytic pathway, and disulfides like cystine may alter thiolic enzymes by thiol/disulfide exchange, the main objective of the present study was to investigate the effect of cystine on PK activity in the brain cortex of developing Wistar rats. We performed kinetic studies and investigated the effects of GSH, a biologically-occurring thiol groups protector, and cysteamine, the drug used for cystinosis treatment, on the enzyme activity. Cystine inhibited the enzyme activity by two different mechanisms, one through the competition with ADP and phosphoenolpyruvate, and the other non-competitively, probably through oxidation of the thiol groups of PK. We also observed that GSH and cysteamine fully prevented and reversed the inhibition caused by cystine.

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125-P

CYSTINE INHIBITS THE *IN VITRO* CREATINE KINASE ACTIVITY IN RAT KIDNEY PROBABLY BY OXIDATION OF THIOL GROUPS

VC Rech, GA Athaydes, P Dornelles, V Rodrigues Jr, C Gantus, A Stefanello, CS Dutra-Filho, ATS Wyse, M Wajner, CMD Wannmacher
Departamento de Bioquímica, ICBS, UFRGS, Porto Alegre, RS, Brazil

Cystinosis, a systemic lysosomal storage disorder caused by deficient cystinosin, usually leads to death by renal failure at puberty, unless treated with cysteamine in the first 2 years of age. The generalized renal tubular damage (Fanconi syndrome), the most prominent feature, suggests an alteration in energy metabolism. Rats loaded with dimethylcystine develop Fanconi syndrome, but mice with mutated cystinosin do not. Considering that creatine kinase (CK) is a thiol-enzyme crucial for energy homeostasis, and that cystine might act on thiol groups, the present study was undertaken to investigate the *in vitro* effect of cystine on CK activity in the kidney of developing Wistar rats, as well as the effects of reduced glutathione (GSH), a thiol group protector, and cysteamine, on cytosolic (Cy-CK) and mitochondrial (Mi-CK) enzyme activities. Kinetic studies were performed according to Lineweaver-Burk. Cystine inhibited CK activity noncompetitively, probably by oxidation of thiol groups of the enzyme, and this inhibition was partially prevented and reversed by GSH or cysteamine, suggesting that inhibition of CK may be one of the mechanisms by which cystine is toxic to the kidney.

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126-A

IN VITRO EFFECT OF METHIONINE ON SOME PARAMETERS OF OXIDATIVE STRESS IN RAT HIPPOCAMPUS

Wyse ATS, Stefanello FM, Kurek AG, Dutra-Filho CS, Wannmacher CMD, Wajner M
Department of Biochemistry, ICBS, UFRGS, Porto Alegre, RS, Brazil

Hypermethioninemia is a rare metabolic disorder caused by deficiency of methionine adenosyltransferase resulting in tissue accumulation of methionine. Affected patients present cognitive deficit, cerebral edema and demyelination, whose underlying mechanisms are poorly known. Oxidat stress seems to play an important role in the pathogenesis of various neurodegenerative disorders, including Alzheimer's disease. Therefore, in the present work we investigated the *in vitro* effect of methionine, at concentrations ranging from 0.2–5.0 mmol/L, on some parameters of oxidative stress (thiobarbituric acid reactive substances (TBARS) and total radical-trapping antioxidant potential (TRAP)) in hippocampus of young rats. We verified that methionine (3.5–5 mmol/L) significantly increased TBARS and decreased TRAP, suggesting that this amino acid induces lipid peroxidation and decreases the non-enzymatic antioxidant defenses in the brain. Therefore we presume that oxidative stress may contribute to the neurological dysfunction occurring in hypermethioninemia.

Financial Support: CNPq, CAPES, PRONEX II, PROPESQ/UFRGS

127-A

ORGANIC ACID ANALYSIS IN THAI INFANTS WITH IEM USING GAS-LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY (GC/MS)

P Wasant, S Liammongkolkul, A Sathienkijakanchai, P Ratanarak
Division of Medical Genetics, Department of Pediatrics, Siriraj Hospital Faculty of Medicine, Mahidol University, Bangkok 10700, Thailand

Disorders of organic acid (OA) metabolism are usually diagnosed by qualitative analysis of organic acids by gas chromatography/mass spectrometry (GC/MS) which was well established in developed countries since 1980s. Clinically patients with organic acid disorders, typically neonates and young infants, presented with lethargy, vomiting, respiratory distress/tachypnea, seizures/coma, hypo/hypertonia, poor feedings, growth failure and delayed development/mental retardation. Biochemical/hematologic abnormalities include metabolic acidosis, hyperammonemia and anemia/pancytopenia. Confirmation of the diagnosis of OA usually is a difficult task in developing countries. Thus normal growth/development, survival into good health is possible if prompt, reliable detection and therapy were available and provided. We have successfully established confirmatory laboratory diagnosis for OA in Thailand in 2001.

Collaboration with Japanese scientists and the donation of GC/MS instrument via JICA (Japan Intergovernmental Cooperation Agency) led to the establishment of such method and training of Thai scientists at Siriraj Hospital in March 2001. During the past 3 years, we have analysed 442 urine samples in 365 patients and identify 23 new cases. These are 1 case of PKU, 7 cases of MSUD, 1 case of IVA, 3 cases of PA, 2 cases of MMA, 1 case of beta ketothiolase deficiency, 4 cases of UCD (ALD, OTC), 1 case of D-glyceric aciduria, 1 case of Glutaric aciduria type 1, 1 case of Fructose intolerance, 1 case of tyrosinemia and 1 case of pyroglutamic acidaemia, 1 case of galactosemia, 1 case of fumarase deficiency, 1 case of multiple carboxylase deficiency, 1 case of glutaric aciduria type II, 1 cases of carnitine palmitoyl transferase I, 1 case of of nonketotic hyperglycinemia. The establishment of GC/MS technology has led to prompt and proper treatment and certainly better outcome in our patients.

128-P

LONG-TERM FOLLOW-UP OF PATIENTS WITH ORGANIC ACIDURIAS. INTEREST OF THE MRI RELATED TO LONG-TERM NEUROLOGICAL PROGNOSIS IN 56 CHILDREN

N Bednarek, G Touati, L Hertz-Pannier, I Desguerre, V Barbier, JM Saudubray
Hôpital Necker-Enfants Malades, Paris et American Memorial Hospital, Reims, France

Aim: To study the neurological evolution of 56 children affected with organic acidurias (OA) (IVA: 15, PA: 18, MMA: 23) born between 1984 and 2002, and to correlate the MRI features according to the type and severity of the metabolic disorder.

Method: Factors such as age at revelation of the disease, severity of the initial coma, time to diagnosis, number of metabolic relapses were studied. The MRI features were analysed with description of the lesions topography. The imaging was correlated with the neurological outcome.

Results: The majority of the children presenting IVA or MMA are well (half of them have a normal outcome). Most children with PA follow an adapted schooling. The forms with severe handicap are equivalent in the 3 groups representing less than 20% of the studied population. Patients with neonatal or late-onset revelation have the same evolution. The time to diagnosis is longer in the group with unfavourable evolution. The MRI abnormalities correlated well with the outcome: normal children have a normal MRI and disabled children have an abnormal MRI. Basal ganglia lesions and cortical atrophy are found in the unfavourable evolution group. A vermian atrophy was found in the group of children carrying moderate disorders of the schooling. Specific hippocampic anomalies are described in the group of children with severe school disorders.

Conclusion: Long-term neurologic prognosis of OAs has clearly improved compared to the reported observations dating from the 1980s. The cerebral MRI is well correlated with the outcome and this study shows specific and frequent hippocampic and vermian anomalies not yet described.

129-P

EEG-ALTERATONS IN PATIENTS WITH PROPIONIC ACIDEMIA

[1]Haberlandt E, [3]Trinka E, [1]Baumgartner S, [1]Konstantopoulou V, [1]Scholl-Bürgi S, [2]Felber S, [1]Zimmerhackl LB, [1]Skladal D
Departments of [1]Pediatrics, [2]Radiology II, [3]Neurology, University of Innsbruck, Austria

Introduction: Propionic acidaemia is a congenital disorder resulting from a defect of propionyl-CoA-carboxylase. There are no data on frequency, type and possible reasons for EEG-alterations in these patients.

Methods: We report nine patients with propionic acidaemia and corresponding EEG findings.

Results: We found diffuse or focal slowing with or without epileptiform abnormalities. In 7 of 9 patients focal and generalized epileptiform activities were found. In addition, 3 patients showed a striking photosensitivity. Four patients suffered from repeated seizures, three of them were treated with antiepileptic drugs. We observerd a varied seizure pattern: myoclonic seizures, absences und tonic-clonic seizures. In addition, we found non-specific EEG-alterations. During metabolic derangement all alterations were accentuated. Two patients had normal EEG-recordings.

Conclusion: Electrophysiologic changes may be caused by one metabolite or a combination of metabolites present in propionic acidaemia and give raise to non-specific derangement of cerebral activity, as well as for epileptiform changes with and without cerebral seizures in these patients.

130-P

EARLY PRENATAL DIAGNOSIS OF Mut^0 MMA BY MOLECULAR AND PROPIONYLCARNITINE AND METHYLMALONIC ACID ANALYSIS

C Cavicchi, S Funghini, G la Marca, S Malvagia, G Poggi, E Pasquini, MA Donati, A Morrone, E Zammarchi

Metabolic and Neuromuscular Unit, Department of Paediatrics, University of Florence, Florence, Italy

Methylmalonyl-CoA mutase (MCM) deficiency is an autosomal recessive inborn error of metabolism, leading to methylmalonic aciduria (MMA). We report the first molecular prenatal diagnosis in an Italian family with a proband affected by neonatal mut^0 MMA. Molecular analysis, performed on cDNA and genomic DNA of the proband, identified the new genetic lesion c643G>A (G215S) in exon 2 of *MUT* gene at an homozygous level. Both parents were heterozygous. On the basis of molecular data, prenatal diagnosis was carried out at 11 weeks of the mother's gestation. Both chorionic villus (CV) and amniotic fluid (AF) samples were used to isolate genomic DNA. PCR amplifications and direct sequences of the foetus' CV and AF samples showed at an homozygous level the presence of the G215S amino acid substitution. The mutation was also confirmed by KpnI restriction enzyme analysis. In addition, organic acids and acylcarnitine profile, carried out on amniotic fluid by GC/MS and LC/MS/MS analysis, showed high levels of methylmalonic acid and propionylcarnitine (C3) respectively. These metabolites were detected only in traces in an amniotic fluid, of the same week of gestation, used as control. These molecular and biochemical data allowed a certain diagnosis and the pregnancy was terminated. Up to now mut MMA prenatal diagnosis has only been performed by biochemical assay. This is the first prenatal diagnosis of mut^0 MMA carried out, both on CV and AF, by DNA, C3 and methylmalonic acid analysis at 11 week of gestation.

The AMMEC Italian association is gratefully acknowledged.

131-O

LONG-TERM OUTCOME IN METHYLMALONIC ACIDURIAS DEPENDS ON THE UNDERLYING DEFECT

F Hörster[1], MR Baumgartner[2], T Suormala[3], GF Hoffmann[1], P Burgard[1], S Kölker[1], ER Baumgartner[3]

University Children's Hospitals Heidelberg[1], Germany, Zürich[2] and Basel[3], Switzerland

Methylmalonic acidurias (MMA) are of heterogenous origin, being caused by methylmalonyl-CoA mutase (MCM) deficiency (mut^0, mut$^-$) or by defects in cobalamin metabolism (e.g. cblA, cblB). Eighty-one patients (age 6–33 years) were included into an cross-sectional multicenter study. Clinical course and outcome were evaluated by standardized questionnaires. The patients were subdivided by detailed enzymatic analysis in cultured fibroblasts into 44 mut^0, 9 mut$^-$, 17 cblA, and 11 cblB deficiencies. Among all patients, 28 (34.5%) died, 28 (34.5%) suffered neurologic disease, whereas 25 (31%) remained neurologically asymptomatic. The outcome was influenced by age at diagnosis and age cohort (i.e. prognosis improved from 1971 to 2003), underlying defect (cblA and mut$^-$ had a better outcome than cblB and mut^0) and quality of therapy. In addition to neurological symptoms, the clinical course was compromised by chronic renal failure, depending on disease duration and underlying defect: 44% of mut^0 patients developed renal failure (GFR <60 ml/h; Schwartz formula) at a mean age of 9 years, in contrast to 25% of cblA patients at a mean age of 11 years. In conclusion, enzymatic analysis and early diagnosis are crucial for therapy and outcome. Chronic renal failure is a severe complication which onset might be delayed (or even prevented ?) in milder forms of the disease. Many questions about the determinants of renal and neurologic disease remain unanswered and have to be addressed in a prospective study.

132-P

MUTATIONAL SPECTRUM OF ISOLATED METHYLMALONIC ACIDEMIA: TWENTY-TWO NOVEL ALLELIC VARIANTS

B Pérez, MA Martinez, A Rincón, LR Desviat, R Navarrete, B Merinero, M Ugarte
Centro de Biología Molecular SO, UAM, Madrid, Spain

In this work we describe the genetic analysis of 25 methylmalonic acidaemia (MMA) affected patients, mainly from Spain, providing essential information to explain the phenotypic variations of this disorder. Using biochemical and cellular approaches our patients have been classified in 13 mutMMA, 7 cblA, 2 cblB and 3 non cblA/cblB deficient patients. Phenotypically, in our series 12 patients exhibited the classical clinical presentation of whom 10 were *mut*^{0} forms and two were *cblB*. Three *mut*$^-$ affected patients show a milder form of the disease. All seven *cblA* had an infantile presentation being B_{12}-responsive. cDNA and genomic DNA sequence analysis of the *MUT, MMAA* and *MMAB* genes have allowed us to identify 27 different changes, 22 novel ones. We have not found any prevalent mutation. The mutational spectrum include 10 missense mutations, (eight in the MUT gene and two in the MMAB gene), three splicing mutations, one in each gene, five nonsense mutations and nine small deletions and duplications all affecting the open reading frame of the corresponding protein. The frameshift mutations are the most common disease-causing mutations in our survey of MMA patients, especially in MMAA affected patients where only this type of mutations are identified. The functional and structural consequences of the amino acid changes identified in the MUT and MMAB genes are discussed in the context of the two crystallized homologous bacterial proteins, the α subunit of MCM of *P. Shermanii* and the ATP:cobalamin adenosyltransferase from *T. acidophilum* respectively.

133-P

BIOCHEMICAL AND MOLECULAR ANALYSES IN METHYLMALONIC ACIDEMIA

E Pospíšilová[1], L Mrázová[1], O Martincová[1], M Elleder[1], J Zeman[1,2]
[1]*Institute of Inherited Metabolic Diseases,* [2]*Centre for Integrated Genomics, General Faculty Hospital and 1st Faculty of Medicine, Charles University, Prague, Czech Republic*

Methylmalonic acidaemia (MMA; McKusick #251000) is a rare inborn error of branched-chain amino acids metabolism with methylmalonate accumulation in blood and urine. MMA may be caused by deficiency of methylmalonyl-CoA mutase (EC 5.4.99.2) apoenzyme (*mut*^{0} or *mut*$^-$) or by defects in the synthesis of adenosylcobalamin (*cblA* or *cblB*). In our study we present the results of biochemical and molecular analyses in eight children with MMA. HPLC method (Kikuchi et al. 1989) was used for estimation of methylmalonyl-CoA mutase activity in isolated lymphocytes and/or cultivated fibroblasts. *Mut* deficiency was demonstrated in five patients. Molecular analyses in four of them revealed that one child is a compound heterozygote for mutation N219Y/H627Q in gene for methylmalonyl-CoA mutase apoenzyme. Two patients are heterozygotes for mutation N219Y and one child is heterozygote for mutation G203R. In these three patients no mutation was found on the other allele, so far. Normal mutase activity in last three patients indicated a possible defect in adenosylcobalamin synthesis. One of them, *cblB* patient, is a compound heterozygote for mutation R186W/c.558(del2/ins1). A novel mutation c.558del2/ins1 leads to a frame shift and 212 amino acids long protein product. Molecular analyses of the other patients are still under way. Conclusion: Mutation N219Y is prevalent in Slavonic population and is as well as mutation G203R associated with *mut*^{0} phenotype; the phenotypic impact of a novel mutation H627Q is unknown.

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134-P

THE URINARY UREA/METHYLMALONIC ACID MOLAR RATIO IS A SIMPLE AND EFFICIENT BIOLOGICAL MARKER TO GUIDE LONG TERM DIETARY TREATMENT IN METHYLMALONIC ACIDURIA PATIENTS

V Valayannopoulos, G Touati, D Rabier, P De Lonlay, JM Saudubray
Necker-Enfanta Malades Hospital, Paris 75015, France

Methylmalonic aciduria (MMA) is an inherited metabolic disorder due to a deficiency of the MMA CoA mutase enzyme. MMA in these patients derives from protein catabolism, odd chain fatty acid oxidation produced by lipolysis and intestinal production by propiogenic bacteria. Long term follow-up and treatment is essential in order to prevent severe complications such as intellectual impairment, growth failure and early progressive renal failure. The basis of the treatment resides on the control of each one of the MMA sources: low protein diet for the protein source, high calory intake or continuous feeding for lipolysis and antibiotic treatment for the intestinal source. We propose here a simple method to determine the contribution of each source by analyzing the urinary urea/MMA molar ratio. Urinary urea reflects the protein catabolism which leads in the case of a total enzymatic bloc in a fixed proportional production of MMA compared to urea. A ratio within the range of 3.4 and 5 mmol/mmol (due to variations in dietary protein composition) suggests a predominant protein source. A very low ratio <1 suggests MMA derived from lipolysis or from bacterial production in the gut. A high ratio >5 is in favour of a residual enzymatic activity. We have applied this method to study the MMA excretion and make dietary and treatment decisions in 10 patients during a period of 4 to 12 years. Our results show that the urinary urea/MMA ratio allows to determine easily the major source of MMA and the possible existence of residual enzymatic activity and thus make efficient therapeutic decisions that contributed to ameliorate the metabolic control in our patients.

134a-P

LIVER TRANSPLANTATION FOR EARLY-ONSET METHYLMALONYL-CoA MUTASE DEFICIENCY

M Spoda[1], F Gennari[2], A Peduto[1], G Bonetti[1], C Acquaviva[3], JF Benoist[3], E Ceratti[2], A Ponzone[1], M Salizzoni[2]
[1]*Department of Pediatrics, University of Torino;* [2]*Liver Transplantation Unit, Molinette Hospital, Torino;* [3]*Service de Biochimie, Hopital R. Debré, Paris, France*

The outcome for children with severe forms of methylmalonic acidemia (MMA) remains poor despite conventional treatment with diet, carnitine and metronidazole. Alternative treatment such as liver transplantation (OLT) has been considered, but this option is not commonly adopted. Here we report on a male patient with classical cobalamin non-responsive early-onset MMA due to 7 methylmalonyl-CoA mutase deficiency (urinary MMA excretion between 6000 and 8000 mmol/mol C; mutase activity in fibroblasts $<10\%$; compound heterozygous for the already reported mutations G158V and R467X at the methylmalonyl-CoA mutase locus). Following the neonatal acute onset, he did not show major problems during the first year of life. However, since the age of 12 months frequent episodes of metabolic decompensations required several hospital admissions. Slight neuromotor retardation occured, but cognitive level was still normal. Because of poor quality of life and poor long-term prognosis under conventional therapy, parents opted for OLT as alternative strategy. Patient entered the transplantation list at the age of 30 months and underwent the OLT at the age of 3 years. No complications were observed during the peri-operative period and the child was discharged after 8 days. At 9 months since intervention, the child shows a marked neuromotor improvement and did achieve full fasting and intercurrent catabolic illness tolerance, gastric drip feeding was no more employed, protein intake is only moderately restricted and a significant MMA clearance has been observed (urinary MMA now ranging from 500 to 1000 mmol/mol C).

135-P

LATE ONSET METHYLMALONIC ACIDURIA AND HOMOCYSTINURIA PRESENTING AS A PROGRESSIVE LEUKOENCEPHALOPATHY

I Moroni, M Bugiani, M Rimoldi, L D'Incerti, G Uziel
Istituto Nazionale Neurologico 'C.Besta', Milan, Italy

Objective: To emphasize the possibility of purely neurological manifestations and diffuse leukoencephalopathy in late-onset methylmalonic aciduria and homocystinuria (MMA-HC) and to demonstrate clues to the diagnosis for a rare treatable disease.
Case report: A girl, 17 years of age, presented with a subacute decline of cognitive performances, followed by a progressive spastic-ataxic quadriparesis leading to the loss of. independent walking 6 months after the onset of symptoms. MRI showed diffuse white matter signal abnormalities sparing U fibers. H-MRS Imaging revealed a mild accumulation of choline and a moderate reduction of N-acetylaspartate. Urinary GC/MS analysis demonstrated an abnormal peak of methylmalonic acid (372 μg/mg creatinine) and plasma homocysteine was markedly elevated (31.3 μmol/L). No other hematological abnormalities were present. Therapy with intramuscular hydroxycobalamin, oral betaine and carnitine was started.
Results: Intellectual performances rapidly improved and normalized in a few months, as well as cerebellar signs and upper limbs involvement. Methylmalonic acid normalized within one month since the beginning of therapy, whereas homocysteine levels did not grossly changed. MRI slightly improved after 12 months of therapy, but a severe paraparesis is still evident after a 3 years follow-up.
Conclusions: MMA-HC usually presents in infancy, and the rarer late onset cases are often misdiagnosed and therefore not promptly treated. Urinary organic acids analysis should always be included in the diagnostic work-up of leukoencephalopathies. This treatable disorder, when not recognized, may run a fatal course while early treatment may partially revert clinical symptoms and recover cognitive functions.

136-A

INHIBITION OF MITOCHONDRIAL CREATINE KINASE ACTIVITY FROM RAT CEREBRAL CORTEX BY METHYLMALONIC ACID

Schuck PF, Rosa RB, Tonin A, Ferreira GC, Sitta A, Dutra-Filho CS, Wyse ATS, Wannmacher CMD, Wajner M
Departamento de Bioquímica, ICBS, Universidade Federal de Rio Grande do Sul, Hospital de Clínicas, Serviço de Genética Médica, Porto Alegre RS, Brazil

Accumulation of methylmalonic acid (MMA) in tissues and biochemical fluids is the biochemical hallmark of patients affected by the methylmalonic acidaemia (MMAemia). Although this disease is predominantly characterized by severe neurological findings, the underlying mechanisms of brain injury are not totally established. In the present study, we investigated the effect of MMA, as well as propionic (PA) and tiglic (TA), whose concentrations are also increased but to a lesser extend in MMAemion total (tCK), cytosolic (Cy-CK) and mitochondrial (Mi-CK) creatine kinase (CK) activities from cerebral cortex of 30-day-old Wistar rats. Total CK activity (tCK) was measured in whole cell homogenates, whereas Cy-CK and Mi-CK was determined in cytosoland mitochondrial preparations from rat cerebral cortex. We verified that tCK and Mi-CK activities were significantly inhibited by MMA at concentrations as low as 1 mM in contrast to Cy-CK which was not affected by the presence of the acid in the incuban medium. Furthermore, PA and TA did not alter CK activities. We also observed that these inhibitions were fully prevented by pre-incubation of the homogenates with glutathione, suggesting that the inhibitory effect of MMA on CK activity is possibly mediated by oxidation of essential thiol groups of the enzyme. Therefore, it could be suggested that deranged energy metabolism may contribute to the brain damage of patients affected by MMAemia.

137-O

INTERNATIONAL CROSS-SECTIONAL STUDY ON OUTCOME AND THERAPEUTIC EFFICACY IN GLUTARIC ACIDURIA TYPE I (GA-I)

Kölker S, Greenberg CR, Leonard JV, Saudubray JM, Kalkanoğlu HS, Walter JH, Lagler F, Schwahn B, Troncoso M, Chalmers RA, Lund A, Koeller D, Burlina A, Wajner M, Christensen E, Zschocke J, Merinero B, Ribes A, Burgard P, Hoffmann GF for the GA-I Study Group
Conducted by University Children's Hospital, Division of Metabolic Diseases, Heidelberg, Germany

The major aim of the present study was to investigate the clinical diversity, outcome, and therapeutic efficacy in glutaric aciduria type I (GA-I). The study was performed using a standardized questionnaire. 261 anonymized patients (f:m = 112:149) with confirmed diagnosis of GA-I were included into the study after giving informed consent. Median age at diagnosis was 12 mo and patients had a median follow-up of 67 months. Diagnosis was suspected due to clinical presentation (*CP*, 79%), previously diagnosed siblings (12%), or newborn screening (*NBS*, 9%). Lysine-restricted diet (40%) or sole protein restriction (46%) were used, whereas 14% received no diet. Furthermore, carnitine (87%) and riboflavine (63%) were administered. The first encephalopathic crisis occured at a median age of 9 months, the oldest patient with a crisis was aged 70 months. Notably, some treated patients still had encephalopathic crises: 11 crises/104 patients on lysine restriction and 43 crises/120 patients on protein restriction. The 'NBS' group had the best (65% asymptomatic, 31% handicapped, 4% dead) and the 'CP' group the worst outcome (5% asymptomatic, 75% handicapped, 20% dead). In conclusion, GA-I is a treatable disease, and outcome can be influenced by early diagnosis and therapy. However, further improvements in the treatment are still needed to prevent neurological complications.

138-P

CLINICAL OUTCOME OF GLUTARIC ACIDURIA TYPE I IN IRELAND

ER Naughten[1], PD Mayne[2], R Walsh[2], P Howard[1], AA Monavari[1]
[1]*The National Centre of Inherited Metabolic Disorders and* [2]*Department of Clinical Chemistry, Children's University Hospital, Temple Street, Dublin, Ireland*

Glutaric aciduria type I (GA I, McKusick 231670) is a devastating condition with a high morbidity. Numerous approaches to treatment are being tried with variable outcome. We present the outcome of 22 patients diagnosed with GA I over a 16 year period (155 treatment years) in Ireland, 11 following clinical presentation and 11 following a high risk screen.

Twenty patients have been managed with diet; 9 have died of whom 7 were diagnosed clinically, 6 with dystonic and one with spastic cerebral palsy. Of the 11 patients who did not have cerebral palsy, 10 were diagnosed following a high risk screen. Six of 7 patients with no abnormal neurological signs have abnormal CT or MRI findings; no case of striatal degeneration has occurred during the past 14 years in the high risk screened group. Those that presented clinically with dystonia following encephalopathy had a poor outcome, 7 of 11 patients dying despite aggressive management, one from a complication of a central venous line without metabolic decompensation. Two of 11 patients, detected following high risk screen and managed aggressively, have died.

Striatal degeneration has not been seen in any of the high risk screened patients over 14 years despite numerous illnesses and surgical procedures. This has been attributed to modifications incorporated into the treatment over the past ten years, in particular aggressive high calorie and carnitine input and meticulous electrolyte and phosphate management.

139-P

CLINICAL AND BIOCHEMICAL FEATURES IN 3 PATIENTS WITH GLUTARIC ACIDURIA TYPE I FOUND IN NEWBORN SCREENING

Shigematsu Y[1], Hata I[1], Naito E[2], Tajima G[3], Sakura T[3], Yorifuji T[4]
Department of Pediatrics, University of Fukui, Fukui[1]; Department of Pediatrics, Tokushima University, Tokushima[2]; Department of Pediatrics, Hiroshima University, Hiroshima[3]; Department of Pediatrics, Kyoto University, Kyoto[4], Japan

Relationship between clinical course and biochemical data were examined in three patients with glutaric aciduria type I found in newborn screening with tandem mass spectrometry (MS-MS).

Concentrations of glutarylcarnitine (GC), glutaric acid (GA) and 3-hydroxyglutaric acid (HGA) in body fluids were measured by stable-isotope dilution methods using MS-MS or gas chromatography-mass spectrometry. Glutaryl-CoA dehydrogenase activities were measured using lymphocytes, and those in three patients were below the detection limit. All patients have been treated with carnitine and protein-restriction diet. Patient MM was delayed in early infancy and showed improvement of DQ 85 at the age of 21 months. Patients HK and CH have been in good health up to the age of 15 months, and 10 months, respectively. HK, 'high excretor' of GA, showed higher levels of GA in CSF and serum and those of HGA in serum than those in MM and CH in early infancy, although the HGA level in CSF was similar to those in MM and CH. While levels of GA in urine and serum of HK remained high, those of MM and CH decreased to the control ranges at the age of 9 months and 2 months, respectively, when HGA and GC levels in CSF of MM and CH remained high. Careful monitoring of the metabolites may be necessary because of the possible discrepancy in metabolism of glutaryl-CoA between CNS and the other organs in GA-I patients.

140-P

LATE-ONSET NEUROLOGIC DISEASE IN TWO PATIENTS WITH GA-I

Kölker S, Bodamer OA, Külkens S, Sauer S, Seitz A, Schulze-Bergkamen A, Zschocke J, Harting I, Hoffmann GF
Departments of General Pediatrics, Neurology, Human Genetics, and Neuroradiology, University Hospitals Heidelberg and Mannheim, Germany, and University Hospital Vienna, Austria

Recently, adult-onset glutaric aciduria type I (GA-I) has been described in one patient, suggesting a distinct manifestation type in adulthood (Bähr et al. 2002). Here we present two further patients with a similar clinical phenotype. Patient 1, a 66-year-old male patient, presented with a progressive neurologic disease of unknown origin. Macrocephaly was recognized in childhood, psychomotor development was unremarkable. Headaches started at age 35 years, followed by tremor in both arms (50 years), tonic clonic seizures (54–62 years), and dementia (since 62 years). MRI showed generalized atrophy and severe leukodystrophy. Diagnosis was confirmed by analysis of acylcarnitines, organic acids ('high excretor' type), mutation analysis in the *GCDH* gene (homozygous for R383C), and by biochemical analysis of a brain biopsy specimen. Patient 2, a 15-year-old male adolescent with macrocephaly, presented with severe diffuse headache, vertigo, and gait disturbance, developing after upper respiratory tract infection. He had an uneventful history. MRI showed temporal hypoplasia and leukodystrophy in frontal and occipital white matter sparing the U fibres. Biochemical analysis ('high excretor' type) and mutation analysis (homozygous for R88C) confirmed the diagnosis. He fully recovered after supplementation with carnitine and moderate protein restriction. In conclusion, adult-onset GA-I should be considered as distinct manifestation form in previously 'asymptomatic' adult or adolescent patients.

141-P

IN VITRO LOADING IN PBMC AND LYMPHOBLASTS: A HELPFUL TOOL FOR THE DIAGNOSIS OF MILD BIOCHEMCIAL VARIANTS

Schulze-Bergkamen A, Okun JG, Greenberg CR, Hoffmann GF, Kölker S
University Children's Hospital, Division of Metabolism. and Endocrine Diseases, Heidelberg, Germany; Department of Biochemistry and Medical Genetics, University of Manitoba, Winnipeg, Canada

In glutaric aciduria type I (GA-I), the biochemically defined subgroup of low excretors (LE) are at risk to be missed by usual diagnostic approach. Thus, we developed an in vitro test using peripheral blood mononuclear cells (PBMC) and lymphoblasts (LB). Loading conditions were varied as follows: (1) palmitate (C16), (2) lysine and tryptophan, or (3) 2-oxo-adipate (2-OA). In analogy, loading was also performed in LB from LE. Furthermore, we performed loading tests in PBMC from patients with other organic acid (OAD) and fatty acid oxidation disorders (FAOD). In GA-I, the most reliable condition was 2-OA. In three GA-I patients with homozygosity for E365K (high excretors) and in two patients with compound heterozygosity for F236L/S259P (LE) increased glutarylcarnitine (Glut) was found after loading PBMC with 2-OA. In LB, one patient with homozygosity for IVS-1+5g→t (LE) could be consistently found by elevated Glut (not diagnosed before by ESI-MS/MS in dried blood spots). Using C16 loading, all patients with FAOD (SCADD, MCADD, VLCADD, LCHADD, n = 2, 7, 5, 2) and classic OAD (IVA, MMA, PA, n = 4, 6, 4) were diagnosed. In conclusion, the standardized in vitro loading test in PBMC and LB is helpful for the diagnosis of GA-I, classic OAD and FAOD. In particular, PBMC loading is suitable for patients with mild biochemical phenotypes that might be missed by standard diagnostic approach. A detection of LE among GA-I patients seems possible by this approach. However, these preliminary findings must be confirmed upon a greater number of patients.

142-P

NOVEL AND RECURRENT *GCDH* GENE MUTATIONS IN GLUTARIC ACIDURIA TYPE 1 (GA1) PATIENTS FROM ISRAEL

SH Korman[1], C Jakobs[3], A Gutman[1], Z Ben-Neriah[2], MS van der Knaap[4], GS Salomons[3]
Departments [1]Clinical Biochemistry and [2]Genetics, Hebrew University, Hadassah Medical School, Jerusalem, Israel; [3]Departments of Clinical Chemistry and [4]Child Neurology, VU University Medical Center, Amsterdam, The Netherlands

GA1 was diagnosed in 1 Jewish and 10 Arab patients from 10 families in Israel. Parental consanguinity was common. One patient was asymptomatic whilst one was mildly affected, one moderately and 8 severely affected. Diagnosis was often delayed or missed, suggesting that the incidence of GA1 may be underestimated and indicating a need for greater awareness of GA1 amongst pediatricians and neuroradiologists. Two patients had normal glutaric but increased 3-hydroxyglutaric acid excretion. Two severely affected patients had unusual MRI findings: one with diffuse cerebral white matter signal abnormalities and one lacking typical Sylvian fissure and temporal lobe abnormalities. *GCDH* mutations were identified at all patient alleles, including 4 novel and 4 previously reported mutations, indicating that GA1 is genetically heterogeneous in this geographic region. Knowledge of the responsible mutation enabled successful prenatal diagnosis on CVS tissue in two subsequent pregnancies. One asymptomatic and 3 severely affected patients were homozygous for the T416I mutation, implying that additional genetic and/or environmental factors must account for the phenotypic heterogeneity. In this context, the presence of unrelated neurological disorders (mental retardation, D-2-hydroxyglutaric aciduria, and anencephaly with multiple congenital malformations) in unaffected siblings in three families may be of significance.

143-P

URINARY UREA TO GLUTARIC ACID RATIO IN GLUTARIC ACIDURIA TYPE I PATIENTS IS RELATED TO LYSINE INTAKE BUT NOT WITH OUTCOME

Williams M[1], Rabier D[2], Touati G[3], Saudubray JM[3]
[1]Erasmus MC – Sophia. Department of Pediatrics, Rotterdam, The Netherlands, [2]Biochemical Laboratory, [3]Department of Metabolic Disorders, Necker Hospital, Paris, France

Glutaric aciduria type I (GAI) is due to a deficiency of glutaryl-CoA dehydrogenase. Eighteen patients were followed up at the Necker Enfants Malades Hospital. A restriction in protein or a lysine limited diet, riboflavin and carnitine is the recommended therapy in this disease. During decompensation, adequate calories but no proteins are given. One patient was treated with a strict low protein diet from the neonatal period onward, the others started treatment at a mean age of 28 months (median 15 months). A low protein diet was not always accepted. A protein restricted diet was discussed as 6 of these patients were neurologically normal. We used the urea excretion as a measure of protein intake/catabolism. Urea and glutaric acid concentrations were determined from the same urine sample (96 samples from 7 patients). A correlation between protein intake and the amount of glutaric acid excreted was made. **Results**: (1) The urea to glutaric acid ratio reflected the amount of lysine/tryptophan in the patients diet ($r^2 = 0.73$). (2) There was no relation between the urea to glutaric acid ratio and fibroblast glutaryl-CoA dehydrogenase activity (determined by E. Christensen, Denmark). (3) There was no relation between the urea to glutaric acid ratio or neurological outcome. These results raise the question to whether a low protein diet is beneficial to patients with glutaric aciduria type I.

144-P

IN VITRO EFFECT OF 3-HYDROXYGLUTARIC ACID ON ENERGY METABOLISM IN RAT STRIATUM AND CEREBRAL CORTEX

Latina A, Rosa RB, Leipnitz G, Scussiato K, Schuck PF, de Assis DR, Maria RC, Dutra-Filho RC, Wajner M
Departamento de Bioquímica, Laboratório de Erros Inatos do Metabolismo, ICBS, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

3-Hydroxyglutaric acid (^{3}HGA) accumulates in glutaryl-CoA dehydrogenase deficiency (GDD), an inherited disorder characterized by neurological symptoms accompanied by fronto-temporal atrophy and striatum degeneration. Considering that it has been recently suggested that energy derangement may be involved in GDD brain damage, we investigated the *in vitro* effect of ^{3}HGA (0.01–1 mmol/L) on important enzyme activities involved with energy metabolism, including the respiratory chain complexes I–III, II–III, II, IV and V, as well as the activities of succinate dehydrogenase, creatine kinase (CK) and Na^+,K^+-ATPase in striatum and cerebral cortex homogenates from 30-day-old-rats. We verified that ^{3}HGA mildly nhibited the activities of complex II and succinate dehydrogenase in cerebral cortex only at 1 mM concentration, in contrast to the other respiratory chain activities, which were not affected by this organic acid. Moreover, this inhibitory effect was not detected in striatum. In addition, ^{3}HGA did not modify significantly the activities of CK and Na^+,K^+-ATPase in both brain structures. The data indicate a slight *in vitro* inhibition of ^{3}HGA on energy metabolism in rat cerebral cortex. Although other parameters of energy metabolism must be searched for before definitive conclusions can be drawn, our results indicate that other mechanisms than mitochondrial impairment, i.e. excitotoxicity and oxidative stress, are mainly involved in the cortical and, particularly, in the striatal.

145-P

ON THE NEUROTOXICITY OF GLUTARIC, 3-HYDROXY GLUTARIC AND TRANS-GLUTACONIC ACID IN GLUTARIC ACIDEMIA TYPE 1

Lund AM[1], Christensen E[1], Kristensen AS[2], Schousboe A[2], Lund TM[2]
[1]Department of Clinical Genetics, Juliane Marie Centre 4062, Copenhagen University Hospital, 9 Blegdamsvej, 2100 Copenhagen, Denmark; [2]Department of Pharmacology, The Danish University of Pharmaceutical Sciences, 2 Universitetsparken, 2100 Copenhagen, Denmark

Glutaric acidaemia type 1 (GA1) is an autosomal recessively inherited deficiency of glutaryl-CoA dehydrogenase. Accumulating metabolites, 3-hydroxy-glutaric (3-OH-GA), glutaric (GA) and trans-glutaconic (TG) acids have been proposed to be involved in the development of the striatal degeneration seen in children with GA1 via an excitotoxic mechanism. We have studied to which extent 3-OH-GA, GA and TG are neurotoxic and whether neurotoxicity is caused by an excitotoxic mechanism in which 3-OH-GA, GA or TG overactivates NMDA receptors. In cultured mouse neocortical neurons all three compounds were weakly neurotoxic possibly through activation of NMDA receptors as determined by the MTT-assay. However, further electrophysiological studies in the rat cortical wedge preparation and with NMDA receptors expressed in Xenopus oocytes could not confirm an interaction of the compounds with NMDA receptors. It is concluded that the metabolites 3-OH-GA, GA and TG are only weak neurotoxins and the neurodegenerative cascade destroying the striatum in patients with GA1 involves mainly mechanisms other than excitoxicity.

146-P

THE BRAIN IS DIFFERENT – TISSUE-SPECIFIC DISTRIBUTION OF 3-HYDROXYGLUTARIC AND GLUTARIC ACIDS IN *GCDH*$^{-/-}$ MICE

Sauer S, Okun JG, Feyh P, Goodman SI, Crnic LS, Hoffmann GF, Koeller DM, Kölker S
University Children's Hospital, Division of Metabolic Diseases, Heidelberg, Germany

Glutaric (GA) and 3-hydroxyglutaric acids (3-OH-GA) are key metabolites in glutaric aciduria type I, however, the exact tissue distribution of these organic acids is poorly characterized. In this study, we investigated the distribution and the concentrations of these metabolites in tissue homogenates (brain, liver, heart, skeletal muscle) from *Gcdh*$^{-/-}$ mice compared with wild-type (*wt*) mice using gas chromatography/mass spectrometry with stable-isotope dilution assay.

Organic acids	Genotype	Brain	Liver	Heart	Muscle	Serum (μmol/L)
GA	Gcdh$^{-/-}$	11.2±5	9.9±3.8	0.1 ±0.04	0.6±0.4	506±223
(nmol/mg protein)	wt	0.03±0.06	0.1±0.1	0.01 ±0.01	0.2±0.1	10.9±6.6
3-OH-GA	Gcdh$^{-/-}$	0.5±0.3	0.4±0.2	0.1±0.05	0.2±0.2	13.3±4.1
(nmol/mg protein)	wt	0.01±0.04	0.03±0.06	0.00±0.00	0.04±0.05	0.0±0.0

In liver, heart, and skeletal muscle of *Gcdh*$^{-/-}$ mice the concentrations of GA and 3-OH-GA positively correlated with the respective GCDH activities, reflecting different metabolic activities of the deficient pathway in these tissues. Strikingly, there are equivalent concentrations of GA respectively 3-OH-GA in the brain (low GCDH activity) and liver tissues (high GCDH activity). This finding can be explained by (1) transport of both organic acids *via* the blood-brain barrier, (2) cerebral *de novo* synthesis, and/or (3) subsequent trapping in the deep compartment of the brain.

147-P

TISSUE-SPECIFIC BIOENERGETIC ANALYSIS IN *GCDH*$^{-/-}$ MICE

S Sauer, DM Koeller, MA Schwab, SI Crnic, LS Goodman, GF Hoffmann, JG Okun, S Kölker
University Children's Hospital, Division of Metabolic Diseases, Heidelberg, Germany

Gcdh$^{-/-}$ mice are an animal model for glutaric aciduria type I (GA-I), revealing the characteristic biochemical phenotype of this disease. An impairment of energy metabolism by accumulating glutaric (GA) and 3-hydroxyglutaric acids (3-OH-GA) has been previously suggested. The aim of this study was to investigate key bioenergetic parameters in tissue homogenates (liver, brain, heart, skeletal muscle) from *Gcdh*$^{-/-}$ and wild-type (*wt*) mice. Furthermore, we investigated the influence of GA, 3-OH-GA, and glutaryl-CoA on respiratory chain, α-ketoglutarate dehydrogenase (KGDH) and pyruvate dehydrogenase (PDHc). The level of ongoing oxidative stress in brain and liver was estimated by analysis of glutathione concentrations. Activity analysis of single respiratory chain complexes I–V did not show any difference between *Gcdh*$^{-/-}$ and *wt* mice. Neither GA nor 3-OH-GA (up to 10 mmol/L) exerted any effect on respiratory chain complexes I-V, PDHc or KGDH. In contrast, glutaryl-CoA inhibited complexes II and III, and KGDH in a concentration-dependent manner (% inhibition at 2 mmol/L glutaryl-CoA; complex II: 45%, complex III 70%, KGDH 80%), whereas PDHc was not affected. Glutathione concentrations in liver and brain of *Gcdh*$^{-/-}$ mice were significantly decreased ($p < 0.002$) compared with *wt* mice. In conclusion, activity of single respiratory chain complexes remained unaffected in *Gcdh*$^{-/-}$ mice, whereas decreased glutathione suggested increased oxidative stress in these mice. Notably, we demonstrated for the first time a potential specific role for glutaryl-CoA in the pathophysiology of GA-I.

148-P

COMPLEX BRAIN MALFORMATION REVEALING A SEVERE GLUTARYL CoA DEHYDROGENASE DEFICIENCY

I Desguerre[1], C Barnérias[1], P Sonigo[2], D Rabier[3], E Christensen[4], JM Saudubray[1]
[1]*Pediatric Department Necker Hospital, Paris.* [3]*Biochemestry Department Necker Hospital, Paris;*
[2]*Radiology Department, Necker Hospital,* [4]*Department of Clinical Genetics Copenhagen, Denmark*

We report the case of the second child of consanguinous parents. At 34 weeks of amenorrhea, prenatal echography revealed severe ventriculomegaly and posterior gyration abnormalities were suspected on the prenatal MRI..The girl was born three weeks later (weight 3500 g, head circumference 39 cm). She had erratic ocular movements without eye contact, severe axial hypotonia and pyramidal signs. She was able to suck and had no respiratory distress. The ophthalmologic examination was normal (fundus, visual evoked potentials and electroretinogramm). The RMI confirmed the abnormal posterior gyration associated with ventriculomegaly, cerebellar and brainstem hypoplasia. Peroxysomal investigations were normal. The chromatography of organic acids suggested glutaric aciduria type I: glutaric acid = 9871 µmol/L, 3OH glutaric acid = 110 µmol/L). The glutaryl CoA dehydrogenase activity on cultured fibroblasts was very low (0.03 µmol/h/g protein for a normal range of 5.0 ± 1.6). At six months of age, the macrocephaly remained important (HC = 52 cm). The girl was very dystonic with pyramidal signs but without any head control. The ocular motricity was better, she was able to follow with the eyes and to smile to the examinator. The genetic study is ingoing. This is the first reported case of prenatal brain malformation due to glutaric aciduria. Two cases of 'prenatal hydrocephaly' have been reported without further investigation.

149-A

IF THESE PATIENTS DON'T HAVE GLUTARIC ACIDURIA TYPE I, WHAT ELSE COULD THEY HAVE?

Fernández-Alvarez E, García-Cazorla A, Póo P, Campistol J, Vilaseca MA[1], Christensen E[2], Ribes A[3]
Neurology Service, Biochemical Unit[1], Hospital Sant Joan de Déu, 'Institut de Bioquímica Clínica[3], Barcelona, Spain; Rigshospitalet[2] Copenhagen, Denmark

Objective: To describe the clinical, biochemical and genetic characteristics of 2 patients strongly suspected of having GA1. **Case reports**: TM is a girl with antecedents of motor delay who developed dystonia and choreoathetosis at 4.5 years. These movements have progressively impaired until now (23 years). AF is a boy with psychomotor delay, macrocephaly and dystonias from the first months of age. At 4 years, he has dystonic-athetoid movements with an autonomous gait. Neither of them was L-Dopa responsive. **Results**: In both patients MRI showed bilateral high intensity images in putamen. Extensive metabolic exams were normal. More in-depth studies of GA1 were performed:

	GCDH activity (μmol/h/g protein)	GCDH gene Allele 1	Allele 2	Glut. (mmol/mol creatinine)	3OHGlut. (mmol/mol creatinine)
TM	1.66 (Controls: 5.0±1.6)	L125P	+	2 (N:2–10)	9(N:2–15)
AF	4.9 (Controls: 5.0±1.6)	Q76R	+	2 (N:2–10)	8(N:2–15)

Conclusions: Clinical manifestations (though atypical in TM) and MRI are suggestive of GA1. In both cases only one mutation was found. GDCH activity of TM was 30% of the controls', whereas it was normal in AF. Should we still expect them to be affected with GAI? Should we think about expanding the diagnostic criteria of this disease?

150-P

3-OH GLUTARIC ACID IS NOT A SPECIFIC MARKER FOR GLUTARIC ACIDEMIA TYPE I

M de Sain-van der Velden, T de Koning, G Visser, P van Hasselt, R Berger, B Dorland
Department of Metabolic and Endocrine Diseases, UMC Utrecht, The Netherlands

Glutaric acidaemia type I (GA-I) is an autosomal recessive disorder of amino acid metabolism resulting from a deficiency of glutaryl-CoA dehydrogenase (GCDH). The diagnosis of GA-1 is made by the presence of increased urinary glutaric acid and 3-hydroxyglutaric acid and is confirmed by a deficiency or absence of GCDH in cultured fibroblasts or leucocytes. Moreover, the presence of 3-OH glutaric acid is supposed to be the diagnostic metabolite of GA-I.

We present a boy with hepatomegaly, convulsions, hypotonia and psychomotor retardation. MRI showed abnormalities consistent with Leigh syndrome. Analysis of CSF showed increased lactic acid, succinic acid and glutaric acid. Plasma amino acid analysis revealed an increased alanine concentration. Urinary analysis showed increased succinic acid, fumaric acid and lactic acid with an intermittend excretion of glutaric acid. In addition, 3-OH glutaric acid (confirmed by mass spectrometry) was present in one of these urines. These abnormal metabolites were suggestive of GA-I. However, glutaryl-CoA dehydrogenase activity in leucocytes appeared to be normal. In addition, muscle biopsy revealed a very severe reduction in the activity of complex II indicating a mitochondrial disorder.

These findings imply that the presence of 3-OH glutaric acid is not always indicative of glutaric acidaemia type I, but can also be seen in a respiratory chain disorder.

151-P

QUANTITATION OF DIMETHYLGLYCINE BY ELECTROSPRAY-TANDEM MASS SPECTROMETRY (ESI-MSMS) IN PLASMA AND BLOOD SPOTS AS AN AID TO THE DIAGNOSIS OF MULTIPLE ACYL CoA DEHYDROGENASE DEFICIENCY (GA2)

NJ Manning, JR Bonham, M Downing, SE Olpin, RJ Pollitt, MJ Sharrard
Department Clinical Chemistry, Sheffield Children's Hospital, Western Bank, Sheffield, S10 2TH, UK

Patients with GA2 have been observed to excrete increased quantities of dimethylgycine (DMG) due to impaired activity of dimethylglycine dehydrogenase (EC 1.5.99.2). We have analysed plasma and urine from a patient with GA2 by a stable isotope dilution GCMS method and found elevated concentrations of DMG (plasma 121 μmol/L ref. < 8, urine 481 μmol/mmol creat. ref. < 16). With such highly increased plasma DMG in our GA2 patient, a blood spot assay by ESI-MSMS of butyl esters was developed using 3mm sample discs. To avoid any isobaric conflicts using MSMS ion pairs m/z 160 > 58 (DMG, β-aminoisobutyrate) and m/z 162 > 60 (d2-DMG, serine), butylated extracts were further trifluoroacetylated thus eliminating the interfering amino acids as TFA-butyl esters whilst conserving the unreactive DMG and d2-DMG as butyl esters. The MSMS assay gave comparable results to those obtained by GCMS; plasma at both the 7 μmol/L and 120 μmol/L levels showed a slight positive bias (8%) when assayed by MSMS. The blood spot MSMS assay demonstrated a clear increase in DMG for the GA2 patient. Anonymous newborn screening (day 6) blood spots gave a mean concentration of 2.9 μmol/L (n = 12) with a range of 0.5–5.4 μmol/L. GA2 patient blood spots (n = 4) gave a mean result of 32.1 μmol/L (CV = 6.5%). This simple assay has potential as an adjunct to a blood spot newborn screening program when GA2 is suspected.

152-P

NEONATAL PRESENTATION OF RIBOFLAVIN-RESPONSIVE MULTIPLE ACYL-CoA DEHYDROGENASE DEFICIENCY

Fletcher JM[1], Ketteridge DB[1], Harrison JR[1], Ranieri E[1], Gerace R[1], Bartlett B[1], Blake J[1], Owens G, Morris S[2], Rhead WJ[3]
Departments of Genetic Medicine[1], Women's and Children's Hospital Adelaide, Neonatology[2] Flinders Medical Centre Australia, and Medical College of Wisconsin, USA[3]

Aim: The sensitivity of tandem mass spectrometry newborn screening for vitamin-responsive fatty acid oxidation defects is not known. We describe 3 infants with riboflavin-responsive multiple acyl CoA dehydrogenase deficiency (MADD), 1 of whom was symptomatic in the neonatal period.

Methods: Butylated acylcarnitines from dried filter paper blood spots were determined on a PE SCIEX API 365 tandem mass spectrometer using 13 stable isotope acylcarnitine standards. Urine organic acids were determined as TMS derivatives using a HP GC/MS. Intact fibroblast radioHPLC profiling of the β-oxidation acyl-^{3}H-carnitine intermediates derived from palmitate, branched chain amino acids and ^{3}H-carnitine were determined in riboflavin-deficient, normal and supra-physiological riboflavin-containing media.

Results: Dried blood spot profiles in all 3 infants revealed elevated C5, C6, C8, C12, C14 and C14:1 acylcarnitines. Urine organic acids demonstrated marked dicarboxylicaciduria, increased ethylmalonic, methylsuccinic, glutaric, 5-OH hexanoic acids, hexanoyl- and suberyl-glycine; these normalised after riboflavin 100 mg/d. Fibroblast analysis demonstrated low acetyl-carnitine and high butyryl- and isovaleryl-/α-methylbutyryl-carnitines, consistent with mild MADD.

Conclusion: Tandem mass spectrometry newborn screening has detected 3 cases of MADD in 100 000 tests, 1 with classical symptoms. All of these have been clinically riboflavin-responsive.

153-P

A BROTHER AND SISTER WITH LIPID STORAGE MYOPATHY DUE TO MILD FORM OF MULTIPLE ACYL CoA DEHYDROGENASE DEFICIENCY

MD Bain, M Pourfarzam, D Turnbull, CMM de Sousa, RA Chalmers
St. George's Hospital Medical School, London and University of Newcastle upon Tyne, UK

A nine year old boy (Mo) was investigated for a 4 year history of weakness, primarily in proximal limbs and neck flexors. Parents were consanguineous. An older (6 years) and younger (3 years) sister were well. Investigations showed: mild elevation of creatine kinase (CK)(543 IU/L, NR 30–250), a myopathic electromyograph, and lipid storage on muscle biopsy. Free carnitine was low and acyl-carnitine elevated in both plasma and urine. Initial urine organic acids showed mild dicarboxylic aciduria (ethylmalonic, glutaric, adipic, hexanoylglycine) but with increased levels in later samples. Fatty acid oxidation studies in cultured fibroblasts (riboflavin supplemented/deficient medium) showed no deficiency. There was symptomatic and biochemical improvement with L-carnitine 100 mg/kg/day and riboflavin 1 g/day. At age 7 the younger sister (Mu) was found to have very mild weakness in a similar distribution to Mo. CK was slightly elevated (196 U/L, NR 15–130). Plasma free carnitine 29.5 μmol/L previously, was now low (12.5, NR 30–38) as was urinary free carnitine. She had a slight dicarboxylic aciduria but not the abnormalities initially seen in Mo. Muscle biopsy showed the same lipid storage myopathy. Riboflavin and L-carnitine were beneficial. Tandem MS of blood spots on both siblings has shown elevated levels of short, medium and long chain acylcarnitines indicative of multiple acyl CoA dehydrogenase deficiency. There has been minimal objective clinical progression of the weakness with treatment over 11 years in Mo with intermittent organic aciduria that may well relate to compliance with therapy.

154-P

MYOPATHY IN ADOLESCENCE AS THE INITIAL PRESENTATION OF RIBOFLAVIN RESPONSIVE GLUTARIC ACIDURIA TYPE II

M Topcu[1], G Haliloglu[1], B Talim[2], D Yalnizoglu[1], T Coşkun[3]
Hacettepe University Children's Hospital, Department of Pediatric Neurology[1], Pathology[2] and Nutrition and Metabolism[3], Hacettepe University, Ankara, Turkey

Objective: Glutaric aciduria type II, or multiple acyl-CoA dehydrogenase deficiency is an autosomal recessively inherited defect of mitochondrial energy metabolism. The disease may be due to deficiency of either electron transfer flavoprotein (ETF) or electron transfer flavoprotein ubiquinone oxidoreductase (ETF-QO). **Methods**: The clinical and biochemical characteristics of a 17-year-old girl with late-onset glutaric aciduria type II is reported. The patient presented at the age of 16 years with the complaints of easy fatique for the last 3–4 months. She had no previous illness, and prenatal, natal and postnatal history was uneventful. Parents were relatives, and the family history was otherwise negative. Neurological examination revealed generalized weakness, muscle atrophy and hypoactive deep tendon reflexes. Initial diagnostic tests revealed elevated liver function tests and serum creatine kinase (CK) level was 2098 U/L (N: <200). Muscle biopsy was consistent with lipid myopathy. Urinary organic acid profile showed excretion of 2-OH-glutaric acid, ethylmalonic acid, 3-OH butyric acid, adipic acid and 2-OH glutaratlactone. Tandem mass spectrometry showed a normal carnitine and acyl carnitine profile. Biochemical investigations in fibroblasts showed that palmitate was oxidized normally and no abnormal acylcarnitines were found in the medium of cells loaded with palmitate. Further mutational analysis is pending. **Results and Conclusions**: The clinical manifestations improved remarkably after the administration of riboflavin therapy. During the course of the treatment liver function tests and CK levels returned to normal. We conclude that patients presenting with unexplained myopathy in adolescence should be screened for inherited metabolic disorders.

155-O

VARIABLE BIOCHEMICAL EXPRESSION IN A FAMILY WITH A HOMOZYGOUS ETFA MUTATION CAUSING MULTIPLE ACYL-CoA DEHYDROGENASE DEFICIENCY (MADD)

Olpin SE[1], Olsen RKJ[2], Andresen BS[2], Clark S[1], Gregersen N[2], Manning NJ[1], Bonham J[1], Leonard JV[3]

[1]Clinical Chemistry, Sheffield Children's Hospital, UK; [2]Research Unit for Molecular Medicine, University of Aarhus, Denmark; [3]Biochemistry Nutrition and Metabolism Unit, Institute of Child Health, London, UK

We describe three infants with MADD in an extended consanguineous kindred presenting with severe cardiomyopathy. The index case died at 3 months. Fatty acid oxidation in fibroblasts with myristate (M) and palmitate (P) was $4 \pm 0.9\%$ and $5 \pm 1.5\%$ of controls (4 assays) respectively. Prenatal diagnosis in the next pregnancy by fatty acid oxidation in cultured CV cells gave $50 \pm 4.0\%$ (M) (3 assays, $n = 8$) and 57% and 56% (P) (2 assays). Maternal contamination was excluded and the progeny AA presented at 6 months with cardiomyopathy. However, fibroblast fatty acid oxidation was 50% and 46% (M) (2 assays) and $47 \pm 7\%$ (P) (3 assays), closely reflecting the CV cells. A distant cousin also presented in the first month with cardiomyopathy. Fatty acid oxidation flux in fibroblasts gave $13 \pm 9\%$ (M) and $11 \pm 6\%$ (P) (4 assays). Genomic DNA-based mutation analysis of *ETFA*, *ETFB* and *ETFDH* genes showed that all three patients were homozygous for a novel G to A substitution located 40 nucleotides upstream of the start codon in the 5′UTR of ETFA gene (–40G > A). This mutation is located 3 nucleotides upstream of the nucleotide thought to acquire the 5′7-methylguanosine cap important in translation. RT-PCR ETFA mRNA was low in all 3 cell lines but with significantly higher amounts in AA. We propose that this mutation results in variable mRNA expression/stability with resultant biochemical heterogeneity.

156-P

VARIABLE PHENOTYPE AMONG CHIDLREN WITH 3-HYDROXYISOBUTYRIC ACIDEMIA

Ngu LH, Choy YS, Pertiwi AKD, Zabedah Y, Keng WT, Chen BC

Genetic and Metabolism Unit, Pediatric Institute, Kuala Lumpur Hospital, Malaysia

3-Hydroxyisobutyric acidemia is a rare inborn error of valine metabolism. The metabolic service in Kuala Lumpur Hospital (the only referral centre for the country) had identified a cohort of 5 patients with 3-hydroxyisobutyric academia by urine organic acids GC-MS over a 5-year period. The incidence was estimated to be 1 per 500 000 based on 500 000 life births a year for the country.

All of them had typical urine organic acid profile revealing large amount of 3-hydroxybutyric acid and 2-ethyl-3-hydroxy propionic acid. Free carnitine was low and esterified carnitine was elevated in all of them. However, they differed in their clinical presentations. Two of them were siblings of consanguineous parents, who presented with failure to thrive, episodic feeding intolerance and febrile illness preceding recurrent episodes of encephalopathy characterized by hypoglycemia, severe ketosis and lactic acidosis since early infancy. Both of them had dysmorphic facies characterized by frontal bossing, hypertelorism and flat nasal bridge. They had mild cognitive impairment and deafness. Both of them responded well to protein restricted diet and carnitine supplementation. The third patient presented with global psychomotor retardation and central hypotonia at 3 years of age associated with feeding difficulties and failure to thrive as well as recurrent aspiration pneumonia without metabolic acidosis. The last two cases were siblings of non consanguineous parents. Both were globally delayed and diagnosed initially as dystonic cerebral palsy. They had episodes of dystonic spasm which responded well to carnitine supplementation.

157-P

DIRECT ASSAY OF 3-METHYLGLUTACONYL-CoA HYDRATASE IN CULTURED HUMAN SKIN FIBROBLASTS: THE DEFECTIVE ENZYME IN 3-METHYLGLUTACONIC ACIDURIA TYPE I

FJ Loupatty, JPN Ruiter, L Ijlst, M Duran, RJA Wanders
Academic Medical Centre, Departments of Clinical Chemistry and Pediatrics, Emma Children's Hospital, University of Amsterdam, Amsterdam, The Netherlands

3-Methylglutaconyl-CoA hydratase (3MGH) deficiency is a rare disorder of leucine catabolism but 3-methylglutaconic aciduria, the metabolic hallmark, is a relatively common observation. In order to differentiate between true hydratase deficiency and secondary organic aciduria we developed a direct nonradioactive assay to determine 3MGH activity in cultured skin fibroblasts. The activity was measured in the reverse direction using 3-hydroxy-3-methylglutaryl-CoA as a substrate. The formation of 3-methylglutaconyl-CoA was quantified by use of reversed-phase HPLC with UV detection at 260 nm. Little variation of 3MGH activity was observed between pH 7.0 and 9.0. The assay was linear in time up to 60 min using 50 μg protein/ml. The intraassay and interassay coefficients of variation were 4.0% (n = 10) and 4.8% (n = 11), respectively. The detection limit was 20 pmol/min/mg protein. Mean (SD) control 3MGH activity was 2.1 (0.7) nmol/min/mg protein (range 1.0–3.6; n = 13). Patients with proven 3MGH deficiency had 3MGH activities below the detection limit. This rapid and nonradioactive enzyme assay allows the specific and sensitive determination of 3-methylglutaconyl-CoA hydratase activity in cultured human skin fibroblasts. The enzyme assay can be used for the reliable diagnosis of 3-methylglutaconic aciduria type I and their differentiation from other forms of 3-methylglutaconic aciduria.

158-P

LEUKOENCEPHALOPATHY IN LATE ONSET 3-METHYLGLUTACONIC ACIDURIA, TYPE I

U Engelke[1], H Kremer[1], J de Jong[1], J Schuuring[1], A van den Bergh[1], F Loupatty[2], R Wanders[2], R Wevers[1]
[1]*University Medical Centre Nijmegen, Nijmegen, The Netherlands,* [2]*University Medical Centre Amsterdam, Amsterdam, The Netherlands*

Introduction: A diagnosis of 3-methylglutaconic aciduria based on a 3-methylglutaconyl CoA hydratase deficiency (OMIM: 250950) was made in a 61-year-old female patient with presenting a slowly progressive leukoencephalopathy over more than 30 years.

Methods: Using one-dimensional and two-dimensional *in vitro* NMR spectroscopy, we measured body fluids of the patient. *In vivo* brain MR spectroscopy was performed on a 3.0 tesla magnet.

Results: Increased concentrations 3-hydroxyisovaleric acid (^{3}HIVA) and 3-methylglutaconic acid (*cis*, *trans* and *cyclic cis*) were observed in the NMR spectra of the patient's urine. In the CSF, the ^{3}HIVA concentration was 10 times higher than in plasma of the patient. Only the *cis*-isomer of 3-methylglutaconic acid was observed in the CSF. The *in vivo* brain NMR spectrum showed an abnormal peak at 1.2 ppm that may be caused by ^{3}HIVA. The diagnosis was confirmed at the enzyme level and hetero allelic mutations were found in the gene.

Conclusions: The patient suffers from a late onset form of 3-methylglutaconic aciduria, type I. In literature, only very few patients have been described and leukoencephalopathy has not been presented before in these patients. A slowly progressive leukoencephalopathy may be a hallmark of the late onset form of this disease.

159-P

MATERNAL 3-METHYLCROTONYLGLYCINURIA DIAGNOSED ON DETECTION OF LOW FREE CARNITINE DURING NEWBORN SCREENING FOR MCAD DEFICIENCY: ABNORMALITIES IN BREAST MILK

MJ Sharrard, M Downing, NJ Manning, CE Hart, M Maloney, J Watkinson, J Allen, A Matthews, C John, JR Bonham
Sheffield Children's Hospital, UK

A female child was born at 37 weeks gestation to a healthy 38 year old. A previous male child was uneventfully breast fed. The girl was breast fed and screened by TMS acyl and free carnitine (C0) analysis for MCAD deficiency in blood spots at day 6. There was C0 depletion (3.5 μmol/L, cut-off <5) with an excess of C5 hydroxy carnitine (C5OH) of 11.97 μmol/L (normal <0.5 μmol/L) but no other abnormal acylcarnitines. At 11 days she had lost 30% of birth weight with severe hypernatraemic dehydration. Urine organic acids (by GC-MS) showed no abnormalities while blood spot abnormalities persisted, with absence of C5OH in plasma. Acyl carnitine profile from the mother showed excess C5OH (16.6 μmol/L) and C0 depletion (4.0) and her urine organic acids showed excess methylcrotonylgycine (3-MCG) and 3-hydroxyisovalerate (3-OHIVA). Breast milk contained excess C5OH (23.7 μmol/L, control 0.07), 3-MCG and 3-OHIVA compared to controls, and massive sodium (117 mmol/L, normal <10). The baby was treated with carnitine, slow rehydration, and formula feed and was normal at 4 weeks, but with elevated blood spot C5OH. The abnormal metabolites in breast milk are at variance with previous reports, although their relationship to clinical condition remains uncertain. Maternal diagnosis arose consequent to the baby's low free carnitine as part of routine MCADD screening.

160-O

3-METHYLCROTONYL-CoA CARBOXYLASE (MCC) DEFICIENCY: MUTATION ANALYSIS OF THE *MCCA* AND *MCCB* GENES INCLUDING PATIENTS DETECTED BY NEWBORN SCREENING

Dantas MF[1,2], Suormala T[1], Coelho D[1], Fowler B[1], Baumgartner MR[1,2]
[1]*Metabolic Unit, University Children's Hospital, Basel, Switzerland;* [2]*Division of Metabolism and Molecular Pediatrics, University Children's Hospital, Zurich, Switzerland*

Isolated biotin-resistant MCC deficiency is an autosomal recessive disorder of leucine catabolism. Introduction of tandem mass spectrometry (TMS) based neonatal screening revealed a surprisingly high incidence of MCC deficiency. The phenotype is variable, ranging from neonatal onset with severe neurological involvement to asymptomatic adults. MCC is a heteromeric mitochondrial enzyme composed of 2 non-identical subunits, a α subunit and a smaller β subunit, encoded by *MCCA* and *MCCB*, respectively. To date 9 *MCCA* and 13 *MCCB* mutant alleles have been reported. Here, we report molecular analysis in a further 21 subjects, 13 of which were asymptomatic and were detected by TMS. All exhibited abnormal organic acid excretion and severely deficient MCC activity in cultured fibroblasts. Six of the subjects have mutations in *MCCA*, and 15 in *MCCB*. We identified 13 novel mutant alleles including missense, nonsense and splice mutations. Transfection of reference MCC deficient transformed cell lines with mutant alleles and measurement of MCC activity confirmed that all missense mutations result in loss of function. Our data demonstrate no clear correlation between genotype and phenotype, suggesting that factors other than the MCC loci must have a major influence on the phenotype of MCC deficiency.

161-P

BIOTINIDASE DEFICIENCY: 31 PATIENTS DETECTED BY FAMILY STUDIES OF PROBANDS

T Baykal, G Gokcay, F Demir, M Demirkol
Children's Hospital, Nutrition and Metabolism Department, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey

Biotinidase deficiency (BD) is an autosomal recessive disorder of biotin recycling. The main symptoms are hypotonia, ataxia, seizures, skin rash, alopecia, developmental delay, vision problems, metabolic acidosis and respiratory abnormalities. The disease can be treated effectively with biotin. We report 31 patients with BD detected by family studies in 109 probands ascertained either through survey of symptomatic individuals ($n = 15$) or from newborn screening programs ($n = 94$). The study group consisted of parents (10 mothers, 3 father) and siblings ($n = 18$). There were 16 patients (3 mothers, 3 fathers, 10 siblings) with profound BD ($<10\%$ of mean activity) and 15 patients (7 mothers, 8 siblings) with partial BD (10–30% of mean activity). The mean $\pm$ SD of biotinidase activity (BA) of the profound and partial BD patients were 0.34 ± 0.17, 1.51 ± 0.39 nmol/min/ml (normal 4.2–8.4), respectively. In profound BD, 3 were symptomatic and all of them were siblings. The symptoms were dermatitis ($n = 1$), microcephaly ($n = 1$), developmental delay ($n = 2$), convulsions ($n = 1$). The patients with partial BD did not have any clinical symptoms except one sibling with borderline IQ score (71) determined with WISC-R Test. None of the parents were symptomatic. All of the patients were treated with biotin. The symptoms resolved in all except one patient. Family investigation of patients with BD is very important for the detection of asymptomatic patients who are at risk of exhibiting symptoms at any age.

161a-A

2-METHYL-3-HYDROXYBUTYRIC ACID INHIBITS BRAIN ENERGY METABOLISM

Rosa RB[1], de Assis DR[1], Schuck PF[1], Dalcin K[1], Maria RC[1],Ribeiro CAJ[1], Leipnitz G[1], Ferreira GC[1], Latini A[1,2], Dutra Filho CS[1], Wyse ATS[1], Wannmacher CMD[1], Perry MLS[1], Wajner M[1,2,3]
[1]Departamento de Bioquímica, ICBS, UFRGS, Porto Alegre – RS, Brasil; [2]Hospital de Clínicas, Porto Alegre – RS, Brasil; [3]Universidade Luterana do Brasil, Canoas – RS, Brasil

2-Methyl-3-hydroxybutyric acid (MHB) accumulates in 2-methyl-3-hydroxybutyryl-CoA dehydrogenase (MHBD) and β-ketothiolase deficiencies. Increased plasma concentrations of lactic acid and ketone bodies are also found in these diseases, particularly during crises. Affected patients predominantly present neurological alterations and mental retardation. Considering that the pathophysiology of these disorders are virtually unknown and that the biochemical findings may suggest an impairment of energy production, the objective of the present work was to investigate the *in vitro* effect of MHB on several parameters of energy metabolism in cerebral cortex from young rats. It was demonstrated that MHB inhibited CO_2 production when glucose, acetate and citrate were used as substrates at concentrations as low as 0.01 mmol/L, indicating a blockage of the Krebs cycle. In addition, an inhibition of the activity of the respiratory chain complex IV was observed, which may possibly explain the reduced CO_2 generation. Moreover, total and mitochondrial creatine kinase activities were also diminished by 0.1 mmol/L MHB. If the *in vitro* derangement of brain energy metabolism detected in this study also occurs *in vivo*, it is tempting to speculate that an impairment of the energy metabolism may contribute, at least in part, to the neurological dysfunction found in β-ketothiolase and MHBD deficiencies.

162-P

ASYMPTOMATIC CHILDREN WITH ISOBUTYRYL-CoA DEHYDROGENASE DEFICIENCY AND 2-METHYLBUTYRYL-CoA DEHYDROGENASE DEFICIENCY FOUND BY NEWBORN SCREENING

JO Sass[1], H Reich[2], U Steuerwald[3], S Sander[3], O Schirrmacher[4], J Zschocke[5]
[1]*Stoffwechsellabor, ZKJ, Universitätsklinikum Freiburg, Germany;* [2]*Kinderarztpraxis Drs Strüber/ König, Sulingen, Germany;* [3]*Screening-Labor, Hannover, Germany;* [4]*St. Marienhospital, Vechta, Germany;* [5]*Institut für Humangenetik, University Heidelberg, Germany*

The clinical relevance of the genetic deficiencies of 2-methylbutyryl-CoA dehydrogenase (MBD) and isobutyryl-CoA dehydrogenase (IBD), enzymes catalysing the first steps of mitochondrial beta-oxidation in the catabolic pathways of isoleucine and valine, respectively, is uncertain. The conditions have been diagnosed in very few patients with variable clinical symptoms. We now report two brothers with MBD deficiency and two unrelated children with IBD deficiency who were detected by extended newborn screening with tandem mass spectrometry. Analysis of urinary organic acids yielded 2-methylbutyrylglycinuria (indicating MBD deficiency) and isobutyryl-glycinuria (indicative of IBD deficiency). The diagnoses were confirmed by metabolism studies in fibroblasts and mutation analyses in the *ACADSB* and *ACAD8* genes, respectively. So far all four children have remained asymptomatic. Although the follow-up period is rather short, our data support the possibility that deficiencies of MBD and IBD (whose equivalent enzyme in leucine metabolism is isovaleryl-CoA dehydrogenase) may be benign biochemical variants at least in some cases. Nevertheless, in view of the limited experience with these 'new' disorders, close monitoring of affected individuals appears prudent.

163-P

CLINICAL AND BIOCHEMICAL VARIABILITY IN THREE FEMALE PATIENTS WITH 2-METHYL-3-HYDROXYBUTYRYL-CoA DEHYDROGENASE DEFICIENCY

J García-Villoria[1], A Baldellou[2], R Ofman[3], A Navarro-Sastre[1], J Ramos[4], MP Ruiz-Echarri[2], M Rodes[1], RJA Wanders[3], A Ribes[1]
[1]*Institut Bioquímica Clinica, Corporació Sanitaria Clinic, Barcelona;* [2]*Hosp. Miguel Servet, Zaragoza;* [3]*University Hospital Amsterdam AMC, Amsterdam;* [4]*Hosp. Torrecárdenas, Almeria*

2-Methyl-3-hydroxybutyryl-CoA dehydrogenase (MHBD) deficiency is a recently described X-linked inborn error in the catabolism of isoleucine. This disorder is characterised by normal early development followed by progressive neurodegeneration in males. Only one affected female with psychomotor and speech delay has been described (Ensenauer et al. 2002).

We present two families with three affected females. Patient 1 was studied because of psychomotor retardation, family history revealed a brother who died during the neonatal period. Patients 2 and 3 were the mother and the grandmother of a male patient who died at 4 months of age. In contrast to males, none of the females showed regression but mild to moderate developmental delay. To establish the diagnosis an oral isoleucine load was performed. Results were clearly positive in two of them (one of each family) but negative in the other. Molecular studies revealed two new missense mutations, family 1: 740A→G (N247S) and family 2: 627C→T (P210S). We conclude that the diagnosis of some female patients might easily be missed by metabolite analysis alone. An accurate diagnosis of females is important in order to prevent the birth of affected offspring.

164-P

MR SPECTROSCOPY IN 3-HYDROXY-3-METHYLGLUTARYL COENZYME A LYASE DEFICIENCY

S Yano, K Moseley, A Lelis, S Bluml
Medical Genetics, Department of Paediatrics, Department of Radiology Childrens Hospital Los Angeles/University of Southern California, USA

Four patients with 3-hydroxy 3-methylglutaryl coenzyme A lyase deficiency were examined with magnetic resonance spectroscopy (MRS). Proton MRS showed an increase in glutamine in the gray matter (GM) and in the white matter (WM) compared with the age matched controls (GM: 8.39 ± 1.5, $n = 3$, controls 4.71 ± 0.64, $n = 9$; WM: 9.17 ± 2.24, $n = 4$, controls 3.39 ± 0.76, $n = 7$). This may be due to an increase of ammonia in the brain tissue although blood ammonia was normal. A decrease in Choline was observed in the WM. ($2.11 + 0.21$, $n = 4$, controls $1.77 + 0.25$, $n = 7$). Two unusual signals at 1.3 ppm and 2.43 ppm were identified in all four patients. The signal at 1.3 ppm was not consistent with lactic acid. Two of the four (age 6 and 12 y) have not had major metabolic decompensation and have normal motor and mental development although the proton MRS abnormalities were comparable to the other two severely affected patients (age 2 and 13). The proton MR spectroscopic abnormalities were reported to correlate with the degree of the disease. However, this study showed that the MRS abnormalities did not correlate with the clinical severity of the disease.

165-P

A NEW CASE OF SUCCINYL-CoA: 3-KETOACID CoA-TRANSFERASE DEFICIENCY (SCOT) – CLINICAL PICTURE IN AN ADULT MALE

J Alkén, R Wibom, J Alm, J Gustafsson, C Marcus
Paediatric Research Laboratory B62, Karolinska University Hospital, 141 86 Stockholm, Sweden

SCOT deficiency is an inborn error of the ketolytic pathway. Affected patients present with ketonemia and metabolic acidosis in infancy. This man presented in 1968 with ketoacidosis at the age of 20 months with metabolic acidosis, pH 7.13 and BE –18 mmol/L. He was treated with buffer and recovered with no permanent sequele. He has been recommended frequent carbohydrate intake since childhood, which has negatively affected his quality of life. His health has been good despite permanent ketosis.

Aim: To explore if this adult patient is SCOT deficient and to investigate if metabolic acidosis or hypoglycaemia can be provoked by a prolonged fast.

Method: SCOT and AAT (acetoacetyl-CoA thiolase) activity was investigated in cultured fibroblasts. During a 48 h fast blood levels of β-hydroxybutyrate, free fatty acids and hormones controlling the glucose homeostasis were followed every third hour. Base excess and pH was continuously measured.

Results: SCOT activity was decreased to 35% of the mean activity in controls lines. Activity of AAT was normal. Age at investigation was 38 yrs, BMI 23 with normal bone mineral content (DEXA). The fasting continued for 40 hours and was terminated due to BE –13, pH 7.27 and that the patient felt dizzy, had problems with vision and nausea. Starting at 0.4 mmol/L of β-hydroxy-butyrate, the levels rose to 7.3 at 40 hours. The patient remained normoglycaemic.

Conclusions: An adult patient with SCOT deficiency can handle a 24-hour fast without developing hypoglycaemia or any other metabolic derangement.

166-P

L-2 HYDROXYGLUTARIC ACIDURIA IN PORTUGAL

J Teixeira[1], M Santos[2], I Carrilho[2], L Diogo[3], P Cabral[4], R Chorão[5], A Sousa Silva[6], R Maré[7], ML Cardoso[8], C Barbot[2]
[1]Hospital Geral de Santo António, [2]Hospital de Crianças Maria Pia, [3]Hospital Pediátrico de Coimbra, [4]Hospital Egas Moniz, [5]Centro Hospitalar de Vila Real, [6]Hospital Srù da Oliveira, [7]Hospital de São Marcos, [8]Instituto de Genética Médica, Porto, Portugal

Objective: To report clinical and MRI data of 16 patients between 16 months and 45 years with L-2 hydroxyglutaric aciduria (L-2-OHG). **Method**: The diagnosis was confirmed by gas chromatography-mass spectrometry (GC-MS) of urinary organic acids and the absolute configuration of the acid was determined. **Results**: All the patients presented with mild mental retardation, slowly progressive cerebellar and pyramidal signs. Other associated symptoms, in variable combinations, were seizures (one inaugural status epilepticus at 45 years), macrocephaly, dystonia and dementia. MRI findings are very suggestive, with high-signal T2-weighted in dentate nuclei in all cases, abnormalities of subcortical white matter with mild swelling of gyri, high-signal of globus pallidus more often than putamina, involvement of external and extreme capsules and insular white matter, and frequent involvement of internal capsule. The extent of white matter abnormalities was divided in three groups, with reasonable correlation with clinical severity. Two patient developed gliomas. **Conclusion**: A good clinical and imagiological evaluation of many cases of L-2-OHG, as well as DNA analysis, should provide further advances in our understanding of this rare disease.

167-P

THE STAFFORDSHIRE BULL TERRIER AS A NATURALLY OCCURING ANIMAL MODEL OF L-2-HYDROXYGLUTARIC ACIDURIA

CJ Abramson[1], SR Platt[2], NM Verhoeven[3], C Jakobs[3]
[1]The Ohio State University College of Veterinary Medicine Ohio, USA; [2]Animal Health Trust, Newmarket, UK; [3]VU University Medical Center, Amsterdam, The Netherlands

Sixteen cases of L-2-HGA have been identified in Staffordshire Bull Terrier dogs in the UK. This represents the first known naturally occurring non-human model of this inborn error of metabolism. Similar to humans affected with this disorder, the dogs present with signs predominantly related to an encephalopathy including seizures and dementia. Some affected animals also show hypertonicity and dysmetria after exercise. Characteristic changes on magnetic resonance imaging (MRI) include hyperintensity of the cerebral, cerebellar, and brainstem grey matter on T2-weighted images. The most prominent areas also show hypointensity on T1-weighted images. The cause of the grey matter abnormalities compared to white matter abnormalities seen in humans is unknown at this time. Organic acid evaluation reveals elevation in L-2-hydroxyglutaric acid in the urine, cerebral spinal fluid (CSF), and plasma similar to affected humans. There are also marked elevations in CSF and urinary lysine in the affected canines which are similar to findings in affected humans. The biochemical similarities of this naturally occurring inborn error of metabolism in the Staffordshire Bull Terrier population makes them a reasonable candidate as an animal model of the human disease.

168-P

ENANTIOMERIC DETERMINATION OF URINARY *D*-2- AND *L*-2-HYDROXYGLUTARATE BY STABLE-ISOTOPE DILUTION LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

EA Struys, EEW Jansen, NM Verhoeven, C Jakobs
Metabolic Unit, Department of Clinical Chemistry, VU University Medical Center, Amsterdam, The Netherlands

The differential diagnosis of *D*-2-hydroxyglutaric aciduria (*D*-2-HGA), *L*-2-hydroxyglutaric aciduria (*L*-2-HGA) and the combined *D/L*-2-hydroxyglutaric aciduria (*D/L*-2-HGA) can only be accomplished by the enantiomeric separation of *D*-2- and *L*-2HG. We developed a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the determination of *D*-2- and *L*-2-HG. Urine samples of 20 µl were mixed with 250 µl of methanol containing the internal standards (3,3,4,4-$^2H_4$-*D/L*-2-HG), and subsequently dried. The analytes were derivatized using diacetyl-*L*-tartaric anhydride (DATAN) to obtain diastereomers, which were separated on an achiral C_{18}-HPLC column and detected by the MS/MS operating in multiple reaction-monitoring mode. The use of DATAN as reagent resulted in baseline separated peaks of the formed diastereomers of *D*-2- and *L*-2-HG, with a total runtime of five minutes. The inter- and intraassay CVs for *D*-2- and *L*-2-HG ranged from 3.4 to 6.2%. Mean recoveries of *D*-2- and *L*-2-HG, performed on two concentration levels were 94%. Detection limit of the presented method was 20 pmol for a sample volume of 20 µl, representing a concentration of 1 µmol/L. Method comparison of the LC-MS/MS method with the GC/MS method, in which *D*-2- and *L* -2-HG were derivatized with R-(–)-butanol, showed good agreement.

169-P

DEMONSTRATION OF *D*-2-HYDROXYGLUTARATE (*D*-2HG) GENERATING TRANSHYDROGENASE ACTIVITY IN HOMOGENATES OF HUMAN LIVER AND LYMPHOBLASTS

EA Struys[1], NM Verhoeven[1], KM Gibson[2], C Jakobs[1]
[1]*Metabolic Unit, Department of Clinical Chemistry, VU University Medical Center, Amsterdam, The Netherlands;* [2]*Department of Molecular Medical Genetics, Oregan Health and Science University, Portland, OR, USA*

The existence of the enzyme hydroxyacid-oxoacid transhydrogenase (E.C. 1.1.99.24) was first described in 1988, and activity was documented in rat liver, kidney and brain. It has never been proven to exist in human. We incubated human liver and lymphoblast homogenates with $^2H_6$-GHB and 2-KG in the absence of co-factors, and detected incorporation of ^2H specifically in *D*-2HG. This indicated the stereo-specific transfer of a deuterium from $^2H_6$-GHB to 2-KG. *L*-3-hydroxybutyrate and *D/L*-3-hydroxyisobutyrate could also serve as proton donors. To investigate the biochemical significance of this enzyme, we measured the levels of *D/L*-2HG in body fluids derived from patients with succinic semialdehyde dehydrogenase (SSADH) deficiency, in which GHB is highly elevated. Mean urine levels of *D*-2HG in SSADH ($n = 14$) were 34 µmol/mmol creatinine (controls < 17), mean plasma levels ($n = 11$) were 3.0 µmol/L (controls < 1), and mean cerebrospinal fluid levels ($n = 8$) were 1.5 µmol/L (controls < 0.4). *L*-2HG was not increased. GHB in physiological fluids derived from *D*-2HG aciduria patients was normal. This is the first demonstration of a human hydroxyacid-oxoacid transhydrogenase in human. The role of this enzyme in SSADH deficiency and *D*-2HG aciduria, and in detoxification of GHB during illicit consumption, requires further investigation.

170-P

GAMMA-HYDROXYBUTYRATE (GHB) METABOLISM TO D-2-HYDROXYGLUTARATE (D-2-HG) AND 4,5-DIHYDROXYHEXANOATE (DHHA): FURTHER PATHOMECHANISMS IN SUCCINATE SEMIALDEHYDE DEHYDROGENASE (SSADH) DEFICIENCY

EA Struys[1], NM Verhoeven[1], EEW Jansen[1], HJ ten Brink[1], TG Burlingame[2], M Gupta[2], LS Quang[3], T Maher[4], AK Goodwin[5], EM Weerts[5], C Jakobs[1], KM Gibson[2]
[1]Clinical Chemistry, VUMC, Amsterdam; [2]Molecular Medical Genetics, Oregon Health and Science University, Portland, OR, USA; [3]Pediatric Pharmacology, Case Western Reserve University, Cleveland, USA; [4]Department Pharm. Science, Massachusetts College Pharm. Health Science, Boston, USA; [5]Behavior Biology, Johns Hopkins, Baltimore, USA

We hypothesized that GHB, a GABA analogue both therapeutically and illicitly consumed and elevated in SSADH deficiency, might be metabolized to potential neurotoxins (i.e. D-2-HG (catalyzed by D-2-HG transhydrogenase) and DHHA (a putative derivative of GABA-derived succinate semialdehyde (SSA)). Murine SSADH-deficient tissues (brain (b), liver (l), kidney (k)) showed increased D-2-HG (0.4–5.2 μmol/100 mg protein (b,l,k); control <0.14, $n = 6$) and elevated DHHA (0.4–5.1 (l,k); control <0.3: 0.10–0.14 (b); control, undetectable). We then delivered acutely the GHB precursors gamma-butyrolactone (GBL) and 1,4-butanediol (1,4-BD;1000 mg/kg i.p.) to rats, and administered chronic GHB (750–1000 mg/kg/d, i.g. or i.v.) to baboons. In rat, GBL and 1,4-BD led to rapid GHB accumulation and increased urine D-2-HG (227–343 mmol/mol creat, $n = 11$; saline vehicle <95). In baboon, urine D-2-HG was 77–2586 ($n = 8$, water vehicle <6) with plasma 12–112 mmol/L (water vehicle <2). D-2-HG and DHHA may represent metabolites of GHB and/or SSA which induce neuropathology through oxidative stress and neuroexcitation.

171-P

SSADH DEFICIENCY: 4,5-DIHYDROXYHEXANOIC ACID INHIBITS UBIQUINONE-DEPENDENT RESPIRATORY CHAIN COMPLEXES I–III

Okun JG[1], Sauer S[1], ten Brink HJ[2], Jakobs C[2], Hoffmann GF[1], Gibson KM[3], Kölker S[1]
[1]University Children's Hospital, Heidelberg, Germany, [2]Department of Clinical Chemistry, VU University Medical Center, Amsterdam, The Netherlands, and [3]Oregon Health and Science University, Portland, OR, USA

Succinate semialdehyde dehydrogenase (SSADH) deficiency shows a variable clinical phenotype, frequently presenting with retarded speech and psychomotor development, hypotonia, and seizures. Biochemically, the disease is characterized by an accumulation of GABA, gamma-hydroxybutyric acid (GHB) and 4,5-dihydroxyhexanoic acid (DHHA). We studied the effect of these metabolites on mitochondrial respiratory chain activity in submitochondrial particles from bovine heart. GABA and GHB did not affect the catalytic activities of complexes I–V. In contrast, DHHA inhibited the ubiquinone-dependent complexes I, II and III in a concentration-dependent way, in particular complex III ($IC_{50} = 2$ mmol/L). We investigated the single enzyme complex I–V of respiratory chain in tissue homogenates (liver, muscle, kidney, cerebellum, cortex, brainstem, hippocampus) of *Aldh5a1*$^{-/-}$mice a mouse model for SSADH deficiency. We could not find any changes in the activities of single respiratory chain complexes I-V between *Aldh5a1*$^{-/-}$and wild-type mice. In addition, *Aldh5a1*$^{-/-}$ mice revealed reduced glutathione levels in liver and brain. In conclusion, these results suggest that beyond GHB-induced effects DHHA-induced inhibition of respiratory chain might play an important role in the neuropathogenesis of SSADH deficiency.

172-A

SUCCINIC SEMIALDEHYDE DEHYDROGENASE DEFICIENCY: CASE REPORT

Szlago M, Jorge L, Chamoles N
Laboratorio de Neuroquímica, Uriarte 2383, 1425 Buenos Aires, Argentina

Background: Succinic semialdehyde dehydrogenase (SSADH) deficiency is a rare disorder affecting CNS γ-aminobutyric acid (GABA) degradation. High level of urinary 4-OH-butyrate is its biochemical marker. Clinical findings include mental retardation with disproportionate language dysfunction. In same cases, brain MRI studies revealed symmetric increased T2 signal in the globus pallidi. Vigabatrin, an irreversible inhibitor of GABA transaminase is proposed as a treatment.

We report two sisters, of 6 and 10 years of age, which present with developmental delay since 6 month and 2 years respectively and a severe speech disturbance. There was not loss of skills during the evolution of the disorder. Physical examination showed macrocephaly, and prominent ears. Bilateral mydriasis, not previously reported to our knowledge, was found. Both cases showed hyperintense signals in the globus pallidus in T2 and FLAIR sequences. One of the girls developed seizures, with good response to valproate. SSADH deficiency was suspected through urine organic acids analysis and confirmed by enzymatic activity assay.

Conclusions: Urine organic acid analysis should be done in patients with mental retardation or neuropsychiatric disturbance of unknown aetiology. Such study will lead to increased diagnosis of SSADH deficiency and a more accurate representation of the clinical spectrum and frequency.

173-A

NEONATAL MEVALONIC ACIDURIA WITH MITOCHONDRIAL RESPIRATORY CHAIN DEFICIENCY

K Mention[1], G Touati[1], D Rabier[1], MO Rolland[2], JM Saudubray[1], P De Lonlay[1]
[1]*Hôpital Necker-Enfants Malades, Paris, France,* [2]*Hôpital Debrousse, Lyon, France*

'A' was born to first cousin Moroccan parents. During the pregnancy an intrauterine growth failure, a ventricular dilatation and cerebellar hypoplasy were noted. After one week of life, the patient presented with fever, increase in size of liver and spleen, failure to thrive, hypotonia and a morbilliform migrant rash. He had dysmorphic features. High white cell counts, no regenerative anemia and thrombopenia were observed. The presenting picture may suggest congenital infections without any biological markers. The organic acids analysis in urines detected an important elevation of mevalonic acid (179 μmol/mmol creatinine), suggesting the diagnosis of mevalonate kinase deficiency. The diagnosis was confirmed by assay of mevalonate kinase in white blood cells with a reduced activity level of 0.03 (control level : 2.9). IgD level was normal. The analysis of the respiratory chain revealed a complex I deficiency in lymphocytes with a normal analysis in cultured fibroblasts. A non steroidal anti-inflammatory drug associated with an anti-oxidative treatment was started at 6 months of age. Despite the treatment, 'A' presented at nine months of age a developmental delay, an hypotonia, an ataxia, an hepatosplenomegaly, a failure to thrive (weight –4 SD) and reccurent episodes of fever with inflammatory biological markers (CRP : 200 mg/l, VS : 90 mm/s, anemia).

This discusses the significance of association with respiratory chain complex I deficiency and the medical treatment, anti-TNF or the interest of a bone marrow transplantation in severe neonatal mevalonic aciduria.

174-P

INHIBITION OF THE ISOPRENOID BIOSYNTHESIS PATHWAY AS THERAPEUTIC OPTION FOR MEVALONATE KINASE DEFICIENCY

MS Schneiders, RJA Wanders, HR Waterham
Laboratory Genetic Metabolic Diseases, Department of Pediatrics and Clinihemistry, Academic Medical Center, Amsterdam, The Netherlands

Hyper-IgD and periodic fever syndrome (HIDS) and mevalonic aciduria (MA) are two autosomal recessively inherited autoinflammatory disorders both caused by a deficient activity of the enzyme mevalonate kinase (MK) due to mutations in the encoding *MVK* gene. Patients of both defects suffer from periodic fever episodes associated with headache, arthritis, nausea, abdominal pain, diarrhea and skin rash. In addition to these symptoms, MA patients also have developmental delay, dysmorphic features and often die in infancy. MK activity in MA is usually below detection levels when measured in cultured skin fibroblasts of MA patients. In HIDS, however, a residual MK activity varying between 1 and 7% of the control value can be measured in fibroblasts from patients. MK is an enzyme of the isoprenoid biosynthesis pathway, which is tightly regulated to allow a constant production of the various isoprenoid molecules and to avoid over-accumulation of toxic intermediates. This regulation includes feedback regulation by eproducts, achieved predominantly through repression of transcription of the genes encoding the enzymes involved in isoprenoid biosynthesis. We here show that specific enzyme inhibitors of the pathway lead to increased *MVK* gene transcription and, as a consequence, increased MK enzyme activity in fibroblasts of MK deficient patients. The inhibitors may provide a therapeutic option for treatment of patients with HIDS and MA.

175-P

TRIAL OF ERYTHROPOIETIN TREATMENT IN A BOY WITH GLUTATHIONE SYNTHETASE DEFICIENCY

J Sykut-Cegielska, A Jurecka, J Taybert, W Gradowska, E Ristoff, E Pronicka
Children's Memorial Health Institute Warsaw, Karolinska Institutet, Huddinge University Hospital

Deficiency of glutathione synthetase (GS) is a rare inborn error of glutathione metabolism. We report 26 month-old boy with GS deficiency identified by selective screening on the basis of GC/MS and MS/MS profiles. Diagnosis was confirmed by enzymatic assay (10% activity of GS in fibroblasts). Since the first day of life respiratory failure with metabolic acidosis and deterioration have been observed in spite of symptomatic treatment. Severe metabolic acidosis, hyperbilirubinaemia and haemolytic anaemia were observed. The diagnosis of 5-oxoprolinuria was established in the sixth day of life. Treatment with natrium bicarbonate, vitamins C and E and N-acetylcysteine was introduced. Three times blood transfusions were required within six weeks. To avoid the increased possibility of hemosyderosis, a trial of erythropoietin treatment was begun at the dose of 100 U/kg s.c. since 7 wks. Next doses were administrated after three days, and then every 7–10th day. No side effects were observed. Additionally ferrum, folic acid and vitamin B_{12} were given. The last dose of erythropoietin was administrated when the patient was 10 months old. Now the patient is doing well, exhibiting normal growth and body weight with normal psychomotor development. As far as we know, such treatment with erythropoietin in the patients with GS deficiency has not been described up to now. The course of disease in our patient suggests that erythropoietin therapy could be reasonable and results in improvement of haematological status.

176-P

HIGH LEVELS OF EMA IN SUSPECTED SCAD PATIENTS ARE IN CERTAIN CASES DUE TO DEFECTS IN THE ETHE1 GENE

[1]Bischoff C, [1]Vang S, [2]Burlina A, [3]Merinero B, [3]Ugarte M, [4]Maropoulos G, [1–5]Andresen BS, [1]Gregersen N

[1]Research Unit for Molecular Medicine, Aarhus University Hospital, Denmark, [2]Department of Pediatrics, University of Padua, Italy, [3]Centre for Molecular Diagnostics, Autonomous University of Madrid, Spain, [4]Children's Hospital of Athens, Greece, [5]Institute of Human Genetics, Denmark

The most common cause of ethylmalonic aciduria is mutations in the SCAD gene. However, the recent finding that ethylmalonic (EMA) encephalopathy can be caused by defects in the ETHE1 gene, has presented another genetic explanation of EMA aciduria. The promoter region and seven exons of the ETHE1 gene in DNA preparations from 20 selected patients that were sent to our laboratory for mutation analysis in the SCAD gene were sequenced. Five were homozygous or compound heterozygous for mutations in the ETHE1 gene. These five patients all exhibited classical clinical presentations of EMA encephalopathy: petechiae, acrocyanosis, hypotonia and diarrhea. The protein encoded by ETHE1 share similarities with the human glyoxalase-II protein. Alignment of the amino acid sequence of the ETHE1 gene product with glyoxalase-II predicts that His79 of ETHE1 is a catalytic active site. Interestingly, one patient is homozygous for His79Leu. The other patients have mutations that most likely affect the synthesis, folding or stability of the protein. In conclusion: Our analysis indicates that mutations in ETHE1 are causative for the specific clinical presentation known as ethylmalonic encephalopathy, but they can not explain the majority of non-SCAD deficient patients presenting with elevated EMA.

177-O

ETHYLMALONIC ENCEPHALOPATHY IS DUE TO MUTATIONS IN ETHE1

Zeviani M[1], Tiranti V[1], D'Adamo P[2], Briem E[1], Mandel H[3], Balestri P[4], Garcia-Silva MT[5], Vollmer B[6], Rinaldo P[7], Hahn SH[7], Leonard J[8], Rahman S[8], Dionisi-Vici C[9], Garavaglia B[1], Gasparini P[2]

[1]National Naurological Institute, Milan, Italy; [2]TIGEM, Naples, Italy; [3]Haifa, Israel; [4]Siena, Italy; [5]Madrid, Spain; [6]Tübingen, Germany; [7]Mayo Clinic, Rochester, USA; [8] London, UK; [9]Hospital Bambino Gesù, Rome, Italy

Ethylmalonic encephalopathy (EE) (OMIM 602473) is an autosomal recessive disorder originally reported in Italian families. Most of the EE patients described thereafter have been, with a few exceptions, of Mediterranean or Arabic descent. EE is characterized by neurodevelopmental delay, recurrent petechiae, orthostatic acrocyanosis and chronic diarrhea, leading to death within the first decade of life. Symmetrical necrotic lesions in the deep gray matter structures are the main neuropathological features of the disease. EE is characterized by persistent lactic academia and markedly elevated urinary excretion of ethylmalonic acid (EMA). The specific activity of cytochrome *c* oxidase is reduced in skeletal muscle, but not in fibroblasts. Using homozygosity mapping, we identified the EE1 locus on chromosome 19q13. By integration of physical and functional genomic data sets, and mutational screening, we then identified *ETHE1* as the gene responsible for EE. We also demonstrated that the *ETHE1* protein product is targeted to mitochondria and internalized into the matrix after energy-dependent cleavage of a short leader peptide. We identified >20 different *ETHE1* mutations in several EE families or singleton patients. The function of ETHE1 is presently unknown; however, the severe consequences of its malfunctioning indicate an important role in mitochondrial homeostasis and energy metabolism.

178-P

NATURAL HISTORY AND THERAPEUTIC TRIAL OF COENZYME Q, CARNITINE AND VITAMINS IN CHILDREN WITH ETHYLMALONIC ENCEPHALOPATHY

Choy YS, Ngu LH, Pertiwi AKD, Zabedah Y, Hussain IHMI, George T
Genetic and Metabolism Unit, Paediatric Institute, Kuala Lumpur Hospital, Malaysia

Ethylmalonic encephalopathy (EE) is a rare metabolic disorder with devastating neurological outcome and characteristic vasculopathy. The ETHE1 gene was recently found to be the cause of this disorder affecting mitochondrial homeostasis and energy metabolism. We had identified 3 patients with EE by the characteristic clinical features and urine organic acids over a 5-year period. The incidence was estimated to be 1 per 850 000. Two patients had consanguineous parents. All of them had markedly elevated urinary excretion of ethylmalonic acid associated with profound psychomotor delay and regression since infancy, acrocynosis and characteristic tortous retinal vessels. All had MRI showing cerebral atrophy and T2 hyperintensities of the caudate nuclei and putamen. All of them had lactic acidosis and paradoxical ketosis with glucose loading. All had low total and free carnitine. Two patients with more severe involvement had recurrent episodes of metabolic crises characterized by petecheal rashes, respiratory distress and diarrhea prior to initiation of therapy. Therapeutic trial of coenzyme Q, carnitine, riboflavin, thiamine, vitamin C and E were given with other supportive therapies including optimization of anticonvulsive therapy and avoiding drugs affecting mitochondrial homeostasis. One patient had his seizure controlled without recurrence of crises for 2 years. One patient succumbed to the illness during crises in a local hospital. One patient had definite improvement in cognitive and developmental milestones.

179-P

TREATMENT AND OUTCOME OF PATIENTS WITH FATTY ACID OXIDATION DEFECTS IDENTIFIED BY EXPANDED NEWBORN SCREENING IN AUSTRIA

KonstantopoulouV[1], Möslinger D[1], Mercimek-Mahmutoglu S[1], Bodamer OA[1], Erwa W[2], Stöckler-Ipsiroglu S[1]
University Children's Hospitals of Vienna[1] and Graz[2], Austria

Objective: To evaluate therapy and clinical course of fatty acid oxidation defects (FOD) diagnosed by expanded newborn screening using tandem mass-spectrometry.

Results: 9 out of 14 patients with FOD diagnosed by newborn screening over a two year period, are followed at the University Children's Hospital, Vienna. FOD include VLCAD (n =1), LCHAD (n = 2), glutaric aciduria type II (GA II) (n = 1) and MCAD (n = 5). Total incidence of FOD was 1:11 000 newborn. With the exception of two patients with GA II and VLCAD, all patients were clinically asymptomatic when diagnosed.

Dietary therapy included restriction of fat intake (all patients) and MCT supplementation (LCHAD, VLCAD) as well L-carnitine. One infant with MCAD developed mild lactic acidosis during gastroenteritis. The patients with LCHAD and VLCAD became symptomatic during their first year of life despite vigorous therapy. At present all of them are metabolically stable. The patient with GA II died at the age of 8 months during an episode of febrile upper airway infection despite vigorous dietary therapy, L-carnitine and riboflavin supplementation.

Conclusion: Although early diagnosis and therapeutic intervention in FOD may be favourable for the overall development, infants with FOD still remain at risk for metabolic decompensation.

180-P

ACYLCARNITINE PROFILES IN STABLE AND UNSTABLE CONDITIONS OF TWO PATIENTS WITH LCHAD DEFICIENCY AND CORRELATION WITH SERUM CK

E Maier[1], B Plecko[1], M Brunner- Krainz[1], W Erwa[2]
[1]Department of Pediatrics, University Hospital Graz, [2]Laboratory for Medical and Chemical Laboratory Diagnosis, Graz, Austria

Long-chain *L* 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency is an autosomal recessive disorder of mitochondrial fatty acid oxidation. Clinically patients present recurrent attacks of hypoketotic hypoglycaemia provoked by katabolic episodes. Acylcarnitine analysis by tandem mass spectrometry enables rapid diagnosis and follow up. We report on the acylcarnitine profiles of two patients with LCHAD deficiency. During stable conditions their diet consists of fat restriction (Patient 1: 17% MCT, 6% LCT; Patient 2:13% MCT, 9% LCT of total energy). In patient 1 mean C16:1OH is 0.042 μmol/L, (reference <0.02 μmol/L) and mean C18:1OH is 0.116 μmol/L, (reference <0.01 μmol/L) ($n = 9$); in patient 2 mean C16:1OH is 0.067 μmol/L and mean C18:1OH is 0.205 μmol/L ($n = 6$). The CK levels are within normal range in both when well. Under unstable conditions, usually caused by infectious disease, we observed clearly elevated values of acylcarnitines especially of C18:1OH and C16:1OH and highly elevated CK levels up to 32 280 U/L in the first patient and 10 560 U/L in the second patient (reference <160 U/L). The increase of CK levels compared to the increase of acylcarnitines is delayed by 24 hours. The higher supply of LCT in patient 2 is reflected by higher mean C18:1OH and C16:1OH levels. The correlation of acylcarnitine levels and clinical parameters, e.g. ejection fraction, retinopathy and the overall clinical course has to be elucidated. Diet should be adjusted to the individual patient.

181-P

4-PHENYLBUTYRATE (4-PBA) DEGRADATION IN HUMAN FIBROBLASTS AND THE IDENTIFICATION OF THE ENZYMES INVOLVED IN 4-PBA BETA-OXIDATION

Wanders RJA, Van Baal M, Ruiter JPN, Ijlst L
University of Amsterdam, Academic Medical Centre, Department of Clinical Chemistry and Pediatrics, Emma Children's Hospital, Laboratory Genetic Metabolic Diseases, Amsterdam, The Netherlands

Background: 4-Phenylbutyrate is used in the treatment of urea-cycle disorders as well as other disorders including X-linked adrenoleukodystrophy (X-ALD). Studies in fibroblasts from X-ALD patients and mutant X-ALD mice show that 4-PBA is capable of normalizing very-long-chain fatty acid levels. Unfortunately, studies in X-linked ALD patients have remained unsuccessful since 4-PBA turned out to be oxidized rapidly so that plasma 4PBA levels remained unappropriately low.

Goal: To study the mechanism of oxidation of 4-PBA and identify the enzymes involved in the degradation.

Results: We synthesized 4-phenylbutyryl-CoA and its various beta-oxidation intermediates and used these to resolve which of the beta-oxidation enzymes handle each of the intermediates.

Making use of mutant fibroblasts with established deficiencies at the level of SCAD, MCAD, VLCAD, LCHAD, MTP, and beta-ketothiolase, we established that 4-PBA is primarily degraded via the sequential action of MCAD, crotonase, SCHAD, and the thiolase component of MTP.

182-P

RAPID AND SIMPLE METHOD FOR DIAGNOSIS OF FATTY ACID OXIDATION DEFECTS USING WHOLE BLOOD: COMPARISON WITH REFERENCE METHOD USING CULTURED SKIN FIBROBLASTS

AF Dessein-Pouchelle[1], M Fontaine[1], A Martin-Ponthieu[1], I Kim[1], D Dobbelaere[2], L Vallée[3], N Porchet[1], G Briand[1,4]

[1]Laboratoire de Biochimie et de Biologie Moléculaire, CHRU-Eurasanté, Lille, France, [2]Unité des Maladies Héréditaires du Métabolisme, Service de Pédiatrie, Hôpital Jeanne de Flandre, Lille, France, [3]Service de Neuropédiatrie, Hôpital R. Salengro, Lille, France, [4]Laboratoire d'Application de Spectrométrie de Masse, Faculté de Médecine, Lille, France

Objective: Development of a method for rapid biochemical diagnosis of fatty-acid b oxidation deficiency enabling targeting of the defect, based on incubation of whole blood with stable labeled palmitate. **Method**: Incubation with $(^2H)_5$-palmitate; specific identification and quantitative measurement of accumulated intermediate $(^2H)_5$-acylcarnitines by tandem mass spectrometry (ESI-MS/MS). Evaluation on two sisters carrying medium-chain acyl-CoA dehydrogenase (MCAD) defect and comparison to Roe's reference method (on fibroblasts). Each assay was performed on patients and healthy subjects. Results were expressed as a ratio of $(^2H)_5$-octanoylcarnitine between patient and control, leading to estimation of residual MCAD activity. **Results**: Consistent estimated residual activities were obtained: respectively 12% and 14% on whole-blood versus 9% and 5% on skin fibroblasts. **Conclusion**: Our preliminary data suggest that this method is reliable and sufficiently sensitive for diagnosis of this defect. Other data concerning two patients suffering from VLCAD deficiency fully confirm these promising results.

183-P

CARNITINE DEFICIENCY WITH HYPOGLYCEMIC CONVULSION INDUCED BY LONG TERM ADMINISTRATION OF PIVALATE-GENERATING PRODRUGS

Ito T[1], Sugiyama N[2], Ohkubo Y[1], Ueta A[1], Sumi S[1], Narita M[3], Ohro Y[3], Mizuno M[3], Togari H[1]

[1]Department of Pediatrics, Neonatology and Congenital Disorders, Nagoya City University, Graduate School of Medical Sciences, [2]Department of Pediatrics, Aichi-Gakuin University, School of Dentistry and [3]Department of Pediatrics, Daido Hospital, Nagoya, Japan

Pivalate (trimethylacetic acid) is used as a prodrug to increase oral absorption of therapeutic agents. The administered pivalate is excreted as pivaloylcarnitine into the urine and decrease of serum carnitine is observed even in a short term therapy of pivalate prodrugs. During a long term administration, side effects such as tiredness, behavioral problems and weakness have been reported in children. We describe here a case with hypoglycemic convulsion after 6 months administration of pivalate-generating antibiotics. **Case**: A normally developed 16 months old boy was hospitalized because of afebrile convulsion for 30 minutes. His blood sugar level was 18 mg/dl and he recovered immediately after a glucose infusion. The same blood sample showed hypocarnitinemia (free: 3.2 μmol/L, total: 4.1 μmol/L). Close questioning revealed that he had happened to be administered pivalate-generating antibiotics because of repeated otitis media and/or upper respiratory tract infections for about 6 months. After stopping the drugs and avoid fasting, he never had hypoglycemia or muscle symptoms. **Conclusion:** Because muscle volume is small and dietary intake is limited, children are at high risk of carnitine deficiency. Physicians should re-realize the possibility of carnitine depletion in the administration of pivalate-generating prodrugs.

184-P

IS URINARY 3-HYDROXYGLUTARATE: A MARKER FOR SCHAD DEFICIENCY?

Cardoso ML[1], Martins E[2], Barbot C[2], Ramos A[1], Cheillan D[3], Vianey-Saban C[3], Vilarinho L[1]
[1]*Instituto Genética Médica Jacinto Magalhães, Porto, Portugal;* [2]*Hospital Maria Pia, Porto, Portugal;* [3]*Hopital Debrousse, Lyon, France*

Short-chain L-3-hydroxyacyl-CoA dehydrogenase (SCHAD) deficency is a rare severe autosomal recessive disorder (OMIM + 601609), which presents with hypoglycaemia and mild to absent ketosis. We present a new case of SCHAD deficiency in an 18 year-old-boy, 6th child of consanguineous Caucasian parents. The pregnancy, birth and neonatal period were uneventful. Since 8 months of age, he had daily myoclonic generalised seizures. A persistent hypoglycaemia was found by the age of 11/12, independently of the nutritional status. At 7 years, he did not have any language, couldn't sit, was hypotonic and had a pyramidal syndrome. Metabolic investigations showed increased ammonemia (58–88 µmol/L normal: 26-47), increased glycine in plasma and urine. Organic acids showed consistent increased excretion of 3-hydroxyglutarate ranging from 12 to 45 µmol/mmol creat (3-hydroxybutyrate and traces of adipate were also present in urine in fasting state). Glutaryl-CoA dehydrogenase activity in cultivated fibroblasts was normal (excluding an atypical case of glutaric aciduria type I) but a markedly decreased SCHAD activity was found (8 nmol/min/mg prot; controls: 111–174). Furthermore a new mutation c.587del C that originates a truncated protein lacking 117 amino acids was present in exon 5 of HADHSC gene. This is the third case of SCHAD deficiency in which a mild increased excretion of 3-hydroxy-glutarate was reported which can be a hallmark of the disorder. We suggest that 3-hydroxyglutaric acid should be carefully evaluated in hypoglycemic patients suspected of metabolic disease.

185-P

INTERFERENCE OF CEFOTAXIME IN PLASMA ACYLCARNITINE PROFILE, MIMICKING AN INCREASE OF 3-HYDROXYPALMITOLEYLCARNITINE ($C_{16:1}$-OH), USING BUTYL ESTERS

Vianey-Saban C[1], Boyer S[1], Levrat V[2], Cheillan D[1], Piraud M[1], Guffon N[2], Maire I[1]
[1]*Service de Biochimie Pédiatrique, Hôpital Debrousse, 2Service de Pédiatrie Maladies Métaboliques, Hôpital Edouard Herriot, Lyon, France*

We routinely perform acylcarnitine profile in plasma using butyl ester derivatives detected with the *m/z* 85 precursor ion scan mode, for the diagnosis of fatty acid oxidation disorders and organic acidaemias. On several occasions, we observed in plasma from neonates and infants, an increase of the signal at *m/z* 470 alone, which could correspond to 3-hydroxypalmitoylcarnitine ($C_{16:1}$-OH). $C_{16:1}$-OH is classically increased in plasma of patients with documented long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency, associated to other long-chain 3-hydroxyacylcarnitines and long-chain acylcarnitines. For one patient, we had the opportunity to analyse (with the consent of parents) acylcarnitine profile on a blood spot collected for the neonatal screening at 3 days of life, before he received any treatment : no increase of the signal at *m/z* 470 was observed. After a systematic investigation of therapeutics to the paediatricians in charge of these patients, cefotaxime (Claforan® treatment was followed by all of them at the time of acylcarnitines analysis. We subsequently analyzed plasma acylcarnitines from an infant before and after treatment by Cefotaxime: the signal at *m/z* 470 was only increased after treatment.

This interference would probably not lead to a false positive diagnosis of LCHAD deficiency, because neither other long-chain hydroxylated acylcarnitines nor long-chain acylcarnitines are increased in such cases. However, it has to be known, since the use of this antibiotic is rather frequent in the treatment of materno-fetal infections in neonatology units.

186-P

OCTN2 DEFICIENCY. WHAT HAPPENED AFTER STOPPING CARNITINE SUPPLEMENTATION BY A 14-YEAR-OLD BOY

HR Scholte[1], R Rodrigues Pereira[2]
[1]Department of Biochemistry, Erasmus MC, POB 1738, 3000 DR Rotterdam and [2]Department of Pediatrics, MCRZ St Clara Hospital, Olympiaweg 350, 3078 HT Rotterdam, The Netherlands

A boy born in 1983, got lower respiratory infection and congestive heart failure at the age of 17 months. Plasma free carnitine (FC) was 1.5 μmol/L and total carnitine (TC) 1.8 μmol/L (Contr >30). Muscle TC was 60 nmol/g (Contr >2500). Isolated muscle mitochondria showed abnormal oxidative phosphorylation with low stimulation by ADP, but he had no myopathy (Rodrigues Pereira R et al., 1988). The high affinity uptake of carnitine in cultured fibroblasts was found to be deficient, and a mutation was found in his OCTN2 gene (homozygote A632G, Tyr211Cys, Vaz FM et al. 1999). After supplementation with $4 \times \frac{1}{4}$ g L-carnitine/(kg/day) heart size and function normalized within 3 months. Plasma TC increased to control levels but muscle TC only to 0.20 μmol/g. Later on, he consumed 8–12 g L-carnitine/day. After the age of 7 years, his plasma FC decreased to an average level of 13 μmol/L. A trace of carnitine was recovered in urine (0.3% of the ingested dose). At the age of 14 years, plasma FC was 1.06 μmol/L and TC 1.11. Showing that he had stopped with carnitine. On request he took 40×330 mg carnitine = 13.2 g/day. FC became 31 μmol/L. After 3 months FC was 1.2 μmol/L and TC 1.2 μmol/L, implying that he stopped again. However, his physical condition, heart size and function remained normal. He did not notice any change after stopping/restarting carnitine supplementation. Although we tried to convince him that he was at risk for severe disease, he is still in good health. Apparently, blood carnitine is not the source of brain carnitine, and doctors may be wrong.

187-P

OCTN2 MUTATION (R254X) FOUND IN SAUDI ARABIAN KINDRED: RECURRENT MUTATION OR ANCIENT FOUNDER MUTATION?

A-M Lamhonwah[1,2], R Onizuka[1,2], SE Olpin[3], F Muntoni[4], I Tein[1,2]
[1]Department of Pediatrics, Hospital for Sick Children, [2]Department of Laboratory Medicine and Pathobiology, University of Toronto, Ontario, Canada; [3]Department of Clinical Chemistry, Sheffield Children's Hospital, Sheffield, UK; [4]Dubowitz Neuromuscular Centre, Department of Pediatrics, Hammersmith Hospital, Imperial College, London, UK

The plasmalemmal carnitine transporter defect is a potentially lethal autosomal recessive disease of childhood. The truncating R254X mutation in the OCTN2 gene results in defective high-affinity carnitine transport and has been described as a founder mutation in the Chinese with an estimated carrier rate of 1 in 125 (Tang et al. Hum Mutat. 2002;20:232). We report a 3-year old girl of Saudi Arabian descent who presented at 6 months of age with cardiomyopathy, gross motor delay, falure to thrive and very low total plasma carnitine concentrations of 7 μmol/L which responded to L-carnitine therapy. **Objective**: Molecular and biochemical analysis of OCTN2 in a kindred. **Methods and Results**: L-[^{3}H]-Carnitine uptake was 1% of controls in cultured skin fibroblasts. Western blot of skin fibroblast lysates from the proband with our specific anti-murine Octn2 antibody revealed absence of the OCTN2 protein. Sequencing of genomic DNA demonstrated a mutation in exon 4 of the *OCTN2* gene (c.760C>T) resulting in a truncated protein, R254X for which the proband was homozygous and her parents heterozygous. **Conclusion**: R254X may be a recurrent mutation that has arisen in diverse genetic backgrounds or else a very ancient founder mutation similar to the R282X and N32S mutations in OCTN2.

188-P

A THIRD HUMAN CARNITINE/ORGANIC CATION TRANSPORTER (*OCTN3*) AS A CANDIDATE FOR THE 5Q31 CROHN'S DISEASE LOCUS (*IBD5*)

A-M Lamhonwah[1], J Skaug[2], SW Scherer[2], I Tein[1]
[1]*Department of Pediatrics and Department of Laboratory Medicine and Pathobiology, and*
[2]*Department of Genetics and Genomic Biology, The Centre for Applied Genomics, The Hospital for Sick Children, The University of Toronto, Toronto, Ontario, Canada M5G 1X8*

Organic cation transporters function primarily in the elimination of cationic drugs in kidney, intestine and liver. The murine organic cation/carnitine (*Octn*) transporter family, *Octn1*, *Octn2* and *Octn3* is clustered on mouse chromosome 11. The human *OCTN1* and *OCTN2* orthologs map to the syntenic *IBD5* locus at 5q31, which has been shown to confer susceptibility to Crohn's disease. **Objective**: To show that the human OCTN3 protein, whose corresponding gene is not yet cloned or annotated in the human reference DNA sequence, does indeed exist and is uniquely involved in carnitine-dependent transport in peroxisomes. **Methods and Results**: We expressed a green fluorescent protein (GFP)-mouse *Octn3* construct in human cells (HepG2). Using biochemical and confocal microscopy analysis, we demonstrated the subcellular localization of the human OCTN3 protein in peroxisomes. Importantly, we showed that the Octn3 antibody specifically detected a human protein of the expected size (63 KDa) in HepG2. We measured L-[^{3}H]carnitine uptake in GFP-mOctn3 transfected HepG2 and determined that the Octn3 protein had a Km of 20 μmol/L for carnitine. **Conclusion**: Our data suggest a unique role for OCTN3 in the maintenance of intracellular carnitine homeostasis. Its functional properties and inferred chromosomal location of hOCTN3 at 5q31 implicate it for involvement in Crohn's disease.

188a-P

PRE-ECCLAMPSIA IN A MOTHER PREGNANT FROM A BOY SUFFERING FROM LETHAL CARNITINE-ACYLCARNITINE TRANSLOCASE (CACT) DEFICIENCY

KE Niezen[3] WB Geven[1], A Timmer[4], A van Loon[2], RJA Wanders[5], FJ van Spronsen[3]
Departments of Pediatrics[1] and Obstetrics[2], Martini Hospital, Metabolic Diseases[3] and Pathology[4], University Hospital Groningen and Metabolic Diseases[5], Academic Medical Center Amsterdam, The Netherlands

Distinct long-chain fatty acid oxidation defects (LC-FOAD) are associated with pre-ecclampsia, haemolysis elevated liver enzymes low platelets (HELLP), and acute fatty liver of pregnancy. We present a case with CACT deficiency in the child and pre-ecclampsia plus four miscarriages in the mother. After a normal pregnancy and birth of a healthy daughter followed by 3 miscarriages, the mother (full cousin of her husband) suffered from pre-ecclampsia at 35 weeks gestation in her 5th pregnancy. Magnesium sulphate infusion was ineffective, and labor was induced. The boy M. had a good start and after 3 hours bottle feeding was taken well. At 27 hours M started grunting, was hypotonic and pale, with a heart rate between 40 and 100 beats/min and normal blood pressure. M was intubated, artificially ventilated, and treated for shock and hypoglycemia (detected by Glucometer®). Despite this treatment, circulation deteriorated very rapidly and the patient died. At autopsy, macroscopic normal organs were found. Microscopy showed small fat droplets (Oro staining) in all cardiomyocytes, macro- and microscopic steatosis in all liver zones and macrovesicular steatosis in the kidney. All other tissues were normal. Cultured fibroblasts showed an abnormal palmitate loading test, elevated palmitoylcarnitine, a clear deficiency of CACT and normal activity in all other enzymes involved. A 6th pregnancy ended again in miscarriage. Therefore, this case provides additional evidence that fetal LC-FOAD are associated with maternal problems in pregnancy. This case, however, puts also questions to a possible relation between fetal fatty acid oxidation and miscarriage.

189-P

INTRONIC MUTATIONS LEADING TO ABERRANT mRNA SPLICING IN A PATIENT WITH CARNITINE ACYLCARNITINE TRANSLOCASE (CACT) DEFICIENCY

M Zater[1], J Pitt[2], B Wilcken[3], A Boutron[1], A Legrand[1], M Brivet[1]
[1]Laboratoire de Biochimie AP-HP, Hôpital de Bicêtre[1], Le Kremlin-Bicêtre, France; Genetics[2], Murdoch Institute, Melbourne and NSW Biochemical Genetics Service[3], Sydney, Australia

CACT deficiency is a rare disease of long chain fatty acid oxidation with bad prognosis. The patient, a girl, died suddenly on day 2. Heart, liver and skeletal muscle showed fatty accumulation. Tandem MS revealed a gross increase in the C16 acylcarnitine species after incubation of fibroblasts with 2D-palmitate and L-carnitine. CACT activity was nil in fibroblasts. Direct sequencing of exons and intron-exon junctions of the CACT gene revealed two intronic mutations c.326delG (maternal allele) previously described and IVS6-3C>G (paternal allele). The IVS6-3C>G mutation affects position −3 in the intron 6 acceptor site of the CACT gene. The nucleotides preceding the AG at the 3′ splice site in the human genome have very different frequencies (CAG 75%, TAG 23%, AAG 2% and GAG <1%), so we speculated that IVS6-3C>G might be a pathogenic mutation causing exon 7 skipping. The entire CACT-cDNA was amplified in three overlapping fragments. Gel electrophoresis revealed aberrant bands corresponding to skipping of exons 3, exons 3+4 (c.326delG) and exon 7 (IVS6-3C>G). Each species was identified by direct sequencing after gel extraction. Skipping of exons 3, 3+4 and 7 can be predicted to lead to the apparition of premature termination codons (140X, 289X and 210X respectively), therefore the two intronic mutations may be considered as disease-causing mutations in the patient.

190-P

BACTERIAL OVEREXPRESSION OF HUMAN CARNITINE ACYLCARNITINE TRANSLOCASE (CACT): SOLUBILIZATION AND PURIFICATION BY NI^{2+7} – AFFINITY CHROMATOGRAPHY

FVentura[1], AS Bravo[1], G Soveral[1], RJ Wanders[2], P Leandro[1], I Tavares de Almeida[1]
[1]CPM-UBMBE, Fac. Farmácia, Univ. Lisboa, Portugal; [2]Departments of Clinical Chemistry and Pediatrics, Laboratory of Genetic Metabolic Diseases, AMC, University of Amsterdam, The Netherlands

The inner mitochondrial membrane carrier CACT is essential in the transport of long-chain fatty acids into mitochondrial matrix for β-oxidation. We aimed to develop a method for high level expression and purification of soluble human CACT in *E. coli*. Human cDNA CACT was cloned in the expression vector pTrcHis and recombinant protein was obtained in high yields (1 mg/L culture) after IPTG induction. Solubilisation of inclusion bodies' proteins was achieved using 2% sarkosyl. The solubilised protein was purified by immobilized metal affinity chromatography using an imidazol gradient (20–300 mmol/L) and a non-fluorescent detergent. A highly purified protein (>90%) with a MM of ≈40 kDa (6 × His-CACT) was eluted at 200 mmol/L imidazol. Peptide pattern, obtained by CNBr cleavage, confirmed the recombinant protein sequence. Fusion partner (6 × His) was efficiently cleaved by enterokinase. Protein stability was followed by intrinsic Trp fluorescence. The pTrcHis vector allowed the overexpression of a non-toxic human mitochondrial membrane protein in *E. coli*. Moreover, the strategy of using a fusion peptide made possible the purification of human CACT by an easy single-step method. The developed protocol for human CACT production is an essential step for further functional studies of human CACT mutant forms.

191-P

METABOLITE STUDIES IN A BABY WITH SHORT/BRANCHED-CHAIN ACYL-CoA DEHYDROGENASE DEFICIENCY: EVIDENCE FOR THE INVOLVEMENT OF THE R-PATHWAY OF ISOLEUCINE CATABOLISM

A Boneh[1], BS Andresen[2], J Pitt[1]
Genetic Health Services Victoria, Royal Children's Hospital, Melbourne, Australia[1], Research Unit for Molecular Medicine and Institute of Human Genetics, Aarhus University, Denmark[2]

Short/branched-chain acyl-CoA dehydrogenase deficiency (SBCADD) is a recently identified inborn error of isoleucine metabolism. We describe an asymptomatic baby, now aged 6 months, with SBCADD detected by newborn screening because of high C5 carnitine levels (1.2 μmol/L, NL <0.8). Urine organic acid analysis showed high levels of 2-methylbutyrylglycine (36.3 h μmol/mmol, NL <2.3) and 2-ethylhydracrylic acid. Most isoleucine catabolism occurs *via* the so-called S-pathway but 2-ethylhydracrylic is an intermediate in the normally minor R-pathway. Further studies confirmed the involvement of the R-pathway in this baby. Mildly increased *allo*-isoleucine was found in plasma (2.6 μmol/L, NL <2.1). In addition, significant amounts of both the R- and S- isomers of 2-methylbutyric acid were excreted in urine as conjugates. The baby was found to be a compound heterozygote for the 1165A>G mutation (a common mutation among the Hmong population) and a novel mutation, 848A>G. We predict that this mutation affects substrate binding. The involvement of the R-pathway in the elimination of accumulating intermediates may provide an explanation for the mild phenotype in SBCADD in comparison with other disorders of branched-chain amino acid metabolism.

192-P

FIRST REPORT OF EQUINE SCAD DEFICIENCY

CM Westermann, MGM de Sain-van der Velden, JH van der Kolk, R Berger, JA Lenstra, DCJ van Boxtel, ID Wijnberg, L Dorland
Faculty of Veterinary Medicine, Utrecht University and Department of Metabolic and Endocrine Diseases, UMC Utrecht, Netherlands

A seven-year-old horse gelding of the so-called Groninger breed was admitted to Utrecht University with a history of moderate pain following exercise. Episodes of muscle pain had been reported previously. Clinical examination revealed depression, sweating, preference for lateral recumbency and reluctance to move, muscle fasciculations, tetraparesis and normal muscle consistency associated with discoloured brown urine. Blood biochemistry showed hyperglycaemia (13.1 mmol/L), elevated lactate (4.7 mmol/L), very high activities of CK (179 000 IU/L), ASAT and LDH and a normal ammonia concentration. Following diagnosis of acute exertional rhabdomyolysis, treatment with saline and NSAID's was started. In two days, the horse deteriorated and was euthanized due to permanent recumbency. Plasma and urine obtained ante mortem were used for a metabolic screening. The results were compared with values from 12 healthy control horses. In urine, GC/MS profiling revealed high concentrations of ethylmalonic acid, methylsuccinic acid and lactate. While (iso)valerylglycine and hexanoylglycine were high, butyrylglycine was strongly elevated. Tandem MS spectrometry revealed elevated free- and acylcarnitine. In addition, carnitine ester profile analysis showed very high concentrations of C4- and C5 carnitine. Furthermore, ethylmalonic acid was found in plasma. These findings are very suggestive of a deficiency of the enzyme short chain acylCoA dehydrogenase (SCAD). Analysis of the SCAD gene is in progress in order to detect a genetic defect.

193-P

THE MUTATIONAL SPECTRUM IN EMA PATIENTS: NEARLY ALL VARIATIONS ARE MISSENSE SCAD GENE VARIATIONS CAUSING IMPAIRED FOLDING OF VARIANT PROTEIN

Winter VS[1], Pedersen CB[1], Bross P[1], Kjeldsen M[1], Corydon TJ[2], Vang S[1], Gregersen N[1]
[1]*Research Unit for Molecular Medicine, Aarhus University Hospital, Skejby Sygehus, Denmark;*
[2]*Institute of Human Genetics, University of Aarhus, Denmark*

In 250 EMA patients with SCAD deficient genotypes the mutational spectrum is comprised of 2 common SCAD gene variations, 625G>A and 511C>T, and 32 rare variations. All the variations are missense variations, except 2 stop-variations. We studied the fate of 30 missense variant proteins in isolated mice liver mitochonria. Compared to wild type SCAD all variant proeins were impaired in folding to the native tetramer and showed prolonged association with the mitochondrial Hsp60 chaperonin as well as increased degradation and/or aggregation. 14 of the variant SCAD proteins were able to form tetrameric enzyme in varying amounts, while the other 16 could not form tetramers. We compared these *in vitro* results with the program PolyPhen: "Predicion of functional effects of human nsSNP's" developed at EMBL, which offer prediction of the effect of missense variations. Among 14 variant proeins, which showed production of tetrameric enzyme, 11 were predicted as probably/possibly daging by the PolyPhen, whereas 3 were preicted as beign. Of the other 16 gene variations, which did not show any tetrameric enzyme, 11 were preicted as probably/possibly damaging and 5 were predicted as benign. In conclusion Polyhen can be used as a first approach to deterine the effect of a given variation, but is still lacking sufficient accuracy.

194-O

DIAGNOSIS OF SCAD DEFICIENCY BASED ON PROTEIN MISFOLDING IN PATIENT FIBROBLASTS

Pedersen CB[1], Winter VS[1], Bross P[1], Corydon TJ[2], Ruiter JP[3], Wanders RJ[3], Gregersen N[1]
[1]*Research Unit for Molecular Medicine, Aarhus University Hospital, Skejby Sygehus, Denmark;*
[2]*Institute of Human Genetics, University of Aarhus, Denmark;* [3]*Department of Clinical Chemistry, Academic Medical Center, University of Amsterdam, The Netherlands*

SCAD deficiency is an autosomal recessive inherited disease in the mitochondrial fatty acid oxidation due to rare mutations as well as common susceptibility variations in the SCAD gene. Previous studies in SCAD deficient mice liver mitochondria showed that protein misfolding is involved in the pathogenesis of SCAD deficiency (Pedersen CB et al. J Biol Chem 2003;278(48):47449–58). We found that some of the rare SCAD mutations resulted in a severely impaired folding with high levels of insoluble SCAD protein. Based on these findings we investigate the aggregation tendency, i.e. the ratio between soluble and insoluble SCAD protein in cultured skin fibroblasts from SCAD patients, and in order to judge this aggregation ratio as a diagnostic measure for SCAD deficiency, we compared SCAD protein levels with SCAD enzyme activities. The study included fibroblasts from four patients homozygous for 319C>T, 529T>C, 511C>T and 625G>A gene variations, respectively. Protein misfolding in patient and control cells was evaluated by western blotting, and SCAD enzyme activities were measured by a HPLC method. Preliminary results indicated increased SCAD aggregation in some of the patient cells compared to control cells. Compatible with the experiments in isolated mitochondria, the modest increase was seen in the 625A/625A cells, whereas the highest amount of aggregated SCAD was found in 529C/529C cells.

195-P

SCAD DEFICIENCY AND RIBOFLAVIN THERAPY

BT van Maldegem[1], HR Waterham[2], M Duran[2], MA van Werkhoven[2], L Ijlst[2], J Ruiter[2], RJA Wanders[2], FA Wijburg[1]
[1]Department of Pediatrics and [2]Laboratory Genetic Metabolic Diseases, Academic Medical Centre, University of Amsterdam, The Netherlands

Riboflavin is a precursor of flavin adenine dinucleotide (FAD), which functions as co-factor for short-chain acyl-CoA dehydrogenase (SCAD). Although it has been recommended as a medicine in SCAD deficiency, the efficacy of treatment has not been studied. Furthermore, the FAD status could be of importance for the development of SCAD symptomatology in patients homozygous for the 625G>A SCAD gene variant. **Methods**: Riboflavin (10 mg/kg, maximum 150 mg/day) was administered to seven patients with SCAD deficiency, 4 with a rare mutation and 3 homozygous for the 625G>A variant. Before and 5 weeks after starting treatment, urine and blood samples were collected for Ethylmalonic acid (EMA), C4-carnitine (C4C) and FAD measurements. **Results and Conclusions**: FAD concentrations were normal in all patients and appear no major contributor to the development of symptoms in patients with the 625G>A variant. Six patients did not respond to riboflavin but in one patient, with a mutation similar to 2 other non-responsive patients, a subjective clinical improvement as well as a clear decrease and normalisation of EMA and C4C levels were found. This suggests that the response to riboflavin is patient specific and should be tested in all individual patients.

196-P

POTENTIAL CONFOUNDERS IN THE DIAGNOSIS OF MEDIUM CHAIN ACYL-CoA DEHYDROGENASE DEFICIENCY (MCADD)

FM Carragher[1], RN Dalton[2]
[1]Department of Chemical Pathology, [2]Department of Paediatrics, Guys Hospital, London, UK

MCADD is the most common of the fatty acid oxidation disorders, diagnosed predominantly by urine organic acid analysis. In the acute situation the biochemical hallmark of the disease is a hypoketotic hypoglycaemia with accumulation of medium chain dicarboxylic acids and the pathognomic acyl glycine metabolites hexanoyl, suberyl, and phenylpropionyl glycine in urine. However, in the non-acute state, diagnosis is often difficult with only the characteristic acyl glycine metabolites present, albeit at lower concentrations.

Hexanoyl glycine was measured by stable isotope gas chromatography-mass spectrometry (GC-MS). The assay was linear to 100 μmol/L, with assay sensitivity 0.1 μmol/L and mean recovery 95.6% in range 1–50 μmol/mmol creatinine. A reference range of <1 μmol/mmol creatinine was established.

To assess potential confounders in the diagnosis of MCADD, the hexanoyl glycine excretion was measured in five common clinical presentations. Hexanoyl glycine excretion (μmol/mmol creatinine) was significantly increased in groups of subjects after controlled fasting (n = 14, mean 0.746, p = 0.0017), on valproate therapy (n = 28, mean 0.756, p <0.0001), on Medium Chain Triglyceride feed (n = 36, mean 0.859, p<0.0001) and with ketosis (n = 52, mean 0.493, p<0.0001). compared with control subjects (n = 290, mean 0.297). In subjects with proven MCADD (n = 3), hexanoyl glycine was 9.4–20.0 μmol/mmol creatinine, clearly distinguishable from other groups.

197-O

MEDIUM-CHAIN ACYL CoA DEHYDROGENASE DEFICIENCY (MCAD): OUTCOME FOLLOWING DETECTION BY NEWBORN SCREENING

B Wilcken[1,3], C Pleffer[1], M Chaplin[2], M Haas[2,4], P Joy[1], V Wiley[1]
The Children's Hospital at Westmead[1], The Centre for Health Economics Research and Evaluation[2], University of Sydney[3], University of Technology4 Sydney, Australia

MCAD is the disorder most used to justify tandem mass-spectrometry newborn screening. Without screening, 25% of all diagnosed MCAD patients die, and many sustain intellectual damage. Adverse outcomes after diagnosis are extremely rare with proper management. In New South Wales we tested 550 000 neonates by MSMS and found 27 with MCAD and 3 older siblings, one symptomatic. One baby died on day 3, before test results. We assessed all 16 patients now aged 2–5 years. Seven had had hospital admissions, (one had 10). Of 19 total admissions, 2 were for decompensations (one on day 3), 2 unrelated to MCAD, and 15 prophylactic. There were 57 outpatient attendances. All children were growing within normal parameters and all but one perform in the normal range in communication, daily living, socialisation and motor development on the Vineland Adaptive Behaviour Scales. In the previous 23 years, 24 patients were diagnosed (20 clinical, 4 family studies). Five died during a first episode, aged 3 days to 30 months, one has severe and 5 mild intellectual handicap. These 24 patients are 23% of expected cases. This preliminary study indicates that screening for MCAD will save a few lives and prevent intellectual handicap in a few children. Costs comparisons are difficult because some never-diagnosed MCAD subjects will have had hospital admissions and other morbidities not able to be assessed.

198-A

IN VITRO EFFECT OF THE MEDIUM-CHAIN FATTY ACIDS ACCUMULATED IN MCAD DEFICIENCY ON CREATINE KINASE ACTIVITIES FROM RAT CEREBRAL CORTEX OF YOUNG RATS

D Reis de Assis, PF Schuck, A Tonin, R de Cássia Maria, CS Dutra Filho, ATS Wyse, CMD Wannmacher, M Wajner
Departamento de Bioquímica, ICBS, Universidade Federal do Rio Grande do Sul, Porto Alegre RS, Brazil

Medium-chain acyl CoA dehydrogenase (MCAD) deficiency is an inherited metabolic disorder biochemically characterized by tissue accumulation of octanoic (OA), decanoic (DA) and cis-4-decenoic (cDA) acids. Clinically, the affected patients present severe neurological alterations during periods of crisis, especially after fasting. Consring that creatine kinase (CK) plays a central role in energy metabolism, especially for high energy consuming tissues such as brain, the purpose of the present study was to investigate the *in vitro* effect of OA, DA and cDA, at concentrations found in blood of MCAD deficient patients, on creatine kinase (CK) activity from cerebral cortex of young rats. DA and OA did not affect this activity, in contrast to cDA, which significantly inhibited (about 40%) in a concentration-dependent manner CK activity in brain homogenates. Furthermore, we also demonstrated that mitochondrial and cytosolic CK activities were also inhibited by cDA.

199-A

INHIBITION OF ENERGY METABOLISM IN RAT CEREBRAL CORTEX BY THE METABOLITES ACCUMULATING IN MCAD DEFICIENCY

DR de Assis, R de C Maria, RB Rosa, GC Ferreira, CS Dutra-Filho, CMD Wannmacher, MLS Perry, ATS Wyse, M Wajner

Departamento de Bioquímica, Laboratório de Erros Inatos do Metabolismo, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

Medium-chain acyl CoA dehydrogenase (MCAD) deficient patients suffer from acute episodes of encephalopathy followed by coma and even death. However, the mechanisms underlying these neurological symptoms are poorly known. In the present study, we investigated the *in vitro* effect of octanoic acid (OA), decanoic acid (DA) and cis-4-decenoic acid (cDA) on $^{14}CO_2$ production from [U-^{14}C] glucose, [1-^{14}C] acetate and [1.5-^{14}C] citrate in cerebral cortex of young rats. OA and DA significantly reduced $^{14}CO_2$ production from acetate by around 30–40%, and from glucose by around 70%. DA significantly reduced $^{14}CO_2$ production from citrate by around 40%, while OA did not affect this parameter. cDA inhibited $^{14}CO_2$ production from all tested substrates by around 30–40%. The activity of the respiratory chain complexes were also tested in the presence of DA and cDA at the same concentrations used for $^{14}CO_2$ production. Both metabolites significantly inhibited cytochrome *c* oxidase activity by 30%, while complex II+III activity was reduced by 25% (DA) and 80% (cDA), respectively. However, only cDA inhibited complex II activity (by 30%). The results suggest that the major metabolites which accumulate in MCAD deficiency compromise brain energy metabolism. We presume that these findings may be of relevance to the understanding of the pathophysiology of the neurological dysfunction of MCAD deficient patients.

200-P

CORRELATION OF GENOTYPE AND BIOCHEMICAL PHENOTYPE IN 106 PATIENTS WITH MCAD DEFICIENCY

S Tortorelli, C Tokunaga[1], AW Strauss[1], J Winters, SH Hahn, D Matern, P Rinaldo

Mayo Clinic College of Medicine, Rochester, MN; [1] Vanderbilt University, Nashville, USA

Between 1999 and 2003 we have diagnosed biochemically 179 new patients with MCAD deficiency. While the number of those diagnosed clinically (75) and by newborn screening (104) were comparable, the proportion of the latter went from 0:9 in 1999 to 52:70 (74%) in 2003. Genotype and biochemical phenotype (median 5–95%ile range of plasma octanoylcarnitine, C8; and urine hexanoylglycine, HG) were established for 106 patients to date:

Group		No.	%	C8 (μmol/L)	HG (μg/mg cr)
Homozygous common mutation	(G/G	32	30	4.9 (0.5–11.0)	16.5 (9.0–67.4)
Compound heterozygote, one 985G	(G/X)	15	14	1.6 (0.6–3.2)	6.5 (1.6–18.9)
Tested only for 985G, one allele found	(G/unk)	19	18	0.5 (0.2–8.7)	9.7 (0.9–83.3)
Tested only for 985G, no allele found	(unk/unk)	12	11	1.5 (0.8–7.1)	18.1 (1.0–112.1)
Two other mutations	(X/X)	4	4	1.7 (1.5–3.2)	8.7 (6.4–9.1)
1 allele 985G, sequencing negative	(G/wt)	17	16	0.2 (0.1–3.0)	0.5 (0.3–27.8)
1 allele non 985G, sequencing negative	(X/wt)	6	6	0.6 (0.3–1.2)	0.6 (0.4–3.0)
No mutations found by sequencing	(wt/wt)	1	1	1.3	3.2
	Reference range:		<	0.45	0.2–1.9

As results of newborn screening, the frequency of the common mutation appears to be further declining. A possible relationship between genotype and phenotype has been observed.

201-P

LOW INTENSITY EXERCISE IN PATIENTS WITH MCAD DEFICIENCY

FA Wijburg, J Schneider, HH Huidekoper, T Westphal, M Duran
Department of Pediatrics, Academic Medical Center, Amsterdam, The Netherland

Introduction: treatment in medium-chain acyl-CoA dehydrogenase deficiency (MCADD), probably the most common fatty acid oxidation (FAO) defect, consists of avoidance of fasting, often in combination with L-carnitine medication. Although FAO is the main source of energy during low intensity exercise, it is not known whether carnitine threrapy is important for MCADD patients during exercise. We studied the efficacy of carnitine in MCADD patients during low intensity exercise. **Materials and methods:** 5 MCADD patients, ages ranging from 9 to 39 years, in good general health, were studied. Three volunteers, ages 28 to 45, were studied as controls. Patients were tested twice, once without, and once with carnitine medication (50 mg/kg/day), after a 12 hr fast, during a 2 hrs. low intensity exercise on a cycle ergometer. Blood was drawn before, during, and 1 hr after the test. Urine was collected before and after test. **Results:** patients and controls were able to exercise for 2 hr at 60% of maximum heart rate. Blood glucose, CK and lactate remained normal. Free fatty acids increased from low values to concentrations > 1 mmol/L, decreasing in the 1 hr after the exercise. Plasma total and free carnitine concentrations remained stable during the test, with low values for MCADD patients without carnitine suppletion. MCADD patients had very high concentrations of carnitine (C8- *and* C0-carnitine) in urine after exercise. **Conclusions:** Prolonged low intensity exercise is well tolerated in patients with MCADD. Despite high losses of carnitine in the urine as a result of exercise, plasma levels are kept constant, also in patients not on carnitine medication. Probably, increased synthesis of carnitine compensates for the losses.

202-O

LONG-CHAIN FATTY ACID OXIDATION DURING EARLY HUMAN DEVELOPMENT

[1]NA Oey, [1]MEJ den Boer, [1]FA Wijburg, [1]M Vekemans, [3]J Augé, [3]C Steiner, [2]RJA Wanders, [2]HR Waterham, [2]JPN Ruiter, [3]T Attié-Bitach
[1]Department of Pediatrics, [2]Department of Biochemistry, Academic Medical Centre, Amsterdam, The Netherlands, [3]Department of Genetics and INSERM U-393, Hôpital Necker-Enfants Malades, Paris, France

Introduction: The high incidence of the gestational complications AFLP and HELLP syndrome observed in mothers carrying a LCHAD/MTP deficient child and the recent reports of fetal hydrops due to cardiomyopathy in MTP deficiency, as well as the high incidence of IUGR in children with LCHAD/MTP deficiency, suggest that FAO may play an important role in the fetal-placental unit during development. Recent studies showed high activity of FAO enzymes in the human placenta. **Materials and Methods:** In this study, using *in situ* hybridization of the VLCAD and the LCHAD genes, we report on the expression of genes involved in the mitochondrial oxidation of long-chain fatty acids during early human development. Furthermore, we measured the enzymatic activity of the VLCAD and LCHAD enzymes in different human fetal tissues. Human embryos (at days 35 and 49 of development) and separate tissues (5–20 weeks of development) were used. **Results and Conclusions:** The results show a strong expression of VLCAD and LCHAD mRNA and a high enzymatic activity of VLCAD and LCHAD in a number of tissues, such as liver and heart. In addition, high expression of LCHAD mRNA was observed in the neural retina and central nervous system. The observed pattern of expression during early human development is well in line with the spectrum of clinical signs and symptoms reported in patients with VLCAD or LCHAD/MTP deficiency.

203-O

HYPOGLYCEMIA IN VLCAD-DEFICIENT MICE AS A RESULT OF IMPAIRED GLUCONEOGENESIS

Spiekerkoetter U[1,2,3], Ruiter JPN[2], Tokunaga C[3], Wendel U[1], Mayatepek E[1], Duran M[2], Wijburg FA[2], Strauss AW[3], Wanders RJA[2]
[1]University Children's Hospital, Duesseldorf, Germany, [2]Academic Medical Center, Amsterdam, The Netherlands, [3]Vanderbilt University, Nashville, TN, USA

Clinical phenotypes in humans with very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency are heterogeneous; stress-induced hypoketotic hypoglycemia being one of them. The pathogenic mechanisms of hypoglycemia in fatty acid oxidation (FAO) defects are still unknown. The VLCAD knockout (KO) mouse similarly exhibits fasting- and cold-induced hypoglycemia, and is a suitable model to study glucose metabolism in FAO disorders. Blood and liver glucose correlate in both wildtype (WT) and KO mice. In KO mice, liver glycogen is significantly reduced under non-stressed conditions. After fasting and cold stress, glycogen is completely used in WT and KO mice, and KO mice exhibit hypoglycemia. The glucose precursors glucose-6-phosphate and fructose-6-phosphate are significantly reduced in KO mouse liver suggesting impaired gluconeogenesis. Glycerol and glycerol-3-phosphate as alternative substrates for glucose production are also significantly lower in KO mouse liver. Activation of alternative glucose production pathways such as the glycerol pathway may not be able to compensate for hypoglycemia in VLCADD. *In vivo* flux studies in our mouse model are necessary to determine at what level gluconeogenesis is impaired and whether inhibitory effects on gluconeogenesis enzymes or substrate deficiency are responsible.

204-P

RHABDOMYOLISIS IS FOUND IN EARLY ONSET VERY LONG-CHAIN ACYL-CoA DEHYDROGENASE (VLCAD) DEFICIENCY, DESPITE NORMAL GLUCOSE AFTER FASTING

HM Engbers, L Dorland, EC Carbasius-Weber, G Visser
Department of Metabolic Disorders, Wilhelmina Children's Hospital, Utrecht, The Netherlands

Introduction: Three clinical types of very long chain acyl-CoA dehydrogenase (VLCAD) deficiency are known: (1) severe early onset presenting with cardiomyopathy and hypoglycemia, (2) early onset with hypoglycemia, without cardiomyopathy, (3) late onset with episodes with rhabdomyolysis and myoglobinuria. We present a patient with early onset VLCAD deficiency without cardiomyopathy, but with rhabdomyolysis despite normal glucose after fasting.

Case: The patient a boy, was admitted the second day of life with a sepsis like presentation. He had hypoglycemia (1.9 mmol/L). Organic acids and acylcarnitine profile suggested VLCAD deficiency, which was confirmed by enzyme assay in lymfocytes and fibroblasts and mutation analysis (272 C>A, 577G>C). No cardiomyopathy was found. The patient recovered quickly and did very well on a VLCAD diet. At the age of one year, a fasting test was performed to evaluate his fasting tolerance. After 12 h glucose levels were still normal (5.1 mmol/L), but CK levels had increased from 223 U/L to 1464 U/L and despite feeding, glucose infusion and carnitine suppletion, rose to 12724 U/L in 5 h and normalised in 2 days.

Conclusion: Muscle damage may occur in early onset VLCAD before hypoglycaemia is found. CK should be monitored for several hours after fasting tests.

205-A

LONG-CHAIN 3-HYDROXY-ACYL-CoA DEHYDROGENASE DEFICIENCY

Belo N[1], Diogo L[1], Mendes C[1], Tavares de Almeida I[2], Duran M[3], Wanders RJA[3], Vilarinho L[4], Garcia P[1]
[1]Unidade de Doenças Metabólicas – Hosp. Pediátrico Coimbra, [2]Centro de Patogénese Molecular – F Farmácia – Lisboa, [3]Laboratory Genetic Metabolic Diseases – AMC University of Amsterdam, [4]Instituto de Genética Médica Porto, Portugal

Since 1989, four cases of LCHAD deficiency were followed in our Metabolic Unit. **Case 1**: a girl born in 1986, was diagnosed at the age of 2, after three acute coma episodes. By the age of 5.5 years, a febrile intercurrence (with very high creatine kinase and aminotransferases levels and normoglycaemia) ended in cardiac arrhythmia and dead. **Case 2**: a boy born in 1989, presented with a Reye-like syndrome at 18 months of age, such as his older sister, who had died at 10 months of age. He is a 14-year-old who strictly complies with treatment and does regular sport activity. He has occasional acute rhabdomyolysis crisis. Regular cardiac and ophthalmologic evaluations have been normal. **Case 3**: a girl born in 2000, healthy until the age of 2 months, when feeding difficulty, hypotonia and encephalopathy arouse. Progressively, liver enlargement with hypoketotic hypoglycaemia, elevated aminotransferases, and cardiomyopathy led to death at 5 months of age. Plasma carnitine level of zero raised the hypothesis of $OCTN_2$ deficiency, which was excluded. **Case 4**: a girl born in 2002, after a pregnancy complicated with HELLP syndrome, presented with hypoglycaemic coma at 8 months of age. In case 1, diagnosis was based exclusively on metabolic profile of organic acids. In the others, enzymatic deficiency in lymphocytes or fibroblasts and homozygosity for the G1528C mutation in the LCHAD gene were demonstrated. There seems to be no correlation between genotype and phenotype, at least in this group of patients.

206-P

OCULAR FOLLOW-UP OF PATIENTS WITH LCHAD-DEFICIENCY

T Tiina, L Päivi, K Tero
Helsinki University Central Hospital, Helsinki, Finland

Progressive pigmentary chorioretinopathy is a major long-term concern in long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency. To evaluate efficacy of the current treatment in preventing progression of retinopathy, we reviewed nine patients with LCHAD deficiency caused by the homozygous G1528C mutation. Clinical examination and electroretinography (ERG) were performed every 6 to 12 months up to the age of 1.5 to 36 years (median 3.2). The patients were treated with a high-carbohydrate, low-fat diet (long-chain triglycerides 5E%) supplemented with essential fatty acids. Seven patients (age 5–12 years, median 3.2) who received early dietary therapy (started at the age of 3–8 months, median 6) had or developed granular pigmentary deposits at the level of the retinal pigment epithelium with or without pigment clumping in the macula (stage 2). None of them have developed chorioretinal atrophy or staphylomas (stage 3 to 4). Two patients (now 12 and 13 years) had visually insignificant lens opacities. In one of them, the opacities have progressed. One patient had mild myopia. Despite deteriorating ERG in two patients, all had normal visual function. Advanced fundus changes (stage 4) of two long-term survivors (now 24 and 36 years) with delayed dietary therapy did not progress further. Normal visual function and limited progression of the chorioretinopathy when dietary therapy was started within two months after the first symptoms is encouraging, although an effect on the growth of the eye and development of myopia at the onset of puberty can not yet be excluded in most of them.

207-P

THE FIRST CASE OF MITOCHONDRIAL LONG-CHAIN 3-HYDROXYACYL-CoA DEHYDROGENASE (LCHAD) DEFICIENCY IN LITHUANIA

B Skerlienė, W Lehnert
Vilnius University Clinic of Children's Diseases, Vilnius, Lithuania; Freiburg University Children's Hospital, Metabolic Unit, Freiburg, Germany

The first case of LCHAD deficiency detected in Lithuania is presented. The family history disclosed an unexpected death of an older brother at day 2. An older sister with arthrogryposis-like deformations, inguinal hernias, episodes of acute cardiopulmonary insufficiency, hepatomegaly, and hypoglycemia during acute infections died at the age of 9 months. The patient, a now 5.5 years old girl, had recurrent episodes of acute metabolic decompensation (AMD) since the age of 10 months, provoked by intercurrent infections with overnight fasting. AMD manifested as Reye-like illness with vomiting, irritability, drowsiness leading to coma, hepatomegaly, cardiomyopathy (CMP), hypoketotic hypoglycemia, metabolic acidosis, hyperammonemia, and elevated transaminases. Clinical and laboratory alterations were variable during following numerous AMD's. Pigmentary degeneration was detected at the age of 1.5 years. Severe acute rhabdomyolysis with myoglobinuria took place twice since the age of 2.5 years. Analysis of urinary organic acids by gas chromatography-mass spectrometry and of acylcarnitines in dried blood spots by tandem-mass spectrometry revealed metabolite patterns characteristic of LCHAD deficiency. The most frequent 1528G > C mutation was disclosed. Both parents were found to be heterozygous for this mutation. Under the frequent feeding using an isocaloric diet low in fat the frequency of AMD slowed down, no AMD appeared since the age of 4.5 years. Signs of CMP and hepatosteatosis resolved. Physical and psychomotor development is normal.

208-P

CLINICAL AND MORPHOLOGICAL FEATURES OF LONG CHAIN 3-HYDROXY-ACYL-CoA DEHYDROGENASE (LCHAD) DEFICIENCY

M Pohorecka, K Iwanicka, W Gradowska, J Sykut-Cegielska, M Pronicki
Children's Memorial Health Institute, Warsaw, Poland

Since 1994, twenty two unrelated children with LCHAD deficiency were identified in Poland. Eight deaths of older siblings occurred. The aim of the study was to analyse clinical course and morphological findings in LCHAD-deficiency before and after appropriate management. There were 8 LCHAD-deficient children at the age of 2–9 yrs identified before the protocol. Two patients showed neurological sequels and five died. Only three are in good condition. Diagnosis was markedly delayed in all cases, rarely established at first ALTE, frequently *post mortem*. In second period (since 2000) 12 of 14 new LCHAD deficient cases were identified during infancy (at mean age of 5.5 months). The diagnosis was established usually at first ALTE, only 4 times it was delayed (*post mortem* or post-episodic neurological sequels).

In six LCHAD-deficient patients morphological data were available (1 liver biopsy, 5 autopsies). Typical pattern of liver changes could not be identified. Diverse patterns of pathological changes were observed including different intensity of steatosis, fibrosis, non-specific inflammation, and cholestasis. The findings were usually reflected the current clinical status.

Conclusions: (1) the protocol consisted of preventive hospitalisation, appropriate diet and cardiological monitoring seems quite effective in the short-term management of the LCHAD-deficient children; (2) early diagnosis is major predictor of good prognosis in LCHAD-deficiency; and (3) LCHAD deficiency should be excluded in each sudden death with liver steatosis at autopsy.

209-P

SEVERE LACTIC ACIDOSIS IN THE ABSENCE OF DICARBOXYLIC ACIDURIA IN A NEWBORN WITH MITOCHONDRIAL TRIFUNCTIONAL PROTEIN DEFICIENCY (MTP)

Ireland JW, Heese BA, Strauss AW, Michels VV, Whiteman D, Hahn SH, Rinaldo P, Matern D
*Mayo Clinic College of Medicine, Rochester, MN; *Vanderbilt University, Nashville, TN, USA*

MTP deficiency is a fatty acid β-oxidation disorder characterized by hypoketotic hypoglycemia, cardiomyopathy, hepatopathy, coma or sudden death. The diagnosis is typically established by blood acylcarnitine and urine organic acid analyses revealing abnormal long chain 3-OH acyl-carnitine species and dicarboxylic and 3-OH dicarboxylic aciduria (DCA), respectively. We report a male newborn who developed severe nonketotic lactic acidosis (25 mM; lactate/pyruvate ratio 57) over the 1st day of life. Initial urine organic acid analysis revealed marked lactic aciduria in the absence of ketones and DCA, leading to a working diagnosis of Pyruvate dehydrogenase (PDH) deficiency. On the next day, however, acylcarnitine analysis of a plasma sample collected at the same time as the urine was consistent with MTP deficiency, which was later confirmed by bio-chemical and DNA studies demonstrating homozygosity for a 5 base pair deletion in exon 4 of MTP's α-subunit gene (Δ274-278; Asp67ter). Respiratory chain enzyme and PDH activities were normal. Despite aggressive treatment, the patient died at 5 weeks old in multiorgan failure. The family history is significant for 'non-immune hydrops' in a previous pregnancy and an affected fetus in a current pregnancy. **Conclusion**: While it remains unclear why DCA was absent in this patient's initial urine specimen, this case underscores the importance of comprehensive laboratory studies in patients with lactic acidosis to avoid incorrect conclusions and treatment efforts.

210-P

BIOCHEMICAL, CLINICAL AND MOLECULAR FINDINGS IN DEFECTS OF MITOCHONDRIAL TRIFUNCTIONAL PROTEIN

Olpin SE[1], Clark S[1], Andresen BS[2], Olsen RKJ[2],Gregersen N[2], Downing M[1], Manning NJ[1], Sharrard M[3], Bonham J[1], Muntoni F[4], Turnbull D[5], Pourfarzam M[6]
[1]Department of Clinical Chemistry and [3]Department of Paediatrics, Sheffield Children's Hospital, UK; [2]Research Unit for Molecular Medicine, Aarhus University, Denmark; [4]Department of Paediatrics and Neonatal Medicine, Hammersmith Hospital, London, UK; [5]School of Clinical Neuosciences, University of Newcastle, [6]James Spence Institute of Child Health, Royal Victoria Hospital, Newcastle, UK

Both isolated LCHAD and general MTP deficiency show a wide clinical spectrum of disease ranging from severe neonatal / infantile cardiomyopathy and early death to mild peripheral neuropathy with rhabdomyolysis. We present biochemical, clinical and mutation data in a range of patients spanning the full spectrum of disease. Fibroblast fatty acid oxidation assays show good correlation with clinical phenotype, as does fibroblast acylcarnitine profiling. Fibroblasts from infants (E474Q homozygous) presenting with severe disease giving $61 \pm 13\%$, $38 \pm 8\%$ and $18 \pm 5\%$ (8 patients) residual flux with tritiated myristate, palmitate and oleate respectively. Corresponding values in an 11 year old boy with MTP deficiency presenting with isolated mild neuro/myopathy were $92 \pm 10\%$, $82 \pm 14\%$ and $53 \pm 13\%$ (5 assays) and for a mild MTP infantile phenotype with hypoglycaemia, respiratory problems and sideroblastic anaemia $92 \pm 7\%$, $61 \pm 9\%$, $47 \pm 10\%$ (4 assays) respectively. Mutation analysis in MTP patients revealed a number of β-subunit mutations. Specific enzyme assay generally correlates poorly with phenotype, probably due to instability of mutant protein in disrupted cells.

211-O

ISOLATED DEFICIENCY OF 3-KETOTHIOLASE: FIRST CASE REPORT

AM Das, S Illsinger, T Lücke, H Hartmann, J Sander[1], RJA Wanders[2]
Department Paediatrics, Hannover Medical School, [1]Screen. Laboratory, Hannover, [2]GMZ, AMC Amsterdam

Introduction: The mitochondrial trifunctional protein (MTP) catalyzes the last 3 steps in the β-oxidation of long-chain fatty acids: 3-hydroxy-acyl-CoA dehydrogenase (LCHAD), 2,3-enoyl-CoA hydratase (LCEH) and 3-ketoacyl-CoA thiolase (LCTH). In the literature several cases with complete dysfunction of MTP or isolated LCHAD-deficiency can be found while there are no reports on isolated LCTH-deficiency so far.

Case Report: The boy was born at 35 wks of gestation as the 3rd child of healthy parents, 1 sibling died at the age of 8 days, family history was otherwise unrevealing. Soon after birth he developed tachydyspnea and needed mechanical ventilation. After neonatal screening suggested a long-chain fatty acid oxidation defect MCT-feeding was started. Temporarily, the clinical state stabilized but finally he developed pulmonary edema and cardiomyopathy and died at the age of 7 weeks.

Biochemical findings: Neonatal screening by tandem-MS at the age of 3 days suggested a long-chain fatty acid oxidation defect with increased peaks of C14:1, C16:1, C16-OH, C18:1-OH. Slight dicarboxylic aciduria was found. Activities of β-oxidation enzymes were assayed in lymphocytes and cultured skin fibroblasts. LCTH-activity (C16 substrates) was found decreased at 8% and 4% of normal values in lymphocytes and fibroblasts, respectively, while the activities of LCHAD and LCEH were normal. Mutation analysis revealed compound heterozygosity for 2 different mutations in the gene encoding for the β-subunit of MTP.

Conclusion: We describe the case of a newborn with isolated long-chain thiolase deficiency.

212-O

FUNCTIONAL CHARACTERIZATION OF ACAD-9, A NOVEL ACYL-CoA DEHYDROGENASE OF MITOCHONDRIAL BETA-OXIDATION AND ITS ROLE IN LONG CHAIN FATTY ACID METABOLISM

Ensenauer RE[1,2], He M[3], Willard JM[1], Corydon TJ[4], Vockley J[3]
[1]Medical Genetics and [2]Laboratory Medicine and Pathology, Mayo Clinic College of Medicine, Rochester, MN, USA; [3]Human Genetics and Pediatrics, University of Pittsburgh Medical Center, PA, USA; [4]Human Genetics, University of Aarhus, Denmark

Objective: Acyl-CoA dehydrogenases (ACDs) catalyze the first step of each cycle of mitochondrial β-oxidation of fatty acids. Recently, a novel ACD that is highly homologous to human very-long-chain acyl-CoA dehydrogenase, ACAD-9, has been identified by large-scale random sequencing. To characterize its biological role, we have used expression in *E. coli* and performed analysis of its substrate specificity. **Methods**: *In vitro* mitochondrial import studies were used to identify the mature amino terminus of ACAD-9. Mature ACAD-9 was overexpressed in *E. coli*, purified, and its enzymatic activity characterized with a variety of short, medium and long chain saturated and unsaturated acyl-CoAs. **Results**: A 37 amino acid-leader peptide is cleaved sequentially by two mitochondrial peptidases, mitochondrial processing peptidase and mitochondrial intermediate peptidase. The mitochondrial localization was confirmed by expression in eukaryotic cells followed by subcellular localization through confocal laser scanning microscopy. ACAD-9 showed preference for long chain unsaturated acyl-CoA substrates. This is consistent with predictions based on molecular modeling. **Conclusions**: These data suggest a unique role for ACAD-9 in the mitochondrial β-oxidation of long chain unsaturated fatty acids.

213-O

IDENTIFICATION AND CHARACTERIZATION OF NEW MEMBERS OF THE ACYL-CoA DEHYDROGENASE (ACD) GENE FAMILY

He M, Vockley J
Departments of Pediatrics and Human Genetics, University of Pittsburgh and the Children's Hospital of Pittsburgh, Pittsburgh, PA, USA

The ACDs are an evolutionarily related family of flavoenzymes involved in fatty acid β-oxidation and amino acid catabolism. Bioinformatics has recently allowed the identification of two new members of the ACD gene family. Expression studies have shown that one is an isobutyryl-CoA dehydrogenase, while the other is involved in the catabolism of unsaturated fatty acyl-CoAs. We have identified an additional group of ACD-like enzymes (ACDX1 and ACDX2) sharing 20–30% homologous to the ACDs but with genomic arrangement that predicts a complicated pattern of in vivo expression. At least two different full length cDNA species of ACDX2 are present in human liver mRNA, apparently generated by differential splicing. One covers 15 exons, encoding only an ACD like protein, while the other includes 20 exons encompassing a predicted aminoglycoside phosphotransferase domain at the amino terminus, but only a partial ACD domain at the carboxy terminus. In vitro mitochondrial import studies of the ACD like domain of ACDX2 show that a 52KD ACD-like precursor protein is imported into mitochondria and assembled into a multimer. Computer modeling of the structure of the ACD domain predicts the catalytic base of ACDX2 to be aspartic acid instead of glutamic acid as seen in other members of this gene family. There are also hydrophilic residues in the ACDX2 substrate binding pocket, previously seen only in glutaryl-CoA dehydrogenase, suggesting a possible decarboxylation function for the ACDXs. Database searches and structural modeling of the ACDX2 substrate binding pocket suggest a role for this enzyme in dicarboxylic acid metabolism, a novel substrate for ACD-like enzymes.

214-P

MITOCHONDRIAL LIVER DISEASE – A MANAGEMENT DILEMMA

P Gissen[1], I Van Mourik[1], A Minford[2], P McKiernan[1]
[1]The Liver Unit, Birmingham Children's Hospital, [2]Department of Paediatrics, Bradford General Hospital, UK

Background: Liver disease due to mitochondrial respiratory chain (MRC) abnormalities carries a poor prognosis. Evidence suggests that patients with multi-system involvement at presentation have a particularly poor prognosis unlike those with a liver specific MRC enzyme defect. We present two cases of hepatic failure in early childhood with MRC abnormalities. **Case 1**: HN presented at 10 weeks of age with failure to thrive and hypotonia. He developed progressive hepatomegaly and liver dysfunction, and was referred for liver transplant assessment. He had raised plasma lactate and muscle biopsy showed reduced cytochrome oxidase (COX) activity. Liver biopsy showed cholestasis and patchy loss of hepatocellular COX on staining. With supportive treatment the patient's liver dysfunction and hepatomegaly resolved. He has continued to make good developmental progress and is well at the age of 4.8 years. **Case 2**: JS presented in infancy with a history of failure to thrive and was found to have lactic acidaemia and deranged liver function tests. Liver biopsy showed cirrhosis and macrovesicular steatosis in regenerative nodules. Muscle biopsy showed gross complex 1 deficiency with normal morphology and no other evidence of extrahepatic disease, despite rigorous investigations. The patient developed progressive decompensation of his liver disease and underwent orthotopic liver transplantation (OLT). 16 months after OLT he is well. **Conclusions**: Medical management and prognosis is difficult in patients with MRC abnormalities causing liver disease. Some cases should be suitable candidates for OLT, but an accurate genetic diagnosis will aid management in the future.

215-O

LONG TERM FOLLOW-UP OF NEONATAL MITOCHONDRIAL CYTOPATHIES

García-Cazorla A, De Lonlay P, Nassogne MC, Touati G, Saudubray JM
Department of Metabolic Diseases, Hôpital Necker-Enfants Malades, Paris, France

Objectives: To determine the long term clinical and biochemical outcome of patients with mitochondrial cytopathies (MC) of neonatal onset.Material and Methods: 57 newborns with MC (24 CI, 12 CI+CIV, 7 CIV, 7 generalised, 3 CII, 1 CIII, 1 CV, 1 CIII+IV and 1 on muscle morphology) presenting in most of the cases with high lactate (50/57) were identified in a retrospective review (1983–2002). Clinical and biochemical characteristics of patients surviving >4 years are analysed.

Results: 33 patients died, 12 were lost in the follow-up and 12 remain alive (6 older than 4 years).

Current age	Complex deficit	Initial symptoms	Initial lactate (mmol/L)	Clinical outcome	Current lactate
4 years	III+IV M	Hypotonia	1.8	MR, hypoacusia	Normal
5 years	Generalised M	T/H/F	15.2	MR, r. tubulopathy	8–10
6 years	I M	Hepatomegaly	9.3	MR	Normal
12 years	I+IV M	T/H/F	6.1	Muscle weakness	Normal
12 years	IV M	T/H/F	1.7	Muscle weakness	Normal
18 years	I+IV hepatic	Hepatomegaly	7.6	Encephalopathy ++	Normal

M, muscle; MR, mental retardation; T/H/F, tachypnea, hypotonia, feeding problems

Conclusions: Neonatal onset MC have a very high mortality and poor prospects. Myopatic outcome cases have a better prognosis. Lactate mostly normalises in months.

216-O

LONG-TERM DCA TREATMENT OF MITOCHONDRIAL PATIENTS

BA Barshop[1], RH Haas[1,2]
Departments of Pediatrics[1] and Neurosciences[2], University of California San Diego, USA

We studied a total of 79 mitochondrial disease patients treated with dichloroacetate (DCA) up to 7 years. A cohort of 37 was treated in an open-label format for 3 wk to 7 yr (mean 3.25 yr) at 25–50 mg/kg/day, and pharmacokinetics, efficacy in lowering lactate, and general safety were shown. A second cohort was treated in a double-blind crossover protocol with 4 courses of relaively high (1000) and low (250 mg/m^2/day) doses alternating every 6 months. Of 42 in the crossover study, 21 reained at >6 months, 19 at >12 months, and 13 completed the full 24 months. Twelve of the 13 patients completing 24 months chose to continue DCA in open-label format; of those, 4 chose the high, 4 the low, and 4 a mid-range dose because they could not distinguish a difference. There were 4 who left the protocol at 12–18 months because they wanted open-label DCA and did not want further blinded treatment (3 of 4 chose low dose). There were 11 deaths, of which 9 were in the first few wk of the first course (6 on high, and 3 on low dose), 1 soon after 6 months (on low dose), and 1 after 12 months (also after changing to low dose). The longest ongoing treatment duration in the crossover study cohort is ~4 yrs. Most common side effects were extremity pain, neuropathy, or tremor, with 13 episodes in 10 patients (4 while on low, and 9 on high dose). A few cases had clear benefit attributable to DCA, including resolution of severe migraines and marked improvement of enduance. In the open-label study, neuroogic invenory showed stabilization or improvement over 1 yr. Frequency of stroke-like events seemed to be reduced. Among 8 open-label patients with 17 events in the prior 0.25–5 yr, there were a total of 2 events over 3-6 yr of DCA. Of 6 in the crossover cohort with prior stroke, 4 had events during DCA (3 soon after entering with low dose). However, more information about natural history is needed to determine how consistently there may be benefit from DCA in altering the outcome or the expression of mitochondrial disease.

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217-O

HYPOGLYCAEMIAS IN RESPIRATORY CHAIN DEFECTS BUT NORMAL LIVER FUNCTIONS: MECHANISMS

F Mochel[1], A Slama[3], G Touati[1], I Desguerre[1], I Giurgea[2], D Rabier[1], M Brivet[3], P Rustin[2], A Munnich[2], J-M Saudubray[1], P De Lonlay[1]
[1]Département de Métabolisme, [2]Département de Génétique and INSERM U-393, Hôpital Necker-Enfants Malades, Paris, [3]Service de Biochimie, Hôpital Bicêtre, Kremlin-Bicêtre, France

Hypoglycaemia occasionally results from oxidative phosphorylation defects, mainly accounted for by liver failure. Conversely, in case of respiratory chain defects preserving normal hepatic functions, the impairment in glucose metabolism remains poorly understood. We report on three unrelated children with hypoglycaemia as a main symptom associated with oxidative phosphorylation defect but normal liver functions. Two patients presented with long fast hypoglycaemias as the presenting symptom, related to complex III and complex IV deficiency. The third patient manifested both short and long fast hypoglycaemias related to complex IV deficiency. The first two patients underwent a fasting test that denoted impairment of neoglucogenesis (progressively increase of plasma lactate and alanine levels during the fast) and fatty acid oxidation (hypoketotic hypoglycaemia contrasting with increase of non esterified fatty acids) respectively. The mechanism of hypoglycaemia was not as clear for the third patient but could be partly related to a growth hormone (GH) deficiency. We suggest that hypoglycaemia can be the main symptom of oxidative phosphorylation deficiency, related, in case of long fast hypoglycaemias, to either secondary neoglucogenesis or fatty acid oxidation impairment – through a decrease in required co-factors – and to GH deficiency in case of short fast hypoglycaemia.

218-P

NEONATAL HYPERAMMONEMIA, HYPOGLYCEMIA AND LACTIC ACIDOSIS IN A CHILD WITH A MITOCHONDRIAL DISEASE

L De Meirleir[1], R Van Coster[2], B De Paepe[2], W Lissens[3], J Smet[1], E Gerlo[4], A Meulemans[3], S Seneca[3]
[1]Department of Pediatric Neurology, [3]Center of Medical Genetics and [4]Department of Clinical Chemistry, AZ-Free University Brussels, B-1090 Brussels, Belgium; [2]Department of Pediatrics, University of Gent, B-9000 Ghent, Belgium

A newborn girl presented with lethargy followed by deep coma at day 2. Severe hyperammonemia (1091 μg/dl), lactic academia (11 mmol/L) and hypoglycaemia were found. Urea cycle disorders and fatty acid and organic acidurias were excluded. The child recovered and remained with intermittent lactic acidosis, without hypoglycaemia or hyperammonemia. During the next two years she developed a Leigh's encephalopathy with typical lesions on brain MRI. She died at the age of 18 months.

OXPHOS investigations were done in muscle, liver and fibroblasts, revealing decreased complex I and II (–2SD of normal) activity. MtDNA analysis of all tRNAs and the subunits of complex I, using DGGE, did not reveal a disease causing mutation. Bleu Native Page gel electrophoresis on fibroblasts was normal. Using immunocytochemical staining with antibodies against complex I (20 kDA), complex II (Ip), complex III (core I), complex IV (subunit I) and complex V (alpha), a mosaisism could be detected for complex I and IV. These results indicated a intra mitochondrial dysfunction, which could also be detected in some mitochondria using anti dsDNA. Although no molecular anomaly has been found yet, the defect must be situated within the intra mitochondrial function.

219-P

SECONDARY IMPACT OF FATTY ACID β-OXIDATION IN RESPIRATORY CHAIN DEFECTS

A Dinopoulos[1], C Roe[2], P Morehart[1], D Roe, B Wong[1], V Mikraki[4], C Hoppel[3], T Degrauw[1]
[1]CCHMC Neurology Cincinnati; [2]IDM Baylor Univercity Dallas; [3]CIDEM Rainbow's Cleveland; [4]University of Larissa

To assess the level of interference and identify patterns of abnormalities between mitochondrial oxidative phosphorylation and fatty acid oxidation (FAO), 53 patients with a clinical suspicion of mitochondria disorder and a degree of respiratory chain (RC) defect were retrospectively reviewed. All patients underwent muscle and skin biopsy. Enzymatic assay of mitochondrial respiratory complex I–IV was performed in muscle homogenate (all in same laboratory). *In vitro* probe of fatty acid oxidation was performed in 41 patients (all in same lab) in cultured fibroblasts. Nine over 41 patients had an abnormality on *in vitro* fatty acid oxidation probe. All 9 revealed mild increased d3 butyrylcarnitine in the oxidation product and 2 of them revealed elevations on d3 C4, C6, C8, C10, C12, C14, C16 and d3 C8, C10, C12 respectively. No urine organic acid abnormalities were noted. 4 patients were found to have complex I, 2 complex III, 1 complex IV, 1 CoQ, and 1 complex II and III deficiency. 4 patients had a 'definite', 4 a 'probable' and 1 a 'possible' diagnosis of RC defect (according the Modified Adult Criteria scheme). Mild abnormalities of β-oxidation are not uncommon on RC defects. We observed a pattern which is similar but not what we typically see with a block in short-chain acyl-CoA dehydrogenase (SCAD) activity. Acylcarnitine elevations were mild, and no organic acid abnormalities were observed. A vulnerability of the SCAD enzyme is speculated with RC impairment. Although the oxidative phosphorylation and FAO pathways have been considered independent source of cellular energy production, interactions exist and clinicians should be aware of this biochemical phenotype.

220-P

MILDER DEFECTS IN RESPIRATORY CHAIN ACTIVITY MAY STILL BE SIGNIFICANT FINDINGS IN THE DIAGNOSIS OF RESPIRATORY CHAIN DISEASE

Sirrs S, Mattman A, Nussbaumer G, Mezei M, Bateman L, Vallance H
University of British Columbia, Vancouver, BC, Canada

Background: Published criteria (Bernier et al. Neurology. 2002:59:1406–11) for the diagnosis of respiratory chain disorders (RCD) weight respiratory chain activity (RCA) of $<30\%$ of normal as a major diagnostic criterion and 30–40% of normal as a minor diagnostic criterion. We evaluated this threshold to see if it distinguished patients with definite RCD from those in whom the diagnosis was thought to be unlikely. **Methods**: 101 patients referred for evaluation of RCD were reviewed and rated as definite, probable, possible or unlikely using the published criteria. Receiver operating characteristic (ROC) curves were plotted for ratios of complex (C) I and IV normalized to citrate synthase comparing RCA for those patients with definite diagnoses (n = 8; 3 with CPEO+myopathy syndromes) and those thought to be unlikely (n = 10). Data are expressed as multiples of median (MOM). **Results**: The distribution of RCA was continuous with no evidence of a threshold level at 30%. CI and CIV MOM values for patients with CPEO+ syndromes with mtDNA deletions (n = 3) were similar to those from other patients with other RCD diagnoses. ROC curves suggest that higher cut-offs in RCA (0.54–0.96 MOM for CI and 0.65–0.78 MOM for CIV) may be optimal. For CI and CIV, values of 0.62 MOM and 0.74 MOM respectively would yield a sensitivity of 50% and specificity of 90%, giving a likelihood ratio (LR) for the diagnosis of RCD of 5. Combining data from multiple centers is needed to validate this approach and allow calculation of LR at other levels of RCA. **Conclusion**: The level of RCA which may be significant in the diagnosis of RCD may be higher than previously reported.

221-P

LACTIC ACIDEMIA IN HUMAN IMMUNODEFICIENCY VIRUS-UNINFECTED INFANTS EXPOSED TO ANTIRETROVIRALS

A Noguera[1], C Fortunya[1], C Muñoz-Almagro[1], E Sánchez[2], MA Vilaseca[1], R Artuch[1], R Jiménez[1]
[1]Hospital Sant Joan de Déu, Universitat de Barcelona, [2]Catalan Agency for Health Technology Assessment and Research, Generalitat de Catalunya, Spain

Background: Exposure to nucleoside analogues in fetal life has been associated with mitochondrial toxic effects. **Objective**: To determine the prevalence, clinical evolution and risk factors for hyperlactatemia (HLA) in our cohort of HIV-uninfected children exposed to antiretrovirals. **Methods**: Prospective observational study in 127 HIV-uninfected infants born to HIV-infected women. Clinical symptoms suggesting mitochondrial dysfunction were analyzed in routine follow-up and LA plasma levels were obtained at 6 wks and 3 months, 6 months and 12 months. **Results.** Most women (85%) received HAART during pregnancy and zidovudine during labor (93%). Most children (96%) received zidovudine alone. HLA was detected in 63 children in at least one of the measurements. Mean LA levels were higher in NRTI-exposed children than controls (2.88 vs 1.61 at 6 wks, 2.78 vs 1.49 at 3 months, 1.89 vs 1.39 at 6 months and 1.71 vs 1.24 at 12 months, $p < 0.0001$; peak levels: 8.06, 10.1, 7.28 and 4.48 mmol/L). Three girls presented a mild self-limited delay in psychomotor development, with LA peak levels of 7.3, 4.0 and 4.6 mmol/L. Only the gestational use of didanosine was associated with a higher risk of HLA ($p = 0.043$). **Conclusions**: In our series, 50% of the children exposed to nucleoside analogues developed benign and self-limited HLA. When symptomatic, nucleoside analogue-induced toxicity affected neurological development.

222-P

RESPIRATORY CHAIN DEFICIENCY IN A CASE OF AICARDI–GOUTIÈRES SYNDROME

I Giurgea[1], C Barnérias[1], L Hertz-Pannier[2], D Chrétien[3], P Rustin[3], I Desguerre[1], P De Lonlay[1]
[1]Departement of Pediatrics, [2]Departement of Pediatric Radiology, [3]INSERM U-393, Hôpital Necker-Enfants Malades, Paris, France

Aicardi-Goutières syndrome (AGS) is an early-onset progressive encephalopathy characterized by calcifications of the basal ganglia, white matter abnormalities, chronic cerebrospinal fluid (CSF) lymphocytosis and/or a raised level of CSF interferon alpha (INF-α). Here, we report on mitochondrial respiratory chain deficiency in one child fulfilling criteria of AGS. The patient presented severe encephalopathy in the first year of age associated with liver enlargement and elevated serum transaminases. White cell count was normal but INF-α was increased in the CSF. Calcifications of basal ganglia were noted on the CT scan and cerebral MRI showed bilateral and symmetric hyperintensity of the posterior white matter. A complex I deficiency of the mitochondrial respiratory chain was found in the skeletal muscle, which was associated with a complex IV deficiency in cultured skin fibroblasts. An elder brother presented mental retardation, cerebellar hypoplasia and white matter abnormality related to complex IV deficiency in cultured skin fibroblasts. The question of whether this oxidative phosphorylation deficiency is primary or secondary in AGS is open to debate. We suggest giving consideration to systematic evaluation of the mitochondrial respiratory chain in skeletal muscle and skin fibroblasts of other AGS patients.

223-P

FROM MIGRAINE TO MITOCHONDRIAL RESPIRATORY CHAIN DISEASE

Almeida D[1], Diogo L[1], Garcia P[1], Grazina M[2], Silva E[3], Tavares de Almeida I[4], Oliveira C[2]
[1]Hospital Pediátrico Coimbra; [2]Centro de Neurociências Univ. Coimbra; [3]S. Oftalmologia HUC; [4]C. Patogénese Molecular- Fac. Farmácia Lisboa – Portugal

Mitocondrial respiratory chain deficiencies (MRCD) have long been regarded as encephalomyopathies. However it has been increasingly recognised that they account for a large variety of clinical symptoms in childhood. We report the case of a 14-year-old girl, who presented at 23 months of age with monthly episodes of abdominal pain, vomiting, phono and photophobia and superficial coma, lasting for several days. Fifteen months later, diagnosis of basilar migraine was evoked. By 4 years of age, long fasting hypoglycemia with hyperlactacidaemia, moderate ketonuria, dicarboxylic aciduria and sub-clinical hypertrophic cardiomyopathy, detected during a severe episode, lead to the suspicion of fatty-acid β-oxidation deficiency. Therapeutic measures were apparently successful, decreasing both frequency and length of crisis. However, β-oxidation enzyme studies in fibroblasts were normal. Upon evolution, cardiomyopathy became symptomatic. At 11-years-old, MRCD was confirmed in liver homogenate (complexes I to V: 7%, 13%, 5%, 5% and 23% of control mean in relation to CS, respectively). Liver histology showed septal fibrosis. Skeletal muscle biopsy evaluation was normal. mtDNA mutations (A3243G; T3271C; T8993G/C, A8344G; T8356C; G8363A) and deletions were excluded. Recently, myopatic symptoms with abnormal electromyography emerged and pigmentary retinopathy and elevated lactate in cerebral spectroscopy were detected. Multisystem involvement is the hallmark of mitochondrial cytopathies. However, it may take several years for it to emerge clinically.

224-A

CONSIDERATIONS ABOUT A CASE WITH CONGENITAL LACTIC ACIDAEMIA AND HIGH EXCRETION OF CITRULLINE, PROLINE, LYSINE AND PIPECOLIC ACID

R Vulturar[1], I Lupea[2], G Benga[1,3]
[1]Department of Cell and Molecular Biology of 'Iuliu Hatieganu' University of Medicine and Pharmacy, Cluj-Napoca, Romania; [2]Department of Neonatology of 'Iuliu Hatieganu' University of Medicine and Pharmacy; [3]1st Laboratory of Genetic Explorations of Cluj County Hospital, Romania

A 2-day-old boy was referred to Neonatology Department of 1st Gynaecology Clinic from Cluj-Napoca for a dramatic clinical presentation after 3 hours from birth with generalized hypotonia, respiratory functional changes, severe hyperlactacidaemia (103.4 mg/dl), hyperpyruvicemia (1.78 mg/dl), hyperammonia (222 μg/dl), elevated anionic gap (26 mEq/L) and increased lactate/piruvate ratio (>35). The couple has another child, born at 36 weeks, with similar presentation, who died in the perinatal period. The transfontanelar ultrasounds demonstrated ventricular enlargement and bilateral perventricular pseudocysts. In spite of repeated administrations of bicarbonate, the metabolic acidosis persisted and the boy died in the 13th day of life. The amino acids analysis performed from urine (by two dimensional thin layer chromatography, Wadman et al. 1980) showed a high excretion of citrulline; were also elevated excretion of proline, lysine and pipecolic acid. Unfortunately, the analysis of urinary organic acids with the ratio 3-hydroxybutyrate/acetoacetate could not be done. In spite of this, we considered that the clinical, biochemical, and imagistic aspects are likely corresponding for type B of deficiency of pyruvate carboxylase.

225-A

EFFECT OF HIGH FAT DIET ON INTRACTABLE SEIZURES IN PATIENTS WITH SUSPECTED MITOCHONDRIAL DISEASES

D Rusli Sjarif
Department of Child Health University of Indonesia School of Medicine, Dr. Cipto Mangunkusumo General Hospital, Jakarta, Indonesia

Ketogenic diet has been used widely to control intractable seizures. This high fat diet produces ketone bodies that act like GABA as an inhibitory neurotransmitter in the brain to prevent seizures. Since the fat content of our patients daily diet is less than 20% of total calories, we tailored modification of ketogenic diet which is allowing us to increase the fat content of total calories started from 50% to 80%. The aim of this study is to evaluate the efficacy and patients tolerance of this high fat diet to control intractable seizures.

We evaluated regularly 6 patients (3 females and 3 males), ages 1 to 12 years, with intractable seizures who came to our pediatric outpatient clinic between July 2001 to July 2003. All these patients have been treated with multiple anticonvulsant drugs without any progress. Every 2 weeks we evaluate urinary ketone, frequency and duration of the seizures after the initiation of high fat diet. The result showed that all patients tolerate the diet well, 4 patients decreased seizures frequency and duration, while another 2 patients had free of seizures within the first 2 weeks when the percentage of fat still 62% of total calories. None of these patients experienced ketonuria. Based on this result we assumed that these patients probably have respiratory chain disorders (complex I deficiency). Unfortunately, we are lack of facilities to performed enzymatic assay to prove the diagnosis.

226-P

BIOCHEMICAL EVALUATION AND DIETARY TREATMENT OF RECURRENT HYPOGLYCEMIC EPISODES IN PATIENTS WITH MITOCHONDRIAL DYSFUNCTION

H Zweers, M De Vries, J Smeitink, E Morava
UMC Nijmegen, The Netherlands

Hypoglycemic episodes have been sporadically reported in patients with mitochondrial oxidative phosphorylation system (OXPHOS) enzyme defects without a clear etiology. We performed a comprehensive metabolic study and a detailed dietary evaluation in four children diagnosed with OXPHOS defects (2 children with decreased pyruvate oxidation rates, one with complex I and IV defect and one with complex I and II defect), normal liver function and recurrent hypoglycemia. We observed a shortened fasting tolerance (glucose <2.8 mmol/L after 3-22 hours of fasting) in three patients. There was an increase in the serum lactate level in two children and a decreased ketone body production by the third child at the time of the hypoglycemia. Further metabolic and endocrine studies were normal. The patients were all dystrophic before the dietary intervention with an insufficient dietary intake (less the 75% requested). We initiated a treatment with complex carbohydrate supplementation in our patients. Additional diet optimization was advised aiming to prevent catabolism and improving the nutritional status in all patients, including tube feeding (during the night) for two children. No more hypoglycemic episodes have been observed in these patients and their nutritional status improved. We hypothesize a multifactorial etiology underlying the shortened fasting tolerance in OXPHOS disorders; insufficient dietary intake, a decreased amount of glycogen in liver and muscle (muscle wasting), a decreased amount of body-fat, and less effective ketone production due to mitochondrial dysfunction.

227-P

TRANSCRIPTIONAL PROFILING IN MITOCHONDRIAL DISORDERS

WJ Craigen, D Deng, F Scaglia, Z Cai, T Sheiko
Department of Human and Molecular Genetics, Baylor College of Medicine, Houston, TX, USA

Because of locus heterogeneity associated with mitochondrial disorders identifying the molecular basis for mitochondrial disorders remains difficult. In order to develop alternative strategies for establishing a diagnosis in a model mitochondrial disorder, Leigh Syndrome (LS), we analyzed the transcriptional profile of fibroblasts from a LS patient and a control subject. Cytochrome c oxidase activity of the LS patient was decreased by 6 fold. Microarray analysis was performed using the 18 K Affymetrix chip and analyzed using three software packages. We found that a candidate gene for LS, the COX assembly factor SURF1, was down-regulated approximately 4 fold and was identified by all three software packages, whereas other known COX related genes such as SCO2, COX10, COX11, and COX15 were unchanged. Mutation screening of SURF1 revealed a deletion within exon 4 on one allele leading to a premature termination codon, along with a nonsense mutation at amino acid position 196 on the other allele. Gene Set Enrichment Analysis (GSEA) showed that expression of genes involved in energy metabolism down stream of the OXPHOS pathway was also altered. For example, hypoxia induced factor-3 alpha (HIF3-alpha), a negative regulator of HIF1-alpha, was up-regulated 4.7 fold, while vascular endothelial growth factor B (VEGFB), normally induced by HIF1-alpha, was down-regulated approximately 5 fold. Thus, transcriptional profiling potentially offers an alternative, less invasive approach to the current established diagnostic tests for clinical cases where the molecular basis of disease exhibits genetic heterogeneity. It also provides novel insights into metabolic aberrations present in these disorders.

228-P

PROGRESSIVE MUSCLE ATROPHY WITH SELECTIVE LOSS OF MUSCLE FIBERS IN A PATIENT WITH MTDNA DEPLETION DUE TO TK2 DEFICIENCY

Roig M, Del Toro M, Zeviani M[1], Castellote A, García-Arumí E, Meseguer A, Vilà MR
Hospital Vall d'Hebron, Barcelona, Spain; [1]Istituto Besta, Milano, Italy

Mutations in thymidine kinase 2 (TK2) gene cause a myopathic form of the mitochondrial DNA depletion syndrome (MDS). Although clinical manifestations in these patients vary, most of them die during infancy and long-term survival is unusual. Here, we report the morphological and molecular findings, as well as a 12-year clinical follow-up in a patient with the myopathic form of MDS in whom pathogenic mutations were identified in the TK2 gene.

Morphological studies were performed in muscle biopsies that were obtained at ages 2, 5, and 12 years. Quadriceps muscle biopsy performed at age 2 depicted an extensive muscle fiber necrosis and phagocytosis, moderate fibrosis and numerous scattered fibers with diffuse increased oxidative activity and neutral lipid accumulation. At age 5, biopsy showed that the OXPHOS system was mildly affected but completely normal at age 12. At this age, biopsy showed a fairly well structured muscle integrated almost exclusively by type I fibers. Other findings included minimal lipid vacuoles and the presence of few, scattered COX negative ragged red fibers. Mitochondrial DNA depletion worsened over the time (from 75% at age 5 to 90% at age 12). TK2 activity in fibroblasts was significantly reduced (7.8% of controls) and molecular analysis revealed the presence of two allelic heterozygous mutations in the TK2 gene (T108M in exon 5 and R192K in exon 8). We propose that mtDNA depleted fibers undergo selective segmental necrosis and, with time, they progressively vanish leading to muscle atrophy and weakness.

229-O

SCREENING FOR MUTATIONS IN *DGUOK* IN PATIENTS WITH MITOCHONDRIAL DNA DEPLETION

Navarro-Sastre A[1], Playan A[3], Lopez-Gallardo E[2], Montoya J[2], Yanes L[2], Ruiz-Pons M[3], Campistol J[4], Pineda M[4], Vilaseca MA[4], Briones P[1], Ribes A[1]
[1]Institut Bioquim. Clin, Corporació Sanitària Clínic, Barcelona, [2]Fac. Veterinaria, Univ. Zaragoza, [3]Hospital Virgen de la Candelaria, Tenerife, [4]Hospital Sant Joan de Deu, Barcelona

The hepatocerebral form of mtDNA depletion has been associated to a deoxyguanosin kinase deficiency. As most of our patients presented this form, we started with the mutational study of *DGUOK* gene. We selected 26 candidate patients according to their clinical and biochemical characteristics. In 18, mtDNA depletion was demonstrated in one or more tissues by Southern blot. *DGUOK* was studied in genomic DNA by PCR and sequencing.We have found mutations in three families. Patient 1 was homozygous for 763dupGATT, a mutation previously reported by Salviati et al (2002). In addition, we have identified a new mutation which is present in homozygous form in four patients of two unrelated families. The new mutation (c677 A>G) changes histidine 226 to arginine (H226R). It is present in heterozygous form in the patients' parents, but was absent in 100 control alleles. Histidine 226 is highly conserved between species and between different deoxynucleotide kinases; its substitution for arginine would change the hydrophobicity leading to incorrect conformation of the protein. Our results have important consequences for the affected families, who may now apply for prenatal diagnosis in future pregnancies. Nevertheless, only a part of our patients presented mutations in *DGUOK*. Therefore, it is necessary to further investigate other genes and mechanisms that may be involved in the disease.

230-P

DETERMINATION OF BLOOD MONONUCLEAR CELL CoQ_{10}: A MARKER OF ENDOGENOUS CoQ_{10} STATUS?

IP Hargreaves[1], JM Land[1], J Poulton[2], SJR Heales[1]
[1]Neurometabolic Unit, National Hospital, Queen Square, London, UK; [2]Nuffield Department Obstetrics and Gynaecology, John Radcliffe Hospital, Oxford, UK

Coenzyme Q_{10} (CoQ_{10}) functions as an electron carrier within the mitochondrial electron transport chain (ETC) in addition to serving as an important cellular antioxidant. Due to the difficulties in measuring CoQ_{10} in plasma a reliable HPLC method has been developed to determine CoQ_{10} in skeletal muscle and blood mononuclear cells (MNC). Furthermore, a novel internal standard was synthesised to improve the accuracy of these determinations. Recently using this HPLC method a 47 year-old female with clear evidence of CoQ_{10} deficiency in skeletal muscle (**33***: 140–580 pmol/mg protein) and MNC (**20***: 37–133 pmol/mg protein) has been identified. Consequently, CoQ_{10} supplementation was instigated at 300 mg/day. The patient has responded well to CoQ_{10} and has moved from being a 'bottom shuffler' to walking upright albeit with some support. This improvement in mobility has also accompanied an increase in MNC CoQ_{10} status to within normal limits (44: 37–133 pmol/mg protein). Assessment of CoQ_{10} status is indicated with known ETC succinate cytochrome c reductase (complex II–III) deficiency or in patients presenting with ataxia, seizures and muscle symptoms. Analysis of muscle biopsies clearly provides a means of identifying patients with suspected CoQ_{10} deficiencies. However, since MNC may represent a reliable marker of endogenous CoQ_{10} status, initial assessment of patients by determination of MNC CoQ_{10} may be appropriate prior to a skeletal muscle biopsy.

231-P

FAMILIAL CEREBELLAR ATAXIA WITH COENZYME Q_{10} DEFICIENCY

R Artuch[1], P Briones[2], A Aracil[1], A Ribes[2], J Pineda[1], M Galván[1], JA Sánchez-Alcázar[3], G Brea[3], P Navas[3], M Pineda[1]

[1]Hospital Sant Joan de Dìu, [2]Institut de Bioquímica Clínica, CSC-CSIC, Barcelona; [3]Universidad Pablo de Olavide, Sevilla, Spain

Case: A one-year-old girl was noted to be clumsy and she fell frequently when walking unaided. At age 5 years she presented slight dysatria with slurring and rapid alternating movements. She was unable to walk with feet in tandem position. Gait speed was slightly reduced with broad base. Her cognitive level was normal. The patient's father referred a history of clumsiness, frequent falls, tremor and nystagmus since his first years of life. On clinical examination slurred speech, and persistent nystagmus with clearly saccadic ocular pursuit were more evident to the left side. **Results**: Laboratory tests for screening of metabolic disorders in blood and urine were all normal. Muscle biopsy was performed and decreased activities of NADH:cytochome c reductase (combined complex I+III) and succinate:cytochrome c reductase (complex II+III of the mitochondrial respiratory chain) were detected, with normal activity of the individual complexes. Muscle Q_{10} concentrations were clearly decreased in the index case (56 nmol/g protein: reference values: 157–488). Q_{10} deficiency was confirmed in fibroblasts in both cases. A decreased incorporation of radiolabeled 4-hydroxy [U-14C] benzoic acid (4-[U-14C]HB), a precursor of the quinone ring in Q_{10} was also observed (40% of control values), suggesting that the primary defect is allocated in the transprenylation pathway of Q_{10} biosynthesis. **Treatment**: After Q_{10} treatment, the girl is now able to walk unaided and cerebellar signs have disappeared. Her father showed also a good treatment response, since no sings of tremor, ocular movements and dysarthria are now present.

232-P

COENZYME Q_{10} DEFICIENCY AS A CAUSE OF NEONATAL LIVER FAILURE, TRANSIENT PANCREATIC INSUFFICIENCY AND LEIGH-LIKE DISEASE?

NI Wolf[1], T Lerman-Sagie[2], J Zschocke[3], JAM Smeitink[4], GF Hoffmann[5], M Lindner[5], T Meissner[5], D Rating[1]

Department of [1]Paediatric Neurology, [3]Human Genetics, [5]Metabolism, University Children's Hospital Heidelberg, Germany; [2]Paediatric Neurology and Neurogenetics Unit, Wolfson Medical Centre, Holon, Israel; [4]Nijmegen Centre for Mitochondrial Disorders, Nijmegen, The Netherlands

We describe the second case of a characteristic mitochondrial syndrome possibly caused by a deficiency of coenzyme Q (CoQ). Our female patient, the first child of consanguineous Turkish parents, presented on day 2 with hyperammonaemia, elevated tyrosine and alanine, and lactic acidosis. Liver function and tyrosine values returned to normal in the following days. Exocrine pancreas insufficiency was diagnosed at the age of 4 months and resolved spontaneously after enzyme substitution for several months. Neuropaediatric assessment at the age of 12 months revealed developmental delay and muscular hypotonia; the child was able to fixate and grasp objects. A cranial MRI showed a Leigh-like pattern. During the second and third year of life the child lost previously acquired skills and developed sensorineural hearing loss. In fibroblasts, activity of complex II+III was reduced whilst the single enzyme activities of complexes II and III were normal, indicating a deficiency at the level of coenzyme Q (CoQ). Respiratory chain studies in muscle were normal. Another case with an identical clinical picture, similar metabolic findings and a deficiency of the CoQ-dependent reactions of the mitochondrial chain (complexes I+III and II+III) *in vitro* has been recently reported [1]. This disease might represent a new mitochondrial disorder with a characteristic clinical phenotype possibly caused by a defect in coenzyme Q biosynthesis.

[1] Leshinsky-Silver E. et al. Neonatal liver failure and Leigh syndrome possibly due to CoQ-responsive OXPHOS deficiency. Mol Genet Metab. 2003;79:288–93

233-P

ADULT ALPERS DISEASE WITH LIVER COMPLEX I AND IV DEFICIENCY

Wiltshire EJ[1,4], Haas L[2], Sadleir L[1], Zuccollo J[3], McEwen A[4], Thorburn DR[5]
[1]Paediatrics, [2]Neurology and [3]Pathology, Wellington School of Medicine, [4]Central Regional Genetics Service, Wellington, New Zealand; [5]Murdoch Childrens Research Institute, Melbourne, Australia

Introduction: We report a young adult with clinical, EEG and autopsy features of Alpers disease, a condition usually occurring in young children, and complex I and IV deficiency. **Case Report**: A 17 year old female presented with occipital seizures and peripheral neuropathy. A materanl aunt had neurological regression in childhood. Initial EEG showed diffuse slowing maximal in the left occipital region. 4 months after her first seizure she developed a progressive encephalopathy with nonconvulsive status epilepticus and a right hemiparesis. Many anti-convulsants, and empiric therapy for infectious, inflammatory and neoplastic processes, were tried with no sustained benefit. Liver dysfunction developed. MRI, initially normal, now showed abnormal signal in the thalamus and frontal lobes. Repeat EEGs had discharges in the left posterior temporal occipital regions. A mitochondrial disorder was considered, raised serum lactate noted and borderline low complex I activity (42%) was found in muscle. Mulitvitamin therapy and high fat low carbohydrate diet had no effect. On-going deterioration led to her death at 17.5 years. Autopsy showed extensive loss of neurones and gliosis, especially in the occipital lobes, and extensive steatosis with some necrosis in the liver. Respiratory chain enzymology in liver showed marked deficiency of complexes I (3%) and IV (6%). **Conclusions**: An Alpers-type phenotype can occur beyond early childhood. Enzyme analysis in an affected tissue is essential. The family history and enzymology suggest a mtDNA (not autosomal recessive) cause. Metabolic disease must be considered in adults with neurological conditions.

234-P

CLINICAL FEATURES AND BENEFICIAL EFFECT OF RIBOFLAVIN IN CHILDREN WITH COMPLEX II DEFICIENCY

M Bugiani, I Moroni, E Lamantea, A Bizzi, G Uziel
Istituto Neurologico 'C. Besta', Milan, Italy

Objectives: To report the peculiar clinical features and the beneficial effect of riboflavin treatment in three children with mitochondrial encephalopathy and complex II defect. **Methods**: At follow-up (mean 40, range 28–62 months), a disability scale was applied to evaluate the clinical course. **Results**: Two children presented acutely with psychomotor regression and progressive spasticity and mental impairment within the first year of life. MRI showed a diffuse cystic leukoencephalopathy. Riboflavin treatment was started. Follow-up showed a stabilization of symptoms in one child, while a partial improvement of motor and cognitive functions in the other. Molecular analysis of the four subunits of complex II gave normal results. The third child presented with failure to thrive and mild motor delay since the first year of life. Plasma lactate was markedly increased. Riboflavin administration was started and after two-years, he is in stable clinical conditions; plasma lactate normalized. A first MRI study, performed at the time of the last observation, is normal. **Conclusions**: Respiratory chain complex II deficiency may present in infancy with MRI features of a leukoencephalopathy characterized by a progressive cystic rarefaction of affected tissue over time. Riboflavin treatment may exert a beneficial effect both in reducing the rate of disease progression in severely affected patients, or preventing the appearance of clinical symptoms in milder phenotypes. The absence of molecular lesions in the genes encoding the four subunits of complex II, together with the beneficial effect of riboflavin, suggest the possibility that this vitamin may play a role in the assembly and stabilization of complex II.

235-P

PHENOTYPES IN 178 CHILDREN WITH COX DEFICIENCY

Bohm M[1], Hansikova H[1], Piekutowska-Abramczuk D[2], Pronicki M[2], Karczmarewicz E[2], Popowska E[2], Vesela K[1], Houštěk J[1], Houstkova H[1], Pronicka E[2], Zeman Jr[1]
[1]Department of Pediatrics, Charles University, Prague, Czech Republic; [2]Children's Memorial Health Institute, Warsaw, Poland

We analysed phenotypes and genotypes in 178 children with cytochrome c oxidase (COX) deficiency from 141 unrelated families living in Poland or Czech and Slovak Republics. **Methods**: The activities of respiratory chain complexes (RC) were measured in muscle and/or fibroblasts spectrophotometrically. Amount and composition of RC were studied by 2D-PAGE. Mutations in mtDNA and SURF1 and SCO2 genes were analysed by cyclic sequencing and PCR-RFLP. **Results**: Isolated COX deficiency was found in 99 children and COX deficiency combined with other RC disturbances in 79 children. The first clinical symptoms developed during the first 18 months of life in 78% of children. The most frequent symptoms were failure to thrive (67%), encephalopathy (90%), hypotony (73%) and cardiomyopathy (24%). 66% of children died in childhood. *SURF1* mutations were found in 46 children with Leigh syndrome, (deletion 841delCT was prevalent in 72%) and *SCO2* mutations were found in 8 children with encephalomyopathy and/or cardiomyopathy (mutation G1541A was prevalent in 80%). MtDNA deletion or depletion was found in 8 children, mutation A3243G in 6 and mutations A8344G, A8348G, G8364A and del9205TA in one child. **Conclusion**: Detailed characterisation of COX on the protein and molecular level is necessary for genetic counseling in affected families. Mutations 841delCT in SURF1 gene and G1541A in SCO2 gene are prevalent, at least in Slavonic population.

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236-P

NEUROLOGICAL PRESENTATION OF COMPLEX IV DEFICIENCY DIAGNOSED IN LIVER

J Panetta, K Gibson, D Kirby, D Thorburn, A Boneh
Genetic Health Services Victoria, Murdoch Childrens Research Institute, Royal Children's Hospital, Melbourne, Australia

Mitochondrial respiratory chain (MRC) complex IV deficiency has a variety of presentations. The diagnosis is usually made by analysis of MRC complex activities in muscle biopsy. We describe 3 patients in whom the diagnosis was based on MRC complex IV deficiency in liver alone. Patient 1 presented with generalised muscle and bulbar weakness, tremor, retinal dystrophic changes, ptosis and external ophthalmoplegia. He also had FTT, growth hormone and ACTH deficiencies and nephropathy. Patient 2 presented with developmental delay, seizures and an abnormal respiratory pattern post anaesthetic. Patient 3 presented with seizures, developmental delay, ataxia and muscle and bulbar weakness. All 3 patients had non-specific white matter changes on MRI. None had evidence of liver disease. Mitochondrial respiratory chain enzyme analysis was performed on both muscle and liver biopsy specimens as previously described (Kirby et al. 1999). Muscle complex IV activity was equivocal (45–78% and 42–64% relative to citrate synthase and complex II activity, respectively). Liver complex IV activity was markedly reduced (9–37% and 10–19% relative to citrate synthase and complex II activity, respectively). These findings illustrate the wide variety of presentations associated with complex IV deficiency. They also demonstrate the importance of MRC enzyme analysis in liver in addition to muscle, even in cases where the primary clinical deficit is neurological and there is no liver disease.

237-P

COMPLEX V SUBCOMPLEXES IN THE PATIENTS WITH OXPHOS DEFECTS

Van Coster R[1], Smet J[1], de Paepe B[1], Lissens W[2], Seneca S[2], De Meirleir L[2]
[1]*Department of Pediatrics, Pediatric Neurology and Metabolism, Ghent University Hospital;*
[2]*Department of Pediatrics and Department of Medical Genetics, Free University Brussels, Belgium*

The oxidative phosphorylation (OXPHOS) generates ATP through passage of electrons along the four complexes of the respiratory chain (I-IV) in the mitochondrial inner membrane. ATP synthase (complex V) is a multi-subunit enzyme consisting of a catalytic portion (F1), a membrane portion (F0) and two stalks linking F1 and F0. Using Blue Native polyacrylamide gel electrophoresis and subsequent catalytic staining for ATPase, only one band corresponding to complex V was detected in most of the patients. In nine patients, however, in addition to the normal band, complex V subcomplexes with lower molecular weight were detected. This phenomenon was seen in skeletal muscle (2 patients), liver (3 patients) and in cultured skin fibroblasts (4 patients). A severe, combined deficiency of OXPHOS complexes (I, III, IV and V) together with normal activity of complex II was seen in two patients, and a less severe, combined deficiency (I and IV) in four patients. Two of the latter patients were carriers of a MERRF point mutation. In three patients no other defects of OXPHOS complexes were found. The exact cause of incomplete complex V synthesis, or decreased stability is not known. One likely hypothesis is a defective intramito-chondrial protein synthesis. The fact that combined OXPHOS complex deficiencies were detected in several of the patients, is in favor of this hypothesis. Remarkably, two of these patients carried a MERRF mutation.

238-P

FUNCTIONAL ANALYSIS OF AN ATP12 MUTATION IN YEAST

Seneca S[1], Van Coster R[3], Smet J[3], Lissens W[1], Alderweireldt V[1], Ackerman S[4], Liebaers I[1], De Meirleir L[2]
Center of Medical Genetics[1] and Department of Pediatric Neurology[2], AZ-Free University Brussels, B-1090 Brussels, Belgium; Department of Pediatrics[3], University of Gent, B-9000 Ghent, Belgium; Department of Surgery[4], Biochemistry and Molecular Biology, Wayne State University School of Medicine, Detroit, Michigan, USA

Complex V (ATP synthase) of the respiratory chain couples the proton gradient, generated by the respiratory chain, to ATP synthesis. The ATP12 gene product is one of the proteins required for assembly of complex V. Here, we report on the functional effect of a W94R mutation, present in the ATP12 gene of a patient with an ATP synthase decreased activity. Multicopy plasmid constructs containing the human and yeast wild type ATP12 gene, as well as the human mutation W94R and the yeast counterpart W103R were prepared. These constructs were introduced in a yeast host strain deprived of his ATP12 function, and as such respiratory deficient. ATP synthase activity of individual yeast transformants was assayed by growth studies on a non-fermentable carbon source and by Blue Native Polyacrylamide Gel Electrophoresis (BN-PAGE) stained for ATP synthase catalytic activity. WT plasmid constructs rescue the respiratory defect of a yeast Δatp12 mutant strain. Growth on a non-fermentable carbon source was strongly impaired for the human W94R mutant, while the growth of the yeast W103R mutant strain was normal. BN-PAGE studies are in progress. Our yeast complementation studies showed clearly that the human W94R mutant does not confer the respiratory competence to an Δatp12 yeast strain and is most probably the cause of the complex V dysfunction in our patient.

239-P

3-METHYLGLUTACONIC ACIDURIA IN TWO PATIENTS WITH QUANTITATIVE DEFICIENCY OF THE MITOCHONDRIAL F_1F_0-ATP SYNTHASE

JA Mayr[1], W Erwa[2], P Kurnik[3], P Covi[1], H Förster[1], J Paul[4], J Houštěk[4], W Sperl[1]
Departments of Paediatrics, [1]Paracelsus Private Medical University Salzburg, [2]University Graz, [3]General Hospital Klagenfurt, Austria, [4]Institute of Physiology Prague, Czech Republic

Two girls presented with varying lactic acidosis (1.4–12 mmol/L) from the neonatal period. Both are developmentally delayed, show psychomotoric retardation and muscular hypotonia. Patient I has a low degree, well compensated dilated cardiomyopathy of the left ventricle at the present age of 3 yrs. A severe hypertrophic biventricular cardiomyopathy and pulmonal hypertonia was found in patient II at birth which stabilised till the present age of 1 yr. A decrease of the oligomycin-sensitive ATPase to 5 and 4% of controls was respectively found in the 2 patients' skeletal muscle and to a lesser extent in fibroblasts. Respirometric investigation of mitochondria showed a reduced respiratory control with ADP. Mitochondrial ATPase was quantitatively reduced $>90\%$ in muscle in both patients determined by Blue Native PAGE. No mutation was found in the mitochondrial genes *ATP6* and *ATP8*. 3-Methylglutaconic acid (3-MGA) was persistently excreted in the urine of both patients in the range of 115-460 µmol/mmol creatinine (normal <10). This finding is in accordance with Holme et al. Ped Res 1992;32:731–5, who described a patient with an isolated ATPase defect and 3-MGA aciduria. Type II-IV 3-MGA aciduria are associated with a disturbed mitochondrial energy metabolism. Defects of mitochondrial F1Fo-ATP synthase seem to be highly associated with 3-MGA excretion, however the metabolic correlations remain to be unravelled.

240-O

NMR SPECTROSCOPY IDENTIFIES BIOCHEMICAL CONSEQUENCES OF PYRUVATE DEHYDROGENASE COMPLEX DEFICIENCY AND ITS TREATMENT IN CULTURED HUMAN FIBROBLASTS

PW Stacpoole, NE Simpson, Z Han, DS Kerr, I Constantinidis
Departments of Medicine, Biochemistry and Molecular Biology, University of Florida College of Medicine, Gainesville, FL 32610 and Department of Pediatrics, Case Western Reserve University, Cleveland, OH 44106, USA

Little is known of the effects of discrete mutations in the pyruvate dehydrogenase complex (PDC) on mitochondrial intermediary metabolism and energetics or of the effects of putative therapeutic agents on these processes. We applied NMR spectroscopy (NMRS) to cellular extracts of fibroblasts cultured from 6 healthy controls and 6 patients with mutations in the E1α subunit of PDC. Confluent cultures were exposed to uniformly labeled ^{13}C glucose for 20 h, followed by extraction using a dual phase technique with methanol/chloroform. We examined the aqueous phase by ^{31}P and ^{13}C NMRS and performed isotopomeric analysis of the ^{13}C data to determine the relative flux of pyruvate entrance into the tricarboxylic acid (TCA) cycle via PDC or pyruvate carboxylase (PC). We also measured the effect of 5 mmol/L dichloroacetate (DCA) on glucose and energy metabolism. Marked differences occurred between healthy and PDC-E1α deficient cells. In patient cells, the ratio of ATP/ADP and the intensity of TCA related metabolites were lower than control cells although PDC and PC activity were variable. DCA treatment shifted carbon flux in favor of PDC and increased TCA metabolite intensity and cellular energy charge. We conclude that NMRS applied to cellular extracts of human fibroblast cultures can provide insight into the biochemical consequences of certain genetic mitochondrial diseases and of potential therapies.

241-P

GENETIC STUDIES IN A PATIENT WITH PYRUVATE DEHYDROGENASE DEFICIENCY REVEAL AN EXONIC SPLICING ENHANCER SEQUENCE IN EXON 7 OF THE PDHA1 GENE

GK Brown[1], PT Keighley[1], RM Brown[1], S Krywawych[2]
[1]Genetics Unit, Department of Biochemistry, University of Oxford and [2]Department of Chemical Pathology, Great Ormond Street Hospital for Children, London, UK

A female with reduced pyruvate dehydrogenase activity and immunoreactive E1α protein in cultured fibroblasts was found to be heterozygous for a nonsense mutation (TAC > TAA, Tyr > stop) in exon 7 of the PDHA1 gene. However, rather than the expected reduced levels of the mutant message due to nonsense-mediated decay, analysis of PDH E1α transcripts in fibroblast RNA extracts revealed the presence of two abnormally spliced products, one in which both exons 6 and 7 had been skipped and one in which only exon 7 was deleted. Segments of the PDH E1α gene from intron 5 to intron 8, with and without the patient's mutation, were inserted into an exon-trap vector and expressed in COS-7 cells. Analysis of the transcripts generated from these constructs confirmed that the mutation causes aberrant splicing. Premature termination codons inserted into the normal sequence upstream and downstream of the site of the patient's mutation affected only the level of message, not the pattern of splicing. The sequence context of the patient's mutation indicated that it could be part of a putative splicing enhancer sequence, in which the cytosine residue of the TAC tyrosine codon would be critical. Expression of a silent mutation (TAC > TAT, Tyr > Tyr) at the position of the patient's mutation generated the same pattern of aberrantly-spliced transcripts, confirming the importance of this sequence for normal splicing.

242-P

A PUZZLING CASE OF MOSAICISM WITH MUTATION LEADING TO NONSENSE MEDIATED mRNA DECAY (NMD) IN A MALE PATIENT WITH PYRUVATE DEHYDROGENASE (PDH) DEFICIENCY

M Mine[1], M Zater[1], H Ogier[3], D Feneux[2], A Legrand[1], M Brivet[1]
Laboratoires de Biochimie[1] et de Cytogénétique[2], AP-HP Hôpital de Bicêtre, Le Kremlin-Bicêtre, France; Neuropédiatrie[3], AP-HP, Hôpital Robert Debré, Paris

This 2-yr-old male had unspecific encephalopathy with microcephaly. Brain MRI revealed cortical atrophy and ventricular enlargement. Lactate was elevated in blood (2.8 mmol/L) and CSF (4.2 mmol/L) with a normal lactate/pyruvate ratio. PDH activity in fibroblasts was at 55% of controls and western blotting was normal. Direct sequencing of PDHA1-cDNA from fibroblasts did not reveal any change. Conversely, at the genomic DNA level, the patient was found heterozygous for a premature termination codon mutation (R263X) creating a DdeI digestion site. PDHA1 gene is X-linked and hemizygosity should have been observed. DdeI digestion of PCR amplified PDHA1 exon 8 from fibroblasts, lymphocytes and hair roots of the patient demonstrated different relative band intensities, indicative of a mosaicism; no mutation was found in the mother's lymphocytes. To explain the absence of the R263X mutation in the patient's cDNA, we speculated that transcripts derived from the defective allele might be eliminated by NMD. Treatment of the patient's fibroblasts with a translation inhibitor (emetine 100 μg/ml in the growth medium for 7 hr) allowed us to amplify the mutated transcript and to reveal the heterozygous R263X mutation. Somatic mosaicism in male patients with PDHA1 deficiency has only been reported twice. Mutations generating NMD may complicate their diagnosis at the transcriptional level.

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243-P

COEXPRESSION OF *PDHA1* AND *PDHA2* GENES IN SOMATIC TISSUE OF A PATIENT WITH PDHc DEFICIENCY – FIRST CASE REPORT

Silva MJ, Rivera I, Tavares de Almeida I
Unidade Biologia Molecular Biopatologia Experimental, Faculdade Farmácia Universidade Lisboa, Portugal

Mammalian pyruvate dehydrogenase complex (PDHc), a mitochondrial multicomponent enzyme, plays a pivotal role in energy metabolism by catalysing the irreversible oxidative decarboxylation of pyruvate into acetyl-CoA. The majority of PDHc deficiencies involve the E1α subunit, which is of particular interest since it contains the cofactor binding site, as well as the phosphorylation site through which the activity of the whole complex is regulated. The human PDH E1α subunit exists as two isoforms encoded by two different genes: *PDHA1*, located on X chromosome and expressed in somatic tissues; *PDHA2*, located on chromosome 4, possesses characteristics of a functional processed gene and is selectively expressed in testis, after the onset of spermatogenesis.

We report the study of a Portuguese female patient whose molecular characterisation of the E1α subunit revealed the simultaneous presence, in circulating lymphocytes, of a normal *PDHA2* transcript and an incomplete *PDHA1* transcript, lacking the 5′ region coding for the signal sequence for mitochondrial import. To extend of our knowledge, this is the first case describing the somatic expression of the *PDHA2* gene. These results may open new perspectives for the study of mechanisms underlying gene expression, namely those regulating temporal and tissue-specific expression patterns.

244-P

PYRUVATE DEHYDROGENASE E1β SUBUNIT DEFICIENCY

RA Head[1], RM Brown[1], II Boubriak[1], JV Leonard[2], NH Thomas[3], GK Brown[1]
[1]*Genetics Unit, Department of Biochemistry, University of Oxford, Oxford,* [2]*Biochemistry, Endocrinology and Metabolism Unit, Institute of Child Health, London,* [3]*Department of Paediatric Neurology, Southampton General Hospital, Southampton, UK*

Pyruvate dehydrogenase (PDH) is a multienzyme complex composed of multiple copies of 5 main subunits: the E1α and E1β subunits of E1 (pyruvate dehydrogenase), E2 (dihydrolipoamide transacetylase), E3 (dihydrolipoamide dehydrogenase) and E3 binding protein. Nearly all cases of PDH deficiency are due to mutation in the X-linked gene for the E1α subunit, though a small number of patients have been identified with mutations in the genes for the E3 and E3BP subunits. In spite of this limited genetic heterogeneity, pyruvate dehydrogenase deficiency is clinically extremely variable, with presentations that include neonatal lactic acidosis, Leigh Syndrome and chronic neurodegeneration. We describe here two unrelated patients with PDH deficiency due to mutations in the E1β subunit. These patients presented with lactic acidosis and muscle hypotonia in infancy and both had marked reduction in enzyme activity and immunoreactive E1β protein. Both patients were homozygous for missense mutations (Y132C and P344S respectively) in the PDHB gene. These mutations are predicted to reduce the stability of the E1 $\alpha_2\beta_2$ tetramer. Activity of the pyruvate dehydrogenase complex was restored in cultured fibroblasts from both patients by transfection and expression of the normal E1β coding sequence.

245-A

E3 DEFICIENCY IN THE NORTH AMERICAN ASHKENAZI JEWISH POPULATION

ME Grace, R Kornreich, C Sansaricq, MM McGovern, RJ Desnick, K Raymond
Department of Human Genetics, Mount Sinai School of Medicine, New York, NY, USA

A 3 year-old male of Ashkenazi Jewish (AJ) descent with repeated episodes of hypoglycemia was diagnosed with E3 deficiency based on a urine organic acid profile with increased lactic acid, α-ketoacids and 2-keto-glutarate. E3 is a key component of three mitochondrial α-ketoacid dehydrogenase complexes. Although the classical form of this disease (OMIM 246900) presents with severe and progressive neurological impairment with persistent lactic acidosis and high mortality, a milder, episodic form with no neurological involvement has been described in AJ patients in Israel (Shaag et al. Am J Med Genet. 1999;82:177–82) who were found to be homozygous for a missense mutation, G229C. The carrier frequency for the G229C mutation in the Israeli AJ population was reported as 1:94. The diagnosis of E3 deficiency in our AJ patient was confirmed by DNA analysis which demonstrated homozygosity for the G229C mutation. A younger sibling, currently asymptomatic, was alsound to have the G229C/G229C genotype. Determination of the carrier frequency of the G229C mutation in AJ individuals from the New York metropolitan area is ongoing. This abstract presents the first case of E3 deficiency in the North American AJ population. If the New York carrier frequency for the G229C mutation is found to be similar to that in Israel, the lack of previously identified E3 patients in the American AJ population suggests that the disease is under diagnosed and screening for the AJ E3 mutation would be recommended. While the Ashkenazi E3 deficiency variant is considered mild, the disease can be lethal if it remains undiagnosed.

246-O

DIHYDROLIPOAMIDE ACETYLTRANSFERASE DEFICIENCY

RM Brown[1], RA Head[1], PT Clayton[2], GK Brown[1]
[1]*Genetics Unit, Department of Biochemistry, University of Oxford, Oxford,* [2]*Biochemistry, Endocrinology and Metabolism Unit, Institute of Child Health, London, UK*

A male with typical features of Leigh syndrome and a raised blood lactate concentration had reduced pyruvate dehydrogenase activity in cultured fibroblasts, but normal levels of immunoreactive protein for the subunits of the complex. No mutations were detected in the genes for the E1α and E3-binding protein subunits, the most common sites for mutations in pyruvate dehydrogenase deficiency. However, sequence analysis of cDNA corresponding to the E2 (dihydrolipoamide acetyltransferase) subunit revealed a 3 bp deletion in exon 2. This was confirmed in genomic DNA and the patient appeared to be homozygous for the mutation. The deletion is in frame and results in deletion of glutamic acid residue 34 of the mature the E2 protein. This amino acid is highly conserved in vertebrate and invertebrate species and some fungi. Direct demonstration of the pathogenicity of this mutation was obtained by functional complementation following transfection and expression of the normal E2 coding sequence. In spite of substantial residual pyruvate dehydrogenase activity and normal levels of E2 immunoreactive protein, significant correction of the defect could be demonstrated in patient fibroblasts. However, recovery of activity took much longer (3–6 days) compared with the 1–2 days required for functional complementation in patients with defects in the E1α subunit, suggesting slower turnover of the E2 subunit.

247-P

LEIGH SYNDROME AND SURF1 GENE MUTATIONS IN RUSSIAN PATIENTS

Zakharova EY[1], Voskoboeva EY[1], Dadaly EL[1], Malmberg SA[2], Ilina ES[2], Fedonyk ID[2], Nikolaeva EA[3], Mikhaylova SV[2]
[1]Research Center for Medical Genetics, Moscow, Russia; [2]Russian Child Hospital, Moscow, Russia; [3]Institute of Pediatrics and Child Surgery, Moscow, Russia

Leigh syndrome (LS) is one of the most frequent forms of mitochondrial disease in infancy and childhood. Etiologies of LS include both mitochondrial and nuclear DNA defects. Since 1999 in our Department of inherited metabolic diseases 67 patients with different forms of mitochondrial diseases have been observed. In 18 cases LS was diagnosed. The diagnosis was made on the basis of typical clinical pictures, biochemical abnormalities and brain magnetic resonance imaging. 14 unrelated Russian patients with LS and unknown molecular defects were screened for mutations in the mtDNA and the SURF1 gene. We found SURF1 mutations in 7 of our patients. 4 patients were homozygotes for common mutation 845delCT and the other 3 were compound heterozygotes for that mutation. In one patient mtDNA T8993G mutation was found. In patient with mtDNA T8993G mutation, onset was earlier and the clinical course and progression more rapid than in patients with SURF1 mutations. These data show that mutations in SURF1 is an important cause of LS in Russian. Mutation 845delCT is prevalent in Russian patients with LS and accounts for 85% of mutant alleles.

248-P

LEIGH SYNDROME IN SIBLINGS CARRYING THE FBSN (FOCAL BILATERAL STRIATAL NECROSIS) 9176T > C MUTATION

G Gray, T Hutchin, A Chakrapani, J Wright
Departments of Clinical Chemistry and Clinical Inherited Metabolic Disorders, Childrens Hospital, Birmingham, UK

A male infant of Greek and British ancestry presented at 9 months of age with a sudden loss of developmental milestones. An MRI scan revealed bilateral altered signals in the caudate nuclei, putamina and globus pallidus consistent with Leigh syndrome. He made slow motor progress and at 30 months has ataxia, central hypotonia, mild dystonia of his arms but no speech or swallowing difficulties and is intellectually normal. His younger sister had normal development until 7 months after which she regressed over a 3-month period. She improved but at 15 months had an acute presentation with hypotonia, encephalopathy and tachypnoeia. She was found to have a plasma lactate of 5.3 mmol/L and renal tubular acidosis-all developmental milestones were lost. Since then she has made slow and steady developmental progress. At 20 months she has central hypotonia, a well-developed pincer grasp, limb ataxia, drooling and some intellectual impairment but no dystonia. Overall she is more severely affected than her brother.

Blood mitochondrial DNA analysis in both siblings revealed homoplasmy for the FBSN 9176T > C mutation with approximately 50% heteroplasmy in the asymptomatic mother. This mutation affects subunit 6 of the ATP synthetase complex and has been reported to date in 9 families with Leigh-like syndromes showing varying severity including severe, slowly progressive and non-progressive phenotypes.

249-P

PYRUVATE UPTAKE IS INHIBITED BY VALPROIC ACID AND 3-KETO-VALPROIC ACID IN INVERTED SUBMITOCHONDRIAL VESICLES

Aires CP[1], Silva MF[1], Soveral G[1], SantosJ[1], ten Brink H[2], Duran M[3], Wanders RJ[3], Almeida IT[1]
[1]Centro Patogénese Molecular, Faculdade Farmácia Univ. Lisboa, Portugal, [2]VU Medical Centre, Metabolic Laboratory, Amsterdam, [3]Academic Medical Centre, University Hospital Amsterdam, The Netherlands

In mitochondria the anticonvulsant drug valproic acid (VPA) is mainly metabolised by β-oxidation generating a complex pattern of intermediates that could potentially interfere with endogenous metabolism at different levels. We have previously reported that oxygen consumption supported by pyruvate (pyr) oxidation was severely decreased by VPA. Aim: this work aims to elucidate the possible inhibition of the mitochondrial pyr carrier by VPA and its β-oxidation metabolite 3-keto-VPA. **Methods**: inverted submitochondrial vesicles (ISMV) were prepared using rat liver mitochondria, according to published procedure. The purity of the membrane vesicle preparation was characterized by the measurement of succinate dehydrogenase activity. The pyr carrier activity was assayed by measuring the uptake rate of [^{14}C]pyr driven by an inwardly directed proton gradient. Initial rate measurements were performed at room temperature by the inhibitor-stop technique, using α-ciano-4-hydroxycinnamate (CHC), followed by a rapid filtration. **Results**: a time-course study of pyr uptake by ISMV suggested an apparent first-order kinetics and an initial rate of 5 seconds was selected for further measures. CHC (1 mmol/L) induced a marked inhibitory effect (70% of control) on pyr transport. VPA and 3-keto-VPA (1 mmol/L) were both found to impair pyr influx (30–40% of control). **Discussion**: our results suggest that either VPA or 3-keto-VPA exert a direct effect on the mitochondrial pyr carrier, accounting for the limited oxidation of this keto-acid and thus to the compromised energy production driven by this substrate.

250-P

RENAL MANIFESTATIONS IN THE MELAS SYNDROME

Ireland, JHE, Rossetti, S, Hahn, SH, Harris, PC
Mayo Clinic 200 First Street SW Rochester, MN 55905, USA

The MELAS syndrome [Mitochondrial myopathy, Encephalopathy, Lactic Acidosis and Stroke] is frequently caused by mutations in the mitochondrial *MTTL1* gene coding for $tRNA^{Leu(URR)}$. Although not in the acronym, renal involvement has been reported. We retrospectively analyzed the records of all patients seen at the Mayo Clinic in the past five years for suspected MELAS syndrome. We report four unrelated adult patients with the A3243G mutation and renal insufficiency. Three of the patients (Patients 1, 2, 3) also had hearing loss and one was previously misdiagnosed with Alport's syndrome (Patient 3). Three patients (Patients 1, 2, 4) had native kidneys had varying degrees of renal insufficiency at presentation (iothalamate clearance of 67 to 29 ml/min) and all three had proteinuria, but not in the nephrotic range. Patient 3 had already received a successful kidney transplant when she presented here. Two pediatric patients with this mutation did not have renal manifestations (ages 4 months and 16 years). We conclude renal manifestations in the MELAS syndrome are common and patients with the A3243G $tRNA^{Leu(URR)}$ mutation should be screened for renal function and proteinuria. Furthermore, care should be taken to control blood pressure, proteinuria and avoid nephrotoxic medications. If living-related kidney transplantation is considered for end-stage renal disease, care should be taken to ensure that the donor is not affected by the same mutation.

251-P

PEROXISOME BIOGENESIS DISORDERS WITH PROLONGED SURVIVAL IN 31 PATIENTS: CORRELATION OF CLINICAL, BIOCHEMICAL AND MUTATIONAL ANALYSIS

BT Poll-The[1], S Ferdinandusse[2], J Gootjes[2], M Duran[2], HR Waterham[2], RJA Wanders[2], PG Barth[1]

University of Amsterdam, Academic Medical Center, Departments, [1]Pediatric Neurology and [2]Clinical Chemistry, Amsterdam, The Netherlands

We studied 31 patients affected by a peroxisome biogenesis disorder (Zellweger spectrum) (PBD-ZeS) with prolonged survival (>1 yr) to obtain data relevant for prognosis. We correlated the observed genotypes with cognitive and motor development, brain MRI, and peroxisomal functions. The neurodevelopmental outcome showed variation from mild to severe handicap. All patients presented elevated plasma very long-chain fatty acids and all but one patient also had elevated plasma di- and trihydroxycholestanoic acids (DHCA, THCA). Plasma phytanic acid and pristanic acid values were normal in a minority. This may in part reflect the effect of diet. Plasmalogen levels in erythrocytes were deficient in about half of the patients. Erythrocyte docosahexaenoic acid levels, however, were decreased in 84%. Twenty-one patients had mutations in the *PEX1* gene (67%). The most common *PEX1* genotypes were 2528 G>A/ 2528 G>A and 2528 G>A /2097insT. Patients homozygous for 2528 G>A generally had a better developmental outcome. Of all biochemical parameters studied, the averaged plasma DHCA and THCA levels correlated best with genotype and developmental outcome. The results indicate that impaired branched chain peroxisomal beta oxidation may have a special effect on the outcome in PBD-ZeS with prolonged survival.

252-P

PEROXISOMAL FUNCTION IN BRAIN IS ESSENTIAL FOR NORMAL NEOCORTICAL MIGRATION BUT NOT FOR THE FORMATION OF THE CEREBELLUM

M Baes[1], L Hulshagen[1], O Krysko[1], P Gressens[2]

[1]Laboratory of Clinical Chemistry, KU Leuven, Belgium and [2]Laboratory of Developmental Neurology, Hospital Robert-Debré, Paris, France

In *Pex5*, *Pex2* and *Pex13* knockout mice, which are mouse models of Zellweger syndrome, the most severe peroxisome biogenesis disorder, malformations of the neocortex were reported analogous to the neuronal migration disorder in human patients. In *Pex2* knockout mice with longer postnatal survival, also severe cerebellar malformations were observed. We investigated whether the abnormalities in brain development were due to the local absence of peroxisomes in brain by creating mice with brain selective deletion of peroxisomes. To this end *Pex5-loxP* mice were crossed with Nestin-Cre mice, yielding *B-Pex5 KO* mice with inactivation of *Pex5* in all neural progenitor cells. We found that import competent peroxisomes were eliminated from brain between E14.5 and E18.5, the period when neocortical migration is proceeding. BrdU birthdating experiments revealed a neuronal migration impairment at birth which was less severe than in generalised *Pex5* knockout mice. At later ages, staining with the neuron specific NeuN antibody was not different in the white matter between control and *B-Pex5 KO* mice. No abnormalities in the formation of the cerebellum were observed despite the complete elimination of peroxisomes from this tissue. These results indicate that absence of peroxisomal function in brain impairs the neocortical neuronal migration but does not affect the formation of the cerebellum.

253-P

EXTREMELY EARLY-ONSET OF SEVERE CHILDHOOD CEREBRAL X-LINKED ADRENOLEUKODYSTROPHY

Steinfeld R, Henneke M, Gärtner J
Department of Pediatrics and Pediatric Neurology, University of Göttingen, Germany

X-linked adrenoleukodystrophy (X-ALD) is the most common inherited peroxisomal disorder affecting the nervous system myelin and the adrenal cortex. It presents with various clinical phenotypes ranging from severe childhood cerebral forms beyond the age of three years to lifelong asymptomatic courses. X-ALD is associated with mutations in a peroxisomal ABC-type transporter (ALDP) which is presumably involved in the uptake of very long chain fatty acids (VLCFA). Hematopoietic cell transplantation (HCT) is presently the only effective long-term treatment when performed prior to the onset of the cerebral disease. We report on a family with three affected boys who carry a novel missense mutation (W524R) within the nucleotide binding fold of ALDP and who disclosed the earliest disease presentation so far communicated for X-ALD. One of the boys showed a slight developmental delay and suffers from myoclonic epilepsy since the age of 16 months. Though, initial neuroimaging was not indicative of a neurodegenerative disorder the boy progressively lost his motor skills. At the age of 22 months, the child presented with spastic movement disorder, severe ataxia, dysphagia, general weakness, and fatigue. Cerebral MRI revealed wide-spread diffuse demyelinating lesions in the entire white matter. Elevated plasma concentration of VLCFA and the mutation W524R in the *ALDP* gene are consistent with X-ALD. Our findings extend the clinical spectrum associated with childhood cerebral X-ALD and imply that X-ALD has to be included in the group of late infantile neurodegenerative disorders. Further, our data argue for an earlier start in diagnostic follow-up of boys with known X-ALD to recognize early signs of the cerebral disease and to be able to perform HCT.

254-P

MUTATIONAL ANALYSIS OF X-LINKED ADRENOLEUKODYSTROPHY IN SIXTEEN RUSSIAN FAMILIES: IDENTIFICATION OF SIX NOVEL MUTATIONS

Lomonosova EZ, Voskoboeva EY, Rudenskaya GE, Shekhter OV, Bukina AM, Dadaly EL, Zakharova EY
Department of Inherited Metabolic Diseases, Research Center for Medical Genetics, Russian Academy of Medical Sciences, Moscow, Russia

X-linked adrenoleukodystrophy (X-ALD) is an inherited disorder of beta-oxidation in peroxisomes. The disease results from mutations in ABCD1 gene leading to deficient or absent activity of peroxisomal ABC half-transporter (ALDP). More than 600 mutations in the gene have been reported. In this study, we present data on ABCD1 mutations in 16 unrelated Russian families with X-ALD childhood and adolescent cerebral forms. The diagnosis was confirmed by serum VLCFA study. Sixteen mutations were detected including eleven missense mutations leading to amino acid substitution, three frameshift mutations leading to a premature stop codon downstream from the mutation, one deletion of three base pairs (CTC) leading to the loss of the leucine residue, and one nonsense mutation. Six of the mutations (fsR117del, L138del, fsS164, T254K, fsF423 and G607D) are novel; ten others (S108L, S108W, W132X, Y181C, Y296C, R152C, R152L, G266R, R554H, S606L) and one polymorphism L516L (c.1548G>A) have been reported previously. Prenatal DNA diagnostics were performed in 3 families.

255-P

X-LINKED ADRENOLEUKODYSTROPHY IN SPAIN. IDENTIFICATION OF 26 NEW MUTATIONS IN ABCD1 GENE IN 80 PATIENTS AND IMPROVEMENT OF GENETIC COUNSELLING IN 162 FEMALES

T Pàmpols, MJ Coll, N Palau, C Camps, M Ruiz, M Girós
Institut de Bioquímica Clinica, Corporació Sanitària Clinic, Barcelona, Spain

X-linked adrenoleukodystrophy (X-ALD) is a severe neurodegenerative disorder presenting various phenotypes that often cooccurs within the same kindred. It is caused by mutations in the ABCD1 gene encoding a peroxisomal ABC transporter, 662 mutations have been found and approximately 75% of them appear to be private of a unique family (http://www.x-ald.nl).

We studied 80 patients from 62 unrelated families. An SSCP change was detected in 59/62 patients.We have identified 53 different mutations 26 of which are novel and 2 non-pathogenic sequence variants previously described. Allelic heterogeneity is important, 47/53 (88.7%) are private and only six mutations (Y174S, G277R FsE471, R518Q, P543L and R554H) have been found in more than one family. FsE471, the most frequent mutation found worldwide (10%) has only been found in 2 (3.4%). In contrast, mutations G277R, P543L and R554H, are most frequent (5%). Most of the families shows phenotypic variability, but in one with a new mutation R120P only adult mild phenotype is present (5 hemizygous). We have detected 80 heterozygous women from a group of 162 by mutation analysis; but only 78/80 showed VLCFA profile altered. Our results extend the espectrum of mutations, enable reliable identification of heterozygous females and also reinforce previous observations in other series of patients, with respect to the difficulty for predicting genotype-phenotype correlation, although few mutations do correlate.

255a-A

EFFECTS OF LORENZO'S OIL TREATMENT ON OXIDATIVE STRESS IN X-ALD PATIENTS

M Deon[3], G Ferreira[3], LR Sirtori[2], D Fitarelli[2], A Sitta[4], AG Barschak, GES Civallero[2], D Coelho[2], M Chiochetta[2], R Giugliani, S Llesuy[4], A Belló-Klein[5], CF Mello[3], M Wajner[2,3], CR Vargas[1,2]
[1]Department of Clinical Analysis, Pharmacy Faculty, UFRGS; [2]Medical Genetics Service, HCPA; [3]Department of Biochemistry ICBS, UFRGS; [4]Department of Biochemistry and Biophysic, University of Buenos Aires, Argentina; [5]Department of Physiology ICBS, UFRGS

X-linked adrenoleukodystrophy (X-ALD) is clinically characterized by central and peripheral demyelination, as well as by adrenal insufficiency. Current treatment for X-ALD with the mixture of glyceroltrioleate/glyceroltrierucate (4:1), named Lorenzo's oil (LO), combined with a VLCFA-poor diet normalizes VLCFA concentrations, but does not reverse or avoid progress of neurological symptoms. Free radicals seem to be involved in a large number of diseases, including Parkinson's disease, Alzheimer's disease and multiple sclerosis, chronic-inflammatory, vascular and neoplasic diseases. In the present study we evaluated the effect of LO treatment on several oxidative stress parameters in plasma and erythrocytes of symptomatic and asymptomatic X-ALD patients before and after LO treatment since these patients seem to have a neuroinflammatory disease. It was verified that TBA-RS was increased in these patients and LO was not able to reverse this increase in plasma of X-ALD individuals. TAR measurement was not altered in plasma of these patients, as well as the erythrocyte enzyme activities CAT, SOD and GPx. These data suggest that LO treatment do not have a protective action upon free radicals damage in X-ALD. Further studies become necessary to verify other parameters of oxidative stress and the effect of others therapies on oxidative stress in X-ALD patients.

256-P

PEROXISOMAL 'D-BIFUNCTIONAL PROTEIN' DEFICIENCY: A REMARKABLE PRESENTATION WITH FAILURE OF PEROXISOMAL SCREENING

Soorani-Lunsing RJ[1], van Spronsen FJ[2], Stolte-Dijkstra I[3], Wanders RJA[4], Poll-The BT[5], Rake JP[2]
[1]Department of Child Neurology, [2]Pediatrics, and [3]Genetics, University Hospital Groningen, [4]Department of Biochemistry and [5]Child Neurology, Academic Medical Centre, Amsterdam, The Netherlands

Objective: To demonstrate the diagnostic pittfalls and present a remarkabe clinical presentation of a peroxisomal 'D-bifunctional protein (DBP)' deficiency. **Patient:** A seven-year-old girl of consanguineous parents showed psychomotor retardation, axial hypotonia, perception deafness and mild facial dysmorfism. At the age of six she was no longer educable and lost sign language and motor skills. **Methods:** Diagnostic metabolic work-up was performed, including peroxisomal screening (very-long-chain fatty acids (VLCFAs), pipecolic, coprostanic and phytanic acid). Recently, peroxisomal investigations were performed in fibroblasts. **Results:** The only abnormal finding was VLCFAs C26/C22 ratio just above the normal upper range (i.e. 0.037 (range 0.011–0.025)) at the age of two. Peroxisomal function tests in fibroblasts showed abnormalities compatible with a DBP deficiency.

Conclusions: Our patient demonstrates a remarkably mild case of DBP deficiency and screening results of VLCFAs were considered to be normal. Therefore, in case of a strong clinical suspicion of a peroxisomal disorder it is our opinion that peroxisomal investigations in fibroblasts are indicated even when screening results are not clearly disturbed.

257-A

DEFICIENCY OF D-BIFUNCTIONAL PROTEIN IN A 6-MONTH-OLD BOY

E Marszal[1], E Jamroz[1], E Gluszkiewicz[1], J Paprocka[1], A Jezela-Stanek[2]
[1]The Children' Neurology Department, Silesian Medical University, Katowice; [1]Department of Medical Genetics, The Children' Memorial Health Institute, Warsaw, Poland

Objective: The authors present a 6-month-old-boy, a child of young, healthy and nonconsanguinous parents. In the family history elder sister died at the age of 14 months because of leucodystrophy of unknown origin. The pregnency period was uneventful. At birth, a profound hypotonia with arreflexia and areactivity, polymorphic seizures and breathing difficulties were noticed. In physical examination craniofacial dysmorphia and failure to thrive were present. In neurological examination large frontal fontanelle, severe axial hypotonia and hypertonia of the limbs were found. **Methods and Results**: MRI performed at the age of 3 and 6 months showed dysmyelination in the occipital lobes. In EEG paroxysmal changes with right temporal lobe predominance were noted. Neurophysiological study detected peripheral neuropathy. In serum and fibroblasts increased level of very long chain fatty acids (VLCFA) was observed. Decreased phytanic acid oxidation, normal level of phytanic acid and decreased number of peroxisomes pointed to deficiency of a single enzyme, deficiency of D-bifunctional protein.

Conclusions: 7-αβ-hydroxysteroid dehydrogenase IV [MIM 261515 and 601860] is involved in the degradation of VLCFA, as well as of the branched-chained fatty acids, pirystanic acid, and the bile - acid intermediates: di-, trihydroxycholestanoic acid. To data, D-bifunctional protein deficiency constitutes one of the most frequently occurring single-peroxisomal-enzyme-deficiency disorders.

258-O

ABSENCE OF PEROXISOMES IN HEPATOCYTES CAUSES MITOCHONDRIAL AND ER ABNORMALITIES BUT NO OXIDATIVE STRESS

R Dirkx[1], I Vanhorebeek[1], K Martens[1], P Van Veldhoven[2], M Baes[1]
[1]*Laboratory of Clinical Chemistry and* [2]*Department of Pharmacology, KU Leuven, Belgium*

Observations in mice and men point out that mitochondrial alterations accompany the absence of functional peroxisomes in liver. In the present study, a mouse model with a hepatocyte selective inactivation of peroxisomes was generated to further study the consequences of the absence of functional peroxisomes in hepatocytes. Both structure and function of mitochondria were found to be affected: the inner mitochondrial membrane was severely altered, while the activities of complex I, complex III and complex V of the respiratory chain was drastically reduced. However, these alterations had only a minor effect on ATP levels and redox state of the liver. Neither oxidative damage to proteins or lipids, nor activation of oxidative stress defence mechanisms were found, invalidating the hypothesis that the observed mitochondrial defects were caused by or accompanied with the production of oxidative radicals. The observed hepatocyte hypertrophy and hyperplasia and the proliferation of the smooth endoplasmatic reticulum was associated with the increased expression of PPARα regulated genes, and suggest that ligands for this nuclear receptor accumulate in peroxisome deficient hepatocytes. Accumulation of lipid droplets and glycogen aggregates were also observed. In conclusion, inactivation of peroxisomes in hepatocytes has widespread consequences on multiple subcellular compartments.

259-P

INTRACELLULAR TRANSPORT OF BILE ACID INTERMEDIATES ACROSS RAT LIVER PEROXISOMAL MEMBRANES

H Gan-Schreier[1], JG Okun[1], C-D Langhans[1], D Kohlmüller[1], HJ ten Brink[3], NM Verhoeven[3], C Jakobs[3], A Voelkl[2], GF Hoffmann[1]
[1]*University Children's Hospital, Heidelberg,* [2]*Department of Anatomy and Cell Biology II, Heidelberg, Germany;* [3]*Department of Clinical Chemistry, Metabolic Unit, VU University Medical Center, Amsterdam, The Netherlands*

The last step of bile acid biosynthesis is the conversion of bile acid intermediates, 3α,7α,12α-trihydroxy- and 3α,7α-dihydroxy-5β-cholestan-26-oic acids (THCA and DHCA), to primary cholic and chenodeoxycholic acids through peroxisomal β-oxidation. The further conjugation with glycine and taurine are presumed to occur exclusively in peroxisomes. To study the intracellular transport of intermediates from cytosol into the peroxisomes, the isolated rat liver peroxisomes were incubated with both a mixture of THCA and DHCA, and their CoA esters. The production of conjugates was followed by means of ESI-MS/MS.

Bile acid conjugates were detectable only when the peroxisomes were incubated with the CoA esters of THCA and DHCA, suggesting that the CoA esters are the preferred substrates for transport into peroxisomes. The production was inhibited by presence of ATP/Mg^{2+} and bovine serum albumin. Furthermore, inhibitory effects were observed when peroxisomes were pre-treated with protease K, histone and Triton X-100. In addition, inhibition of bile acid transport by VLCFA CoA esters was found to be competitive. It was shown that the novel model is a useful tool for investigation of peroxisomal bile acid transport. Our data suggest the existence of a bile acids CoA esters transport system across peroxisomal membranes similar to that of VLCFA.

260-P

ANALYSIS OF LEUKOTRIENES AND THEIR METABOLITES IN BILE OF PATIENTS WITH PEROXISOMAL OR MITOCHONDRIAL β-OXIDATION DEFECTS

T Meissner[1], S Ferdinandusse[2], RJA Wanders[2], E Mayatepek[1]
[1]*Department of General Pediatrics, University Children's Hospital, Düsseldorf, Germany, and*
[2]*Laboratory for Genetic Metabolic Diseases, Academic Medical Center, Amsterdam, The Netherlands*

Background: Cysteinyl leukotrienes (LTs) are potent lipid mediators predominantly eliminated via bile. The aim of this study was to present for the first time a complete profile of LTs in human bile and to investigate bile LTs in case of a peroxisomal or a mitochondrial β-oxidation deficiency. **Methods**: Cysteinyl LTs and their oxidation metabolites were analysed after HPLC separation by specific immunoassays or gas chromatography-mass spectrometry. **Results**: Under physiological conditions LTs are found in human bile (n = 8) in the nmolar range with LTD_4 predominating. In bile of a patient with Zellweger syndrome (ZS) LTE_4 was found to be slightly increased, whereas the w-oxidation metabolites of LTE_4 were highly increased (up to 18 times). The β-oxidation metabolite β-carboxy-tetranor-LTE_3 was below the detection limit (<0.1 nmol/L; controls 1.4 ± 1.2 nmol/L). In contrast, patients with X-ALD, MCAD as well as VLCAD deficiency did not show any differences in their biliary profile of LTs compared to controls. **Conclusions**: Increased levels of LTs in the bile of patients with ZS demonstrate an impaired degradation of LTs that might be of pathophysiological significance, e.g. for liver injury. In addition, our data confirm that the β-oxidation of cysteinyl LTs *in vivo* occurs in peroxisomes and not in mitochondria.

261-O

REINVESTIGATION OF TRIHYDROXYCHOLESTANOIC ACIDAEMIA REVEALS A PEROXISOME BIOGENESIS DISORDER

S Ferdinandusse[1], J Gootjes[1], F Skovby[2], E Christensen[2], RJA Wanders[1]
[1]*Laboratory of Genetic Metabolic Diseases, Academic Medical Center, Amsterdam, The Netherlands;*
[2]*D93 Department of Clinical Genetics, Copenhagen University Hospital, Copenhagen, Denmark*

We reinvestigated a patient with ataxia, dysarthric speech, dry skin, hypotonia and absent reflexes, who was previously diagnosed with an isolated peroxisomal β-oxidation defect due to a deficiency of the enzyme trihydroxycholestanoyl-CoA oxidase [1]. This diagnosis was based on the pattern of accumulating metabolites solely. New insights into the peroxisomal β-oxidation system and the development of novel methods to measure the activity of the different β-oxidation enzymes in skin fibroblasts prompted us to reinvestigate the underlying defect in the reported patient. An isolated β-oxidation defect in this patient was excluded by measurement of the various β-oxidation enzymes. Instead, we found that the patient was suffering from a peroxisome biogenesis disorder (PBD) caused by mutations in the *PEX12* gene, although all peroxisomal functions in cultured skin fibroblasts were normal. Clinically this patient is among the mildest PBD patients reported. Our investigation of this patient shows that even when all peroxisomal functions in fibroblasts, which are routinely used to diagnose PBDs, are normal a PBD cannot be excluded and additional studies are required. In addition, our findings imply that there is no longer evidence for the existence of trihydroxycholestanoyl-CoA oxidase deficiency as a distinct disease entity.

[1] Christensen, E et al. *J. Inherit. Metab. Dis.* 1990, 13:363-366

262-A

PRENATAL SCREENING FOR PEROXISOMAL DISORDERS: DETERMINATION OF VERY LONG CHAIN FATTY ACIDS IN AMNIOTIC FLUID BY LC/APCI-MS

Korall H[1], Heilbronner H[2], Wallner S[1], Löffler M[1], Trefz FK[1], Gärtner J[3]
[1]*Metabolic Centre, zfs Reutlingen, Wörthstr. 47, D-72764 Reutlingen, Germany,* [2]*Institute for Clinical Genetics, Olgahospital, Bismarckstr. 3, D-70176 Stuttgart, Germany,* [3]*Department of Pediatrics, Georg-August-University, Robert-Koch-Str. 40, D-37075 Göttingen, Germany*

Prenatal diagnosis of peroxisomal disorders has been accomplished up to now by different methods in cultured amniocytes and chorionic villus material. These methods are partially time-consuming. We established a new method for prenatal screening by determination of very long chain fatty acids (VLCFA: C22:0, C24:0, C26:0) in amniotic fluid. Free fatty acids were obtained by hydrolysis of 200 µl amniotic fluid with methanolic KOH followed by extraction with heptane. Nonadecanoic acid (C19:0) was used as internal standard. VLCFA were separated by a reversed phase column and detected as $(M\text{-}H)^-$ peaks in negative-ion mode by LC/APCI-MS using a PE-SCIEX tandem mass spectrometer. Sample preparation is easily performed within 2.5 h, run time per sample is only 10 min. C22:0, C24:0, C26:0 and the ratios C24:0/C22:0 and C26:0/C22:0 of amniotic fluid from fetus with peroxisomal disorders clearly stand out from normal values. Data confirm that LC/APCI-MS together with a rapid and simple sample preparation is a very specific method for high throughput prenatal diagnosis of peroxisomal disorders. This method will accelerate and facilitate prenatal diagnosis of peroxisomal disorders.

263-P

A NEW METHOD ALLOWING COMPLEMENTATION ANALYSIS OF FIBROBLASTS FROM PATIENTS WITH PEROXISOME MOSAICISM AND THE IDENTIFICATION OF A FREQUENT MUTATION IN *PEX12*

Gootjes J, Schmohl F, Mooijer PAW, Dekker C, Mandel H, Topcu M, Huemer M, von Schutz, Marquardt T, Smeitink JA, Waterham HR, Wanders RJA
[1]*Clinical Chemistry and* [2]*Pediatrics, Academic Medical Centre, University of Amsterdam, The Netherlands,* [3]*Rambam Medical Centre, Haifa, Israel,* [4]*Hacettepe University School of Medicine, Ankara, Turkey,* [5]*Department of Pediatrics, Feldkirch, Austria,* [6]*Kinderkrankenhaus Auf der Bult, Hannover, Germany,* [7]*Kinderheilkunde, Munster, Germany,* [8]*University Medical Centre, Nijmegen, The Netherlands*

Background: The disorders of peroxisome biogenesis (PBDs) show marked clinical and genetic heterogeneity, caused by mutations in one of at least twelve different *PEX* genes, which code for proteins involved in peroxisome biogenesis. Complementation analysis is an essential step in the identification of the molecular defect in any PBD patient. Mildly affected patients often show a mosaic catalase immuno-fluorescence pattern with positive and negative cells, which has obstructed complementation analysis so far. **Goal**: To develop a method allowing complementation analysis in patients with peroxisome mosaicism in fibroblasts, followed by molecular analysis. **Results**: We have developed a new method, which involves growth of fibroblasts at 40°C, which leads to a remarkable shift in the percentage of catalase negative versus catalase positive cells in fibroblasts from peroxisome mosaicism patients when compared to 37°C thus allowing complementation analysis to be done. We have applied this method to a large cohort of peroxisome mosaicism patients and were able to assign 8 patients to CG3 caused by mutations in the *PEX12* gene. Interestingly, a single homozygous mutation p.S320F(c.959C>T) was identified in these patients. **Conclusion**: We have developed a new method allowing complementation analysis to be done in peroxisome mosaicism patients and identified a frequent mutation in the *PEX12* gene.

264-P

RHIZOMELIC CHONDRODYSPLASIA PUNCTATA AND ALKYLGLYCEROL SUPPLEMENTATION

AM Mengerink[1], R Duran[2], J Gootjes[2], F Valianpour[2], P Brites[2], RJA Wanders[2], BT Poll-The[1]
University of Amsterdam, Academic Medical Centre, Emma Children's Hospital, Pediatric Neurology[1] and Laboratory of Genetic Metabolic Diseases[2]

Rhizomelic chondrodysplasia punctata (RCDP) is an inherited disease of peroxisomal metabolism, characterized by facial dysmorphia, cataracts, rhizomelia, joint contractures, failure to thrive and severe psychomotor delay. RCDP is genetically heterogeneous, consisting of three groups of patients with distinct genetic defects. Biochemically there is a deficiency of plasmalogens in all three groups. Plasmalogens are a special group of phospholipids present in all cell membranes but especially well represented in myelin. The first two steps in plasmalogen synthesis occur in peroxisomes. Alkylglycerol is an intermediate product in the synthesis of plasmalogens. In order to investigate whether alkylglycerol supplementation can restore plasmalogen levels in patients suffering from RCDP, fibroblast cell lines of 6 patients and 1 controle were grown in the presence of alkylglycerol. We measured plasmalogens by direct injection ESI-MS. Normalisation of both plasmenylethanolamines and -cholines occurred in the cell lines of all RCDP patients. There seemed to be a preference of incorporation of alkylglycerol in plasmalogen species containing an arachidonic acid molecule. In the past year 6 patients suffering RCDP have started oral alkylglycerol supplementation. In order to evaluate therapy several diagnostic tests were performed and will be repeated in the future. Although results are still preliminary, so far all patients show a small increase in plasmalogen levels in erythrocytes. Further evaluation of the effects of alkylglycerol supplementation will be nescessary to see if benefits are reproducible.

265-P

TWO SIBLINGS WITH RHIZOMELIC CHONDRODYSPLASIA PUNCTATA: AN UNUSUAL OUTCOME

R van Dijken-Visser[1], RJA Wanders[2], H Waterham[2], FJ van Spronsen[3]
[1]Institute Mental Retardation, Boejenoord, Assen, [2]Laboratory of Metabolic Diseases, Academic Medical Center Amsterdam, [3]Department of Metabolic Diseases, University Hospital Groningen, The Netherlands

Introduction: Rhizomelic chondrodysplasia punctata (RCP) is a severe peroxisomal disease, usually with a early fatal outcome.

Objective: to present 2 siblings with RCP with a greater survival.

Case 1: female, firstborn of healthy, non-consanguineous parents in 1969. She had growth restriction, secondary microcephaly, early severe psychomotor delay, bilateral cataract, blindness, shortening of the long bones and epiphyseal stippling, kidney stones, convulsions. Her phytanic acid was 55 μmol/L, DHAPAT, phytanic acid oxidation and plasmalogen snthesis were severely decreased. DNA analysis showed only 1 mutation (L292X). She now is 34 yrs: urinary infections, PEG for feeding problems and a menomethorrhagia are major problems.

Case 2: male (1978) with more or less the same problems who died at 16 yrs of age because of pneumonia. Pytanic acid 61 μmol/L with identical enzyme activities.

Conclusion: both patients had the classical form with clinical, radiological, and biochemical abnormalities typical for classical RCP. Despite these findings they survived longer than expected. These cases show that the natural history is more variable than reported before, and that in case of shortening of the long bones and epiphyseal stippling even at later ages, the diagnosis of RCP should be considered.

266-P

BILE ACID ANALYSIS IN D-BIFUNCTIONAL PROTEIN (DBP), L-BIFUNCTIONAL PROTEIN (LBP) AND DBP/LBP DOUBLE KNOCKOUT MICE

S Ferdinandusse[1], H Overmars[1], L Van Eeckhoudt[2], V Cuyt[2], S Denis[1], RJA Wanders[1], M Duran[1], M Baes[2]
[1]*Laboratory of Genetic Metabolic Diseases, Academic Medical Center, Amsterdam, The Netherlands;* [2]*Laboratory of Clinical Chemistry, KULeuven, Leuven, Belgium*

Bile acid analyses were performed using HPLC-electrospray tandem mass spectrometry in bile, plasma and liver from D-bifunctional protein (DBP), L-bifunctional protein (LBP) and DBP/LBP double knockout mice. DBP and LBP are responsible for the second and third step of the peroxisomal β-oxidation process. Studies in patients with a deficiency of DBP and previous studies in the DBP knockout mouse have shown that DBP is involved in bile acid biosynthesis, because there is an accumulation of the bile acid intermediates di- and trihydroxycholestanoic acid (DHCA and A). However, DBP-deficient patients do not have a deficiency of the primary bile acid cholic acid (CA) and chenodeoxycholic acid (CDCA). In case of DBP deficiency, LBP could possibly be involved in a rescue pathway for the synthesis of CA and CDCA. We s in LBP-deficient pups (14–15 days old) that there is a normal bile acid profile, whereas in DBP-deficient pups there is not only an accumulation of mainly THCA:1 but also a severe deficiency of the primary bile acids. In DBP/LBP double knockout pups theficiency of the primary bile acids was comparable to DBP-deficient pups, but they accumulated mainly THCA in stead of THCA:1. Our results did not provide any evidence for a role for LBP in the biosynthesis of bile acids.

267-P

THE PEROXISOMAL ABC-TRANSPORTER ALDP IS PREDOMINANTLY HOMOMERIC IN MOUSE LIVER

CP Guimarães[a,b], P Domingues[c], P Aubourg[d], F Fouquet[d], A Pujol, G Jimenez-Sanchez[f], C Sá-Miranda[a,g], JE Azevedo[a,b]
[a]*IBMC, Porto, Portugal;* [b]*ICBAS, Porto;* [c]*Department Química Un. Aveiro, Portugal;* [d]*Hôpital St Vincent de Paul, Paris, France,* [e]*IGBMC, Strasbourg, France;* [f]*Department Biological Chemistry, Johns Hopkins, Baltimore, USA;* [g]*IGMJM, Porto, Portugal*

The ATP-binding cassette (ABC) transporters are molecular pumps that transport a wide range of substrates using ATP hydrolysis as the driving force. The ABC-transporters can be divided into two subclasses according to their structure: half- and full-transporters. Full-transporters have two transmembrane and two nucleotide-binding domains. For this reason, it is assumed that half proteins need to homo or heterodimerize to become a functional unit. In the peroxisomal membrane four half ABC-transporters are known but their native structure was never established. Since defects in the ALDP transporter are associated with a human disorder (known as X-ALD) this subject is of utmost importance. In order to characterise the quaternary structure of ALDP and PMP70 we have used purified peroxisomes from mouse liver as the starting material. The proteins were solubilized using digitonin and the protein complexes were characterized by using sucrose density gradient analysis or preparative immunoprecipitation experiments. Our results clearly indicate that, *in vivo*, both half ABC-transporters exist predominantly (if not exclusively) as homomeric proteins, most probably dimers. This conclusion narrows the number of possible substrates transported by these proteins, which may have implications on the understanding of the pathogenesis of X-ALD.

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268-P

MOUSE MODELS FOR X-LINKED ADRENOLEUKODYSTROPHY

S Forss-Petter[1], H Werner[2], S Pangratz-Fuehrer[3], R Höftberger[1], H Lassmann[1], K-A Nave[2], J Berger[1]
[1]Brain Research Institute, Medical University Vienna, Vienna, Austria; [2]Neurogenetics, Max-Planck-Institute for Experimental Medicine, Göttingen, Germany; [3]Clinic of Internal Medicine I, University of Veterinary Medicine, Vienna, Austria

X-ALD includes several clinical variants, differentiated by the extent of cerebral inflammation, neurodegeneration (demyelination, spinal cord and peripheral nerve involvement) and adrenocortical insufficiency. All forms arise from mutations in the peroxisomal membrane transporter, ALDP/ABCD1, resulting in accumulation of very long-chain fatty acids (VLCFA). To study the pathomechanism of X-ALD, we previously generated *Ald*-deficient mice, which show similar defects in fatty acid metabolism as human X-ALD patients. On a C57BL/6 background, aging *Ald(–/y)* mice show no signs of demyelination or inflammation in the CNS. By quantifying APP accumulation in axonal spheroids, increased axonal degeneration was noted in spinal cord of 2-yr-old mutants, suggesting that VLCFA build-up alone predisposes for slow axonal damage with some resemblance to human adrenomyeloneuropathy. Overexpression of the related peroxisomal membrane protein, ALDRP/ABCD2, can restore VLCFA metabolism in X-ALD cells. To determine whether *Aldr* compensates for *Ald*-deficiency in mice, we generated *Aldr* and *Ald/Aldr* knockout mice. These develop normally and lack severe phenotype as young adults. Although VLCFA levels are normal in *Aldr(–/–)* mice and comparable to *Ald(–/y)* in the CNS of double-deficient mice, both lines developed motor coordination and nerve conduction deficits at 1 yr of age.

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269-P

ACCUMULATION OF VERY-LONG CHAIN FATTY ACIDS DOES NOT ALTER MITOCHONDRIA IN THE X-ALD MOUSE MODEL

Oezen I[1], Rossmanith W[2], Forss-Petter S[1], Voigtländer T[3], Kemp S[4], Moser-Thier K[2], Wanders RJA[4], Bittner RE[2], BergerJ[1]
[1]Brain Research Institute and [2]Neuromuscular Research Department, Institute of Anatomy and [3]Institute of Clinical Neurology, Medical University Vienna; [4]Laboratory for Genetic Metabolic Diseases, Academic Medical Centre, Amsterdam

X-linked adrenoleukodystrophy (X-ALD) is a neurodegenerative disease, which is characterised by accumulation of very-long chain fatty acids (VLCFA). It has been suggested that mitochondrial abnormalities contribute to X-ALD pathology. The X-ALD mouse model does accumulate VLCFA in all tissues investigated including the skeletal muscle. Interestingly, the peroxisomal as well as the mitochondrial β-oxidation was normal in isolated organelles of the skeletal muscle from X-ALD mice. No difference was observed in polarographic investigations of the respiratory chain in isolated muscle mitochondria between X-ALD and control mice. Moreover, electron-microscopic investigations revealed normal size, structure and localisation of mitochondria in muscle of both groups. In spite the accumulation of VLCFA in the skeletal muscle, no accumulation was found in the mitochondria of X-ALD mice.

Thus we conclude that accumulation of VLCFA *per se* does not cause mitochondrial abnormalities as well as mitochondrial abnormalities are not responsible for the accumulation of VLCFA in X-ALD mice.

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270-P

β-OXIDATION IN HEPATOCYTE CULTURES FROM MICE WITH SINGLE OR MULTIPLE PEROXISOMAL ENZYME DEFECTS

R Dirkx[1], M Baes[1], P Van Veldhoven[2]
[1]*Laboratory of Clinical Chemistry and* [2]*Department of Pharmacology, KU Leuven, Belgium*

In order to better delineate the substrate specificities of peroxisomal β-oxidation, mouse models were analysed with (1) complete inactivation of peroxisomes in hepatocytes (*L-Pex5* KO mice), () MFP-1 deficiency or (3) MFP-2 deficiency. Since in *L-Pex5* KO mice, severe structural and functional alterations of the mitochondrial inner membrane were observed, an additional question was whether this affected mitochondrial β-oxidation. Previous experiments using liver homogenates of these mice indicated that β-oxidation rates did not reflect the inactivation of peroxisomal enzymes. Therefore, hepatocyte cell cultures were optimised which allowed to incubate intact cells with the radiolabelled substrates for 6 h. Despite the severe inactivation of several complexes of the electron transport chain, the degradation of [1-^{14}C]-palmitic acid was not impaired in *L-Pex5* KO mice. As expected, the oxidation of 2-methyl- [1-^{14}C]-hexadecanoic acid and [26-^{14}C]-3,7,12-trihydroxycholestanoic acid, known substrates for peroxisomal β-oxidation, was less than 15 and 5% in *L-Pex5* KO mice as compared to control mice. Interestingly, the degradation of the dicarboxylic acid [1,14-^{14}C]-1,14-tetradecanedioc acid, for which the site of β-oxidation has not been firmly established, was 75% lower in peroxisome deficient hepatocytes as compared to controls. Experiments with MFP-1 and MFP-2 knockout hepatocytes indicated that MFP-2 is responsible for the degradation of branched chain fatty acids and bile acid intermediates, whereas MFP-1 has a role in the degradation of dicarboxylic acids.

271-P

AGE DEPENDENT FATTY ACID COMPOSITION IN ALD MOUSE TARGET TISSUES. COMPARATIVE STUDY WITH TRANSGENIC ABCD2 GENE OVEREXPRESSED/ALD MOUSE

M Girós[1], M Ruiz[1], C Camps[2], A Pujol[2], T Pampols[1]
[1]*Institut de Bioquimica Clínica, Corporació Sanitària Clínic, Barcelona, Spain;* [2]*Institut de Genétique et de Biologie Moléculaire et Cellulaire, CNRS/INSERM/ULP Strasbourg, France*

X-linked adrenoleukodystrophy mouse model, ABCD1 KO mouse (ALD), accumulates VLCFAs in target organs and develops AMN-like phenotype (Pujol et al. 2002). We presented the fatty acid evolution: saturated, w9, w6 and w3 series, in brain (BR), spinal cord (sC), sciatic nerve (sn) and adrenal glands (AG) of the: ALD mouse (ALD); ALD mouse with transgenic overexpression of an ALDrelated gene (ABCD2/ALDR) (ALDtg); wild mouse (WT) and WT with ALDR overexpression (WTtg). Our results were: (a) in 8 days old ALD C26 increase was null in BR and incipient in SC; at 3, 8 and 18 months the C26 increase was similar in all tissues compared with WT (2–6 times), but the % was always the lowest in BR (0.1–0.3%) and the highest in SN (0.3–4.1%). (b) ALDtg rescues the C26 storage at any age in all tissues, but only partially in SC at 18months. (c) The most relevant poliunsaturated fatty acids (PUFAs) in each series were not affected in ALD compared with WT in target tissues. PUFA alterations, in w3 and w6, were found in ALDtg and WTtg. These changes were subtle in BR, SC and SN and did not affect C22:6w3 and C20:4w6, except for AG. An explanation could be an age and tissue dependent lipid enzyme composition (eg. elongases, desaturases). These results demonstrate the usefulness of ALD mouse as a model for therapeutic approaches and can contribute to elucidate theALDR function.

272-P

IN VITRO EFFECTS OF LORENZO'S OIL ON OXIDATIVE STRESS PARAMETERS ELICITED BY HEXACOSANOIC AND TETRACOSANOIC ACIDS IN RAT CEREBRAL CORTEX

M Deon[3], G Ferreira[3], LR Sirtori[2], D Fitarelli[2], S Landgraf[2], Sitta A[2], GES Civallero[2], D Coelho[2], M Chiochetta[2], S Llesuy[4], A Belló-Klein[5], M Wajner[2,3], CR Vargas[1,2]

[1]Department of Clinical Analysis, Pharmacy Faculty, UFRGS, [2]Medical Genetics Service, HCPA; [3]Department of Biochemistry, ICBS, UFRGS; [4]Department of Biochemistry and Biophysic, University of Buenos Aires, Argentina; [5]Department of Physiology ICBS, UFRGS

X-linked adrenoleukodystrophy (X-ALD) is an inherited disorder biochemically characterized by tissue accumulation hexacosanoic ($C_{26:0}$) and tetracosanoic ($C_{24:0}$) acids, which is associated with central and peripheral demyelination and adrenal insufficiency. Therapy for X-ALD consists of oral administration of a mixture (4:1) of $C_{18:1}/C_{22:1}$ mixture, named Lorenzo's oil (LO) combined with a VLCFA-poor diet. Considering that the mechanisms underlying the brain damage in X-ALD are poorly known in the present study we evaluated the *in vitro* effects of $C_{26:0}C_{24:0}$ acids on various oxidative stress parameters in cerebral cortex of young rats.We also studied the effect of LO on the altered parameters. We observed a significant increase of chemiluminescence and TBA-RS in rat cerebral cortex added with $C_{26:0}C_{24:0}$ acids, and the addition of LO did not alter these effects. TAR measurement and the antioxidants enzyme activities CAT and GPx were not altered by $C_{26:0}C_{24:0}$ acids, in contrast to SOD activity which was increased by $C_{26:0}C_{24:0}$ acids but $C_{18:1}/C_{22:1}$ mixture did not reverse this effect. These data strongly suggested that oxidative stress could be involved in the pathophysiology X-ALD and that LO treatment is ineffective in preventing the *in vitro* induction of oxidative stress.

273-P

OMEGA-OXIDATION OF PHYTANIC ACID: A NEW STRATEGY TO TREAT REFSUM DISEASE?

JC Komen, M Duran, RJA Wanders

University of Amsterdam, Academic Medical Centre, Departments of Clinical Chemistry and Pediatrics, Emma Childrens Hospital, Laboratory for Genetic Metabolic Diseases, Amsterdam, The Netherlands

Adult Refsum disease (ARD) is characterized by deficient peroxisomal a-oxidation, which is caused by mutations in the gene coding for phytanoyl-CoA hydroxylase in the majority of ARD patients. As a consequence, phytanic acid accumulates in tissues and body fluids, which is the main biochemical marker for ARD and believed to be the major cause of the pathology of the disease. The symptoms include retinitis pigmentosa, peripheral neuropathy, and cerebellar ataxia. Treatment of ARD consists of a diet low in phytanic acid. This study focuses on an alternative route of phytanic acid degradation, i.e. ω-oxidation. The first step in ω-oxidation is hydroxylation at the ω-end of the fatty acid. In order to study this first step, the formation of hydroxylated intermediates was studied in human liver microsomes incubated with phytanic acid and NADPH. Two hydroxylated metabolites of phytanic acid were identified using gas chromatography mass spectrometry (GC/MS) analysis, viz. ω- and (ω-1)-hydroxyphytanic acid (ratio of formation 20:1). Formation of the two hydroxylated phytanic acid analogues was NADPH dependent, linear in time up to 60 min, and linear with the amount of protein until 1 mg/ml. These results indicate that phytanic acid undergoes ω-hydroxylation in human liver microsomes. Upregulation of this alternative ω-oxidation pathway may decrease phytanic acid levels in ARD patients and can therefore be considered as a new approach in the treatment of the disease.

274-O

IDENTIFICATION OF FATTY ALDEHYDE DEHYDROGENASE IN THE BREAKDOWN OF PHYTOL TO PHYTANIC ACID

DM van den Brink, JNI van Miert, G Dacremont, J-F Rontani, A Gerbert, RJA Wanders
Departments of Clinical Chemistry and Pediatrics, University of Amsterdam, Academic Medical Center, Emma Children's Hospital, Meibergdreef 9, 1105 AZ, Amsterdam, The Netherlands

Phytanic acid is a branched chain fatty acid that plays a role in a number of metabolic disorders. In Refsum disease, an accumulation of phytanic acid caused by a defective breakdown is thought be the direct cause of disease. Treatment of patients therefore consists of prescribing a diet low in phytanic acid. However, a natural source of phytanic acid that has been largely overlooked is its precursor, phytol, which is abundantly found in nature as part of the chlorophyll molecule. Therefore, we studied the metabolism of phytol, of which little is known in humans.

Upon analysis by GC-MS of fatty acids in human fibroblasts cultured in the presence of phytol, phytenic acid was identified as an intermediate of the conversion of phytol to phytanic acid. This conversion most likely involves two subsequent enzymatic reactions catalyzed by an alcohol dehydrogenase and an aldehyde dehydrogenase. Fibroblasts derived from patients suffering from Sjögren-Larsson syndrome (SLS), characterized by a deficiency of fatty aldehyde dehydrogenase (FALDH), were found to be deficient in the production of phytenic acid when cultured in the presence of phytol. In addition, fibroblast homogenates of these patients, incubated with phytol in the presence of NAD^+ did not produce any phytenic acid. This indicates that FALDH is involved in the breakdown of phytol.

275-P

INVESTIGATION OF PHYTOL-INDUCED CHANGES IN THE EXPRESSION OF FATTY ACID METABOLIZING ENZYMES IN MICE

J Gloerich, GA Jansen, S Denis, JPN Ruiter, MA van Werkhoven, M Duran, RJA Wanders, S Ferdinandusse
Laboratory of Genetic Metabolic Diseases, Academic Medical Center, Amsterdam, The Netherlands

Objective: In many patients with a peroxisomal disorder, there is an accumulation of branched chain acids. Feeding mice a diet enriched in phytol mimicks the situation in these patients, because it results in increased levels of phytol metabolites, such as the branched chain fatty acids phytanic and pristanic acid. These fatty acids are ligands of peroxisome proliferator-activated receptor α (PPARα) *in vitro*, but little is known about their effects *in vivo*. **Methods**: Wild type and PPARα$^{-/-}$ mice were fed either a control diet or a phytol enriched diet. Enzyme activity measurements and immunoblot analysis of various peroxisomal and mitochondrial enzymes involved in fatty acid metabolism were performed. **Results**: A PPARα dependent increase in protein expression and/or activity of almost all peroxisomal β-oxidation enzymes was observed. In contrast, for catalase and phytanoyl-CoA hydroxylase, and also for straight chain acyl-CoA oxidase, 3-ketoacyl-CoA thiolase, and carnitine octanoyltransferase a PPARα independent increase was observed. Of the mitochondrial fatty acid metabolizing enzymes, a PPARα dependent increase in protein expression and/or activity of short chain and long chain acyl-CoA dehydrogenase, and carnitine palmitoyltransferase 2 was observed. **Conclusions**: In peroxisomes, both PPARα dependent and independent effects of the phytol enriched diet were observed, whereas in mitochondria only PPARα dependent effects were observed.

276-P

IDENTIFICATION OF THE PEROXISOMAL β-OXIDATION ENZYMES INVOLVED IN THE DEGRADATION OF LONG-CHAIN DICARBOXYLIC ACIDS

S Ferdinandusse[1], S Denis[1], CWT van Roermund[1], RJA Wanders[1], G Dacremont[2]
[1]*Laboratory of Genetic Metabolic Diseases, Academic Medical Center, Amsterdam, The Netherlands;* [2]*Department of Pediatrics, University of Ghent, Ghent, Belgium*

Dicarboxylic acids (DCAs) are ω-oxidation products of monocarboxylic acids. After activation by a dicarboxylyl-CoA synthetase, the dicarboxylyl-CoA esters are shortened via β-oxidation. Although it has been extensively studied where this β-oxidation process takes place, the intracellular site of DCA oxidation has remained controversial. Making use of fibroblasts from patients with defined mitochondrial and peroxisomal fatty acid oxidation defects, we show that peroxisomes, and not mitochondria, are involved in the β-oxidation of C16DCA. Additional studies in fibroblasts from patients with X-linked adrenoleukodystrophy (X-ALD), straight-chain acyl-CoA oxidase (SCOX) deficiency, D-bifunctional protein (DBP) deficiency and rhizomelic chondrodysplasia punctata (RCDP) type 1, together with direct enzyme measurements with human recombinant L-bifunctional protein (LBP) and DBP expressed in a *fox2* deletion mutant of *Saccharomyces cerevisiae*, show that the main enzymes involved in β-oxidation of C16DCA are SCOX, both LBP and DBP, and sterol carrier protein X (SCPx) possibly together with the classical 3-ketoacyl-CoA thiolase. This is the first indication of a specific function for LBP, which has remained elusive until now.

277-P

CLINICAL IMPLICATIONS OF MUTATION ANALYSIS IN PRIMARY HYPEROXALURIA TYPE 1

CS van Woerden[1], JW Groothoff[1], FA Wijburg[1], C Annink[2], RJA Wanders[2], HR Waterham[2]
[1]*Emma Children's Hospital AMC,* [2]*Laboratory Genetic Metabolic Diseases, Department of Clinical Chemistry, Academic Medical Center, University of Amsterdam, PO Box 22660, 1100 DD Amsterdam, The Netherlands*

Background: Primary hyperoxaluria type 1 (PH1) is an inborn error of glyoxylate metabolism with an extensive clinical and genetic heterogeneity. The aim of this study was to determine relationship between genotype and clinical outcome.

Methods: AGXT mutation analysis and assessment of biochemical characteristics and clinical outcome of patients from a Dutch PH1 cohort.

Results: 33 of a cohort of 57 PH 1 patients were analyzed. Ten different mutations were found. The most common mutations were the Gly170Arg, Phe152Ile and the 33InsC mutations. Homozygous Gly170Arg and Phe152Ile mutations were associated with pyridoxine responsiveness and a preserved renal function over time when treatment was timely initiated. All patients homozygous for the 33InsC mutation had end-stage renal disease before the first year of age. A novel Gly82Arg mutation was found in 3 patients with adverse outcome in 2 of them.

Conclusion: Early detection of Gly170Arg and Phe152Ile mutations in PH1 has important clinical implications because of their association with pyridoxine responsiveness and clinical outcome. The association of a homozygous 33insC mutation with severe infantile ESRD, resulting in early deaths in 2 out of 3 cases, warrants a choice for prenatal diagnostics in affected families.

278-O

PROTEOMICS OF THE LYSOSOMAL MEMBRANE REVEALS DIVERSE ORIGINS OF THE ORGANELLE

RD Bagshaw[1,3], JW Callahan[2,3], DJ Mahuran[1,3]
Department Laboratory Medicine and Pathobiology[1], Department Biochemistry[2], University of Toronto, Metabolism Programme[3], Research Institute, The Hospital for Sick Children, Toronto, Canada, M5G1X8

Lysosomes are dynamic, endocytic subcellular compartments which contribute to degradation and recycling of cellular material. From our proteomic model of lysosomes (rat liver Triton WR1339-filled lysosomes) we have identified 254 proteins attached to the lysosomal membrane, 219 of which are found in the integral membrane. We have used a combination of 2D-IPG-PAGE:mass spectrometry and Ion-exchange chromatography:LC-MS/MS as protein identification strategies. About half of the proteins identified are known endosomal/lysosomal constituents and involved in membrane trafficking. The remainder are those originally associated with other compartments such as Golgi, ER, and Plasma membrane indicating diverse origins of the lysosomal membrane. The Alzheimer disease-associated γ-secretase complex (Nicastrin, Presenilin, and APP) was enriched and displayed an acidic-pH optimum in the lysosomal membrane. Many integral membrane proteins (25%) were poorly defined as to function. One of these displayes features of the ARF family of G-proteins involved in protein trafficking. It localizes with lysosomal markers (NPC1, LAMP1) by immunofluorescence, and is expressed in all tissues, suggesting it has a fundamental role in the endosomal/lysosomal system. Several function-unknown proteins display features of transporter proteins.

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279-P

DIAGNOSIS OF LYSOSOMAL STORAGE DISORDERS IN DRIED BLOOD SPOTS ON FILTER PAPER

Kyosen SO[1], Micheletti C[1], Mendes CSC[1], Rand MH[1], Azevedo RA[1], D'Almeida V[1], Chamoles NA[2], Martins AM[1]
[1]Centro de Referência em Erros Inatos do Metabolismo (CREIM)-UNIFESP/EPM; [2]Fundación para el Estudio de Las Enfermedades Neurometabólicas (FESEN), Brasil

Introduction: The definitive diagnosis of lysosomal storage sisorders (LSD) is made by enzyme assays in blood samples (plasma or leukocytes) or fibroblasts culture but their storage and transport to reference centers are difficult. The enzyme assay in dried blood on filter paper (DBFP) is possible in 15 enzymes leading to the diagnosis of 20 different LSD. **Objectives**: The CREIM-UNIFESP/EPM-Sao Paulo, Brazil in cooperation with the FESEN-Buenos Aires, Argentina investigated brazilian patients with clinical suspicion of LSD by the enzyme assay in DBFP. **Methods**: In the period of December/2000 to December/2003, we sent 87 blood samples of patients who had clinical suspicion of LSDs or had this group of diseases as part of the differential diagnosis by airmail to FESEN where the enzyme assays were performed. **Results**: LSD diagnosis were confirmed in 59 (68%) patients, 40 (67.9%) mucopolysaccharidoses, 2 (3.4%) mucolipidosis, 6 (10.1%) Gaucher disease, 4 (6.8%) Niemann-Pick A or B disease, 6 (10.1%) G_{M1} gangliosidosis and 1 (1.7%) Pompe disease. **Conclusion**: The presented methodology is not only reliable and sensitive for measuring lysosomal enzyme activities in DBFP samples, but easier, faster and less expensive than the leukocyte assay. An aditional advantage of this methodology is it's easier storage and mailing facilities specially in large distances as we have in Brazil.

280-P

HYPOMYELINATION IN INFANTILE ONSET LYSOSOMAL DISEASES

Biancheri R, Rossi A[1], Battagliese A[2], Pessagno A[3], Allegri AEM[4], Filocamo M[5], Lualdi S[5], Di Rocco M[4]

Neuromuscular Disease Unit, University of Genoa, Istituto G. Gaslini, Genoa, [1]Department of Pediatric Neuroradiology, Istituto G. Gaslini, Genoa, [2]Department of Paediatrics, Federico II University, Naples, [3]Department of Child Neuropsychiatry, Istituto G. Gaslini, Genoa, [4]Second Unit of Pediatrics, Istituto G. Gaslini, Genoa, [5] Laboratorio Diagnosi Pre-Postnatale Malattie Metaboliche, Istituto G. Gaslini, Genoa, Italy

White matter impairment occurring in lysosomal diseases has traditionally been considered the result of Wallerian degeneration due to neuronal storage. However, some animal models suggest that metabolic effects on oligodendrocytes may directly contribute to delayed or abnormal myelination. In Krabbe disease, the accumulation of galactosylsphingosine (psychosine) is known to be highly cytotoxic and responsible for death of myelin-forming cells with a rapid disappearance of myelin. A similar mechanism has been suggested, in cultured neurons, for glucosylsphingosine in type 2 Gaucher disease and for sphingosylphosphorylcholine in type A Niemann-Pick disease.

We report three patients with infantile onset of lysosomal diseases (Krabbe disease, acute neuronopathic Gaucher disease, and type A Niemann-Pick disease) whose MRI shows a pattern of hypomyelination. Our study demonstrates that hypomyelinating leukoencephalopathy may represent a common neuroradiological finding of lysosomal storage diseases with onset in the first months of life, when myelination is particularly active.

281-O

HUMAN CENTRAL NERVOUS SYSTEM STEM CELLS (hCNS-SC) TRANSPLANTATION TO TREAT THE NEURONAL CEROID LIPOFUCINOSES

P Huertas, N Uchida, S Tamaki

StemCells, Inc, Palo Alto, California, USA

Human central nervous system stem cells (hCNS-SC) may be purified, banked and expanded reproducibly. When transplanted into the brains of an immunodeficient NOD-SCID neruronal ceroid lipofuscinosis (CLN1) model, hCNS-SCs engraft, migrate, differentiate, express missing enzyme and decrease the autofluorescent lysosomal storage burden. Engraftment was found to be durable and reproducible showing extensive migration throughout the brain including the olfactory bulb, cerebral cortex, basal ganglia, hippocampus, pons/medulla and cerebellum. The progeny of hCNS-SC differentiated into neurons, astrocytes and oligodendrocytes in a site-specific manner with no evidence of tumor formation.

We are in the process of obtaining regulatory approval for a clinical trial of hCNS-SC transplantation in patients with selected forms of NCLs.

282-P

MUTATION ANALYSIS OF THE PATIENTS WITH CLASSIC LATE-INFANTILE NEURONAL CEROID LIPOFUSCINOSIS IN RUSSIA

AMBoukina[1], IV Tsvetkova[2], SV Mikhailova[3], ES Ilyna[3], LI Semikina[3]
[1]Inherited Metabolic Disease Laboratory, Research Centre for Medical Genetics, Moskvorechie St, 1, 115478 Moscow, Russia. [2]Institute of Biomedical Chemistry RAMS,119832 Moscow, Russia; [3]Department of Neurology and Psychiatry, Russian Federation Hospital for Children, Leninskijj prt, 117, 117513 Moscow, Russia

The late-infantile form of neuronal ceroid lipofuscinosis (LINCL) is a progressive and ultimately fatal neurodegenerative disease of childhood. The defective gene in this hereditary disorder, CLN 2, encodes a lysosomal tripeptidyl peptidase 1 that removes tripeptides from the amino-terminus of peptides. We present data on CLN2 mutations in 12 unrelated patients who were diagnosed with LINCL by classical clinical criteria (age at onset, clinical progression). LINCL diagnosis was confirmed by measurement of activity in homogenate of leucocytes of tripeptidyl peptidase 1 (TPP1). Both mutant alleles were identified in all patients with the exception of one. The mutation g3670C>T(Arg208Stop) was identified in 18 mutant alleles: seven probands were homozygous and four patients were heterozygous for it. Four mutations: g6152-6153delCT leading to the loss of the serine residue, missense mutation g3665G>A (Arg206His), splicing mutation g1536G>C (IVS2+5) and one polymorphism g5412C>T(Ser433Phe) – are novel. One patient was founded to be homozygous for IVS2+5 and other one was identified as heterozygous for this mutation.

283-P

MUTATION OF THE GLYCOSYLATED ASPARAGINE RESIDUE 286 IN HUMAN CLN2 PROTEIN RESULTS IN LOSS OF ENZYMATIC ACTIVITY

K Tsiakas[1], S Storch[1], R Steinfeld[1], J Ezaki[2], Z Lukacs[1], E Kominami[2], A Kohlschütter[1], K Ullrich[1], T Braulke[1]
[1]Department of Biochemistry, Children's Hospital, University Hospital, Hamburg Eppendorf, Martinistrasse 52, Bldg W23, D20246 Hamburg, Germany; and [2]Department of Biochemistry, Juntendo University School of Medicine, Tokyo 113, Japan

Late infantile neuronal ceroid lipofuscinosis (LINCL) is caused by the deficiency of the lysosomal tripeptidyl peptidase-I encoded by CLN2. We previously detected in two LINCL patients a homozygous missense mutation, p.Asn286Ser, that affects a potential N-glycosylation site. We introduced the p.Asn286Ser mutation into the wild-type CLN2 cDNA and performed transient expression analysis to determine the effect on the catalytic activity, intracellular targeting, and glycosylation of the CLN2 protein. Expression of mutant p.Asn286Ser CLN2 in HEK293 cells revealed that the mutant was enzymatically inactive. Western blot analysis demonstrated that at steady state the amounts of expressed p.Asn286Ser CLN2 were reduced compared with wild-type expressing cells. The rate of synthesis and the sorting of the newly synthesized p.Asn286Ser CLN2 in the Golgi was not affected compared with wild-type CLN2 protein. The electrophoretic mobility of the immunoprecipitated mutant p.Asn286Ser CLN2 was increased by approximately 2 kDa compared with the wild-type CLN2 protein, whereas deglycosylation led to the generation of polypeptides of the same apparent size. The data suggest that mutant p.Asn286Ser CLN2 lacks one oligosaccharide chain resulting in enzymatic inactivation.

284-P

DIAGNOSIS OF NEURONAL CEROID LIPOFUSCINOSES: TWO-YEARS EXPERIENCE WITH DRIED BLOOD SPOT TESTS FOR CLN1 AND CLN2

Z Lukacs, A Keil, A Kohlschütter
Department of Pediatrics, Metabolic Laboratory, University Hospital Hamburg-Eppendorf, Germany

Neuronal ceroid lipofuscinoses (NCL or CLN) are a heterogenous group of at least eight genetic neurodegenerative disorders now classified as CLN1 trough CLN8 that share certain clinical findings (dementia, retinopathy) and histological features (storage of ceroid lipofuscin). For some of these disorders, molecular genetic analysis is possible but diagnosis frequently remains cumbersome. In some cases electron microscopy is necessary. CLN1 is caused by a deficiency of the lysosomal enzyme palmitoyl protein thioesterase 1 (PPT1) and CLN2 by a deficiency of the lysosomal tripeptidyl peptidase 1 (TPP1). Measurement of these enzyme activities in leukocytes or fibroblasts is a reliable diagnostic tool but fraught with several practical problems. To overcome these we developed assays for both enzymes on the basis of dried blood spots which can easily be mailed (Lukacs et al. Clin Chem. 2003;49:509–11). We now report on two-years experience with these tests used as a diagnosic first tier assay in suspected cases of NCL. Patients who tested positive on the initial sample were retested using either fibroblasts or leukocytes. We found 12 patients with PPT deficiency and 11 patients with TPP deficiency, all of whom were confirmed by further biochemical and clinical work-up. No false negative results were noted. Enzyme activities were stable for several months. Dry blood testing for PPT1 and TPP1 deficiencies proved a reliable and practical method in the initial diagnostic work-up of suspected cases of NCL.

285-O

DEFECTIVE ER-RESIDENT MEMBRANE PROTEIN CLN6 CAUSES NEURODEGENERATIVE LYSOSOMAL DISORDER

C Heine[1], B Koch[1], S Mole[2], DN Palmer[3], A Kohlschütter[1], S Storch[1], T Braulke[1]
[1]Children's Hospital Biochemistry, University Hospital Hamburg, Hamburg, Germany; [2]Department Pediatrics and Child Health, University College London, UK; [3]Animal and Food Sciences Division, Lincoln University, Canterbury, New Zealand

The neuronal ceroid lipofuscinoses (NCLs) are a group of autosomal recessive neurodegenerative diseases characterized by the accumulation of lipopigment and by progressive cell death in the brain and retina. Mutations in the *CLN6* gene result in variant late infantile NCL and have also been described in sheep (OCL6) and *nclf* mice. Recently, the *CLN6* gene was identified encoding a 311 amino acid transmembrane protein of unknown intracellular localization and function. Western blot analysis of *CLN6* cDNA transfected BHK cells demonstrated the expression of a ~27 kDa protein that can form dimers. Immunofluorescence microscopy showed that the endogenous and the overexpressed CLN6 colocalize with the ER-marker protein PDI. The translocation into the ER was demonstrated by the endo H-sensitive glycosylation of a mutant CLN6 (p.Ile153Ser) containing a neo-glycosylation site. The replacement of potential RRR and RKK ER-retention motifs by alanine residues did not alter the ER localization of the mutant CLN6. Additionally, various fusion protein constructs showed that the 19 N-terminal amino acid residues of CLN6 are sufficient to prevent the exit from the ER. In fibroblasts of CLN6 patients, *nclf* mice and OCL6-sheep the degradation of endocytosed arylsulfatase A was strongly reduced in all mutant CLN6 cell lines compared with controls. These data suggest that defects in the CLN6 protein lead to lysosomal dysfunctions which may result in lysosomal accumulation of storage material.

286-P

HAPLOTYPIC VARIABILITY FOR NORMAL AND MUTANT *CLN6* GENE IN PORTUGUESE FAMILIES

Bessa C[1], Teixeira CA[1], MacDonald ME[2], Boustany RM[3], Sá Miranda MC[1,4], Ribeiro MG[1,4]
[1]Unidade de Biologia do Lisossoma e do Peroxissoma, Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal; [2]The Molecular Neurogenetics Unit, Massachusetts General Hospital, Charlestown, MA 02129; [3]Departments of Pediatrics and Neurobiology, Duke University Medical Center, Durham, NC 27710, USA; [4] Unidade de Enzimologia, Instituto de Genética Médica, Porto, Portugal

In the Portuguese population, 5 non-related CLN6 families with 8 affected patients have been previously described. In this cohort of patients two mutations were identified. A common gene defect, the mutation c.460-462delATC leading to the in-frame deletion of I154, was found in 81% (13/16) of CLN6-chromosomes. This led to the hypothesis of an independent founder effect in the Portuguese population. To test this possibility we have conducted a linkage disequilibrium study of multiallelic markers segregating with the *CLN6* gene at loci MADH3gt, D15S1015, MAP2K5ca, and D15S983. A core haplotype, 107–274, at markers D15S1015 and MAP2K5ca was established in all I154del chromosomes. A statistically significant association ($p<0.001$) was observed between I154del mutation and alleles at the 4 loci examined, particularly at D15S1015 (χ^2 56.13, $p<0.001$). The core haplotype observed on I154del chromosomes was not present on disease chromosomes bearing the second Portuguese mutation.

In conclusion, haplotype analysis of normal and mutant CLN6 chromosomes provided evidence for at least two independent mutational origins of vLINCL in the Portuguese population. Since the core haplotype observed in I154del chromosomes spans less than 1.6-Mb, the mutation is likely to have been introduced by an ancient founder probably more than 500 years ago.

287-P

COMPARISON OF CYSTINE DETERMINATION IN MIXED LEUKOCYTES VERSUS POLYMORPHONUCLEAR LEUKOCYTES FOR DIAGNOSIS OF CYSTINOSIS AND MONITORING OF CYSTEAMINE THERAPY

HJ Blom, M Wilmer, A de Graaf-Hess, L van den Heuvel, L Monnens, E Levtchenko
University Medical Centre St Radboud, Nijmegen, The Netherlands

Background: Cystinosis is a rare inborn error of cystine transport, leading to accumulation of cystine within the lysosomes. To diagnose cystinosis and to monitor treatment with cysteamine, adequate measurements of intracellular cystine content are required. Historically, cystine has been measured in mixed leukocyte preparations (ML), although it preferentially accumulates in phagocytic blood cells. Therefore we compared the differences in intracellular cystine content of ML and polymorphonuclear leukocytes (PMN) and finally switched to the determination in PMN cells.

Methods: ML and PNM were isolated from blood of 15 heterozygotes of cystinosis, 12 patients treated with cysteamine, 4 patients at diagnosis and 8 controls. Cystine was by HPLC.

Results: Mean intracellular cystine content (nmol cystine/mg protein) was lower in ML compared to PMN cells in obligate heterozygotes (0.07 versus 0.27, $p<0.001$) and patients treated with cysteamine (0.15 versus 0.94, $p<0.001$). At the time of diagnosis cystine values of ML in two patients were within the normal range, while they were elevated in PMN and in cultured fibroblasts. After the switch from ML to PMN the dose of cysteamine had to be increased in 80% of the patients under cysteamine therapy to keep their cystine level within heterozygote range.

Conclusions: We provide evidence that cystine should be measured in PMN leukocytes and not in ML. The use of ML can lead to delay of the diagnosis of cystinosis and inadequate adjustment of cysteamine dose.

288-P

ATP DEPLETION AND INCREASED OXIDISED GLUTATHIONE IN CULTURED CYSTINOTIC FIBROBLASTS

HJ Blom, E Levtchenko, M Wilmer, A de Graaf-Hess, J Koenderink, L Monnens, L van den Heuvel
University Medical Centre St Radboud Nijmegen, The Netherlands

Background The link between the accumulation of cystine and clinical symptoms of cystinosis is still missing. The aim of the study was to investigate whether intracellular glutathione and ATP content were depleted in cystinotic fibroblasts. **Methods**: Skin fibroblasts of 8 patients with cystinosis and 8 controls were cultured. Intracellular cystine, total and oxidised glutathione were measured by HPLC. ATP content was determined by the luciferin-luciferase assay. The activity of Na,K-ATPase was determined by ^{86}Rb uptake. The activity of mitochondrial respiratory chain complexes (I-IV) was measured in isolated mitochondria. **Results**: Median fibroblast's cystine content of patients with cystinosis was 9.3 nmol/mg protein compared to 0.4 in healthy controls ($p < 0.01$). Total glutathione content did not differ between cystinotic and control fibroblasts, while oxidised glutathione was higher in the patients (median 0.9 versus 0.23 nmol/mg protein, $p < 0.05$). Median ATP content was lower in cystinotic fibroblasts compared to controls (38.8 versus 51.5 nmol/mg protein, $p < 0.05$). The uptake of ^{86}Rb and the activity of mitochondrial respiratory chain complexes did not differ between the patients and the controls. **Conclusion**: ATP depletion in cystinotic fibroblasts did not result in decreased activity of Na,K-ATPase and was not caused by a defect in one of the complexes of mitochondrial respiratory chain. The increase of oxidised glutathione points to oxidative stress, which might be responsible for cell damage in cystinosis.

289-P

INFANTILE FREE SIALIC ACID STORAGE DISEASE. FOLLOW UP OF TWO SIBLINGS INTO LATE CHILDHOOD

Durán G, Hernandez M, Cavagnaro F
Pontificia Universidad Católica de Chile. Departamento de Pediatría. Santiago, Chile

Free sialic acid storage disorders are autosomal recessive disturbances in lysosomal membrane transport caused by mutations in the SLC17A5 gene which product is a lysosomal membrane protein called Sialin involve in the transport of free sialic acid across the membrane. It has been described an heterogeneous clinical presentation with two principal allelic phenotypes, Salla disease a milder presentation and Infantile sialic acid storage disease (ISSD) of early onset, characterized by failure to thrivhepatosplenomegaly, hypopigmentation of skin and hair, coarse facial features, hypotonia and death during first years of life. **Case reports**: These are two brothers with ISSD diagnosed at 3 years of age. Patients are 15 and 14 years old, have a characteristic phenotype of the disease but different severity of presentation. The oldest one developed mild proteinuria since 3 years of age. At 9 years of age an steroid resistant nephrotic syndrome variety was diagnosed that progress towards end stage renal disease at 11 years of age. He is currently on chronic peritoneal dialysis. He also developed non-responsive severe anemia and self-injury behavior. The youngest one presents autistic behavior but he does not have renal or hematologic complications. **Conclusion**: We report these patients because of the unusual long survival and the development of renal failure, a previously unreported complication.

290-P

TWO SIBS WITH ASYMPTOMATIC SIALURIA

MF Mulder[1], TJ de Koning[2], L Dorland[2], G van der Bruggen-Vaarkamp[2], MGM de Sain-van der Velden[2], L Klomp[2], R Berger[2]
[1]Free University Medical Centre, Amsterdam and [2]University Medical Centre, Utrecht, The Netherlands

We report on two of six children of healthy consanguineous parents who are both healthy, normally developed and without abnormalities on physical examination, with a persistent sialuria. In the male infant (M.H.) of 5 months of age metabolic screening was performed because of an acute life threatening event, which in the course of the evaluation was assigned to a gastro-esophageal reflux. The screening revealed an increased urinary excretion of free sialic acid (670 μmol/mmol creatinine). M.H, the sixth child, was born at term after an uneventful pregnancy. Development is normal. Physical examination showed no abnormalities. In the follow-up the increased excretion of free sialic acid persists (ranging from 408 to 655 μmol/mmol creatinine). Evaluation of the family members showed normal urinary free sialic acid excretion in the parents and in four children. The fifth child, however, L.H, showed increased excretion of free sialic acid (249 μmol/mmol creatinine), which is persistent. L.H. is a girl of 10 years of age, with a normal development, attending normal school, with some extra help in gymnastics, and normal findings on physical examination. Urinary free sialic acid excretion in Salla disease/ISSD (McKusick 269920) ranges from 32 to 225 μmol/mmol creatinine. A few patients have been reported with sialuria (McKusick 269921). In these patients urinary free sialic acid ranged from 2150 to 13 200 μmol/mmol creatinine. In our two sibs the urinary excretion of free sialic acid is between the values of Salla/ISSD and sialuria, the condition is asymptomatic, and the inheritance unclear. Mutation analyses by PCR in these two patients are in progress.

291-P

A TURKISH INFANT WITH FARBER LIPOGRANULOMATOSIS AND CMV HEPATITIS

S Unal, N Kandemir, H Topaloğlu, HS Kalkanoğlu, M Ceyhan, T Coşkun
Hacettepe University Faculty of Medicine, Department of Pediatrics, 06100, Ankara, Turkey

The deficient activity of acid ceramidase in Farber lipogranulomatosis results in the accumulation of ceramide and this is manifested clinically with painful joint swelling and contractures, nodules in the periarticular skin and larynx, psychomotorretardation, recurrent pulmonary infections, axial hypotonia, hoarse cry and occasionally hepatosplenomegaly. A 3-month-old male infant exhibiting painful joint contractures, hoarse cry, swallowing difficulty, hepatomegaly, oral moniliasis, developmental retardation and hypotonia admitted to our ward. Acid ceramidase was found as undetectable level and the patient was diagnosed as Farber lipogranulomatosis. Because of the elevated liver function tests, CMV serology was studied and CMV IgM and IgG positivities were further investigated by CMV PCR, urine CMV inclusion body analysis and avidity, resulting 151 (normal < 1), positive and low avidity, respectively. We continued ganciclovir treatment for 21 days for CMV hepatitis and liver function tests declined significantly by the end of the treatment. The high incidence of recurrent pulmonary infections in Farber Lipogranulomatosis can be attributed to the hypotonia of the patient which is also affecting the respiratory muscles, as well as the defective functioning of lymphocytes and histiocytes laden with ceramide. The oral moniliasis, recurrent pulmonary infections and the CMV hepatitis in our patient can be clues for the presence of immunological abnormalities in Farber lipogranulomatosis.

292-P

QUANTITATIVE ASSESSMENT OF URINARY SULFATIDES IN DIFFERENT FORMS OF METACHROMATIC LEUKODYSTROPHY

A Sürken, A Kohlschütter, Z Lukacs
Department of Pediatrics, Metabolic Laboratory, University Hospital Hamburg-Eppendorf, Germany

Metachromatic leukodystrophy (MLD) is caused by a deficiency of arylsulfatase A (ASA) which also serves as a diagnostic marker. Diagnostic confusion may arise from low ASA activities caused by the frequent ASA pseudodeficiency and in MLD variants with normal ASA activities from a cofactor (saposin) deficiency. Measuring accumulated sulfatides in urine can overcome these difficulties. It is usually done by qualitative thin-layer chromatography (TLC) which may lead to ambiguous results because of low sulfatide excretion or co-migrating lipid bands. We have therefore adapted and optimized a TLC procedure for the quantitative assessment of urinary sulfatides and have investigated patients with infantile, juvenile and adult forms of MLD. To reduce interfering substances, lipid extracts were saponified and purified through a RP-18 column. Sulfatide, sphingomyelin and cholesterol standards as well as samples in different concentrations were spotted on a TLC plate with an automatic system (Sarstedt). Plates were stained, scanned and bands quantified using software to evaluate the darkness of each band. Our procedure yielded rapid and reproducible results. Sulfatide/sphingomyelin ratios allowed the best differentiation of MLD patients (results: 2–11) from controls (results: <1). Absolute concentrations of sulfatides in samples from the same patient on different days showed large variations (e.g. 69–245 µg/ml) whereas the sulfatide/sphingomyelin ratio was less variable (e.g. 2–3.3). Sulfatide excretion did not differ significantly in patients with infantile, juvenile or adult onset of MLD.

293-P

GALLBLADDER POLYPOSIS AND SULFATIDURIA IN TWINS HETEROZYGOUS FOR MLD AND FOR ASA PSEUDODEFICIENCY

Kohlschütter A[1], Lukacs Z[1], Sürken A[1], Wild F[2], Harzer K[3], Mayrhofer H[3], Gieselmann V[4]
Klinik für Kinder- und Jugendmedizin, Universities of [1]Hamburg and [3]Tübingen; [2]Klinikum Neuburg/Donau; [4]Institute of Physiological Chemistry, University of Bonn, Germany

Metachromatic leukodystrophy (MLD) is caused by deficiency of arylsulfatase A (ASA) and has long been known to be associated with gallbladder disease (deficient contractility or polyposis). We report on twin boys with sonographically identified gallbladder polyposis. Sonography was performed because one of them had abdominal pain. The boys were examined for premorbid MLD. Neither neurological symptoms nor neuroradiological signs of white matter disease were present, but biochemical analyses in different laboratories resulted in interesting findings in both patients: the ASA activity was decreased in leukocytes and fibroblasts, and the excretion of sulfatides, measured on four occasions by quantitative thin-layer chromatography, was elevated and was in the lower range of true MLD cases. Molecular genetic analysis of the *MLD* gene was therefore performed and revealed compound heterozygosity for a known mutation that is associated with MLD and for a mutation in the ASA pseudodeficiency allele (P426L/PD). This combination, as observed before, does not seem to be associated with leukodystrophy (Penzien et al. Am J Hum Genet. 1993;52:557–64). **Conclusion**: Although the patients presented showed one of the clinical signs of MLD (gallbladder polyposis), low ASA activities, sulfatide accumulation and the compound heterozygosity described, they are not considered to have premorbid MLD. The consequences for family counselling are obvious.

294-P

DETERMINATION OF ARYLSULPHATASE A IN DRIED BLOOD SPOTS

Tan MAF, Hopwood JJ, Meikle PJ
Department of Paediatrics, The University of Adelaide, South Australia and Lysosomal Diseases Research Unit, Departmen. of Genetic Medicine, Women's and Children's Hospital, South Australia

The deficiency of arylsulphatase A (ASA) is the major cause of metachromatic leukodystrophy (MLD). Biochemical diagnosis of MLD is obtained by enzymatic analysis of ASA activity in peripheral blood leukocytes and cultured fibroblasts. However, methods available to specifically measure the activity of this enzyme are cumbersome and complicated by the high frequency of a pseudo-deficiency allele.

We have developed 2 immune-based assays to distinguish MLD affected individuals from controls. This is achieved through the use of an immune-quantification assay for the ASA protein and an immune-capture assay for the ASA activity. Both assays are performed on 3mm dried blood spots on filter paper. The median concentration of ASA protein in control individuals was 29.2 pg/μl of whole blood (range 21–46 pg/μl, $n = 10$), while MLD affected individuals ($n = 3$) had no detectable protein and pseudo-deficiency homozygotes ($n = 3$) had a range of 13–17 pg/μl. Median activity levels in control individuals were 44 nmol/min/μl of whole blood (range 25–72 nmol/min/μl), while MLD had no detectable activity.

The ease of transport of the dried blood spots on filter paper combined with the sensitivity and specificity of these assays provide for a powerful approach to the diagnosis of MLD and raise the possibility of newborn screening for MLD.

295-P

METACHROMATIC LEUKODYSTROPHY: A HOMOZYGOTE FOR 1204+1G>A MUTATION PRESENTS WITH A LATE-INFANTILE TYPE AND A RARE PATTERN OF MRI APPEARANCES

A Lugowska[1], K. Szymanska[2], T Kmiec[3], I Tarczynska[2], B Czartoryska[1], A Tylki-Szymanska[4], E Jurkiewicz[5]
[1]Department of Genetics, Institute of Psychiatry and Neurology, Warsaw, Poland; [2]Department of Neurology, Institute of Mother and Child, Warsaw, Poland; [3]Department of Neurology, [4]Department of Metabolic Diseases, and [5]Department of Magnetic Resonance, The Children's Memorial Health Institute, Warsaw Poland

The metachromatic leukodystrophy (MLD) – causing mutation 1204+1G>A damages an intron-exon splice site recognition sequence. This results in a complete loss of mRNA and enzymatic activity of arylsulfatase A (ARSA) protein molecules. We have found a late-infantile type MLD patient to be homozygous for this mutation, what was not reported earlier. Interestingly, the cerebral magnetic resonance imaging (MRI) in this patient displayed linear or punctuate structures radiating jin the demyelinated white matter, which were resembling the patterns described in Pelizaeus-Merzbacher disease (PMD). The so-called 'tigroid' or 'leopard-skin' appearances have, already, been observed in MLD patients suffering from different types of the disorder but were not described in a homozygote for 1204+1G>A mutation. It should be emphasized, that whenever a cerebral MRI demonstrates the 'tigroid' or 'leopard-skin' demyelination pattern not only PMD but also metachromatic leukodystrophy diagnosis should be considered; this suggests the necessity of ARSA activity estimations in patients with such specific MRI pattern.

296-O

HEMATOPOIETIC STEM CELL TRANSPLANTATION IN GLOBOID CELL LEUKODYSTROPHY: NEUROLOGICAL OUTCOME

G Uziel[1], M Bugiani[1], I Moroni[1], M Morbi[1], B Bertagnolio[1], A Bizzi[1], P Corti[2], A Rovelli[2]
[1]Istituto Nazionale Neurologico "C. Besta", [2]Clinica Pediatrica, Università di Milano-Bicocca, Italy

Objective: To evaluate the efficacy of hematopoietic stem cell transplantation (HSCT) in patients affected by late onset globoid cell leukodystrophy (GCL). **Methods**: Three children selected according to strict clinical and neuroradiological criteria underwent allogenic HSCT from unrelated donors. Age at transplant was 6.4, 9.1 and 6.3 years (8 months, 1 and 4 years from the onset of symptoms), respectively. Patients were transplanted with positively selected bone marrow CD34+ cells. **Results**: None of the patients developed major early or late complications. Permanence of engraftment with complete chimerism was demonstrated after a period ranging from 20 to 30 months, with a reconstruction of enzymatic activity to normal levels in all patients. Neurological follow-up documented a stabilization in two children, while one had a severe increase in seizures frequency with cognitive decline 9 months after HSCT but then stabilized when seizures were partially controlled. These data were compared with those obtained in an age-matched untreated patient who run a relentlessly progressive course. **Conclusions**: Data reported are still preliminary and follow-up too short to draw definite conclusions on the beneficial effects of HSCT in these patients. In all patients MRI and neurophysiological evaluations showed that the progressive demyelinating process was halted. Seizures frequency deeply interfered with cognitive functions and influenced the overall disability. Epilepsy should therefore be considered as a negative prognostic factor in the neurological outcome of patients with GCL.

297-P

SIGNIFICANTLY HIGH RECURRENCE OF LATE ONSET KRABBE'S LEUKODYSTROPHY IN SICILY: NATURAL HYSTORY

Fiumara A, Bertagnolio B, [2]Barone R, Meli C, Pavone P, Sorge G
Regional Referral Center for Inborn Errors of Metabolism, Department of Paediatrics, University of Catania, [2]Istituto Neurologico Besta, Milano, Italy

Krabbe disease, is considered a rare form of leukodystrophy and even more its late onset forms. Since 1991, in a European survey made by Lyon et al, our experience concerning Sicily, accounted for a unexpectedly high occurrence in our area. Up to now, by β-galactocerebrosidase assay, we have detected 20 patients: 6 early onset, 13 late onset cases and one adult patient. Interestingly, 11/13 of the late infantile cases belong to families not related each other but coming from the same area, north of Catania. Moreover, consanguinity among parents of each patient is extremely rare. These findings suggest a high concentration of healthy carriers. On the contrary all early onset cases were born to related parents and came from different places, including two from Tunisia and one from Brasil. We have observed that 6/13 patients started to complain progressive vision failure some months before the onset of motor signs, 7/13 had acquired hemiplegia and the parents were alerted by frequent falls and motor clumsiness. MRI pattern, with deep white matter lesions mainly interesting occipital and parietal lobes and centrum semiovale, is always more severe than expected on a clinical ground, vanishing any hope of bone marrow treatment. Indeed, our impression is that motor deterioration is extremely rapid and, in 6 to 10 months. generalized spasticity, speech and vision impairment become severe. Later on, the course is apparently stable or slowly progressive for several years. Early diagnosis remains crucial for future treatments.

298-P

HAEMORHEOLOGY IN GAUCHER DISEASE

BE Bax[1], L Richfield[2], MD Bain[1], AB Mehta[2], RA Chalmers[1], MW Rampling[3]
[1]*St George's Hospital Medical School,* [2]*Royal Free Hospital,* [3]*Imperial College, London, UK*

In Gaucher disease a deficiency of glucocerebrosidase results in the accumulation of glucocerebroside within the lysosomes of the monocyte-macrophage system. Prior to the availability of enzyme replacement therapy, splenectomy was often indicated. Haemorheological abnormalities could be expected in view of the anaemia and abnormal lipid metabolism in these patients and the role of the spleen in controlling erythrocyte quality. We report here a study of the haemorheology in Gaucher patients. **Methods**: Blood was collected from 15 healthy controls, 24 Gaucher patients with spleens, 16 asplenic Gaucher patients and 6 healthy asplenic non-Gaucher subjects. Whole blood viscosity was measured at shear rates of $0.277s^{-1}$ and $128.5s^{-1}$ at native haematocrit and at a haematocrit of 45%. Plasma viscosity was measured at high shear rates. Erythrocyte aggregation indices were obtained at stasis and at low shear. Erythrocyte deformability was assessed by measuring the rate of erythrocyte transit through 5 μm pores. **Results**: The following significant differences were found between the controls versus asplenic Gaucher patients and asplenic non-Gaucher subjects respectively: MCHC (33.0 ± 0.2 v 32.1 ± 0.2 and 31.8 ± 0.3), viscosity at 45% haematocrit measured at low shear (49.2 ± 2.1 v: 58.8 ± 3.5 and 59.4 ± 3.3), relative viscosity (34.9 ± 1.1 v 39.3 ± 1.8 and 40.4 ± 2.5) and red cell aggregation index at low shear (10.3 ± 0.5 v 11.7 ± 0.3 and 12.1 ± 0.1). Additional differences observed between the controls and asplenic Gaucher patients were MCV (92.9 ± 1.1 v 97.8 ± 1.0), viscosity at 45% haematocrit measured at high shear (4.1 ± 0.1 v 4.6 ± 0.2) and relative filtration rate (0.651 ± 0.006 v 0.581 ± 0.011). **Conclusions**: The erythrocytes of both asplenic Gaucher patients and asplenic controls were more aggregable than those of controls, indicating that these differences are due to the effects of splenectomy. The only haemorheological differences which could be attributed to Gaucher disease were increased cell size and decreased cell deformability.

299-A

CONGENITAL PRESENTATION OF GAUCHER DISEASE TYPE II

Durán G[1], González A[1], Kattan J[1], Toso P[1], Pastores G[2]
[1]*Pontificia Universidad Católica de Chile, Departamento de Pediatría, Santiago, Chile;*
[2]*Neurogenetics Unit, Department of Neurology and Pediatrics, New York University School of Medicine, New York, USA*

Gaucher disease, the hereditary glucocerebrosidase deficiency, has a vast spectrum of clinical presentation. The severe congenital presentation is a subgroup of low frequency and bad prognosis disease. We report two siblings with severe congenital Gaucher type II which was misdiagnosed as Histiocytosis in the first child. **Case report**: second male child of young, healthy and non-consanguineous parents. At birth he presented hepatosplenomegaly, echymoses, petechiae, skin desquamation and opisthotonus posture. He developed severe neurologic deterioration, thrombocytopenia, leukopenia, cholestasis, hepatic disfunction. Liver and bone marrow biopsy were done which inicially were interpretated as hystiocitosis. The history of an older sister died during the first month of age with the same clinical presentation and diagnosis of Histiocytosis, misleading the study to the wrong way. The liver electron microscopy showed the characteristic storage of Gaucher disease, which was confirm later by enzimatic and molecular assay on fibroblasts. The child died at 20 days of age because of multiorganic failure, after cordon transplant. **Conclusion**: It is very important to rule out all possible ethiologies, including Gaucher type II, in the newborn with visceromegaly and histiocytes in tissue biopsy.

300-P

EVIDENCE OF NEUROLOGICAL MANIFESTATIONS IN PATIENTS WITH TYPE I GAUCHER DISEASE

S Mercimek-Mahmutoglu, S Gruber, Ch Wöber, E Moser, S Stöckler-Ipsiroglu
Children's Hospital, Vienna University, Austria

Gaucher disease (GD) is an inborn error of lysosomal glycosphinglipid storage disorder. GD has been classified into three clinical subtypes: type I is characterized by pancytopenia, hepatosplenomegaly and bone involvement. In this study we investigated, whether there is also involvement of central and peripheral nervous system in patients with type I GD.

Materials and Methods: Four patients (39–64 years old) with type I GD were measured using a 3D-SI sequence with PRESS-preselection at a 3 Tesla whole body system. All voxels were processed using LCModel. Further investigations included nerve conduction velocity, SSEP, myelin antibody profile in serum, serum protein electrophoresis, vitamin B_{12} and HBA1c.

Results: All spectroscopic findings in 4 patients with type I GD showed decreased ratio of NAA/Cr and increased ratio of Cr/Cho in voxels extracted out of the white matter in brain. In one patient electrophysiological signs of axonal neuropathy and in another patient signs of long sensory spinal tract involvement was found. Monoclonal gammopathy and metabolic causes were excluded.

Conclusion: High level of choline and low level of NAA is typical marker for the myelin turnover/breakdown and axonal involvement, respectively. These results together with electrophysiological abnormalities suggest that patients with type I GD may have mild central and peripheral nervous system involvement.

301-A

ORAL SUBSTRATE REDUCTION THERAPY (SRT) WITH MIGLUSTAT FOR PATIENTS WITH MILD TO MODERATE GAUCHER DISEASE (GD) WHO ARE UNSUITABLE FOR ENZYME REPLACEMENT THERAPY (ERT): INITIAL RESULTS

DA Hughes, R Baker, S Goodwin, A Milligan, L Richfield, A Mehta
Royal Free and University College Medical School, Rowland Hill Street, London, NW3 2PF, UK

Objective: To assess the effects of oral miglustat treatment in patients with mild to moderate GD previously treated with i.v. ERT. **Methods**: Case notes review. **Results**: Three patients assessed as unsuitable for ERT, according to the Advisory Council to the European Working Group on Gaucher Disease (EWGGD) position statement, have started oral SRT with miglustat. Patient 1, a 54-year-old male, had poor venous access and, now retired, travelled regularly outside mainland UK to visit his family. Initial flatulence and diarrhoea ameliorated with dose reduction from 100 mg t.i.d. to 100 mg b.i.d. and introduction of a lactose-free diet. Resumption of t.i.d. dosing after 2 months was associated with flatulence but not diarrhoea. Patient 2, an 81-year-old male, had persistent problems with ERT due to tremor associated with Parkinson syndrome. After 6 weeks miglustat, 100 mg t.i.d, chitotriosidase levels remained stable and neurological status was unchanged. Patient 3, a 23-year-old female, had frequent problems with self cannulation. Chitotriosidase levels, which reduced from >25 000 to 2756 nmol/hr/ml after 7 years of ERT, were 2433 nmol/hr/ml after 2 months of treatment with miglustat, 100 mg t.i.d. **Conclusions**: Our initial experience with 3 patients with GD and poor venous access, including the oldest patient for whom miglustat treatment has been reported, is consistent with the EWGGD position statement and indicates that oral miglustat provides an effective alternative for patients unsuitable for ERT.

302-P

MIGLUSTAT TREATMENT OF AN ELDERLY PATIENT WITH GAUCHER DISEASE (GD) AND PARKINSON SYNDROME

DA Hughes, R Baker, S Goodwin, A Milligan, L Richfield, A Mehta
Royal Free and University College Medical School, Rowland Hill Street, London, NW3 2PF, UK

Objective: To report an interesting case of an elderly patient with GD, whose progressive concurrent pathology necessitated switching from i.v. enzyme replacement therapy (ERT) to oral substrate reduction therapy (SRT) with miglustat. **Methods**: Case notes review. **Results**: The patient, a Lithuanian Ashkenazi male, first presented aged 67 with suspected myelodysplastic syndrome and refractory anaemia. Further investigations revealed a 17q11–q12 karyotype; trilineage dyserythropoiesis and Gaucher cells in bone marrow; genotype N370S/N370S; and leukocyte β-glucosidase activity of 0.88 mmol/hr/mg protein. Seven years later, the patient complained of persistent fatigue, his blood chitotriosidase level was 2748 nmol/hr/ml, and ERT was commenced. At this time, Parkinsonian signs were evident. Co-careldopa treatment was started shortly afterwards, but within 2 years the patient became unresponsive to anti-Parkinson therapy; he was subsequently diagnosed with multiple system atrophy. At this time, cannulation was becoming increasingly difficult due to Parkinsonian tremor. The patient, now aged 81, was assessed as suitable for SRT with miglustat, which was started at 100 mg t.i.d. in February 2004. In the brief follow up since therapy switching, chitotriosidase levels are essentially stable, the patient is satisfied with his treatment, and his neurological profile is unchanged. **Conclusions**: Miglustat, a new oral treatment for GD, provides an effective alternative in patients with, for example, concurrent neurological movement disturbances who are unsuitable for ERT.

303-P

BONE DISEASE AND ZAVESCA™ (MIGLUSTAT) – NEW INSIGHTS AND RESULTS

C Hollak
Academic Medical Centre, University of Amsterdam, The Netherlands

The first clinical trial of Zavesca took place in 28 adult patients with type 1 Gaucher disease (7 of whom had undergone splenectomy) who were unable or unwilling to receive enzyme therapy. Zavesca was given orally at a starting dose of 100 mg TID and, initially, treatment lasted 1 year. An extended treatment protocol allowed patients to continue to receive Zavesca after completing the initial study. Bone marrow involvement was assessed every 12 months for patients enrolled at the Academic Medical Centre in Amsterdam with Dixon quantitative chemical shift imaging (Dixon-QCSI) which is an MRI technique for measuring the displacement of fatty marrow by Gaucher cells. Low bone marrow fat fractions have been found in patients with Gaucher disease and reflect the degree of infiltration with Gaucher cells. In 2 previous studies it has been shown that fat fractions are usually low in patients with clinically relevant bone complications and that enzyme therapy results in significant early increases in bone marrow fat fractions in patients with adult type 1 Gaucher disease compared with patients with untreated Gaucher disease. Two patients have now received Zavesca therapy continuously for more than 5 years. Patient 201 had mild hepatosplenomegaly and cytopaenia, and bruised easily at the start of treatment. Patient 202 had a splenectomy and complete bilateral hip prostheses 25 years ago. She also suffered from mild anaemia and fatigue. Patient 201 showed a progressive improvement in bone marrow fat fraction over 6 years with a trend towards the normal range, indicating the clearance of storage cells from the bone marrow. Patient 202, with very severe bone disease, showed an improvement until month 36 and remained stable at months 48 and 60. These results show the beneficial effects of Zavesca therapy on the infiltration of bone marrow by Gaucher cells in type 1 Gaucher disease.

304-P

SUCCESSFUL OUTCOME OF A PREGNANCY WHILST ON N-BUTYLDEOXYNOJIRIMYCIN (NBJ, MIGLUSTAT) FOR TYPE I GAUCHER DISEASE

R Heitner, B Ross
Department of Paediatrics, University, Witwatersrand, South Africa

Objectives and Methods: miglustat is an imino-sugar that inhibits the first step in glycosphyngolipids (GSL) synthesis by inhibiting ceramide specific glucosyl transferase. Miglustat has been demonstrated to cross the blood brain barrier and to reduce accumulation of GSL in animal models of GSL diseases. Embryo-foetal toxicity studies have not demonstrated any impact on development of newborns, but a increase in pre- and post-implantation loss and reduction in litter weight. However, potential risk in humans is unknown, leading to contra-indication of miglustat during pregnancy. We report a case of pregnancy started on miglustat and its oucome. **Results**: patient with Gaucher type 1, aged 32, treated with miglustat, 100 mg tid, in a clinical trial. After $2\frac{1}{2}$ years without drug interruption, she presented with a hemiplegic migraine, whose investigation led to discover she was 9 weeks pregnant. Miglustat was immediately discontinued. She was counselled with respect to termination of the pregnancy, but declined, and subsequently had a normal pregnancy. She delivered (at term, by caesarean section) a healthy female baby (2.72 kg, 47 cm, skull circumference 35.5 cm). There was neither dysmorphic features nor obvious congenital abnormalities. The baby has since been closely monitored. At 14 months she is completely well, with normal physical and neurological examinations (both gross and fine testing). She has followed all normal motor and social developmental milestones. Her mother has resumed miglustat. **Conclusion**: despite this single case with favourable outcome, and in view of the preclinical data available today, pregnancy remains contraindicated with miglustat.

305-P

DOSAGE OF ENZYME REPLACEMENT THERAPY IN CHILDREN WITH GAUCHER DISEASE

E Miebach, E Mengel, L Paruthiyil, A Nicol, M Beck
Children's Hospital, Gutenberg-University, Langenbeckstrasse 1, 55101 Mainz, Germany

Introduction: Enzyme Replacement is a sufficient therapy for patients with non-neuropathic Gaucher disease. The current recommended dosage may be effective, but not the optimal dose for every patient. The lowest (cost-effective) but still sufficient dose has not yet been evaluated. We compared the clinical outcome in children treated with different dosages of ERT.

Patients and Methods: We examined 23 patients at an age of 3 to 17 years (mean age 10 years), who received ERT for at least two years. 3 patients were treated with high dose therapy (60 units/kg/14 days). 18 patients received an individual adapted dosage (mean 38 ± 12 units/kg/14 days). Therapeutical aims for these patients were normalization of growth velocity, absence of skeletal pain, no relevant lysosomal storage in bone marrow and an activity of chitotriosidase < 500 nmol/ml/h (mean 211 ± 239 nmol/ml/h). Two patients received a low dose therapy (20 units/kg/14 days).

Results: No patient developed pathological blood count, bleeding or skeletal involvement. Body height was normal in nearly all patients besides of the 2 receiving low dose therapy. Those patients also developed pulmonary involvement during therapy. Additionally, they showed continuous chitotriosidase activity > 1000 nmol/ml/h.

Discussion: Possible risks of a low dose therapy are a diminished body height, already occurring in childhood, and a progressive lung disease. Chitotriosidase activity is a significant biochemical parameter to indicate partially ineffective dosage of ERT.

306-P

CASE REPORT: NON COMPLIANCE TO ENZYME SUPPLEMENTATION THERAPY IN GAUCHER DISEASE TYPE I; EARLY SIGNS OF RECURRENCE OF DISEASE MANIFESTATIONS

M de Fost[1], M Maas[2], JMFG Aerts[3], JEM Groener[3], CEM Hollak[3]
Department of Internal Medicine, Clinical Hematology[1], Radiology[2], and Biochemistry[3], Academic Medical Center, Amsterdam, The Netherlands

Gaucher disease type I was the first of the lysosomal storage disorders that could be treated using enzyme supplementation therapy (EST), leading to a dramatic clinical response in the majority of patients. Despite the benefits of EST, some patients discontinue therapy, of which we here present an example. After approximately 7 years of EST with a very satisfying response, our patient became non compliant for private reasons. During the period thereafter, a deterioration of his clinical condition could be detected by close monitoring of several disease parameters. Hexosamidase showed a steep increase, rising above pre-treatment levels. CCL18 novel marker for Gaucher cell burden, increased after a period of stability. Liver and spleen volumes increased by 35% and 72% respectively, and platelet count dropped to 65×10^9/L. Bone marrow fat fraction (Quantitative Chemical Shift Imaging, QCSI) showed a dramatic decline from 39% to 24% in 13 months. Interestingly, the patient did not notice any changes in his physical state.

This case report shows that discontinuation of EST can result in a serious worsening of the clinical condition. Close monitoring is essential for early recognition, with biochemical markers that cause no clinical symptoms per se, such as chitotriosidase, hexosaminidase and CCL18, preceding changes in clinical parameters. As for monitoring of bone marrow involvement, changes are quickly detected by a decrease in fat fraction.

307-P

GAUCHER DISEASE IN A PEDIATRIC POPULATION: GROWTH, DENSITOMETRIC AND LOCAL BONE CHANGES DURING A LONG PERIOD OF ENZYME REPLACEMENT THERAPY

G Ciana, R Addobbati, M Nevijel, V Guerci, Pittis MG, B Bembi
Unità di Malattie Metaboliche, Istituto di Ricerca e Cura a Carattere Scientifico 'Burlo Garofolo', Trieste, Italy

Objective: Gaucher type I disease shows to be more aggressive in children than in adults. Growth is affected in about 50% of cases and puberty is often delayed. Skeletal involvement has a particular importance, being the most disabling aspect in the adult population.Treatment during the pediatric age may prevent further skeletal complications and permit a normal growth. **Methods**: Ten children (7 M, 3 F, age range 4–16 years) received enzyme replacement therapy (ERT) for a period ranging 3–9 years. 6 of the patients had failure to thrive; regarding skeletal involvement, 2 patients had femoral head osteonecrosis, 6 had various focal/local lesions and 8 had altered bone density (z score <1) measured by DEXA. **Results**: None of the patients experienced bone crisis during follow up, the 2 patients with osteonecrosis experienced recurrence of pain after 8 and 9 years of treatment respectively. The z score increased from baseline to the last reading for 9 of the 10 patients, normalizing (Z score >-1) in 3 patients. No improvement of radiological bone lesions was observed. Patients with growth delays achieved normal growth rates within 1–3 years after starting ERT. **Conclusions**: ERT improves growth and BMD in pediatric patients, local/focal bone lesions remain unchanged. This effect may prevent serious complication in adult life, such as fractures and vertebral compression due to a low BMD.

308-P

MODIFICATION OF CYSTEINE RESIDUES IMPROVES THE PLASMA STABILITY OF β-GLUCOCEREBROSIDASE

T Edmunds, H Hughes, M Kudo, V Butnev, W Canfield, S VanPatten
Genzyme Corporation Cambridge Massachusetts USA

Deficiency of the lysosomal enzyme β-glucocerebrosidase (GCase) results the accumulation of glycocerebroside in the lysosome leading to Gaucher disease. Enzyme replacement therapy has been used to successfully treat Gaucher disease for over a decade. During this time several second generation protein therapeutics have been developed using technologies such as PEGylation and protein engineering. These technologies generally rely on increasing the plasma half-life to increase the bioavailability of protein therapeutics. Before a similar approach can be applied to GCase it is necessary to overcome the loss of enzyme activity that occurs upon exposure to neutral pH. We have identified the mechanism by which GCase is inactivated at neutral pH. Incubation of GCase at neutral pH or above in phosphate buffer, serum or plasma resulted in rapid loss of activity which correlated with the generation of protein aggregates along with a loss of reactive thiols. Chemical modification of the free thiols also resulted in loss of enzymatic activity but prevented the formation of aggregates, suggesting two thiol mediated mechanisms for the inactivation of GCase. Modification with the active site inhibitor condruitol B epoxide (CBE) offered partial protection against chemical modification of thiols. Peptide mapping of the CBE modified protein indicated that partial modification of all free cysteine residues had occurred. These studies demonstrate a role for free thiols in the activity and stability of GCase and provide a mechanistic understanding of the instability of the protein in plasma and its inhibition by thiol reactive compounds.

309-P

CORRELATIONS AMONG CYTOKINES AND BONE TURNOVER MARKERS IN PATIENTS WITH TYPE I GAUCHER DISEASE

Watanabe Y[1], Tokunaga Y[1], Ida H[2], Kobayashi M[2], Ohashi T[2], Eto Y[2], Yoshino M[1]
[1]*Department of Pediatrics and Child Health, Kurume Univ. School of Medicine, Fukuoka, Japan;*
[2]*Department of Pediatrics, Jikei Univ. School of Medicine, Tokyo, Japan*

In Gaucher disease patients, monocytes/macrophages, typically Gaucher cells, are activated due to accumulation of glucosylceramide. Accordingly, some individual cytokines are known to increase in blood. In the present study, we evaluated changes in multiple cytokines and related compounds in Gaucher disease patients, and further studied possible correlation among such cytokines and bone turnover markers to explore possible role of cytokines in bone disease.

Nine serum cytokines and related compounds (IL-1β, IL-6, IL-8, IL-18, TNF-α, sFas, sCD14, M-CSF, TGF-β1) were measured in four patients with type I Gaucher disease 20 to 50 years of age who had been on enzyme replacement therapy (ERT) with imiglucerase. Of these cytokines and related compounds, concentrations of Il-18 and TGF-β1 were found to be elevated in these patients. We then examined correlation among concentrations of cytokines and related compounds, and bone turnover markers (bone-specific alkaline phosphatase [BALP], osteocalcin, deoxypyridinoline [DPD], total acid phosphatase [t-AcP], N-telopeptide to helix in serum [s-NTx], and in urine [u-NTx]), and found that each of TGF-β1 and TNF-α concentrations correlated with t-AcP, and IL-18 concentration correlated with BALP ($p < 0.05$, $r > 0.95$). These results suggest that increases in these cytokines play a role in pathogenesis of bone disease in type I Gaucher disease.

310-A

MONITORING GAUCHER DISEASE WITH CHITOTRIOSIDASE

L Paruthiyil, E Mengel, M Ries, E Schäfer, A Gal, M Beck
Children's Hospital, University of Mainz, Langenbeckstr. 1, 55101 Mainz, Germany

Objective: The enzymatic activity of chitotriosidase is elevated in patients with Gaucher disease. Monitoring of serum chitotriosidase activity is useful to obtain insight in changes in body burden on storage cells. The usefulness of chitotriosidase is limited to a small degree by recessively inherited 24-bp-deletion that is present in nearly 6% of the population. This study investigated the correlation between severity of disease and chitotriosidase serum activity regarding this 24-pb-duplication.

Results: Before starting any therapeutic intervention chitotriosidase activity was measured in 53 patients as described by Hollak et al. 1995. 24-bp-deletion in homozygous or heterozygous status was detected by PCR methods. In 2/53 patients (4%) without chitotriosidase activity homozygous 24-bp-deletion was found. 19/53 patients (36%) were heterozygous. Respectively 32/53 (60%) patients do not carry the mutation. In the heterozygous group the chitotriosidase activity was measured significantly lower ($p < 0.001$) than in the non-carrier group. We found a significant correlation ($p = 0.008$) between disease severity (neurological involvement) and chitotriosidase activity in the patient group with no mutation in the chitotriosidase gene.

Discussion: Chitotriosidase activity reflects total burden of storage cells and disease severity in Gaucher disease. The heterozygous 24-bp-deletion in a patient leads to a remarkably reduced enzyme activity. These findings should be kept in mind for therapeutic decisions and monitoring therapy efficacy.

311-P

MOLECULAR SCREENING RUSSIAN PATIENTS WITH GAUCHER DISEASE

Boukina TM[1], Tsvetkova IV[2], Semechkina AN[3], Dadaly EL[1], Galkina VA[1]
[1]*Research Centre for Medical Genetics RAMS, Moscow, Russia;* [2]*Institute of Biolmedical Chemistry RAMS, Moscow, Russia;* [3]*Institute of Pediatrics and Child Surgery RAM, Moscow, Russia*

Gaucher disease (GD), the most common lysosomal storage disorder, is caused by mutations in the gene encoding glucocerebrosidase. The deficiency of the enzyme activity impairs the lysosomal degradation of glycosphingolipids, leading to the accumulation of the glucosilceramide. It is an autosomal recessive disorder. The gene for human glucocerebrosidase is located on chromosome 1q21. A pseudogene for glucocerebrosidase, sharing 97% exonic sequence homology. The presence of this homologous sequence complicates mutation detection strategies, since many mutant alleles are point mutations which derive from the pseudogene sequence. So PCR amplification of genomic DNA for sequencing must selectively amplify the functional gene.

We have performed molecular analyses of a 70 patients with GD. Fourteen different mutations were found. Out of 140 alleles screened, 63 were N370S, 32 were L444P. There were 5 homozygotes for point mutation N370S and 4 homozygotes for point mutation L444P. Mutation L444P occurred alone on 24 alleles, and as part of recombinant allele NciI on 8 alleles. Eleven rare mutations were identified: R120W (3/140), R170C (1/140), W184R (6/140), G202R (2/140), P236T (1/140), L288 (2/140), W381X (1/140), A384D (3/140), P391S (1/140), del55 (1/140), R463C (1/140) and five mutations from it were novel: P236T, L288P, W381X, A384D and P391S.

312-P

ANALYSIS OF FIVE 1Q21 MOLECULAR MARKERS IN COLOMBIAN CHROMOSOMES BEARING MUTATION N370S

Barrera ALA, Wilches R, Vega FH
Instituto Errores Innatos del Metabolismo. Bogota Colombia

Gaucher disease is a pan-ethnic condition characterized by glucosilceramide accumulation in macrophages due to glucocerebroidase deficiency. The main cause of the disease in western populations, including Colombia, is the mutation N370S, in the *GBA* gene mapped to 1q21. Samples of 17 Colombian Gaucher patients bearing the N370S mutation, some of their closest relatives and 30 control individuals were taken to asses the degree of association between this mutation and the alleles of five *STRs* enclosing the *GBA* locus. The loci: *D1S305, D1S2624, D1S2777, ITG6.6.2* and *5GC3.2* were amplified using PCR. Allele frequencies were calculated and haplotypes inferred in eleven N370S chromosomes belonging to nine patients for whom gametic phase was available. A consensus N370S haplotype consisting of the alleles 222-314-260-301-172 (bp) of the markers 5GC3.2 *ITG6.6.2, D1S2777 D1S2624* and *D1S305* respectively was found. There was a statistically significant linkage dissequilibrium (LD) between the alleles 222, 314, 260, 301 and N370S mutation. A conserved fraction of the haplotypes, present in all of them suggests that the allele N370S, might have either occurred *de novo* (Recurrence) or have entered into the Colombian population within a single ancestor chromosome. Since the information provided for the generated haplotypes is limited, the inclusion of other *STRs* or different sorts of molecular markers can result in haplotypes informative enough to test the previously proposed hypotheses.

313-P

412 CASES OF GAUCHER DISEASE DIAGNOSED IN BRAZIL

R Giugliani[1,3], K Michelin[2,3], A Wajner[2,3], FTS Souza[2,3], AS Mello[3], MG Burin[2,3], ML Pereira[2,3], RF Pires[3], JC Coelho[2,3]
Genetics[1] and Biochemistry[2] Department, UFRGS, and Inborn Errors of Metabolism Laboratory[3] – Medical Genetics Service – Hospital de Clínicas de Porto Alegre, Porto Alegre, RS Brazil

Gaucher disease (GD) is a sphingolipidosis characterized by the storage of glucosylceramide in cells of the reticulo-endothelial system. The aim of this study is to characterize the GD in Brazil. The diagnosis of GD is based on the measurement of β-glucosidase activity (β-glu) and assisted by a biochemical marker: chitotriosidase activity (CT). Our sample was composed by 1081 cases that were sent to the Medical Genetic Service due to a clinical suspicion of GD from 1982 to October 2003. These samples were from several regions of Brazil (64.8% from south-east, 16.5% from south, 11.2% from north-east, 4.4% from north, and 3.1% from center-west). During this period, 412 cases were diagnosed as GD (38.1%). Mean age at the diagnosis was 19.2 ± 15.6 years, ranging from 7 months to 72 years of age and 57.6% were females and 42.4% were males. Information on consanguinity among parents were obtained from 239 individuals, and consanguinity rate was established to be 3.1%. Mean β-glu in leukocytes from patients with GD was 1.29 ± 1.09 nmol/h/mg of protein and CT was $17\,740 \pm 18\,135$ nmol/h/ml of plasma. Since the introduction of enzyme replacement therapy, the search for the diagnosis of these patients showed the real levels of lysosomal disease in our country. Through a wide divulgation and relative's investigation, we intend to be able to reach an earlier diagnosis of patients with GD.

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314-P

AAV-MEDIATED GENE THERAPY OF A MURINE MODEL OF GAUCHER DISEASE

J Marshall, K McEachern, J Nietupski, J Cavanagh Kyros, G Grabowski, SH Cheng
Genzyme Corp, Framingham, MA 01701, University of Cincinnati, OH 45229, USA

Gaucher disease is caused by mutations in the gene encoding the lysosomal hydrolase, glucocerebrosidase (GC). Deficiency of this enzyme results in the accumulation of glucosyl-ceramide (GL-1) in tissue macrophages primarily of the liver and spleen. A murine model of Gaucher (D409V/null) was recently generated that displayed several of the abnormalities shown associated with the human disease. The D409V/null mice exhibited approximately 5% of normal levels of the enzyme in the visceral tissues and consequently, elevated levels of GL-1 in the liver, spleen, lung and bone marrow. Associated with the abnormal storage of GL-1 was the appearance of enlarged macrophages similar to those observed in Gaucher patients. To evaluate the potential of gene therapy for treating this disease, an AAV 2/2 and 2/8-pseudotyped vector encoding the human GC were constructed. Since secretion of GC from transduced cells occurs only in cells overexpressing the enzyme, efforts were made to optimize its expression. A codon-optimized and CpG-reduced synthetic cDNA for human GC was placed under the transcriptional control of an a1-antitrypsin promoter to which was appended two copies of the α1-microglobulin enhancer (DC172). Studies showed that expression from the DC172 promoter was hepatic-restricted. Intravenous administration of either the AAV2/2 or AAV2/8 vector into D409V/null mice resulted in hepatic transduction and subsequent secretion of supraphysiological levels of GC into the circulation. Expression of GC in animals administered the AAV2/8 vector was >50-fold higher than from the corresponding AAV2/2 vector and remained undiminished at 4 months. In contrast to the untreated mice, which displayed characteristic Gaucher cells in the lungs, liver and spleen, treated animals were devoid of these cells and harbored normal tissue levels of GL-1. The absence of elevated GL-1 levels in the tissues of treated animals confirmed that the hepatically-produced enzyme was in a form that was conducive to recapture by the affected cells and that importantly, the levels attained were sufficient to prevent the development of disease manifestations.

315-P

WOLMAN DISEASE AND TREATMENT BY CHOLESTYRAMINE AND HMG-CoA REDUCTASE INHIBITOR

K Mention[1], P De Lonlay[1], G Touati[1], P Benlian[2], JM Saudubray[1]
[1]*Hôpital Necker-Enfants Malades, Paris, France,* [2]*Hopital St Antoine, Paris, France*

Teoman is the first child of consanguineous Turkish parents. He presented with gastrointestinal symptoms with vomiting, anorexia, failure to thrive, abdominal distension and constipation, in the first week of life. At one month, the examination showed an hepatosplenomegaly with an abdominal distension and a weight growth retardation. The diagnosis of subocclusion syndrome was suspected and a X-ray and ultrasound abdominal examination were performed. Mesenteric adenopathy and splenomegaly were noted associated with bilateral calcifications of the adrenals, confirmed by the abdominal tomography, in favor of diagnosis of lipase acid deficiency. Vacuolated lymphocytes were noted.

Definitive diagnosis was confirmed by the measurement of lysosomal acid lipase activity in lymphocytes from peripheral blood, reduced to 0.6 mkat/kg protein (reference value: 59). Initially total cholesterol was low 2.4 mmol/L (N: 3.2–6), with a low level of LDL cholesterol and a normal level of HDH cholesterol. A moderate elevation of transaminases (X 2/N), a cholestasis and an elevation of LDH were noted. HLA type of parents were semi-compatible with the patient, excluding the possibility of a bone marrow transplantation.

The interest of this case report was to analyse the clinical and biological evolution of the patient after treatment with cholestyramine and an HMG-CoA reductase inhibitor. Present medical therapy has poor or no effect on clinical and biological features. Cholestyramine allowed to improve the intestinal symptoms of the disease and the comfort of the patient.

316-P

INTERMEDIATE NEUROLOGICAL INVOLVEMENT IN SPHINGOMYELINASE-DEFICIENCY

E Mengel, K Harzer, M Vanier, K Kim, M Bajbouj, L Paruthiyil, R Santer, M Beck
Children's Hospital, Gutenberg-University of Mainz, Langenbeckstrasse 1, 55101 Mainz, Germany

Patients with sphingomyelinase-deficiency (SMD) were classified according Crooker to Niemann-Pick disease type A (NPA) and type B (NPB). In NPA progressiv neurodegeneration leeds to death before the age of 4 years, whereas NPB patients show no or very mild neurological signs. In a prospective study we investigated 26 patients with SMD in the age of 1–58 years. 10/26 patients (38%) aged 4–26 years could not be classified to NPA or NPB.

In these patients following neurological signs are found: mild–severe mental retardation, cerebralparesis, peripheral neuropathy and neuroretinal involvement. 7/10 patients carried the pointmutation Q292K in the SMPD1-gene. Furthermore in 3/10 patients echography detected aortic insufficiency. However hepatosplenomegaly is marked, but in school age the visceral involvement seemed hardly progressive.

The classification according Crooker demonstrates not the clinical spectrum and the high incidence of intermediate neurological involvement in SMD. Intermediate neurological involvement is characterised through survival up to school-age, pronounced neurological signs, typical genetical findings and heart valve involvement in some cases. We propose a new classification with attention to the heterogenous neurological involvement. Our findings must also be considered, when studies for future ERT with rhASM in SMD will be designed.

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CHITOTRIOSIDASE AND CCL18 IN TYPE B NIEMANN-PICK DISEASE

J Brinkman[1], FA Wijburg[1], JEM Groener[2], M Verhoek[2], RG Boot[2], JMFG Aerts[2]
[1]*Department of Paediatrics;* [2]*Department of Biochemistry, Academic Medical Centre, Amsterdam*

Introduction and Objectives: Niemann-Pick disease type B (NPD B) is a nonneuronopathic lysosomal storage disorder due to a deficiency in lysosomal sphingomyelinase and characterized by accumulation of sphingomyelin-laden macrophages. The availability of plasma markers for storage cells may be of great value to facilitate therapeutic decisions. Given the similarity of storage cells in NPD and Gaucher disease, we studied Gaucher plasma markers (chitotriosidase and CCL18) in two siblings with NPD B. **Patients and Methods**: NPD B was confirmed in two Turkish siblings by mutation analysis, revealing a homozygous R228C mutation. Plasma chitotriosidase was determined using 4-methylumbelliferyl-β-D-chitotriose as substrate. CCL18 was determined by ELISA. Normal values were obtained for different age groups. **Results:** Analysis of plasma specimens obtained from the oldest child, revealed a 50-fold increased level of chitotriosidase and a 30-fold increased level of CCL18, as compared to age-matched controls. The child, at that time 1.5 years old, showed marked hepatosplenomegaly and pulmonary involvement. In the youngest, asymptomatic, child an abnormal high plasma level of chitotriosidase and CCL18 was seen almost immediately after birth, which rapidly increased further.

Conclusion: Plasma chitotriosidase and CCL18 may serve as markers for the formation of pathological lipid-laden macrophages in NPD B, in analogy to Gaucher disease. Sensitive plasma surrogate markers may serve as an important tool to document the natural course of NPD B and to monitor the efficacy of enzyme supplementation therapy that is currently developed.

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EFFICACY OF AAV-MEDIATED EXPRESSION OF ASM IN NIEMANN-PICK B MICE

SH Cheng, RJ Ziegler, CM Barbon, S Bercury, RJ Desnick, E Schuchman
Genzyme Corp, Framingham, MA 01701, Mt. Sinai School of Medicine, NY 10029, USA

Niemann-Pick B disease (NPD) is an inheritable lysosomal storage disease in which the deficient activity of acid sphingomyelinase (ASM) results in the accumulation of sphingomyelin in myeloid-lineage cells in the bone marrow, lung, liver, and spleen. Systemic administration of either an AAV2/1 or 2/8 pseudotyped vector encoding human ASM under the transcriptional control of a liver-restricted promoter into ASM knockout mice (ASMKO) resulted in expression of therapeutic levels of the enzyme. Expression, which was primarily hepatic-restricted, was approximately 50–100-fold higher with the AAV2/8 than with the AAV2/1 vector. Subsequent extrahepatic uptake of the secreted enzyme, presumably via the mannose and mannose 6-phosphate receptors, was associated with a reduction in sphingomyelin levels in all the affected tissues, including the lung. Since the levels of expression attained with the AAV2/8 vector were significantly higher, the kinetics of sphingomyelin clearance was more rapid than with the AAV2/1 vector. Biochemical and histological analysis of ASMKO mice at days 60 and 120 post-administration demonstrated a profound reversal of lung pathology concomitant with substrate depletion to basal levels. Correction was also associated with reduced levels of the proinflammatory chemokine, MIP-1a, and normalization of the cellularity and cell differentials in the bronchoalveolar lavage fluids (BALF) of treated animals. In contrast to the severe storage inclusions and enzyme-uptake defects shown associated with alveolar macrophages from ASMKO BALF, those isolated from AAV2/8-treated mice demonstrated improved phagocytic activity and displayed morphologies that were more akin to normal macrophages. Hepatic-restricted expression of the human ASM also promoted immune tolerance to the expressed enzyme in the ASMKO mice. No antibodies to the human enzyme were detected in the animals throughout the 120-day study. Hence, AAV2/8-mediated, hepatic-restricted expression of ASM was capable of correcting the visceral storage disease and abrogating the chronic pulmonary inflammatory manifestations shown associated with NPD.

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EVALUATION AND COURSE ASSESSMENT IN NIEMANN-PICK C DISEASE

J Kuhn, E Mengel, K Baron, M Beck
Children's Hospital, Gutenberg-University of Mainz, Langenbeckstrasse 1, 55101 Mainz, Germany

Introduction: By reason of its rareness and complicated diagnosis methods NPC is diagnosed (5–15) years after observation of first symptoms in most cases. To evaluate easier and faster parameters for diagnosis and course assessment were our motives for the study within which we examined NPC patients focussing on early signs and objectiv disease parameters.

Patients and methods: All NPC patients up from the age of 3 years until 41 years were included in the study as far as their state of disease allowed. A thourough PE, history of disease, neurological examination including evoked potentials and neuro-ophtalmologic tests as well as biochemical parameters were part of our investigations.

Results: 18 out of 19 patients showed presumed increasement of chitotriosidase activity (mean: 725 nmol/ml/h, range 0–4326 nmol/ml/h). One patient had no enzyme activity. 16/18 patients had typical vertical paralysis of gaze. Pathological horizontal sacchades were seen in only in 5 patients. In 10 patients measurement was not possible. Prolonged interpeak latancies mainly in VEP but also in SSEP and BAER correlates with more severe neurological findings.

Discussion: As a significant amount of patients had chitotriosidase activities increased by the factor 10–100, it can be considered to use this parameter for screening purposes. Chitotriosidase is easy, fast and cheap to check. So the period of time between first symptoms of NPC and diagnosis could be minimized in the future. Furthermore our findings suggest that evoked potentials may be an objective parameter to measure neurological involvement. We hope to prove that those can be established as assessment parameters, when therapy will get available.

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ATAXIA WITH OCULAR APRAXIA AS LEADING SYMPTOM IN JUVENILE NIEMANN-PICK C

Niezen-Koning KE, Smit GPA, van Spronsen FJ, Rake JP, Ruijter GJG[1], van Diggelen OP[2], Brunt ERP
Univ Hosp Groningen, [1]Department Pediatr, LUMC Leiden, [2]Department Clinical Genetics, Erasmus UMC Rotterdam, The Netherlands

A supranuclear vertical gaze paralysis, foam-cells and sea-blue histiocytes in bone marrow, and hepatosplenomegaly are consistent with Niemann-Pick type C. We present 2 siblings with ataxia and especially ocular apraxia as the leading symptom.

Patient 1, ♀ 23 yrs. Apparently normal development until age 20 yrs. Since age 21 she developed a notable headsway when looking downward. At age 22 she became psychotic. During neurological examination at age 23, she showed a slightly slurred dysarthria, and somewhat clumsy gait. Eye movements showed notable difficulty in initiating downward saccades, while vertical oculo-cephalic reaction was preserved. Electronystagmography confirmed the absence of volitional downward vertical eye movements. Laboratory investigation confirmed the diagnosis of juvenile Niemann-Pick type C.

Patient 2, ♂ 25 yrs, older brother of patient 1. Since age 10 slowly progressive gait and limb incoordination and since age 17 slowly progressive dysarthria without mental changes. Brain CT and EEG were normal. At age 20 a peculiar headsway when looking upward, supposedly a motor tic, was noticed. Brain MRI showed cerebellar atrophy. Neurophysiological investigations confirmed vertical ocular apraxia and polyphasic mups together with slowed tibialis SEP. Neuropsychological examination revealed early mental retardation. Initially a diagnosis of ataxia with ocular apraxia was suspected, but following the diagnosis of his sister, Niemann-Pick type C was also confirmed by laboratory investigations.

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NIEMANN-PICK DISEASE TYPE C PRESENTING AS CHOLESTATIC JAUNDICE IN INFANTS – IS BONE MARROW ASPIRATION A USEFUL INVESTIGATION FOR CONFIRMING THE DIAGNOSIS?

AF Rodrigues, RGF Gray, MA Preece, U Baumann, PJ McKiernan
Liver Unit, Birmingham Children's Hospital, Birmingham, UK

Introduction: Niemann-Pick disease type C (NPC) is a fatal, autosomal recessive lysosomal storage disease which may present in infancy with cholestatic jaundice. In cholestatic patients with splenomegaly, a bone marrow aspirate has been advocated as a relatively accessible source to demonstrate storage phenomena. Typically in patients with NPC, macrophages with abnormal cholesterol storage, so called foam cells, can be detected in the bone marrow. **Aim**: To review our experience of bone marrow aspiration in children with NPC. **Methods**: A retrospective analysis of 10 consecutive children (8 males) from Birmingham Children's Hospital with NPC presenting with neonatal cholestasis was undertaken. The diagnosis of NPC was confirmed in all cases by demonstrating undetectable or low rates of cholesterol esterification and positive filipin staining for free cholesterol in cultured fibroblasts. **Results**: The median age at presentation was 6 weeks (range 2–44 weeks). The liver biopsies revealed histological features consistent with neonatal hepatitis in 5 children, biliary features in 1 child and NPC disease in 4 children. 4 children did not show storage cells in their bone marrow biopsies. **Conclusions**: The sensitivity of a bone marrow aspirate in diagnosing NPC disease in our patients presenting as cholestatic jaundice was low. This is in contrast to previous reports and could be due to technical difficulties in the assessment of the bone marrow. Further investigations are necessary as to why the detection rate with BMA was low. Complementary investigations such as plasma or serum chitotriosidase should be evaluated further.

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PRIMARY AND SECONDARY ELASTIN BINDING PROTEIN DEFECT LEADS TO IMPAIRED ELASTOGENESIS IN G_{M1}-GANGLIOSIDOSIS

A Caciotti, MA Donati, A Boneh[1], A d'Azzo[2], T Bardelli, V Kimonis[3], R Parini[4], D Antuzzi[5], A Federico[6], E Zammarchi, A Morrone

Pediatrics, Meyer Hospital, Florence, Italy; Metabolic Service, Victoria Australia[1], Genetics St. Jude H, Memphis, USA[2], Children's Hospital, Boston, USA[3], Neurology, Siena, Italy[4], Catholic University, Rome, Italy[5], Pediatrics, Monza, Italy

G_{M1}-gangliosidosis (GM1) is caused by a deficiency of β-galactosidase (GLB1). *GLB1* gene gives rise to the GLB1 lysosomal enzyme and to the elastin binding protein (EBP), a component of the non-integrin receptor complex. GLB1 forms a complex with PPCA, NEU1 and GALNS inside lysosomes, while EBP is bound to PPCA and NEU1 on cell surface. The clinical, biochemical and genetic characterisation of 11 GM1 patients enabled a genotype/phenotype correlation. Expression studies and Western Blot analyses showed that the identified mutations affect GLB1 enzyme activity and/or stability. In addition, some *GLB1* gene mutations do not appear to be stabilized by PPCA probably because these mutations hamper the interaction between GLB1/EBP and PPCA inside multyenzyme complex. Impaired elastogenesis in fibroblasts of some patients with infantile form, as detected by elastic fibers assembly by immunofluorescence studies, might be associated with a primary defect of EBP function, given that its normal amount remained unchanged. In a patient with the juvenile form, who shows large urinary keratan sulfate excretion and mutations affecting only GLB1 enzyme, a mild EBP reduction and a decreased elastin deposition were present. These data suggest that the keratan sulfate accumulation leads to secondary EBP deficiency with impaired elastogenesis.

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CEREBRAL FINDINGS IN 8 PATIENTS WITH TYPE III GM1 GANGLIOSIDOSIS

Brenner C, Brum JM, Speck-Martins C, Pinto MTI, Rizzo IPO, Carod-Artal FJ

Sarah de Hospitais do Aparelho Locomotor, SMHS !.501, Brasilia, Brazil

GM1 gangliosidosis is inborn error of sphingolipid metabolism. It is classified into type I (infantile), II (juvenile) or III (adult or chronic), according to age of onset and clinical manifestations. Type III GM1 gangliosidosis can present sometimes in childhood, despite its name. Main clinical findings are extra-piramidal signs, particularly dystonia, dysarthria, and mild disostosis multiplex. There are limited reports on the literature on MRI findings in this form GM1 gangliosidosis.

The aim of this report is to provide MRI data on this form of disease. All patients showed progressive gait disturbance due to dystonia, and impairment of speech. There is no evidence of cognitive deterioration in any patient. All individuals had deficient leukocyte β-galactosidase, normal excretion of glycosaminoglycans and had the diagnosis of MPS IV ruled out.

We review neuroradiologic findings of 8 patients diagnosed in our network of hospitals. All patients, including two siblings, underwent conventional brain magnetic resonance imaging at 1.5 T. All patients had abnormal signal intensity (hyperintensity on T2 weighted images) in the caudate nucleus and putamen, bilaterally and symmetrically. Three patients, including the siblings, had bulging contour of the ventricle walls secondary to caudate nucleus atrophy. One patient had mild cerebral atrophy.

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LATE-INFANTILE GM-1 GANGLIOSIDOSIS: CLINICAL FEATURES AND UNIQUE ULTRASTRUCTURAL AND MAGNETIC RESONANCE SPECTROSCOPY (MRS) FINDINGS

DL Renaud, JD Port, JE Parisi, JF O'Brien
Division of Child and Adolescent Neurology, Mayo Clinic, Rochester, MN, USA

A 2-year-old girl presented for evaluation of developmental regression, hypotonia and seizures. Six months of developmental regression was preceded by a plateau in development beginning in late infancy. At the time of presentation, she was unable to attend visually. No cherry red spot was identified. Diffuse hypotonia and a head lag were present. She had mild coarsening of her facial features and marked thickening of the gums. A skeletal survey revealed localized dysostosis multiplex consisting of anterior beaking of the L1 vertebra and anterior wedging of L2 and L3. The clinical diagnosis of late-infantile GM1-gangliosidosis was confirmed by markedly low B-galactosidase activity in leukocytes and skin fibroblasts. Sialic acid was not present in the urine and the neuramidase activity in skin fibroblasts was normal. The ultrastructural and MRS features of this disorder have not been previously described. Electron microscopy (EM) of skin and conjunctival biopsies revealed numerous membrane-bound vacuoles containing flocculent material. Rare zebra bodies were also noted in the conjunctival biopsy. MRI imaging of the head demonstrated increased T2 signal in the subcortical white matter of both cerebral hemispheres and in the parieto-occipital regions bilaterally. Mildly increased T1 signal was also present in the thalami bilaterally. MRS demonstrated decreased N-acetylaspartate and increased choline. A unique spectroscopy peak was identified as scyllo-inositol. This study demonstrates the diagnostic potential of EM and MRS in the investigation of presumed storage disorders.

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STUDY OF NATURAL HISTORY OF JUVENILE GM2 GANGLIOSIDOSIS

[1]GHB Maegawa, [2]M Tropak, [2]DJ Mahuran, [2]JW Callahan, [3]R Giugliani, [3]C Barros, [4]F Kok, [1]JTR Clarke
[1]Division of Clinical and Metabolic Genetics, [2]Metabolism Program, Research Institute, Hospital for Sick Children, 555 University Avenue, Toronto, ON, Canada; [3]Servico de Genetica Medica, Hospital de Clinicas, Porto Alegre, Brazil; [4]Centro de Estudos do Genoma Humano, Universidade de Sao Paulo, Brazil

The juvenile GM2 gangliosidosis (jGM2) is a rare neurodegenerative disorder characterized by slowly progressive ataxia, dystonia, pyramidal signs, dysarthria and psychiatric symptoms, caused by lysosomal enzyme β-hexosaminidase A deficiency. We report a study of 11 patients from 7 unrelated families, 6 diagnosed with Tay-Sachs variant (TSV) and 5 with Sandhoff variant (SV). All patients were diagnosed by hexosaminidase enzyme studies in serum, leukocytes and fibroblasts. The mean age of onset was 7 years and 3 months (2 to 16 years), 5 males and 6 females. The most common symptoms at onset were ataxia which was found in 5 of 11 jGM2 patients. Interestingly, 4 of 6 TDV patients presented speech delay and only 2 presented initially with ataxia. Among the SV patients, ataxia was the most common presenting symptom occurring in 4 of the 5 patients. On first clinical assessment, the most common physical findings were dysarthria (9/11), pyramidal signs (9/11), ataxic gait (7/11) and muscle atrophy (7/11). Two of 5 SV patients showed height and weight below the third percentile, and 3 of 5 were noted to have some extra-ocular movement impairment. Sensory loss was present in 2 of the 5 SV patients. Later all jGM2 presented some degree of mental deterioration from mild to severe and dysarthria. Psychiatric symptoms such as psychotic episodes, aggressive and compulsive behavior and also schizophrenia developed in 8 patients. Four patients developed seizures. Acroparesia (4/5), visual and hearing problems and poor height/weight gain (3/5) were more common in patients with SV. However, SV patients developed tongue fasciculation (3/5), sensory loss (4/5) and atrophic skin changes (2/5). The juvenile form of GM2 is described as homogenous (Brant et al. 1984). However, we observed some clinical heterogeneity in the clinical presentation as well as in the progression of this condition.

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5-YEAR-OLD GIRL WITH NEURODEGENERATION AND DECREASED HEX B ACTIVITY

[1]GHB Maegawa, [2]M Tropak, [2]DJ Mahuran, [1]S Hewson, [2]JW Callahan, [1]JTR Clarke
[1]Division of Clinical and Metabolic Genetics, [2]Metabolism Program, Research Institute, Hospital for Sick Children, 555 University Avenue, Toronto, ON, Canada

We report a 5-year-old girl who presented with neuro-regression at 4 years of age with a history of dysarthria, unsteady gait, some abnormal posture movements and intentional tremor. She became progressively dependent on others and her fine motor skills deteriorated. There was no history of seizures, appetite changes, swallowing problems, behaviour changes. The family history was non-contributory. At 5 years of age, she had dysarthria, a full range of extra-ocular movements and no nystagmus. The muscle bulk was slightly reduced with decreased power in the lower limbs. She showed dystonic posturing of the arms with some clasp knife rigidity. She also had dysmetria and a mild intention tremor. Her deep tendon reflexes were normal. She was wheelchair bound. Two brain MRIs done at 5 month interval were normal. Her EEG showed slowing of background rhythms in the posterior areas. Total hexosaminidase activity in plasma revealed that total hexosaminidase activity was 724 nmol/h/ml (normal 2400+488) with 16%Hex-B heat stable. Hexosaminidase activities in parents were normal. The total hexoaminidase in leukocytes was 351 nmol/h/mg, Hex-B was 168 and %Hex-B was 48 (30–45 normal range). Total Hex activity in fibroblasts was 24 605 nmol/h/mg, with 21% of the heat stable Hex-B. The GM2 activator in fibroblasts was normal. The sequencing for the *HEXA* gene was normal. The clinical presentation resembles juvenile GM2 gangliosidosis, however, the percentage of heat stable Hex-B is not typical for this condition. We are sequencing the *HEXB* gene to examine the possibility that this patient has a mutation producing a heat-labile Hex-B.

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NEWBORN SCREENING FOR FABRY DISEASE

Spada M, Pagliardini S
Metabolic and Newborn Screening Units, Regina Margherita Children's Hospital, Torino, Italy

Fabry disease, an X-linked lysosomal storage disorder due to α-galactosidase A (α-gal A) deficiency, is considered a rare sfingolipidosis and its birth prevalence has been estimated around 1:100 000, following retrospective series. Here we report on the first newborn screening study performed to clarify the real epidemiology of the disorder. From 1 July 2003 to 31 March 2004 a cohort of 13 392 consecutive male newborns from Piemonte Region, Italy, underwent α-gal A activity measurement in filter paper blood spots, an automated technique suitable for large screening procedures (Spada M, Pagliardini S. New Screening approach for Fabry disease. In: First European Roundtable on Fabry disease. Nice, France, 15 June 2001, 17). Newborns with deficient blood spot α-gal A activity (cut-off level $<25\%$ of the control value of 7.7+1.3 nmol/h/ml of blood) were requested for a second measurement and a recall rate of 0.15% was observed, showing a test specificity of 99.8%. Five out of the 13 392 screened newborns were found to have absent blood spot α-gal A activity and underwent plasma α-gal A determination for definite diagnosis. Severe plasma enzyme deficiency was confirmed in all the 5 newborns (plasma α-gal A activity ranging from 0.76 to 1.1 nmol/h/ml; control value: 14.8+3.4 nmol/h/ml). These results point out that the birth prevalence of Fabry disease is around 1:2600, a frequency much higher than 1:100 000, as commonly reported. This figure is similar to that observed in congenital hypothyroidism (1:3000) or in phenylketonuria (1:8000), two inborn disorders routinely included in newborn screening programs. Present findings show that Fabry disease is largely misunderstood among the medical community and, as effective enzyme therapy is now available, new efforts should be adressed to implement screening strategies in selected patients as those suffering from chronic renal disease, cardiomyopathy or cerebral vasculopathy to offer treatment and genetic counselling.

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SCREENING FOR FEMALE FABRY DISEASE PATIENTS USING WHOLE BLOOD SPOTS FAILS TO IDENTIFY ONE-THIRD OF PATIENTS

GE Linthorst[1], AC Vedder[1], JMFG Aerts[2], CEM Hollak[1]
Department of Internal Medicine/Clinical Haematology[1], and Biochemistry[2], Academic Medical Center, Amsterdam, The Netherlands

Introduction: Fabry disease is a rare X-linked lysosomal storage disorder, characterized by the deficiency of the enzyme α-Galactosidase A. The availability of a treatment has greatly renewed interest in the disorder, which has led to screening for unidentified patients, such as patients on dialysis or with cardiomyopathy. Recently dried whole blood spots have been proposed as source for mass screening [1]. This method has already been used by some for screening for α-Gal A deficiency in several populations [2] We studied the sensitivity of this test in identifying female carriers. **Methods**: 12 male and 21 female Fabry disease patients participated. All had a documented diagnosis of Fabry disease, determined as reduced α-Gal A activity in leukocytes, a mutation in the α-Gal A gene, or both. From EDTA-anticoagulated blood, spots were generated. **Results**: Dried whole blood spots were available from 9 controls, 12 male and 21 female Fabry patients. As expected all males showed reduced α-Gal A activity on the bloodspot, whereas only 13/21 (63%) of female patients did. **Conclusion**: One-third of female carriers will not be picked up during screening making bloodspots not a reliable tool for screening.

[1] Chamoles et al. Fabry disease: enzymatic diagnosis in dried blood spots on filter paper. Clin Chim Acta. 2001;308:195–6
[2] Spada M, Pagliardini, S. Screening for Fabry disease in end-stage nephropaties. JIMD. 2002;25(Suppl. I):113

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REVIVAL OF HAIR-ROOT ANALYSIS IN DIAGNOSING FABRY DISEASE IN FEMALES

AC Vedder, JEM Groener, W Donker-Koopman, CEM Hollak, JMFG Aerts
Department of Internal Medicine and Biochemistry, Academic Medical Center, Amsterdam. Meibergdreef 9, 1105 AZ Amsterdam, Netherlands
E-mail: A.C.Vedder@amc.uva.nl

Objective: Fabry disease is an X-linked disorder caused by deficiency of the lysosomal enzyme α-Galactosidase A (α-Gal). Diagnosis is made by measuring α-Gal activity in leucocytes (males) or DNA mutation analysis (females). Diagnostic tests in a 20-year old female in whom a diagnosis of Fabry disease was suspected revealed a marginally decreased enzyme activity in leucocytes and a mutation in the untranslated region of the α-Gal-gene. As the clinical relevance of the mutation was unclear we decided to confirm the diagnosis by means of hair-root analysis.

Methods: Hair samples were obtained from the patient, 1 hemizygote, 2 heterozygotes and 4 controls. α-Gal activities in individual hair-roots were measured and compared with β-hexosaminidase, another not affected- lysosomal enzyme. As a result of cellular mosaicism, due to random X-inactivation in heterozygotes, single hair-roots reveal a normal, deficient or intermediate α-Gal-activity.

Results: Analysis of 20 randomly picked hair-roots of obligate carriers and hemizygote male revealed α-Gal/β-Hex ratios of 0.25–3.7 and <0.2 respectively, whereas in normal controls the ratio was 1.4–6.3. Hair-root analysis of the patient showed enzyme activity in the normal range (1.5–3.7), without a single hair with deficient activity. This led to rejection of a diagnosis of Fabry disease.

Conclusion: Due to increasing awareness and growth of diagnostic referral, new mutations will be discovered of which the clinical relevance is not always clear. In these cases hair-root analysis could be considered a helpful and useful diagnostic tool.

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FABRY DISEASE: AN EXPERIENCE WITH A MULTI SYSTEMIC DISEASE EMPHASIZING ANGIOKERATOMAS IN THE SKIN AND ESOPHAGIC MUCOSA, CENTRAL NERVOUS SYSTEM INJURIES, UNCOMMON LOCATIONS AND TREATMENT

PC Aranda
Hematology Department, Hospital Evangélico, 1313, Faria Lima Avenue, Londrina-Pr, Brazil
E-mail: paulo.aranda@sercomtel.com.br

Fabry disease is an X-linked inherited metabolism disorder due to mutations in the gene encoding alfa-galactosidase A, a lysosomal enzyme. The enzymatic defect leads to the systemic accumulation of incompletely metabolised glycosphingolipids, primarily the substrate globotriaosylceramide (GL 3), in plasma and lysosomes in the vascular endothelium and numerous tissues, throughout the body. This progressive glycosphingolipid accumulation leads to life-threatening clinical sequelae in skin, renal, cardiac and cerebrovascular systems. Without renal dialysis or kidney transplantation, the average age of death for patients with classical Fabry disease is 41 years. Our object in this case will be on angiokeratomas in the skin and esophagic mucosa, central nervous system injuries, carotid artery damage and angiokeratomas in uncommon locations like fingers, shoulder and eyelid, including a heterozygous patient, focusing yet on the nonspecific treatment when we used many drugs and methods for the different symptoms and specific treatment for this disease, specially using enzyme replacement therapy with agalsidase beta. All the patients have got a good response for it.

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HETEROGENEITY OF SYMPTOMS IN DUTCH FABRY PATIENTS

GE Linthorst, AC Vedder, E Ormel, JMFG Aerts[1], CEM Hollak
Department of Internal Medicine/Clinical Hematology and Biochemistry[1], Academic Medical Center, Amsterdam, The Netherlands

Objective: The natural history of Fabry disease, a lysosomal disorder caused by the deficiency of α-Galactosidase A is poorly understood. We studied symptoms of Dutch Fabry disease patients. **Methods**: Patients were seen at the outpatient clinic and completed quality of life and painscore questionnaires, underwent physical examinations, blood- and urine and renal function tests, as well as MRI, EKG and hearing evaluations. In addition, historical patient records were analysed from patients with a documented diagnosis of Fabry disease. **Results**: We identified 35 males, mean age 36.1 (6–65) and 56 females, mean age 43.4 (8–76) and 22 deceased patients. Current median life expectancy is 58 yrs (range 31–65) for males and 73 yrs (36–76) for females. Most common cause of death was of cardiac origin in males (8/16) and non Fabry-related in females (4/11). Pain scores (BPI) and quality of life (SF-36) did not differ between males and females. Vascular complications were markedly heterogeneous. Older patients had more cardiac, cerebral or renal complications. Hearing loss was the most prevalent organ complication in males (12/17 tested) and cardiac hypertrophy in females (13/16 tested). Renal or cardiac variants could not be identified. There was no clear correlation between residual enzyme activity and the presence of symptoms. **Conclusion**: Even when Fabry disease patients are evaluated in a uniformal way there is a marked heterogenous expression of symptoms, which cannot be explained by residual enzyme activity. Detailed studies on the natural course of Fabry disease using uniformal evaluation of symptoms in an unbiased cohort of patients (especially females) are eagerly awaited.

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CLINICAL PHENOTYPE OF CHILDREN WITH FABRY DISEASE: DATA FROM FOS – THE FABRY OUTCOME SURVEY

U Ramaswami[1], R Parini[2], C Whybra[3], M Beck[3]
[1]Department of Paediatrics, Addenbrooke's Hospital, Cambridge, UK; [2]Centro Malattie Metaboliche, Clinica De Marchi, Milan, Italy, [3]Department of Paediatrics, University of Mainz, Mainz, Germany – On behalf of the European FOS investigators

Background: The Fabry Outcome Survey (FOS) – is a unique European database of the natural history of Fabry Disease (FD) and the effects of enzyme replacement therapy (ERT) with agalsidase alpha.

Method: Demographic information from 82 children (40 males and 42 females) below 18 years of age who were enrolled in FOS, with confirmed FD, were evaluated in this study.

Results: The median age at FOS entry was 12.5 and 13.2 years for males and females respectively. 60–80% of these children had symptoms including dyshydrosis, acroparaesthesiae, altered temperature sensitivity, abdominal pain and altered bowel habits. Tinnitus, vertigo, fatigue and angiokeratoma were present in over 40% of patients. These symptoms were noted in early childhood and with a similar frequency in both sexes. Data from FOS revealed a 10–20 year delay in diagnosis from the age of onset of symptoms ($p < 0.05$).

Conclusion: To our knowledge this is the largest cohort of children with FD that has been studied. The childhood presentation is often missed or misdiagnosed. Life-threatening complications of FD including renal failure and strokes are rare in childhood. Early diagnosis and treatment with ERT could potentially prevent or delay the onset of irreversible organ damage.

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HEALTH RELATED QUALITY OF LIFE (HRQOL) IN PATIENTS WITH FABRY DISEASE UNDER ENZYME REPLACEMENT THERAPY: FOS – THE FABRY OUTCOME SURVEY

B Hoffmann[1], A Garcia de Lorenzo[2], M Beck[3], A Mehta[4], R Ricci[5], U Widmer[6] on behalf of the European FOS Investigators Group
[1]Department of General Pediatrics, University Children's Hospital Duesseldorf Germany; [2]Dr. Formación Médica Continuada Hospital Universitario, La Paz, Madrid, Spain; [3]Department of Pediatrics, University of Mainz, Germany; [4]Department of Haematology, Royal Free Hospital, London, UK; [5]Institute of Clinical Pediatrics, UCSC, Rome, Italy; [6]Department of Medicine, University of Zurich, Switzerland

Background: Fabry disease (FD) is an X-linked metabolic disorder caused by deficiency of lysosomal α-galactosidase A and subsequent accumulation of Gb3 in various organs (e.g. heart, kidney and nervous system). Enzyme replacement therapy (ERT) has been reported to show beneficial effects on clinical symptoms. We evaluated HRQOL in patients with FD under ERT with agalsidase alpha who are enrolled in FOS.

Methods: Assessing HRQOL using the EQ-5D, and measuring pain with the Brief Pain Inventory (BPI).

Results: Median baseline EQ-5D-Score was 0.76 ± 0.32 ($n = 120$, 47 females, 73 males), and thus was significantly lower than in a normative population ($p < 0.05$). 12 months treatment with agalsidase alpha significantly improved EQ-5D-Scores ($p < 0.05$). After 24 months of treatment these results could be sustained. Additionally there were significant improvements in 'pain on average', 'worst pain' and 'pain now' collected by BPI ($p < 0.05$) after 24 months ERT.

Conclusion: Besides its reported clinical effects, ERT with agalsidase alpha significantly improves quality of life and simultaneously reduces pain in FD.

334-P

THE FABRY REGISTRY: NATURAL HISTORY OF RENAL DISEASE AND EFFECTS OF ENZYME REPLACEMENT THERAPY

DP Germain on behalf of the European Board of Advisors
Clinical Genetics Unit, Department of Genetics, Paris, France

Fabry disease results from the deficient activity of α-galactosidase A (α-Gal A) which leads to progressive renal, cardiac and cerebrovascular complications. The Fabry Registry is a global, observational, voluntary program open to all patients with a confirmed diagnosis of Fabry disease. We evaluated renal function based on estimated glomerular filtration rate (GFR, using the Modification of Diet in Renal Disease equation) among patients enrolled in the Fabry Registry. As of April 2004, 666 patients were enrolled in the Fabry Registry (404 males and 262 females). Mean age at baseline (date of first infusion with Fabrazyme) was 39.4 (±13.4) years. Mean (SD) estimated GFR values prior to a patient's first Fabrazyme infusion were 76.8 (±41.2) ml/min/1.73 m^2. Estimated GFR values are negatively correlated with age, untreated male patients had a faster decline in estimated GFR compared to females (GFR slope –2.15 ml/min/1.73 m^2/year versus –0.67 ml/min/1.73 m^2/year). The estimated GFR slopes remained stable pre- and post-enzyme replacement therapy (ERT) among male patients with Fabry disease (n = 106). There was an early improvement in estimated GFR slopes among female patients with Fabry disease post-ERT (n = 36). Estimated GFR values appeared to stabilize post-ERT among patients with GFR values <60 ml/min/1.73 m^2. Preliminary observations suggest that enzyme replacement therapy may stabilize renal function among patients with Fabry disease. Long-term follow up is needed to evaluate the beneficial effects of enzyme replacement therapy.

335-P

HOSPITAL, HOME OR INDEPENDENCE FOR AGALSIDASEβ INFUSIONS – THE PATIENTS VIEW

A Cole[1], A Cousins[1], S Morgan[2], P Lee[1]
[1]*National Hospital for Neurology and Neurosurgery, London, UK;* [2]*Genzyme Homecare, Oxford, UK*

Introduction: Patients have been receiving Agalsidaseβ infusions in our unit since 1999. Since 2002 Homecare (HC) have enabled patients to receive nurse provided treatment at home or training for independence. **Aim**:To review the thoughts of patients regarding their choices for treatment setting, transition and future options. **Method**: Phone call questionnaire was carried out with 20 patients. **Results**: Of the 20 patients, 75% are receiving treatment from HC nurses, 10% are independent and 15% receive ERT in hospital. The transition from hospital to HC occurred between infusion 6 and 82 (individual variations and clinical trial restrictions). 82% felt it was the right time to transfer to home and in retrospect 76% viewed it a 'very positive' experience. 85% of hospital infusions take >6 hr of the persons time. Home infusions take 2–4 hr. 6/12 no longer need to take time off work when treated at home and transport costs are eliminated. HC and independent infusions are reported as most convenient (80%). 55% reported the ability to infuse independently would be best for quality of life and ability to commit long term to therapy. 3 patients are independent, 41% would like to be and view it as achievable. 59% wish to remain on HC/hospital infusions. **Conclusion**: The majority of patients receive treatment at home and report benefits of this. Independent or nurse supervised home infusions are not the choice of every patient but with options available we can best meet their individual needs.

336-P

EXPERIENCE WITH HOME TREATMENT FOR FABRY DISEASE: PRACTICE GUIDELINES

GE Linthorst, AC Vedder, E Ormel, JMFG Aerts[1], CEM Hollak
Department of Internal Medicine/Clinical Hematology and Biochemistry[1], Academic Medical Center, Amsterdam, The Netherlands

Objective: Experience with home treatment with enzyme supplementation therapy in lysosomal sotrage disorders is restricted to Gaucher disease. Recently, chronic supplementation with α-Galactosidase A has been approved as treatment for Fabry disease. The aim of the current study was to investigate the feasibility of home therapy for Fabry disease during a two year period and to make a proposal for practice guidelines. **Methods**: Based on experience in previous clinical trials an algorithm to allow patients to perform home treatment was developed. Patients were allowed to perform home treatment according to the algorithm. **Results**: Twenty-two of the 26 patients eligible for home treatment received in total 652 infusions at home (median 22 infusions, range 2–69), during the period of March 2001 to 31 December 2003. Mean age was 43.8 years (18–63) of which 15 (68%) were male. The majority of patients (19/22, 86%) at home-treatment received 0.2 mg/kg. Seven male patients developed an infusion associated event, of which three developed these at home. All patients with an infusion associated event were IgG positive. None of the events was life-threatening or necessitated urgent admittance. All events were managed conservatively or with dexamethasone 5 mg orally one hour before infusion. **Conclusion**: Using our algorithm, home treatment with rh-αGal A for Fabry disease with 0.2 mg/kg is feasible and safe and reduces burden related to chronic intravenous therapy and health care costs. Whether this can also be applied for male patients treated with 1.0 mg/kg is currently being studied.

337-P

A RANDOMISED TRIAL OF ALPHA-GALACTOSIDASE A REPLACEMENT THERAPY IN MALES WITH ADVANCED FABRY DISEASE CARDIOMYOPATHY – A PILOT STUDY

A Cole, [1]JS Shah, [1]PM Elliott, P Lee
Charles Dent Metabolic Unit, The National Hospital for Neurology and Neurosurgery, London, UK; [1]The Heart Hospital, University College London, UK

Background: Cardiomyopathy in Fabry disease (FD), an X-linked lysosomal storage disorder, is common. The 'cardiac variant' may account for 3–9% of patients with unexplained left ventricular hypertrophy. The role of enzyme replacement therapy (ERT) in the cardiac predominant FD remains unclear. **Methods and Results**: 6 males (median age = 55.5years) were enrolled into a double blind randomised pilot study, comparing standard dose (1 mg/kg) to high dose (2 mg/kg) ERT (Fabrazyme®) for 1 year. Patients were evaluated every three months with clinical assessment, ECG and echocardiography. Pre-treatment, 1 patient reported hypohidrosis and GI symptoms, 1 patient complained of tinnitus and none had angiokeratomas. 3 patients reported exertional dyspnoea, 2 patients reported anginal chest pain. The median maximal left ventricular (LV) wall thickness was 1.8 cm with a median LV mass index of 213.6 gm/m^2. 1 patient was withdrawn due to a presumed drug induced pneumonitis. At one year, 3 had pacemakers implanted for bradyarrhythmia. Two patients required medical management for symptomatic heart failure. There was no change in maximal LV wall thickness or LV mass index (mean difference = 0.02 cm, 95% CI = 0.03, –0.03; 0.4, 95%CI = 47.3, –46.6; p = NS respectively), this was consistent for both high and low doses. **Conclusion**: These results suggest that high dose ERT has no role in patients with advanced FD cardiomyopathy. Early diagnosis and treatment remains important for these patients.

338-P

ERT INFUSION ASSOCIATED REACTIONS IN FABRY DISEASE

A Cole, A Cousins, PJ Lee
Charles Dent Metabolic Unit, National Hospital for Neurology and Neurosurgery, London, UK

Introduction: Little has been documented about the prevalence of infusion associated reactions (IAR) that occur during enzyme replacement therapy (ERT) for Fabry disease. Since initial use of ERT 5 years ago the incidence and management of such events have evolved. **Aim**: To report changes in the frequency and management of IARs from a single unit. **Method**: Retrospective review of medical records from 22 ERT (agalsidase β) treated Fabry patients. **Results**: From 913 infusions, 51 IARs occurred (5.58%) in 10 male patients. Four were treated in the pivotal trial (started 1999). All four had reactions, accounting for 27 of the 51 (53%) IARs. Of the remaining 18, six experienced an IAR. 77% of the reactions consisted of feeling cold and shivery (often concurrent). Reactions occurred between 50–90 min from the start of infusion. All resolved. None led to a serious adverse event. Treatment of IARs in all patients consisted of no intervention (29%), minimal intervention (50%) and medication (21%). Of these the ERT 1999 group accounted for 100% of the medication administered, 39% minimal intervention and 63% no intervention. More recently 61% are treated with minimal intervention. **Conclusions**: No female experienced an IAR. IARs that occurred were primarily dealt with by non-medical intervention. The incidence of IARs was small in comparison to the number of infusions administered. In the past two years the occurrence of IARs per patient has significantly fallen and management both before infusions and during a reaction has become more conservative. Other influences on IAR occurance are manufacturing process, residual enzyme activity, pre-existing ailments and infusion specific detail.

339-A

FABRY DISEASE – ENZYME REPLACEMENT THERAPY IN SINGAPORE

Tan ES, Lo YL, Teo SH
KK Women's and Children's Hospital, Singapore

We report a case of Fabry disease in a 17-year-old Chinese patient who is undergoing enzyme replacement therapy. This the the first case of enzyme replacement therapy in Singapore. The patient first presented at 11 years of age with episodes of excruciating pain over both feet. Initial autoimmune workup was negative. Radiological survey revealed generalised osteopenia which was confirmed on dual-energy X-ray absorptiometry bone mineral densitometry. Electromyography and nerve conduction studies performed were normal. However, quantitative sensory testing revealed the possibility of small fibre sensory polyneuropathy. Sympathetic skin reflex of the lower limbs revealed sudomotor autonomic dysfunction. Clinical examination was normal with no typical skin features of Fabry disease.

Enzyme assays in leukocyte specimen revealed low alpha-galactosidase activity (2.66 nmol/mg prot/h) Enzyme assay in cultured skin fibroblast also confirmed the diagnosis of Fabry disease. (alpha-glactosidase 0.02 nmol/min/mg protein)

He was started on enzyme replacement therapy in August 2003. Till date, he has undergone 12 enzyme infusions at 2 weekly intervals.He was closely monitored in the hospital for adverse reactions. He tolerated all transfusions well with no adverse reactions. His pain and quality of life has improved significantly.

This case reflects the significance of the advancement in medical therapy of Fabry disease in the form of enzyme replacement therapy.

340-P

LEUCOCYTE β-D-GLUCURONIDASE (GCR) AS A MARKER OF ENZYME REPLACEMENT THERAPY (ERT) IN FABRY DISEASE

Burlina AB, Goi G, Massaccesi L, Baquero Herrera CJ, Tognana G,Burlina AP, Lombardo A
Departments of Pediatrics, Neuroscience, Padua; Department of Medical Chem Biochem Biotech, Milan, Italy

Fabry disease results from deficient activity of α-galactosidase A and subsequent accumulation in lysosomes of neutral glycosphingolipids. Recently, ERT has become available as a treatment option for this disease. Till now, no good biochemical parameters for monitoring the therapy are available. **Objective**: The purpose of our study was to investigate whether lysosomal enzymes may detect biochemical changes induced by ERT. **Methods and Patients**: We studied 5 male patients, age range 26–43 years, treated with agalsidase A (Replagal, TKT) and agalsidase B (Fabrazyme, Genzyme). All patients were monitored by clinical and biochemical assessment. About every 2 months several lysosomal glycohydrolases were measured in leucocytes by a standardized microfluorimetric method and expressed as mU/g of protein; all data are expressed as mean ± SD. **Results**: The most sensitive among the considered enzymes was GCR. Before the first administration of ERT, in all patients GCR was markedly increased (7770 ± 1360) with respect to age-matched controls (3520 ± 1340). After 15 days of ERT, GCR levels dropped in all patients (3383 ± 1281). For the patients who regularly followed the therapy, the GCR values remained stable for the next 9 months. Interestingly, in the case of one patient who interrupted the therapy for two months (due to personal reasons) the value of GCR drastically increased and, after the restarting of ERT, returned to the control range. **Conclusion**: Our preliminary results demonstrate that GCR assay in leucocyte is a reliable marker to monitor the ERT.

341-P

EFFICACY FOLLOWING AAV2/8-MEDIATED EXPRESSION OF α-GALACTOSIDASE A IN FABRY MICE

J Marshall, RJ Ziegler, CM Barbon, S Bercury, SH Cheng
Genzyme Corp, Framingham, MA 01701, USA

Fabry disease is an inherited disorder that is caused by a deficiency of the lysosomal enzyme α-galactosidase A. This results in the accumulation of the glycosphingolipid, globotriaocylceramide (GL-3) in the lysosomes of the endothelial vasculature. Previously, we showed that intravenous administration of a AAV2 vector encoding human α-galactosidase A under the control of a liver-restricted promoter (DC190) into Fabry mice resulted in expression of therapeutic levels of the enzyme that was sustained for 12 months. Treatment resulted in a reduction of GL-3 in the liver, spleen and heart to basal levels and in the kidney by approximately 40% at 8 weeks. To improve the therapy to the kidney, different AAV serotypes (1, 5, 8) with reportedly higher hepatic transduction activity were evaluated. A modest improvement in expression levels was seen with the AAV pseudotypes 2/1 and 2/5. However, the corresponding AAV2/8 vector generated an approximately 100 fold greater levels of the enzyme than the AAV2/2 vector. Intravenous administration of the AAV2/8 virus into Fabry mice resulted in higher levels of expression of the enzyme and more rapid clearance of GL-3 from the affected tissues than with a 10 fold higher dose of the corresponding AAV2/2 vector. Clearance of GL-3 from the AAV2/8-treated tissues including kidney was complete by 4 instead of 8 weeks. Furthermore, an improvement in the thermal sensitivity of the treated Fabry mice was also realized. Fabry mice demonstrate a delayed response to placement on a hot plate that is equilibrated to 55°C when compared to unaffected age-matched normal mice. The response time of untreated Fabry mice became progressively worse over a period of 8 months. In contrast, Fabry mice treated at 5 weeks of age with the AAV2/8 vector demonstrated response times that were similar to those for age-matched normal 129/Sv mice throughout the study. Finally, we demonstrated that the immune tolerance to α-galactosidase A observed previously using an AAV2/2 vector harboring a liver-restricted promoter also applied to the AAV2/8 vector. Together, these data support the continued development of AAV2/8-mediated gene therapy for Fabry disease.

342-P

MORBUS FABRY: REDUCED ACTIVITIES OF RESPIRATORY CHAIN ENZYMES WITH REDUCTION OF 'ENERGY-RICH' PHOSPHATES IN FIBROBLASTS

AM Das, E Schmidt, W Höppner, S Illsinger, T Lücke
Department of Paediatrics, Hannover Medical School, D-30625 Hannover, Germany

Aims: M. Fabry (MF, MIM 301500) is an X-chromosomal inherited multiorgan lysosomal storage disease due to deficiency of alpha-galactosidase A. The exact pathogenesis of clinical symptoms is unclear. Besides mechanical storage biochemical factors might play a role.

Methods: Cultured skin fibroblasts from 4 patients with MF and 10 healthy age-matched controls were washed with HEPES-buffer and incubated with glucose for 15 min. Cells were broken by sonication and the activities of respiratory chain enzymes I-V were measured spectrophotometrically. Concentrations of 'energy-rich' phosphates (ATP, ADP, creatinephosphate CP) were assayed luminometrically.

Results: Activities of respiratory chain enzymes I, IV and V were significantly ($p<0.01$) lower in MF cells. Complex II+III-activity was reduced, but not significantly ($p = 0.05$). The activity of the mitochondrial marker enzyme citratesynthase was normal in both groups of cells.

Compared to control cells, levels of CP and ADP were significantly reduced ($p<0.01$), the concentration of ATP was slightly but not significantly lower in MF cells ($p = 0.05$).

Conclusions: Symptoms in MF may not be explained by mechanical storage of glycosphingolipids alone. Lysosomal storage may lead to mitochondrial dysfunction; this could cause compromised cellular energy supply and secondary cell damage.

343-A

INFORMED CONSENT IN ENZYME REPLACEMENT THERAPY (ERT) TRIALS OF PATIENTS WITH MUCOPOLYSACCHARIDOSIS

L Kalakun, AC Azevedo, IV Schwartz, TA Vieira, AC Puga, LLC Pinto, MV Muñoz, R Giugliani
Medical Genetics Service, Hospital de Clínicas de Porto Alegre. Porto Alegre, RS, Brazil
E-mail: lkalakun@hcpa.ufrgs.br

Mucopolysaccharidoses are a group of lysosomal diseases, especially significant because of the serious clinical features they give rise to, such as, progressive learning difficulties, corneal clouding, respiratory and cardiac diseases, facial and skeletal deformities, and joint stiffness. The aim of this study was to understand the experience with the informed consent (IC) process of families undergoing clinical trials with ERT. Seven families with children with MPS VI and 18 families with children with MPS II were relocated from their houses to the State of Rio Grande do Sul with their children for them to receive ERT or placebo. Some families came from different states of Brazil and other even from different countries of Latin America. A retrospective description of parents' perceptions of the circumstances of their child's MPS diagnosis and of the informed consent process were evaluated. Parents described feelings of tremendous stress in the early phase of their child's illness regarding the diagnosis and the lack of treatment options. A sense of constraint and lack of control were common among these families during the IC process. Overall, parents did understand their children's disease and experienced an urge to treat them. This influenced in the agreement of the IC, but not necessarily it reflected a comprehension of the study protocol, as treatment and/or research. The authors suggest that the IC process should be conducted by a multidisciplinary team, focusing mainly in the psychosocial profile of these families.

Support: BioMarin and TKT

344-P

PROSPECTIVE ORAL HEALTH SURVEILLANCE IN PATIENTS WITH MPS DISORDERS: RESULTS FROM A 3-YEAR FOLLOW-UP STUDY

P Farge, M Mille, A Fouillhoux, P Cochat, N Guffon
Metabolic Disease Clinic, Department of Paediatrics, Hôpital Edouard Herriot, Lyon, France

Oral care surveillance is an important issue in maintaining the quality of life of patients with lysosomal storage disorders. Since year 2000, a paediatric dentist has been added to the medical multidisciplinary team in charge of MPS patients. The dental team provides a complete oral diagnosis procedure and follows-up on all dental and oral procedures performed. 32 MPS patients were examined for 3 years. Of these patients, 19 had MPS I, 10 had MPS II, 2 had MPS III and 1 had MPS IV. Macroglossia and other usual oral phenotypic features, such as dental diastema, gingival enlargements and hypersalivation, were present in all MPS I and II patients. The main oral treatment needs were: specific oral hygiene maintenance programs in 8 patients, caries treatment were in 5 cases, chronic oral candidosis treatment in 2 cases. Non inflammatory gingival hyperplasia was noted in a total of 6 patients with MPS I and MPS II. Among the 32 patients, 13 (9 MPS I and 4 MPS II) underwent BMT. Pre-transaplantation oral examinations were provided and oral care was given in the immediate post-transplantation period. In these patients, the BMT corrected hypersalivation, macroglossia and dental diastema. However, non-inflammatory gingival enlargement and delayed eruption were not modified. Long term clinical data will be needed to assess a clinical attitude towards dental inclusion cysts and the prevention of infectious flare-ups. These results emphasize the need for comprehensive oral surveillance in a multidisciplinary set up for MPS patients and suggest a differential effect of the bone marrow transplant on oro-facial structures.

345-P

α-L-IDURONIDASE DEFICIENCY: MICROPLATE ADAPTION OF AN ENZYME ASSAY IN DRIED BLOOD SPOTS SUITABLE FOR NEWBORN SCREENING

A Muehl, S Stöckler-Ipsiroglu, O Bodamer
Unit of Biochemical Genetics, University Childrens Hospital, Vienna, Austria

Mucopolysaccharidosis type I (MPS I) is due to deficiency of α-L-iduronidase. The clinical phenotype may be heterogenous depending on residual enzyme activity and additional genetic and epigenetic factors (Hurler, Scheie). Early diagnosis is essential as bone marrow transplantation and enzyme replacement therapy are available.

We describe the adaption of an enzymatic assay [1] for a microplate procedure suitable for large scale newborn screening programs. A 3 mm dried blood filter paper disk was incubated with 40 µl 50 mmol/L formate buffer (pH 2.8) containing 0.3 µg D-saccharic acid-1,4-lactone and 20 µl 2 mmol/L 4-methylumbelliferyl-α-L-iduronide (Glycosynth, UK) in a microplate. The plate was wrapped in aluminium foil and incubated for 24 hr at 37°C. 250 µl of glycine-carbonate buffer (0.085 mol/L, pH 10.5) was added to each well and fluorescence (365/450 nm) was measured.

Enzyme activity in 220 newborn control samples was 0.75 ± 0.22 µmol/L/h. Patients with MPS I had virtually no enzyme activity (< 0.1 µmol/L/h).

The microplate adaption of the enzyme assay is simple and may be suitable for large scale pilot newborn screening programs.

[1] Chamoles et al. Diagnosis of α-L-Iduronidase deficiency in dried bloot spots on filter paper: the possibility of neborn diagnosis. Clin Chem 2001;47:780–1

346-O

A FIVE YEAR STUDY OF ALDURAZYME FOR TREATMENT OF MPS I

J Muenzer[1], S Swiedler[2], G Cox[3], A Jonas[4], M Sifuentes[4], G Tiller[5], L Waber[6], J Belmont[7], M Lipson[8], A McDuffee[9], D McMahon[10], B Barshop[11], V Proud[12], R Loge[13], K Dveirin[14], G Eames[15], E Kakkis[2]

[1]Univ of N. Carolina, [2]BioMarin, [3]Genzyme, [4]Harbor-UCLA Medical Center, [5]Vanderbilt, [6]University of Texas, [7]Baylor, [8]Kaiser, [9]Huntsville Hospital, [10]Carolinas Medical Center, [11]UCSD, [12]E. Virginia Medical School, [13]Pioneer Med Specs, [14]AZ Community Physicians, [15]Cook Children's Medical Center

Objective: To demonstrate safety and efficacy of Aldurazyme (laronidase) in the reduction of lysosomal storage in Mucopolysaccharidosis I (MPS I). **Methods**: A Phase 1/2 open-label, multicenter study in 10 patients. Patients received 100 U/kg (0.58 mg/kg) laronidase weekly for 269 to 288 weeks. The primary endpoint was $\geqslant 50\%$ reduction of uGAG (urinary GAG) levels. **Results**: Mean age was 12.3 years (range 5–22) and 80% had Hurler-Scheie syndrome. Seven patients completed over 5 years on study and 6 of 7 patients were compliant with weekly infusions. Mean reduction in uGAG excretion from pretreatment to end of study for 6 patients was 73.3%. Four of 6 compliant patients had uGAG levels approaching normal range for age. One patient had 68.5% reduction in uGAG levels at 3 years, but became non-compliant during the next 2 yrs and uGAG levels returned to baseline. Data show that uGAG levels increase by 6% if one infusion is missed, and increase by 12% if two consecutive infusions are missed. Most frequently reported AEs occurred during infusion, i.e. urticaria, headache, edema, pruritus. All patients developed antibodies to laronidase though there was no apparent relationship between antibody levels and changes in urinary GAG excretion. **Conclusion**: Compliance with weekly infusions of Aldurazyme results in a consistent reduction of uGAG levels and an acceptable long-term safety profile.

347-P

MPS1-ENZYME REPLACEMENT THERAPY WITH ALDURAZYME® IN PORTUGAL – FIRST FIVE PATIENTS: BASELINE CHARACTERISTICS AND TREATMENT OUTCOME

Garcia P[1], Amaral O[2], Caseiro C[2], Diogo L[1], Sá Miranda MC[2]

[1] Unidade de Doenças Metabólicas do Hospital Pediátrico de Coimbra, [2]Unidade de Enzimologia do Instituto de Genética Médica Dr Jacinto de Magalhães do Porto Portugal

In Portugal, enzyme replacement therapy to treat mucopolysaccharidosis type I (MPS1) (Aldurazyme, laronidase) has been registered in June 2003, and has been available since September 2003 on a named patient basis. We will report the first clinical experience with laronidase in 5 patients (3 females; ages 21 months to 9 years) currently treated in the Pediatric Hospital in Coimbra. All are genotyped and all but one represent the severe MPS I spectrum with bone disease, visceral, cardiac, ophthalmologic and CNS involvement (except for a Hurler-Sheie girl). Three had already lost the ability to walk and speak. Treatment with Aldurazyme (100 U/kg, weekly) was started between September and October 2003. Baseline and follow up measurements to determine the treatment efficacy and safety after overall six months follow-up include: history, clinical assessment, vital parameters, liver, heart and spleen ultra sound measurements, ECG, ophthalmology testing, 6 minute walk test, a-Iduronidase leukocyte level and urinary GAGs excretion. The enzyme was well tolerated, with no side effects. Subjective improvements were reported shortly after the first 2 infusions, especially an increase in energy and mobility. The main clinical results were reduction of liver and spleen size, disappearance of bone pain, weigh gain, better sleep quality, capacity to walk in one and reappearance of language in two. The biochemical hallmark was an increase of Iduronidase to subnormal levels and a progressive reduction on urinary GAGs (dermatan and heparan sulphate). No positive signals were seen on eyes and heart.

348-A

ENZYME REPLACEMENT IN TWO PATIENTS WITH MPS1

L De Meirleir[1], A Benatar[2], W Lissens[3]
[1]Department of Pediatric Neurology and [2]Pediatric Cardiology, [3]Center of Medical Genetics AZ – Free University Brussels, B-1090 Brussels, Belgium

Two patients with MPS 1 are evaluated after six months of enzyme replacement treatment (Aldurazyme®). The first patient is a four year old girl with Hurler's disease, diagnosed at the age of two years. Although already having central nervous system involvement with psychomotor delay, she is still an active child with good communication skills. Echocardiography shows a mitral valve insufficiency and thickening of pulmonal valve. After 6 months of treatment there was a marked improvement of her general physical condition, with diminution of liver and spleen, less ENT infections and progression in developmental skills. Echocardiography was unchanged.

The second patient is an intelligent boy with Scheie disease. He started enzyme therapy at the age of 15 years; at that time he had severe pains in his hands with stiffness, which made writing difficult. His echocardiography showed thickening of aorta and mitral valve, both with insufficiency. Liver and spleen were enlarged. After six months of treatment, the pains in the hands have disappeared, his echocardiography showed a less thickening of the valves, without insufficiency, liver and spleen normalized in size and mobility in his hands improved.

In conclusion: even after only 6 months of ERT we can conclude that quality of life definitively improves in the Hurler patient, and in the Scheie patient ERT leads to a marked physical amelioration and improvement of cardiac function. A longer follow-up is needed to have the real clinical course of these diseases under ERT treatment.

349-A

ENZYME REPLACEMENT THERAPY IN TWO PATIENTS WITH MUCOPOLYSACCHARIDOSIS TYPE I

Barisic I[1], Huzjak N[1], Petkovic G[1], Tokic V[1], Fumic K[2], Mrsic M[2]
[1]Children's University Hospital Zagreb, Klaiceva 16, 10 000 Zagreb, Croatia, [2]Clinical Hospital Center Rebro, Kispaticeva 12, 10 000 Zagreb, Croatia

Objective: to determine the efficacy of the enzyme replacement therapy with rh-α-L-iduronidase (Aldurazyme) in two patients with Mb. Hurler, the most severe form in the clinical spectrum of Mucopolysaccharidosis type I.

Methods: rh α-L-iduronidase has been administrated weekly, 100 IU/kg.

Results: At the beginning of the treatment, both patients, a nine-year-old boy and an eight-year-old girl, were at the advanced stage of the disease. After 13 months of the therapy, the boy showed an improvement in general physical condition, respiratory function and mobility, as well as the reduced hearing impairment. The sleep-apnea syndrome disappeared, together with all other sleeping disorders. The results of the urinary GAGs became normal and the leukocyte α-L-iduronidase reached the therapeutic level. The girl showed a similar improvement in the first six months of the treatment, but a relative stagnation in her clinical condition followed. A gradual decrease of leukocyte α-L-iduronidase concentration was also noted.

Conclusion: The enzyme replacement therapy has demonstrated positive clinical results in our patients, but a constant monitoring of the clinical and biochemical parameters is necessary for a proper evaluation of the treatment efficacy.

350-P

MPS1 (HURLER SYNDROME): ENZYME REPLACEMENT THERAPY PRIOR TO BONE MARROW TRANPLANTATION

A Chakrapani[1], J Motwani[1], J Wright[1], G Gray[1], A Cooper[2], JE Wraith[2], S Lawson[1], P Darbyshire[1]
Birmingham Children's Hospital[1], Birmingham, United Kingdom and Willink Biochemical Genetics Unit[2], Manchester, UK

We report a patient with MPS1H who has undergone recombinant enzyme replacement therapy (ERT) prior to haematopoietic stem cell transplantation (HSCT). **Case Report**: The male infant presented with facial dysmorphism and developmental delay aged 15 months. MPS1H was diagnosed by enzyme analysis. Hepatosplenomegaly, cardiac valvular abnormalities and cardiomyopathy were present at diagnosis but there was no evidence of sleep apnea. A total of 14 doses of recombinant α-iduronidase 100 IU/kg as weekly infusions were given pre-HCST and a further 4 infusions after HCST. Urine glycosaminoglycans were nearly normal after 4 weeks of ERT. At the time of the transplant the cardiomyopathy and hepatosplenomegaly had considerably improved. A successful matched unrelated donor transplant was performed 14 weeks after commencing ERT following conditioning with busulphan, cyclophosphamide and fludarabine. The post transplant course was relatively uncomplicated. Full donor engraftment was achieved 2 weeks after transplantation and the leucocyte α-iduronidase levels have been at the lower end of normal since. **Conclusion**: Based on these very promising results, we recommend pre-transplant ERT as it significantly reduces clinical symptoms. A predicted benefit should be a reduction in transplant-related morbidity and mortality.

351-P

CAN ENZYME REPLACEMENT THERAPY FOR MPS I BOOST CLINICAL OUTCOME AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION?

N Guffon
Departement de Pediatrie, Hopital Edouard Herriot, Lyon, France

Treatment options for children younger than 2 years of age suffering from the severe type of MPS I syndrome ('Hurler disease') include hematopoietic stem cell transplantation (HSCT) and enzyme replacement therapy (ERT – Aldurazyme®). HSCT is commonly offered to these patients if a suitable donor is available. However post-HSCT, still a variety of clinical problems persist. We report the first MPS I patient ever who received Aldurazyme as adjunctive therapy after HSCT. The transplant was performed at the age of 18 months after full conditioning therapy. 1 year postransplant, chimerism was 30%. However, the clinical evolution of the child was not in line with the expectations. Twenty months after transplantation, weekly infusions with 100 U/kg Aldurazyme were started to reinforce the effect of the HSCT. The average urinary GAG value dropped from 35 μg/mg creatinine post HSCT but before ERT to 18 μg/mg creatinine post ERT (normal GAG value for 3 to 7 years: 6–23 μg/mg creatinine). Leukocyte α-L-iduronidase activity raised. Three months after start of ERT, the patient tested positive for antibodies against the enzyme (1/200). The patient improved clinically especially his speech capacities. Patient started speech therapy short after transplantation and hearing aids were placed at the time of ERT initiation. Enzyme replacement therapy after hematopoietic stem cell transplantation may be an option to improve clinical outcome in patients with severe MPS I disease. In our patient, GAG levels normalised, and the combination therapy was safe. More data are needed before more definite conclusions can be drawn.

352-P

MONTHLY INTRATHECAL ENZYME THERAPY FOR CANINE MPS I

P Dickson[1], M McEntee[2], C Vogler[3], S Le[1], B Levy[3], P Belichenko[4], W Mobley[4], S Hanson, M Passage[1], E Kakkis[1,5]
[1]Division Medical Genetics, Harbor-UCLA REI, Torrance, CA; [2]Department of Pathology, College of Veteterinary Medicine, University of Tenn; Knoxville, TN; [3]Department of Pathology, St. Louis University, MO; [4]Department of Neurology, Stanford University Medical Center, Stanford, CA; [5]BioMarin Pharmaceutical Inc, Novato, CA, USA

Intravenous recombinant human α-l-iduronidase (rhIDU) does not cross the blood brain barrier. Weekly doses of ~1 mg of rhIDU given intrathecally (IT) have been shown to penetrate the CNS and reduce glycosaminoglycan (GAG) storage in canine mucopolysaccharisosis I (MPS I). Two MPS I dogs received 4 monthly doses of ~1 mg IT rhIDU. Iduronidase levels reached 23-fold normal in the brain, 8-fold in the spinal cord, and 294-fold levels in the meninges of dogs treated monthly, vs. 23, 13, and 300-fold in 4 dogs treated weekly. Brain GAG reached normal levels in both regimens. With monthly treatment, we observed a 48% reduction in brain GAG storage (vs. 46% with weekly), a 16% reduction in spinal cord GAG (vs. 32%), and a 65% reduction in meningeal GAG (vs. 57%) compared with 4 untreated MPS I dogs. There was no significant difference in iduronidase or GAG levels with monthly vs. weekly IT rhIDU. One dog developed a lymphoplasmacytic infiltrate in the meninges and a mild antibody response in blood and CSF. The other dog had been made tolerant to rhIDU using a novel method and had little or no detectible immune response in blood and CSF and a very mild meningitis. Both dogs had diminished leptomeningeal and perivascular GAG storage histologically. Monthly IT rhIDU may be as effective as weekly in correcting the lysosomal storage in brain and meninges of canine MPS I.

353-A

IDUA GENE MUTATION 134DEL12 IS ASSOCIATED WITH THE MPS I INTERMEDIATE PHENOTYPE: A REPORT ON 3 BRAZILIAN PATIENTS

Giugliani R[1], Norato D[2], Schwartz I[1], Matte U[1], Muñoz MVR[1]
[1]Medical Genetics Service, HCPA, Porto Alegre, Brazil; [2]UNICAMP, Campinas, Brazil

Mucopolysaccharidosis type I (MPS I) is an autosomal recessive disorder caused by the deficiency of the enzyme alpha-iduronidase (IDUA) and is associated with a wide clinical and allelic heterogeneity. Nonsense mutations will cause severe MPS I disease if present on both IDUA gene alleles. The clinical consequences of mutations that are not clearly null can be predicted more accutely only by looking at the phenotype of the patient in which these mutations have previously been identified We present three Brazilian unrelated patients with intermediate clinical phenotype who have in common the IDUA mutation 134del12 in one allele combined with W402X, Q70X and N350I mutations in the other allele. Seven other MPS I patients presenting 134del12 have been previously reported. In five of these patients mutation 134del12 was present in combination with other IDUA mutations (134del12/474-2A; 134del12/E178K; 134del12/Q584X; 134del12/unknown and 134del12/unknown), and two patients presented 134del12 on both alleles. Clinical phenotype varyed from intermediate to severe. Interestingly, homozigous patients 134del12/134del12, W402X/W402X and Q70X/Q70X and heterozigous W402X/Q70X all present the severe phenotype, while the herein reported genotypes 134del12/W402X and 134del12/Q70X conferes an intermediate phenotype. This findings support the view that the cause of clinical heterogeneity in MPS I should be multifactorial.

354-A

A FOLLOW-UP OF 20 BRAZILIAN PATIENTS WITH HUNTER SYNDROME IN A MEDICAL GENETICS SERVICE: PRELIMINARIES RESULTS

Pinto LLC[1] Puga AC[1], Kalakun L[1], Vieira T[1], Schwartz IV[1], Brustolin S[1], Munoz VR[1], Barrios P[2], Vedolin L[3], Marinho D[4], Esteves P[4], Boy R[5], Santos ES[5], Monlleó I[5], Fontes M[5], Ribeiro E[5], Kim CA[5], Valadares E[5], Damaso M[5], Kahn E[5], Norato D[5], Sobrinho RO[5], Giugliani R[1]
[1]Medical Genetics Service, HCPA, Porto Alegre, Brazil; [2]Cardiology Service, HCPA, Porto Alegre, Brazil; [3]NeuroImaging Diagnostic Service, MDC, [4]Ophthalmology Service, HCPA, Porto Alegre, Brazil; [5]Brazilian MPS II Study Group

Mucopolysaccharidoses are a group of inherited metabolic disorders of lysosomal storage that are characterized by deposits of glycosaminoglycans. Hunter syndrome, inherited in an X-linked recessive manner, is caused by deficiency of the enzyme iduronate sulphatase. Twenty MPS II Brazilian patients were invited to participate in a prospective study at the Medical Genetics Service, Hospital de Clínicas de Porto Alegre, Brazil. Patients were enrolled from all geographic regions of Brazil after signing an informed consent. Neurological, cardiac, pneμmological and ophthalmologic screenings, as well as, routine lab testing and neuroimaging exams has been performed within this two-year study. Age at time of biochemical diagnosis ranged from two to five years old (mean 4.6 years). Patients had been diagnosed after a long period of evident clinical manifestation and a earlier recognition of the disease has important implications for the patient and his family. A multi-disciplinary approach to management is necessary due to the varied and progressive evolution of the disease.

355-P

DETERMINATION OF BONE MINERAL DENSITY IN PATIENTS SUFFERING FROM THE SEVERE FORM OF HUNTER DISEASE

D Rigante, G Segni, R Ricci, P Caradonna[1]
Department of Pediatric Sciences, [1]Department of Internal Medicine – Università Cattolica Sacro Cuore, Rome, Italy

Mucopolysaccharidosis type II also known as Hunter disease (Hd) results from the absence of iduronate sulphatase: the clinical presentation may be extremely heterogeneous, but progressive neurodegeneration results in a vegetative existence for most patients suffering from its severe form. We have submitted two patients with the severe form of Hd to dual energy X-ray absorptiometry (DEXA, Hologic QDR 2000) in order to quantify bone mineral density (BMD) and determine its relationship with disease severity. Here are features and results for our patients expressed as Z-scores.

Pts	Age	Onset	BMI	Bone changes	Deambulation	Paraparesis	Epilepsy	l-BMD	Z-score	f-BMD	Z-score
A	22	3.5 ys	15.3	present	absent	present	present	0.401	–6.49	0.281	–6.29
B	21	5 ys	17.0	present	limited	absent	absent	0.638	–4.34	0.454	–4.73

Patients with Hd usually present mental/physical disability and a wide spectrum of functional bone impairment due to bone storage of undegraded mucopolysaccharides, inadequate body weight, malabsorption of calcium, decreased serum 25-hydroxyvitamin D, absent physical exercise and sex hormone deficiency. Lumbar and femoral neck BMDs were found to be very severely reduced in the two patients, with differences attributable to hypogonadal state and use of antiepileptic drugs disturbing vitamin D metabolism. Bone integrity needs to be overlooked in order to ameliorate the quality of life of these patients: we strongly believe that DEXA determinations are useful for screening patients with disorders electively leading to osteopenia such as Hd and that dietary supplements of calcium/vitamin D might slow skeletal demineralization, while physiotherapy might contribute to BMD amelioration and pain alleviation.

356-P

MUCOPOLYSACCHARIDOSIS TYPE II: IDENTIFICATION OF 24 NOVEL MUTATIONS AMONG SOUTH-AMERICAN PATIENTS

I Schwartz, L Lima, L Scherer, T Dieter, M Ribeiro, J Mota, A Acosta, P Correia, D Horovitz, I Monlleó, M Fontes, A Fett-Conte, R Oliveira Sobrinho, D Norato, A Paula, C Kim, A Duarte, R Boy, P Mabe, M Ascurra, M de Michelena, K Tylee, G Besley, A Martins, M Garreton, M Burin, R Giugliani, S Leistner

Department of Genetics, UFRGS and Medical Genetics Service, Hospital de Clínicas de Porto Alegre, Brazil; Willink Biochemical Genetics Unit, Royal Manchester Children's Hospital, Manchester, UK; MPS II South American Study Group

In this study, 60 unrelated South American patients with mucopolysaccharidosis type II (MPS II) were investigated aiming at the identification of the iduronate-2-sulfatase (IDS) gene mutations and the analysis of the genotype-phenotype correlation. The strategy used for the genotyping involved the identification of the previously reported inversion/disruption of the IDS gene by PCR, and the screening for other mutations by PCR/SSCP. The exons with altered mobility on SSCP were sequenced, as well as all the exons of patients with no SSCP alteration. Adopting this strategy, we were able to find the pathogenic mutation in all but one patient. The inversion/disruption and partial/total deletions of the IDS gene were found in 13/60 (21.6%) patients. Small insertions/deletions/indels (<22 bp) and point mutations were identified in 46/60 (76.6%) patients, including 24 novel mutations. Except for a higher frequency of small duplications in relation to small deletions, the frequencies of major and minor alterations found in our sample are in accordance to those described in the literature. As to the genotype-phenotype correlation, it is clear that MPS II phenotype is reliably predicted by only a small number of IDS gene mutations (NORD, CAPES)

357-P

BRAIN GLUTAMATERGIC METABOLISM IN THE MOUSE MODEL OF SANFILIPPO B DISEASE

D Cheilla[1,2], C Malleval[1], R Froissart[2], A Cressant[3], I Maire[2], MF Belin[1], M Touret[1]

[1]Inserm U433, Faculté de Médecine Laennec – 69008 Lyon France; [2]Laboratoire de Biochimie Pédiatrique, Hôpital Debrousse – 69005 Lyon France; [3]RTG, Institut Pasteur – 75015 Paris, France

Sanfilippo B disease is a lysosomal storage disorder. The first symptom of the disease is a developmental delay later complicated between 3–4 years by severe behavioural and sleep disturbances followed by a progressive demence leading to death. Mouse model of Sanfilippo B developed by the group of E. Neufeld will be very useful to better understand patho physiology of this disease. Our objective was to evaluate glutamatergic metabolism in different brain areas of the sanfilippo B mouse model.

We have studied 3 glutamate transporters (GLAST, GLT-1, VGLUT-1) and 2 astrocytic enzymes (GS, GDH) by western blot analysis in 5 brain areas: cortex, caudoputamen, hippocampus, thalamus, colliculi and cerebellum. 5 affected mice and 5 controls aged of 3 months were studied. Statistical analysis was performed by Student t test.

No statistical differences in GLAST, GLT-1, GS and GDH expression were seen in the different brain areas. However, a lower expression of the neuronal vesicular transporter VGLUT-1 was observed in the caudoputamen and in the thalamus. These results show a possible involvement of excitotoxicity processes in the sanfilippo B disease. To better understand this mechanism, it will be of a great interest to study glutamate transporters expression during natural history of the disease.

358-P

IDENTIFICATION OF TWO NOVEL MUTATIONS IN GALNS GENE IN MUCOPOLYSACCHARIDOSIS IV A PATIENTS FROM COLOMBIA

Sarmiento P[1], Morales I[1], Tomatsu S[2], Barrera L[1]
[1]*Instituto de Errores Innatos del Metabolismo, Pontificia Universidad Javeriana, Bogotá, Colombia;*
[2]*Department of Biochemistry, School of Medicine, Saint Louis University, MO, USA*

Introduction: Morquio A Syndrome is an autosomal recessive lysosomal storage disease of the mucopolysaccharides. It is caused by the deficiency of N-acetylgalactosamine 6-sulfate sulfatase (GALNS) resulting in storage of keratan and chondroitin sulfate. The main clinical aspects are related with bone deformities and its effects on the peripheral nervous system. The gene was located in chromosome 16, contains 14 exons, 1566 nucleotides that code for a protein of 522 amino acids. **Purpose**: to determine the gene mutations responsible for the pathologic phenotype in Colombian patients with Morquio A. **Materials and Methods**: Nine patients were evaluated and enzyme activity was measured in leukocytes in the IEIM from Javeriana University. In some cases the enzyme deficiency was confirmed in fibroblasts and KS determination in urine and plasma using an ELISA sandwich method. For some patients sequencing of the 14 exons of GALNS gene, was done after PCR On physical examination, all patients had a severe form, growth retardation, joint laxity, and bone dysplasia and all of them were neurologically normal? The mutation A75G (GCC to GGC) was found in two brothers but was not found in the other patients although they are from the same geographical region. **Discussion**: We have characterized the mutations of 3 patients and have shown the allelic heterogeneity reflecting the wide spectrum of clinical phenotypes. A new mutation the A75G was identified. In a previous article, the haplotype analysis of Colombian MPS IVA from another region showed two new mutations S162F and G301C this mutaion was associated with a founder effect.

359-A

FAMILY ASSESSMENT OF CHILDREN WITH MPS VI FACING ENZYMATIC REPLACEMENT THERAPY

L Kalakun, TA Vieira, AC Azevedo, IV Schwartz, R Giugliani
Medical Genetics Service, Hospital de Clínicas de Porto Alegre. Porto Alegre, RS, Brazil
E-mail: lkalakun@hcpa.ufrgs.br

In this work, eight children with MPS VI, aged eight to thirteen years old, under clinical trial with recombinant N-acetylgalactosamine 4-sulfatase are being followed for their disease. The trial was set to last for 6 months. Seven children and their mothers come from different states of Brazil and one child comes from Chile. Taking into consideration the necessity of separation from their whole family during this time, and also due to culture differences, our research group decided to evaluate their social and psychological needs, in order to facilitate their adaptation process during treatment. Therefore, a total of seven families with eight children with MPS VI were assessed to identify key relational patterns within their family dynamics. Genogram was used to evaluate these characteristics. Self-differentiation, defined as cognitive and emotional responses, was categorized as low level for three families, moderate level for two families, and moderate to good level of differentiation for the other two families. Families were also classified in three different socio-economical groups: one family was considered upper class, two families were middle class, and the remaining four families belonged to lower class. Other important factors analysed were the total number of brothers and sisters within the families, the number of deceased children in the families, presence of blindness in the MPS child, and communication skills. Although the number of families in this qualitative approach is small, the level of differentiation and the social-economic status were found not to correlate to each other. The first factor, however, may be perceived as the characteristic that facilitates the adaptation process within the treatment requirements.

Support: BioMarin.

360-O

UPDATE ON CLINICAL TRIAL RESULTS IN MPS VI

P Harmatz, R Giugliani, I Schwartz, AC Azevedo, N Guffon, C Sá Miranda, E Teles, E Wraith, M Beck, Y Amraoui, L Arash, C Whitley, R Steiner, B Plecko, P Kaplan, D Ketteridge, L Keppen, S Swiedler, J Hopwood
Children's Hospital and Research Center at Oakland, Oakland, CA, USA

Mucopolysaccharidosis VI (MPS VI; Maroteaux-Lamy syndrome) is a lysosomal storage disease caused by a deficiency of the enzyme N-acetylgalactosamine-4-sulfatase (ASB). Weekly treatment with recombinant human ASB (rhASB) has been studied in two clinical trials: a randomized, double-blind, two-dose Phase I/II study (3 males, 4 females; age 7–16 years), and a Phase II, open-label, single-dose study (3 males, 7 females; age 6–22 years). Week 96 data for the Phase I/II study and week 48 data for the Phase II study are presented here. All subjects tolerated the weekly enzyme infusions and 15 subjects continue treatment. There were 23 Serious Adverse Events (SAEs), 1 drug-related and 1 possibly drug related. Biochemical response was documented by sustained reduction in urinary excretion of glycosaminoglycans (GAGs) by more than 70%, which was not significantly affected by rhASB antibody production. Improvements in endurance and mobility have been noted: the 6-min walk test yielded a mean increase of 96% in the Phase I/II study, the 12-min walk test showed a mean increase of 139% in the Phase II study; the 3-min stair climb in the Phase II study improved by a mean of 147%, and shoulder flexion increased by a mean of 14% in the Phase I/II study. In conclusion, rhASB treatment has been well tolerated with positive clinical and biochemical responses. A report of further results through weeks 144 and 72 for the Phase I/II and Phase II studies, respectively, and preliminary results for the Phase III trial will be presented at the meeting.

Sponsored by BioMarin Pharmaceutical Inc, Novato, CA

361-A

IMPORTANCE OF CEREBRAL NUCLEAR MAGNETIC RESONANCE IN THE EARLY DIAGNOSIS OF MUCOPOLYSACCHARIDOSIS TYPE VI

JS Marques, C Gonçalves, R de Luca, M Vila Real, S Aires Pereira
Serviço de Pediatria do Centro Hospitalar Vila Nova de Gaia, Portugal

A five-month-old boy was admitted in pediatric outpatient department because of axial hypotonia. He is the third child of unrelated healthy parents. He has two brothers: 8 and 4 years old. He was born by normal delivery. Birth weight and stature was more than 95 percentile and head circunference on 75 percentile. On physical examination we found axial hypotonia, coarse face, hirsutism, umbilical and left inguinal hernia, pits on sacrum region and limitation on hips movements. Hips X-ray confirmed bilateral dislocation. Magnetic resonance of the lombar–sacrum region showed sacrum dislocation. Cerebral nuclear magnetic resonance confirmed enlargment of ventricular system and peri-vascular and cerebral space, with strong suspicious of mucopolysaccharidosis. Urine glycoaminoglycans was high: 47 mg/nmol (4–31). Leucocytes and skin fibroblast revealed total absence of arylsulphate B. These results are consistent with the diagnosis of mucopolysaccharidosis type VI or Maroteaux–Lamy syndrome. After confirming the diagnosis, the patient was screened for further associated diseases and we found thickness of aortic and mitral valves, cornea hypotransparency and reduced transmission of sounds caused by serous otitis. The enzymatic study of the family showed an 8-year-old brother with low levels of arylsulphatase in leucocytes.

362-P

A GENOTYPE-PHENOTYPE CORRELATION STUDY IN SIX BRAZILIAN MPS VI PATIENTS CARRIERS OF THE 1531-1553DEL23 MUTATION

I Schwartz, A Azevedo, M Petry, A Behregaray, J Sebben, K Nonemacher, M Burin, A Paula, C Kim, S Leistner-Segal, R Giugliani
Medical Genetics Service, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil

Mucopolysaccharidosis type VI (MPS VI) is an autosomal recessive lysosomal storage disorder caused by the deficiency of enzyme N-acetilgalactosamine-4-sulfatase (ARSB). The ARSB gene has been mapped to chromosome 5q13-14. There is great variability in the clinical manifestations of the disease, probably due to the existence of allelic heterogeneity. In this study, we have evaluated the genotype–phenotype correlation for 6 Brazilian carriers of the 1531-1553del23 mutation. The genotypes found were 1531-1553del23/1531-1553del23 (n: 3), 1531-1553del23/R315Q (n: 2). and 1531-1553del23/ ? (n: 1). All patients had been diagnosed before the age of five. 1/6 patient died at age six due to respiratory insufficiency, and the age of the remaining patients span from 9 months to 15.5 years at evaluation. All patients presented with different levels of sleep apnoea syndrome, as well as mitral insufficiency and severe visual deficit. Mean ARSB activity in leukocytes was 4.1 nanomoles/h per mg protein (VR = 72–176). Patients presented urinary excretion of glycosaminoglycans 2–6 times higher than the upper limit for the age. Results suggest that carriers of mutation 1531-1553del23 usually do not present an attenuated form of MPS VI, and that intrafamilial variability is associated with the genotype 1531-1553del23/R315Q. A more precise genotype/phenotype correlation will only be possible to be determined when other patients with these genotypes are identified.

CNPq/Biomarin/PRONEX

363-P

ARYLSULFATASE B MUTATIONS IN PORTUGUESE MPS VI PATIENTS

AA Dias, E Pinto, I Ribeiro, MC Sá Miranda
IGMJM, Unidade de Enzimologia – Porto and IBMC, Unilipe – Universidade do Porto, Portugal

Mucopolysaccharidosis type VI (MPS VI, OMIM 253200) is a rare autosomal recessive disorder characterized by the deficient activity of arylsulfatase B (ARSB, EC 3.1.6.12). In Portugal, the birth prevalence of the rare MPS VI is 0.42/100 000. With the emerging availability of promising enzyme replacement therapy for this disease, mutation analysis becomes an important tool not only for the genetic counselling of individuals at risk, but also in the prognosis of the disease and identification of cases which might benefit of an early therapeutic intervention.

In this work we present the preliminary results obtained through mutation analysis of 12 Portuguese patients. This study involved PCR amplification and direct automated sequencing analysis of exons and intron boundaries. The identification of seven mutations is reported: two recently described deletions, three missense mutations (two of them new), one novel nonsense mutation and also one new splicing mutation. Additionally, two previously described point mutations were detected in the form of a complex allele. Seven patients were homozygous for various mutations, while the remaining five were compound heterozygotes. Interestingly, three mutations seem to have an increased frequency in the Portuguese sample studied jointly c.1533del23 (Petry et al. 2003), c.427delG (Karageorgos et al. 2004) and R315Q (Villani et al. 1999) represent 58% of the patients alleles.

In some of the cases Western blot analysis was also carried out. The impact of the various mutations at the protein level and the resulting phenotypic implications are discussed.

364-P

MENTAL DEVELOPMENT IN PATIENTS WITH INFANTILE ONSET POMPE DISEASE (IOPD) TREATED WITH ENZYME REPLACEMENT THERAPY (ERT)

A Skrinar[1], D Corzo[1], P Kishnani[2]
[1]*Genzyme Corporation, Cambridge, MA,* [2]*Duke University Medical Center, Durham, NC, USA*

Methods: An open-label study of ERT in eight patients with IOPD is ongoing. Preliminary results of mental development evaluations conducted using the Bayley Scale of Infant Development, 2nd edition (BSID-II) are discussed. **Results**: At baseline, mean chronological age for all patients was 6.0 months (range 2.5–14.6) with a mean BSID-II mental performance age equivalent of 4.5 months (range <1–14). After a mean duration of treatment of 15.2 months (range 3.2–25.2 months), the mean chronological age was 20.1 months (range 8.2–29.5 months) with a mean BSID-II mental performance age equivalent of 15.6 months (range 7–23 months). Improvement in raw scores from baseline was observed in all patients. These changes corresponded to increases in mental performance age equivalents. **Conclusions**: Increases in BSID-II raw and mental performance age equivalent scores indicate a continuous acquisition of new skills, although the improvement does not place these patients at the same functional level of age-matched normally developing children. The complexity of items involving integrated oral-motor and fine-motor skills required for the administration of BSID-II may contribute to this finding. However, the acquisition of new skills in patients with IOPD can be effectively monitored by evaluating changes in raw scores and mental performance age equivalents.

365-A

THIS ABSTRACT HAS BEEN WITHDRAWN

366-O

ENZYME REPLACEMENT THERAPY (ERT) IN INFANTILE-ONSET POMPE DISEASE (IOPD): LONG TERM FOLLOW-UP RESULTS

P Kishnani[1], M Nicolino[2], B Schaefer[3], E Kolodny[4], R Heitner[5], YT Chen[1]
[1]Duke University Medical Center, [2]Hopital Debrousse, Lyon, [3]University of Nebraska Medical Center, [4]New York University School of Medicine, New York, NY, [5]Morningside Clinic, Johannesburg, South Africa

Methods: Eleven patients with IOPD received ERT with CHO-cell derived rhGAA. Efficacy is compared to the natural history of the disease in 168 IOPD cases from a retrospective chart review (Am J Hum Genet. 2003;73:455). **Results**: The median age at ERT was 4.2 months (range 2.4–14.6). The median duration of ERT is 33.9 months (range 3.8–54.9). In the ERT group, 100% of patients were alive and 72.7% were ventilator free at 1 year; 72.7% were alive and 54.5% were ventilator free at 2 years; 45.4% were alive and 27.2% were ventilator-free at 3 years. In the historical cohort, 25.7% were alive at 1 year, 9.0% were alive at 2 years and 7.1% were alive at 3 years. Eleven out of 11 (100%) of patients showed improvement in cardiomyopathy, and 3/11 (27.2%) achieved independent ambulation. Eight patients are deceased (unrelated to rhGAA). All patients developed anti-rhGAA antibodies. Most patients have mild infusion-associated reactions. Conclusions. ERT in IOPD have demonstrated to prolong survival and ventilator-free survival, as compared to a historical cohort. Long-term follow-up is required to further evaluate the safety and efficacy of rhGAA.

367-A

FREQUENCY OF CDG AMONG CHILDREN WITH NEUROMOTOR DELAY/MENTAL RETARDATION (NMD/MR)

JM Brum[1], C Brenner[1], C Speck-Martins[1], MTI Pinto[1], IPO Rizzo[1], MMM Navarro[1], WD Mello[1], ER Valadares[2], J Gurgel-Giannetti[2], C Micheletti[3], S Kyosen[3], AM Martins[3]
[1]Rede Sarah de Hospitais do Aparelho Locomotor, SMHS Q.501, Brasilia, Belo Horizonte e São Luis, Brazil; [2]Departamento de Pediatria da UFMG; [3]Departamento do Pediatria/Genética, UNIFESP

Congenital disorders of glycosylation (CDG) is an heterogeneous group of disorders that affect mainly CNS. It is thought to be underdiagnosed due to variability of signs and symptoms and severity. There are two groups of CDG, according to isoelectric focusing of serum transferrin (TfIEF). There are little data on the literature on frequency of CDG among patients with NMD/MR. The aim of this work is to determine the frequency of CDG in this group of diseases in Brazil.

We performed TfIEF in sera of 287 patients with signs of neuromotor delay/MR of any degree. Abnormal patterns on TfIEF were found in 10 patients: CDG I pattern was found in 4 patients, CDG II pattern in 5 and in 1 patient there was a mild increase in trisialic band and was considered to be doubtful. This patient is the brother of a clearly type II pattern and has the same clinical features and neuroimaging findings of his affected brother. Ten children (3.5%) were considered to have CDG. One patient was considered to have false-negative test. We did not find any false-positive.

We found a frequency of 3.5% of CDG patients among children with neuromotor delay/mental retardation, by associating clinical data and serum TfIEF, and using this wide criteria of inclusion. Using more restrictive criteria, the frequency will increase.

368-P

ENCEPHALIC ABNORMALITIES AND CDG

JM Brum[1], C Brenner[1], C Speck-Martins[1], MTI Pinto[1], IPO Rizzo[1], EVM Borigato[1], MMM Navarro[1], WD Mello[1], ER Valadares[2], J Gurgel-Giannetti[2], C Micheletti[3], S Kyosen[3], AM Martins[3]

[1]Rede Sarah de Hospitais do Aparelho Locomotor, SMHS Q.501, Brasilia, Belo Horizonte e São Luis, Brazil; [2]Departamento de Pediatria da UFMG; [3]Departamento do Pediatria/Genética, UNIFESP

Congential disorders of glycosylation (CDG) is an heterogeneous group comprising at least 12 diseases. They are all multisystemic, frequently severe, autosomal recessive diseases. Clinical features are variable, but the main findings are FTT, psychomotor delay/MR, clotting disorders, dysmorphisms, cerebellar hypoplasia/atrophy, hypotonia and strabismus. MRI findings often show cerebellar hypoplasia or atrophy, and cerebral atrophy, among other features.

In order to gather information on encephalic abnormalities, we reviewed MRI findings in 8 Brazilian patients diagnosed as CDG (3 CDG I (patients 1–3) and 5 CGD II (patients 5–8)) by clinical findings and serum transferrin IEF abnormal pattern.

Brain stem atrophy was recorded in 6/8 patients (patients 1, 2, 4, 5, 7 and 8). Cerebellar atrophy was seen in 6/8 patients (patients 1, 2, 3, 6, 7 and 8). Patient 6 had severe myelination arrest and corpus callosum disgenesis. Medial cerebral artery infarction was present in patient 3, whereas patient 2 had superior cerebellar artery stroke. Cerebral atrophy was recorded in patients 6 and 7.

Our results show that the most common encephalic abnormalities seen in this group of diseases are cerebellar and brain stem atrophy.

369-P

TEN NEW PATIENTS WITH CONGENITAL DISORDERS OF GLYCOSYLATION (CDG) IDENTIFIED BY SELECTIVE SCREENING

C Pérez-Cerdá, MJ Ecay, B Merinero, B Pérez, M Ugarte

Centro de Diagnóstico de Enfermedades Moleculares, Centro de Biología Molecular-SO, UAM, Madrid, Spain

During the last two years, we have extended selective screening to include CDG disorders by determining %CDT in plasma/serum of patients with suspected metabolic disease. Samples from 2300 patients were investigated and 16 cases were identified with high serum %CDT values (4.9%–42%, normal <3%). All cases showed abnormal serum transferrin isoforms by IEF analysis. Eight patients showed type 1 sialotransferrin pattern that probably indicates a defect in the assembly of N-linked glycans (CDG-I). They are currently under mutation analysis of PMM2 gene. One case showed type 2 pattern, suggesting a Golgi glycosylation defect (CDG-II). Other case who died at two-months old with multiorgan failure, presented altered, non type 1, sialotransferrin pattern (CDG-X). We have also identified newborn galactosemic twins, and one two-years girl with fructose intolerance that presented type 1 pattern. Transferrin variants were confirmed in three cases after parents study and neuraminidase digestion of serum samples. IEF of serum α-1-antitrypsin also showed altered profile with hypoglycosylated bands in six CDG cases. Age at diagnosis of the ten CDG cases ranged from 2 months to 10 years. They were investigated due to hypoglycemia, hypertransaminemia, development delay, cerebellar atrophy, hypotonia, deep femoral thrombosis and macro/microcephaly. The broad clinical spectrum of CDG emphasizes the need to screen for this large group of disorders in patients with an unexplained clinical syndrome. Determination of serum %CDT has proven to be a reliable test to screen CDG disorders.

370-A

BIOCHEMICAL AND CLINICAL CHARACTERISTICS OF CONGENITAL DISORDERS OF GLYCOSYLATION TYPE Ix IN THREE POLISH PATIENTS

Adamowicz M[1], Wevers RA[2], Rokicki D[1], Hennet T[3], Chmielinska E[1], Tylki-Szymanska A[1]
[1]Children's Memorial Health Institute, Warsaw, Poland, [2]University Medical Centre Nijmegen, The Netherlands, [3]Institute of Physiology, University of Zurich, Switzerland

Patients with type I pattern of serum sialotransferrins in isoelectric focusing (IEF) and abnormal transferrin SDS-PAGE in whom secondary disturbances of N-glycosylation and defined CDG variants has been excluded are classified as CDG Ix. We report on three patients (two sisters and unrelated boy) with subtype I and an as yet unidentified defect in N-glycan biosynthesis. Their clinical presentations were very similar with moderate developmental retardation, nystagmus, optic atrophy and axial hypotonia with peripheral spasticity. All children were born after uneventful pregnancy with only slight facial dysmorphic features. From infancy their psychomotor development was markedly retarded. In all transaminases were elevated and coagulation factors, AT III, protein S and protein C were decreased as well as LDL-cholesterol. Cathodal serum sialo- transferrins, expressed as %CDT were in range 40–50% (N 2.3–8.6%). Transferrin SDS-electrophoreses were abnormal as expected for patients with type 1 IEF pattern. Normal activities of phosphomannomutase (PMM), phosphomannose isomerase (PMI) and profile of [^{3}H]mannose-labeled lipid-linked oligosaccharides (LLO) in fibroblasts excluded most of known CDG I subtypes. Secondary defect of O-glycosylation was also ruled out on the basis of normal pattern of serum apoCIII isoforms. In conclusion, further work using new methods is needed to identify the glycosylation defect in our patients.

371-P

CDG TYPE I IN A LARGE TURKISH PEDIGREE – A NOVEL DISORDER?

Hagerty B, Holub M, Tuschl K, Bodamer OA
Department of Paediatrics, University Children's Hospital Vienna, Austria

We report a large Turkish pedigree including 5 children and juveniles (2, 2.5, 11, 17 and 18 years of age) with CDG (congenital disorder of glycosylation) type I. Four of the affected are the offspring of the same consanguineous healthy parents and are first degree cousins of the fifth child, also of consanguineous origin. Isoelectric focusing (IEF) of serum transferrin showed a reproducible type I pattern in all affected individuals while obligate carriers showed an unremarkable IEF pattern. CDG Ia (phospho mannomutase deficiency) and Ib (phospho mannose isomerase deficiency) was ruled out in the index patient by demonstrating normal enzyme activity in fibroblasts. Mutation analysis for CDG type 1c, d, e, f and g was performed and did not identify any changes in the coding region of the respective genes. The clinical phenotype shows considerable intra-familial variability including different degrees of mental and psychomotor retardation (n = 5), microcephaly (n = 3), high palate (n = 2), ataxia (n = 2), seizures (n = 2), facial dysmorphism (including prominent nasal root and retrognathia)(n = 1), Budd-Chiari syndrome (n = 1). None of the patients exhibited protein losing enteropathy. Linkage analysis is currently undertaken to identify the locus of interest.

372-P

ABOUT A COMPLEX MOLECULAR DIAGNOSIS OF A CDG Ia FRENCH FAMILY

C Le Bizec, S Vuillaumier-Barrot, A Barnier, T Dupré, G Durand, N Seta
French CDG Research Network INSERM GISMR0308, Biochimie A, Hôpital Bichat-Claude Bernard, 75877 Paris cedex 18, France

Congenital disorders of glycosylation (CDG) Ia is an autosomal recessive disorder, characterised by a central nervous system dysfunction and multiorgan failure associated with phosphomannomutase (PMM) deficiency related to mutations in the *PMM2* gene. We report a complex molecular diagnosis of a CDG Ia French family. The affected child was diagnosed as CDG Ia, according to the typical western blot patterns of different serum N-glycoproteins, and leucocyte PMM deficiency (0.5, N > 4.2 U/g TP) associated with a composite heterozygoty R141H (mother) / IVS7 –23 A–>G/3'UTR +52C–>T (father). The mother's PMM activity was lowered (1.7), contrarly to the father's one which was normal (4.4). We hypothesised that paternal mutations are associated with an unstable mRNA retaining large intron 7 suspected by loss of heterozygoty when primers in exon 8 were used in RT-PCR. Thus, we performed a relative quantification of mRNA by fluorescent primer extension assay which showed an allelic loss of the IVS7–23A–>G/3'UTR +52C–>T paternal allele at the cDNA level. The non expression of the mutated allele in the father could explain his normal activity. The CDG Ia patient expressed the two alleles but the paternal allele was less expressed than the R141H maternal allele. The paternal allele with exon 8 observed in the CDG Ia patient could be explained by differential splicing enzymatic machinery between father and son which counterpart the R141H null allele.

373-P

FIVE PATIENTS WITH CDG 1a AND DECREASED PLATELETS

Tamminga RYJ, Rake JP, Smit GPA, J Jaeken[1], van Spronsen FJ
University Hospital Groningen, The Netherlands, [1]University Hospital Gasthuisberg, Leuven, Belgium

Introduction: CDG (Congenital Disorders of Glycoslylation) type 1a is known with increased risk of trombotic events. Increased platelet counts were reported [1]. **Objective:** to report on reduced platelet counts in CDG 1a. **Methods/Results:** in 2 Dutch CDG 1a patients, a reduced platelet count with macrocytic anemia and low neutrophil count was noted. The onset was gradual but platelets were < 50/nl at 3 and 13 yrs of age without signs of folic acid or vit B_{12} deficiency. In one patient HbF was 23% without signs of cell destruction, and thrombopoietin was high in both. Bone marrow (BM) studies (cytology, histology and stemcell culture) in both showed normal cellularity with reduced erythropoiesis and dysplasia in myelopoiesis in one without megakaryopoiesis in the other, and variably reduced colony formation in both. Chromosome breakage studies showed no abnormalities. In the mean time, a third Dutch and a fourth Belgium CDG 1a patient showed reduced platelet counts. In patient 4, pancytopenia was seen at $2\frac{1}{2}$ years with persistent low platelets at 8 years. BM showed increased myelopoiesis. **Discussion/Conclusion**: This is the first report on reduced platelet counts in CDG 1a. Based on our findings, especially an ineffective hemopoiesis is to be considered. In congenital dyserythropoietic anemia type II, a disturbed glycosylation of band-3 protein is described. Similarly, a disturbed glycosylation of messenger molecules (cytokines, molecules involved in intracellular signal transduction) or of membrane components (adhesion molecules, ligands, receptors) may explain the decreased platelet counts.

[1] Lancet. 1987;2:1938.

374-P

3 SIBLINGS WITH CONGENITAL DISORDER OF GLYCOSYLATION TYPE 1a WITH A MILD INTELLECTUAL PHENOTYPE

Coman D[1], McGill J[1], Morris D[3], Klingberg S[3], Matthijs G[4], Jaeken J[5], Appleton D[2]
Department of Metabolic Medicine[1], Neurology[2], The Royal Children's Hospital, Brisbane, Australia; [3]The Queensland Health Pathology Service, Brisbane, Australia; [4]Centre for Human Genetics, University of Leuven, Herestraat 49, B-3000 Leuven, Belgium; [5]Department of Pediatrics, Centre for Metabolic Disease, University Hospital, Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium

We report 3 siblings (1 male and 2 females) recently diagnosed with CDG 1a in their mid twenties. They experience mild mental retardation but manage to function independently in society. Their professions range from library assistant, professional artistic painter and secretarial work. All 3 siblings have cerebellar hypoplasia and ataxia, but are able to ambulate easily. Two of the siblings have required strabismus surgical repairs. All have antithrombin III deficiency, osteoporosis and mild dysmorphic features. Hypertrophic hypogonadism was a feature of the two female siblings. A type 1 sialotransferrin pattern and phosphomannomutase deficiency have been demonstrated. They are compound heterozygotes for R141H and L32R mutations in the PMM2 gene. L32R has been demonstrated to be a mild mutation with 40–45% residual activity. While there is clinical heterogeneity in CDG 1a, we believe that our patients are among of the mildest intellectually affected CDG 1a patients reported to date.

375-P

A MOUSE MODEL FOR CONGENITAL DISORDER OF GLYCOSYLATION Ia

C Thiel, J Rindermann[1], T Lübke[1], G Matthijs[2], K von Figura[1], C Körner
Universitätskinderklinik Heidelberg, Abteilung I, Im Neuenhimer Feld 153, D-69122 Heidelberg; [1]Georg-August-Universität Göttingen, Zentrum Biochemie und molekulare Zellbiologie, Abteilung Biochemie II, Heinrich-Düker-Weg 12, D-37073 Göttingen, Germany; [2]Center for Human Genetics, University of Leuven, UZ Gasthuisberg O&N6, Herestraat 49, B-3000 Leuven, Belgium

Congenital disorders of glycosylation (CDG) comprise a rapidly growing group of inherited multisystemic disorders, which affect the biosynthesis of glycoproteins. The most common form of CDG, CDG-Ia, affects more than 300 patients world wide. They suffer from a multisystemic disorder with severe neurological impairment. CDG-Ia is caused by mutations in the gene encoding for phosphomannomutase 2 (PMM2) which catalyzes the conversion of mannose-6-phosphate to mannose-1-phosphate in the cytosol.

To elucidate the molecular pathology of CDG-Ia and to study therapeutic strategies we are currently generating a mouse model for this disease. We found that disruption of the *PMM2* gene by the insertion of a neomycin cassette led to early embryonic lethality. The lethal event occurs around day 2.5. Mating of heterozygous PMM2-deficient male and female mice with WT mice revealed that the maternal transmission of the *PMM2* null allele is severely impaired. Since the complete knock-out of PMM2 in mice is not compatible with life we are currently generating two different Cre/loxP mouse models carrying mutations which should lead to a partial loss of PMM2-activity as it was observed in all known cases of CDG-Ia.

376-P

ABOUT ANOTHER CDG IJ FAMILY

L Leniaud[1], S Vuillaumier-Barrot[1], C Le Bizec[1], J Dancourt[2], T Dupré[1], H Journel[3], V Cormier-Daire[4], SEH Moore[2], N Seta[1]
French CDG Research Network INSERM GISMR0308, [1]Biochemistry, Hôpital Bichat, [4]Genetics, Hôpital Necker, Paris, [2]INSERM U504, Villejuif, [3]Genetics, CH Chubert, Vannes, France

Congenital disorders of glycosylation I (CDG I) are a family of inborn errors of metabolism, in which N-glycosylation is affected. Up to now, 11 different subtypes have been characterised at the protein and molecular level (CDG Ia to Ik). They mainly affect the nervous system and many other organs with variable clinical presentation. CDG Ij, is one of the latest discovered, with only one patient so far described. It is caused by deficiency of UDP-GlcNAc: dolichyl-phosphate N-acetylglucosamine 1-phosphate transferase, associated with mutations on the corresponding gene (h*ALG7*). We report here a second family with CDG Ij. The patients are two siblings. The first child (7 years old) has convulsions, psychomotor retardation, hypotonia and eye anomalies. The second child (2 years old) was asymptotic at birth but developed progressively the same pathology. Screening of CDG was performed by Western-Blot analysis of different serum N-glycoproteins, and patterns typical of CDG I were observed. *hALG7* sequencing revealed in both siblings two mutations: paternal 890A–>T (I297F) in exon 6 and maternal IVS1–8G–>A splicing mutation associated with an aberrant transcript lacking part of exon 2. These mutations were not found in 50 normal control individuals. The formation of [6-^{3}H]GlcNAc-PP-dolichol from UDP-[6-^{3}H]GlcNAc and dol-P using microsomal proteins from the patient's and parents' lymphoblasts was reduced from 13% for the patient and 54% and 53% for the father and the mother respectively, compared to control cells. These results are in favour of a new CDG Ij family.

377-P

LONG TERM EFFICACY OF MANNOSE TREATMENT IN CDG Ib PAEDIATRIC PATIENTS

K Mention[1], D Dobbelaere[2], G Touati[1], JM Saudubray[1], N Seta[3], P De Lonlay[1]
[1]Necker EM Hospital, Paris, [2]Jeanne de Flandre Hospital, Lille, [3]Bichat Hospital, Paris

Phosphomannose isomerase deficiency (CDG Ib) is the only form of glycosylation disorder treatable by mannose supplementation. The aim of this study was to evaluate the long term efficacy of the mannose supplementation in 3 patients (P1, P2, P3) with a maximal follow-up of 6 years. At diagnosis, P1 and P2, both 2 months old females, presented with anorexia, vomiting, profuse diarrhea, liver involvement and hyperinsulinemic hypoglycemia, with an enzymatic level of 0.3 U/g of protein. P3, a 3 year old female, presented with severe hyperinsulinemic hypoglycemia and mild liver involvement without any digestive symptoms, with an enzymatic level of 0.8 U/g of protein. Plasma factor XI and antithrombin III levels were decreased in all patients. Mannose treatment was started at 4 months in the two patients and at 6 years in the other one, with a dose of 0.17 g/kg/4 h. Mannose plasma levels varied between 25 to 150 µmol/L at T0 and 50 to 300 µmol/L at T60 mn. Glucose levels returned to normal within one month in all patients. Digestive symptoms resolved after 12 and 18 months of treatment in P1 and P2. Biological coagulation abnormalities were normalized after 1, 4, 30 months in P 2, 3 and 1 respectively. **Conclusion**: mannose treatment in CDG1b is a successful treatment, improves digestive symptoms, hypoglycemia, and coagulation abnormalities. On the other hand, mannose supplementation does not completely correct protein glycosylation (abnormal sialotransferrin electrophoretic profile) and the liver damage. In fact, liver disease of P1 evoluated in cirrhosis, probably depending of the severity of the disease at diagnosis, variable between patients.

378-O

CLINICAL AND BIOCHEMICAL PRESENTATION OF SIBS WITH A MULTIPLE GLYCOSYLATION DISORDER: Cog 7 DEFICIENCY

LJM Spaapen[1], JA Bakker[1], SB van der Meer[2], HJ Sijstermans[2], RA Steet[3], RA Wevers[4], J Jaeken[5]
[1]Department of Biochem. Genetics, Academic Hospital Maastricht, [2]Department of Pediatrics, Atrium Medisch Centrum, Heerlen, The Netherlands, [3]Department of Internal Medicine, Washington University School of Medicine, Saint Louis, MO, USA, [4] Laboratory of Pediatrics and Neurology, University Medical Center, Nijmegen, The Netherlands, [5]Center for Metabolic Diseases, Universitair Ziekenhuis Gasthuisberg, Leuven, Belgium

Congenital disorders of glycosylation (CDG) represent a group of inherited multi-organ diseases caused by defects in the biosynthesis of glycoproteins.

Objective: We report on clinical and biochemical presentation of two dysmorphic siblings with severe liver disease who died at the age of a few weeks.

Methods: Analyses of lysosomal enzymes, plasma total sialic acid, isoelectric focusing (IEF) of sialotransferrins and of apo-lipoprotein CIII were done by established methods.

Results: Activities of lysosomal enzymes in plasma were found 3 to 10 times increased, though total sialic acid in plasma was strongly decreased. IEF of serum sialotransferrins showed a type 2 like CDG pattern. Some of the known CDG subtypes were excluded. O-glycosylation was investigated by IEF of apo-lipoprotein CIII which showed increased fractions of hypoglycosylated isoforms.

Conclusion: This is the first report on patients with a new variant of CDG, due to a multiple Golgi defect, involving both N- and O-glycosylation. In a parallel study a defect in the Conserved Oligomeric Golgi complex on the level of subunit COG 7 was established.

379-O

MUTATION OF THE COG COMPLEX SUBUNIT GENE *COG7* CAUSES A LETHAL CONGENITAL DISORDER OF GLYCOSYLATION

HH Freeze[1], X Wu[1], RA Steet[2], O Bohorov[1], E Eklund[1], J Bakker[3], J Newell[1], M Krieger[4], L Spaapen[3], S Kornfeld[2]
[1]The Burnham Institute, La Jolla, CA 92037, USA; [2]Washington University School of Medicine, Saint Louis, MO, USA; [3]Academic Hospital Maastricht, Maastricht, The Netherlands; [4]Massachusetts Institute of Technology, Cambridge, MA USA

The congenital disorders of glycosylation (CDG) are characterized by defects in *N*-linked glycan biosynthesis that result from mutations in genes encoding proteins directly involved in the glycosylation pathway. We found a fatal form of CDG caused by a mutation in the gene encoding COG-7, a subunit of the conserved oligomeric Golgi (COG) complex. The mutation impairs integrity of the COG complex and alters Golgi trafficking, resulting in disruption of both the N- and O-glycosylation pathways. The mutations reduce the activity of multiple glycosyltransferases and nucleotide sugar transporters. These cases represent a new type of CDG in which the molecular defect lies in a protein that affects the trafficking and function of the glycosylation machinery. CHO cells with defects in other COG complex proteins, COG-1 or COG-2, also show reduced activities of multiple glycosyltransferases and nucleotide sugar transporters. These severely reduce glycosaminoglycan chain assembly and sulfation. Intracellular trafficking of sialyltransferase, ST3GalI, between ER and Golgi is also abnormal. Identifying other patients with defects in intracellular trafficking proteins will require analysis of serum transferrin glycosylation.

380-O

A NEW TYPE II CONGENITAL DISORDER OF GLYCOSYLATION WITH EPI-METAPHYSEAL DYSPLASIA

Jaeken J[1], Mills PB[4], Körner C[5], Callewaert N[6], Van Breuseghem I[2], Carchon H[1], Schollen E[3], Matthijs G[3]
Centre for Metabolic Disease[1], Department of Radiology[2] and Centre for Human Genetics[3], University Hospital Gasthuisberg Leuven, Belgium; Institute of Child Health at Great Ormond Street Hospital, University College London, UK[4]; Biochemie II, Georg-August-Universität Göttingen, Germany[5]; Institute of Microbiology, ETH, Zürich, Switzerland[6]

This boy, born in 1990, had a sister with a similar disorder who died at 15 months. Psychomotor development was moderately retarded and length growth was delayed from birth. At 11 years he showed dwarfism (height 19 cm < 3rd centile) with obesity, waddling gait, dysmorphy (midface hypoplasia, lowset ears, pigeon chest, thoracolumbar scoliosis, O-legs and varus feet) and joint hyperlaxity. RX skeleton showed pronounced generalized osteoporosis, thin cortex and epiphyseal deformation, and MRI of the skeleton showed epi-metaphyseal dysplasia with cartilage herniation. On brain MRI there was vermis hypoplasia. Serum AST, CK, LDH and IgA were increased with normal ALT. Serum transferrin IEF indicated a type 2 pattern and on serum transferrin glycan analysis with MALDI-TOF-MS there was evidence for a galactosylation defect, and there was a reduced level of tri-antennary N-glycans. However galactosyltransferase 1 activity and UDPgalactose transport were normal in fibroblasts, and on mutation analysis there was no evidence a deficiency of galactosyltransferases 4 and 7, and N-acetylglucosaminyl-transferases 3 and 4 deficiency.

Conclusion: epi-metaphyseal dysplasia is a new presentation of CDG; its primary defect remains to be determined.

381-P

COMBINED DEFECTS OF N- AND O-GLYCOSYLATION IN A PATIENT WITH UNUSUAL CLINICAL PHENOTYPE

Adamowicz M[1], Mierzewska H[1], Wevers RA[2], Wopereis S[2], Morava E[2], Chmielinska E[1], Ginalska-Malinowska M[1], Pronicka E[1]
[1]The Children's Memorial Health Institute, Warsaw, Poland, [2]University Medical Centre Nijmegen, The Netherlands

The number of CDG patients with an unidentified glycosylation defect is constantly growing. The part of them is classified as CDG IIx on the basis of transferrin patterns in IEF and SDS-PAGE. Clinical phenotypes among this group are extremely heterogeneous. We present a boy aged 10 years, born (after 7 years of hormonal mother's treatment) with intrauterine asphyxia, bilateral inguinal hernia, micropenis and small testes migrans. At the age of 3 years – hypermetropia, at 6 years – overweighting, at 7 years – the hypothyrotic goitre and hypoacusis were noticed. Hepatopathy with elevated transaminases were noted at 8 years. At the age of 9 years truncal and femoral obesity with tight subcutaneous fatty tissue was present as well as dysmorphic features: upslanting palpebral fissures, bilateral epicanthal folds, thin nasal bridge, mild prognathism and clinodactyly. His mental development was normal. USG revealed enlargement of liver and steatosis of pancreas. Biochemical examinations showed slightly elevated transaminases and diminished activity of protein S. Activity of AT III and protein C were normal. Abnormal IEF profiles of transferrin, α_1AT, TBG and normal transferrin SDS-PAGE suggested CDG type II. The result of the new test, serum apoCIII isofocusing, which exclusively demonstrates O-glycan biosynthesis defects, revealed that our patient has a rare combined defects of N- and O-glycosylation.

382-P

A COMBINED DEFECT IN THE BIOSYNTHESIS OF N- AND O-GLYCANS IN PATIENTS WITH CUTIS LAXA

S Wopereis[1], E Morava[1], S Grünewald[2], P Mills[3], B Winchester[3], P Coucke[4], K Huijben[1], R Wevers[1]
[1]University Medical Center Nijmegen, The Netherlands; [2]University hospital of Essen, Germany; [3]University College London, UK; [4]Center for medical genetics, Ghent, Belgium

Introduction: Based on a previous observation of abnormal glycosylation in a cutis laxa patient five patients with this syndrome were analyzed for congenital defects of glycosylation (CDG).

Methods: Transferrin and apolipoproteinC-III (apoC-III) isofocusing (IEF) were used to screen for defects in the biosynthesis of N-glycans and mucin core 1 type O-glycans respectively. Mass spectrometric N-glycan analyses were performed to reveal the primary defect in patients with abnormal IEF patterns and mutation analysis of the fibulin-5 gene was performed.

Results: Three out of five cutis laxa patients showed an abnormal transferrin IEF profile as well as an abnormal apoC-III IEF profile. In these three patients no mutations were detected in the fibulin-5 gene. Mass spectrometric analysis revealed an increase of N-glycans lacking sialic acid and N-glycans lacking sialic acid and galactose residues.

Conclusions: The autosomal recessive type of cutis laxa in our three patients is not caused by mutations in fibulin-5. The patients have a primary genetic defect affecting both N- and O-glycosylation. Our data narrow the options for the position of the primary defect down to a step in the sialylation or galactosylation of N- and O-glycans. The defect leads to a clinical phenotype with cutis laxa in early childhood.

383-O

EVOLVING PHENOTYPE IN PATIENTS WITH CUTIS LAXA AND A COMBINED DEFECT OF GLYCAN BIOSYNTHESIS

E Morava[1], S Wopereis[1], M Hogeveen[1], P Coucke[2], R Wevers[1], J Smeitink[1], S Grünewald[3]
[1]UMC Nijmegen, The Netherlands, [2]University of Ghent, 3University Hospital of Essen, Germany

Congenital cutis laxa is a genetically heterogeneous condition presenting with loose and redundant skin folds, decreased elasticity of the skin, connective tissue involvement and a highly variable spectrum of associated features. The most common forms are inherited in an autosomal recessive or dominant fashion. Fibuline 5 and elastin mutations were detected in some of these patients, but in most cases the etiology is not known.

We performed a screening for disorders of protein glycosylation including a recently developed test for defective O-glycosylation in five unrelated patients with cutis laxa syndrome. Three out of the five patients had an inborn error of glycan biosynthesis affecting the synthesis of both N-linked and O-linked glycans.

Here, we describe the clinical features and the evolving phenotype of cutis laxa syndrome in these three offspring from consanguineous marriages. The children presented with a severe neonatal cutis laxa, skeletal and joint involvement, microcephaly, delayed closure of the fontanel, normal growth, developmental delay and neurological findings. All patients had an evident progress in the psychomotor development. A significant improvement of the skin findings was observed with the development of fat-pads at an older age. Two of the three children were diagnosed with pachygyria and seizures and one with severe sensoneural deafness.

We describe a distinct clinical phenotype with a combined glycosylation defect. This is the first clinical description of abnormal glycosylation implicated in the etiology of cutis laxa syndrome.

384-P

A NOVEL MUTATION IN TURKISH NEWBORN WITH CONGENITAL GLUCOSE-GALACTOSE MALABSORPTION

Kurt I[1], Gok F[2], Aydin HI[3], Gokcay E[3], Maeda M[4], Kasahara M[4,5]
[1]Department of Clinical Biochemistry, [2]Department of Pediatrics, [3]Division of Pediatric Nephrology, Gülhane Military Medical Academy and Medical Faculty, Ankara, Turkey; [4]Laboratory of Biophysics, School of Medicine and [5]Genome Research Center, Teikyo University, Hachioji, Tokyo, Japan

Congenital glucose-galactose malabsorption(GGM), which caused by defects of SGLT1, is an autosomal recessive disease characterized by selective malabsorption of glucose and galactose. Only ≈200 individuals worldwide are currently known to be affected by GGM. More than 30 mutations of SGLT1 responsible for GGM have been identified so far. Patients with GGM present with neonatal onset of severe, watery, acidic diarrhea while on glucose- or galactose-containing diets and it can be life-threatening if not diagnosed and treated. Herein we report a novel missense mutation (Thr460Pro) in a girl patient suffering from severe watery, acidic diarrhea and hypernatremic dehydration in the neonatal period. The patient's clinical picture was typical of GGM. The sequence analysis of patient showed that she has a homozygous Thr460Pro mutation (AAC→CCC) on exon 12. The sequence analysis of the father and mother of the patient indicated the presence of heterozygous Thr460Pro mutation at this position. The mutation identified in the patient has not previously been detected in other GGM patients.

385-P

RENAL GLYCOSURIA AND AMINO ACIDURIA IN TWO SIBLINGS WITH HETEROZYGOSITY FOR A *GLUT2* MUTATION

A Peduto[1], M Spada[1], R Santer[2], A Ponzone[1]
[1]Department of Paediatrics, University Children's Hospital Turin; [2]Department of Paediatrics, University Children's Hospital Hamburg, Germany

GLUT2 is a facilitative glucose transporter, primarily involved in glucose homeostasis. Genetic defects in GLUT2 are responsible for the Fanconi-Bickel syndrome, a rare condition that presents early in infancy with failure to thrive, rickets, renal tubular dysfunction, hepatic glycogen storage and impaired glucose and galactose metabolism. We report clinical and genetic findings in two sisters with marked renal glycosuria associated by amino aciduria. The siblings' age is now 14 and 10 years, respectively. Glycosuria was revealed at a routine investigation in the neonatal period and has persisted since now at a rate of 25–30 g/day. Furthermore, low grade amino aciduria (thr, ser, ala, cit, cys, lys, arg) has been detected. Otherwise, the siblings are completely asymptomatic, with adequate growth, absence of hepatomegaly, normal glomerular function, normal glucose and galactose tolerance. Analysis of the *GLUT2* gene by direct sequencing revealed heterozygosity for an exon 6 *nonsense* mutation (1213 C>T) in both siblings.

Hereditary renal glycosuria is defined by urinary glucose excretion in the presence of a normal blood glucose concentration and the absence of any signs of a generalized renal tubular dysfunction. It has been proposed recently that the primary defect is localized within the gene for the sodium-dependent glucose transporter 2 (*SGLT2*) which is expressed in proximal renal tubular cells. To date, there has been no report of severe glycosuria and aminoaciduria in individuals who are heterozygous for *GLUT2* mutations. Fanconi-Bickel syndrome shows a recessive trait; parents of patients are asymptomatic. A single case of mild glycosuria after an OGTT test in a heterozygote for a *GLUT2 missense* mutation was reported. Our two patients represent an unusual case of mild Fanconi-Bickel phenotype associated by heterozygosity for a *GLUT2* nonsense mutation. Additional molecular studies of the *GLUT2* and *SGLT2* gene are pending.

386-P

IS HIGH-RISK SCREENING FOR CLASSICAL GALACTOSAEMIA IN IRELAND EFFECTIVE?

S Mohamed, P Mulhair, C O'Neill, E Naughten, S Yap, EP Treacy, PD Mayne
National Centre for Inherited Metabolic Disorders and The National Newborn Screening Laboratory, Children's University Hospital, Temple Street, Dublin 1, Ireland

Introduction: A national newborn screening programme for classical galactosaemia (gal) was introduced in Ireland in 1972 to screen all infants between 72 and 120 h after birth using a bacterial inhibition assay. In 1996, a formal day one high-risk screen for gal was introduced using a fluoremetric assay for GALT (Beutler) for siblings of known cases of gal and infants born to the traveller community (incidence 1/450). A galactose free diet was commenced pending results.

Objectives: To review the results of the newborn screening programme for gal in Ireland between 1989 and 2003 and to assess the effectiveness of the formal high-risk screening strategy.

Method: Retrospective review of the newborn screening programme and the case notes.

Results: 52 infants were diagnosed with classical gal (incidence 1/14 400 live births). Since 1996, 30 infants were diagnosed with classical gal, 16 by the high-risk screen and 14 by routine assay. Between 1989 and 1996, 22 infants were diagnosed with classical gal, of whom 11 were born to traveller families, or with a positive family history of gal, 4 were detected by the high-risk screen. The remainder were diagnosed by routine screen between day 5 and 10. Before implementation of the formal day 1 high-risk screen the majority of high-risk patients had clinical signs at diagnosis. With the change in policy 88% of the high-risk patients were asymptomatic at diagnosis.

Conclusions: The formal high-risk strategy has improved the efficiency of newborn galactosaemia screening in Ireland.

387-P

CLASSICAL GALACTOSEMIA IN ESTONIA: INCIDENCE AND SELECTIVE NEONATAL SCREENING

Õunap K[1,2], Andreson R[3], Laht TM[4], Krabbi K[5], Metspalu A[1,3]
[1]Tartu University Clinics, United Laboratories, [2]Department of Pediatrics and [3]Department of Biotechnology, University of Tartu, Tartu; [4]Tallinn Technical University, [5]Central Laboratory of Chemistry, Health Protection Inspectorate, Tallinn, Estonia

The purpose of our study was to establish the incidence of classical galactosemia caused by galactose-1-phosphate uridyltransferase (GALT) deficiency in Estonia and to evaluate the effectiveness of selective screening for GALT deficiency.

Since 1995 we have done urinary screening tests in all newborns admitted to two main centralized hospitals with suspicion of metabolic diseases (~3000 newborns (2.6%) from total of 115 913 newborns born during 1995–2003). In 6 cases the GALT deficiency has been confirmed by biochemical and enzymatic tests. We also analyzed the DNA from 1019 newborns born in one month from all regions of Estonia and found 6 heterozygotes for Q188R mutation (0.6%), and 158 heterozygotes and 8 homozygotes for N314D mutation (Duarte allele, 16%). From 10 independent chromosomes of Estonian classical galactosemia patients, 50% of them carry the Q188R mutation in GALT gene. The mutations in other alleles are not specified yet. Although the group of the patients is very small, but if we expect that 50% of the alleles carry Q188R and 50% carry other mutation, it gives the incidence of GALT deficiency 1 per 30 000 newborns. The results of selective screening gives even higher incidence (1 per 20 000 newborns), which shows that with very high probability we have not missed any case and that this program has been very effective.

388-A

THIS ABSTRACT HAS BEEN WITHDRAWN

389-P

BIOMARKERS OF BONE METABOLISM IN PATIENTS WITH CLASSIC GALACTOSAEMIA

Prochazkova D[1], Pohlidal A[4], Slabik D[4], Nemeckova J[1], Mrazova J[3], Francova H[2], Kozak L[2]
[1]University Hospital Brno, Czech Republic, 1st Department of Paediatrics, [2]Centre for Molecular Biology and Gene Therapy, [3]Department of Radiology, [4]University Hospital Ostrava Poruba, Department of Biochemistry, Czech Republic

The objective of this presentation is to perform a survey about diminished BMD in prepubertal patients and adolescents with galactosaemia. **Methods**: Six treated patients (five females, one male, aged 5–20 years) had BMD determined by dual energy X-ray absorptiometry. Two measurements were performed: an areal measurement of the total body and a volumetric measurement of femoral neck or spine. Dietary calcium intake, blood calcium, phosphate, vitamin D, parathormone and markers of bone formation (bone alkaline phosphatase, osteokalcin) and bone resorption (N-telopeptide; NTx in urine and C-telopeptide; CTx in blood cross-linked collagen type I) were performed. **Results**: All patients had significantly diminished BMD. Mean Z score of the volumetric BMD was –1.55 (range 0 to –2.7) and of areal BMD –1.7 (range –0.1 to –3.0). Dietary calcium intake was decreased. Blood calcium, phosphate, and parathormone were normal. Bone alkaline phosphatase was normal except of one case, vitamin D metabolites were incresead in three patients, and osteocalcin was increased in all but one patient. Markers of bone resorption were higher (NTx in five and CTx in three patients). **Conclusion**: Early changes in markers of bone resorption (NTx, CTx) may predict long-term changes in BMD in galactosaemics.

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GALACTITOL AND GALACTONATE IN RED BLOOD CELLS OF (D/G) GALACTOSEMIC PATIENTS

C Ficicioglu, C Yager, S Segal
Children's Hospital of Philadelphia, Section of Metabolism, Philadelphia, PA, USA

Objective: With many newborns now being identified as D/G galactosemia,we measured galactitol and galactonate in the red blood cell (RBC) to elucidate the biochemical phenotype of treated and untreated D/G galactosemics at different ages. **Methods/Subjects**: The RBC galactitol, galactonate and gal-1-P were measured by isotope dilution GC/MS. The RBC galactonate, galactitol and gal-1-P were quantified in 14 DG galactosemic newborns on a regular formula, 8 DG galactosemic newborns on a galactose-free formula and 18 DG galactosemic patients between 1 year to 2 years of age that were on a regular diet. The results were compared with those of non-galactosemic healthy newborns/ nfants. **Results and Conclusion**: In the D/G newborns on a regular formula, the levels of RBC galactitol, galactonate and Gal-1-P were significantly higher than those of D/G newborns on a diet treatment and non-galactosemic newborns. There was no difference in the levels of RBC galactitol, galactonate and gal-1-P between D/G galactosemic newborns on a galactose-free diet and the control group. There appears to be two different responses to dietary galactose intake in D/G children. The first group of D/G children on a regular diet had higher RBC galactitol, galactonate levels than those of non-galactosemic children. The second group of D/G children on a regular diet had normal levels of RBC galactitol and galactonate. The levels of RBC gal-1-P were normal in both groups of D/G patients. The alternative pathway products may be important additional markers for monitoring metabolic control in D/G children. The importance and exact nature of alternative pathways should be demonstrated in future studies.

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PSEUDOTUMOR CEREBRI AS A PRESENTING SYMPTOM IN A NEONATE WITH CLASSICAL GALACTOSEMIA

Augoustides-Savvopoulou P[1], Kambouridou P[2], Michelakakis E[3], Kozeis N[4], Tsepkentzis K[5], Papadopoulos F[6]
1 University A'Pediatric Department–Biochemistry Lab, 4 Ophthalmology Department, General Hospital of Thessaloniki 'Hippocration', [2,5,6] Pediatric Clinic, General Hospital of Thessaloniki 'G Gennimatas', [3] Institute of Child Health, Athens, Greece

Classical galactosemia (MIM#230400) is an autosomal recessive potentially lethal inborn error of carbohydrate metabolism due to galactose-1-phosphate uridyltransferase (GALT) deficiency usually presenting with poor feeding, vomiting, hepatomegaly, jaundice, cataracts soon after the initiation of milk feedings. A neonate with galactosemia and brain edema is described with the objective of highlighting a rare manifestation of a wellknown disease with reversible symptoms. The proband is a female infant the only offspring of unrelated parents born after uncomplicated gestation and birth. She was referred when one month old because of weight loss and hepatomegaly. Clinical examination revealed an underweight, floppy infant with a bulging anterior fontanelle and massive hepatomegaly. Head circumference was > the 97th percentile (5 cm increase in 30 days). A brain scan showed diffuse cerebral oedema with complete obliteration of the ventricles. Ocular examination revealed mild bilateral cataract. A metabolic workup revealed gross galactosuria, generalized aminoaciduria and zero activity of GALT in RBC. A lactose-free diet was initiated and one week later there was no further increase in head circumference and liver size had decreased significantly. Lens opacities showed improvement after a month. This report serves as a reminder that classical galactosemia can be a cause of cerebral edema in neonates.

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MILD PHENOTYPE IN A CLASSICAL GALACTOSEMIA PATIENT WITHOUT GALACTOSE RESTRICTION

B Panis[1], B Kuijper[1], JA Bakker[2], LJM Spaapen[2], JP Sels[3], ME Rubio-Gozalbo[1,2]
Department of [1]Pediatrics, Metabolic Diseases, [2]Genetic Metabolic Diseases, [2]Endocrinology University Hospital Maastricht, The Netherlands

Introduction: Classical galactosemia is an AR disorder of galactose metabolism. It presents in the neonatal period with an acute toxicity syndrome. Treatment consists of dietary restriction of galactose. Despite treatment long-term complications occur. We report a classical galactosemia patient with a mild phenotype despite no galactose restriction. Possible explanations are discussed.

Case report: A Caucasian male (age 34 yrs), homogenous for Q188R mutation (1.5% rbc GALT activity) experienced a sepsis as neonate. Since the age of 3 years, galactose restricted diet was withdrawn. He has normal intellectual skills, and no visual or neurological problems. Plasma gal-1-P and galactitol concentrations are 0.41 μmol/gHB (<0.58 in treated patients) and 9.0 μmol/L (12–21 in treated patients) respectively. Urinary galactitol was 83 μmol/mmol creat (59–355 in treated patients) and urinary galactose was 4 μmol/mmol creat (0.2–25.6 in healthy controls). Analysis of the patients' diet showed an intake of 6–9 g galactose a day.

Discussion: This patient does not show long-term complications of galactosemia despite no galactose restricted diet. A Medline database search provided us reports of three more Q188R homozygous patients with a mild phenotype. Phenotype seems not solely dependant on genotype and rbc GALT activity. Alternative pathways must exist to prevent rising of metabolites. Two well known possibilities are the galactitol and galactonate pathways. Furthermore residual rbc GALT activity of 1.5% or residual GALT activity in other tissues might still be responsible for a considerable part of galactose metabolism and are possible 'protective' mechanisms.

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BONE MINERAL DENSITY IN CHILDREN AND ADOLESCENTS WITH GALACTOSAEMIA

MJR Kershaw, N Crabtree, A Chakrapani, A MacDonald, E Elias, NJ Shaw
Birmingham Children's Hospital, Birmingham, UK

Bone mineral density in post-pubertal females with galactosaemia and premature ovarian failure is reduced. Two previous reports suggest bone mineral density is reduced in pre-pubertal children of both sexes. Failure to achieve normal peak bone mass has implications for bone health in later life.

Objective: To determine growth and bone mineral apparent density (BMAD) in children and adolescents with galactosaemia and compare with paediatric reference data.

Methods: Spinal and whole body DXA bone density measurements were performed in 20 children and adolescents (11 male, 9 female, mean age 13.0 years, range 5–22) with galactosaemia. BMAD Z scores were calculated using normative paediatric data established for the Lunar Prodigy scanner. Local ethical approval was obtained.

Results: Lumbar BMAD z scores were reduced for age (mean Z-score –0.62, SE = 0.24, $p = 0.02$), however only one adolescent had a BMAD more than 2 standard deviations below the mean. The mean whole body bone density Z score was –0.93, SE = 0.18, $p < 0.0001$, with two individuals with Z scores <–2.0. There was no relationship between calcium intake and BMAD. Height SDS scores were significantly reduced (mean Z-score –1.2, SE = 0.23, $p < 0.0001$) when compared to the 1990 UK standards. Pubertal status was appropriate for age in all but two patients. No significant gender difference in bone density was observed.

Conclusion: Bone density in the majority of children with galactosaemia is within the normal range for age. Growth during childhood and adolescence may be compromised as indicated by the significantly reduced height SDS in this group.

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NORMAL PREGNANCY OUTCOME IN POORLY CONTROLLED GALACTOSAEMIA

K Gupta, A MacDonald, A Daly, MA Preece, A Green, A Chakrapani
Birmingham Childrens Hospital, Birmingham, UK

Several pregnancies in well controlled classical galactosaemia have previously been reported with good outcomes. We report here a 22 year old M279R homozygous female, poorly controlled till 34 weeks of gestation who had a normal fetal outcome after an uneventful pregnancy.

The patient was born to first cousin parents and was treated prospectively as a previous affected sibling had died following acute neonatal presentation. She had short attention span, behaviour problems, social immaturity and speech difficulties during childhood. A Wechsler Intelligence Scale for children scored a full scale IQ of 64 at 9 yrs. Compliance was variable during childhood and adolescence with elevated gal-1-phosphate levels detected on multiple occasions. FSH and LH were elevated at 13 years but she went into puberty spontaneously and achieved menarche at 17.

She conceived spontaneously whilst poorly compliant with her diet and presented at 28 weeks gestation, when her galactose-1-phosphate levels were found to be 593 μmol/L (1.73 μmol/g Hb) (well controlled galactosaemia <150 μmol/L or <0.6 μmol/g Hb). Liver function tests were normal. Dietary compliance improved through the rest of pregnancy and at 34 weeks the galactose-1-phoshate levels were 257 μmol/L (1.11 μmol/g Hb). Relatively good biochemical control was achieved by 38 weeks (galactose-1-phosphate levels 121 μmol/L and (0.93 μmol/g Hb). She delivered a normal unaffected infant at term.

Conclusions: Poorly controlled galactosaemia can be compatible with a normal pregnancy and fetal outcome.

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LONG-TERM DIETARY MANAGEMENT AND OUTCOME OF GALACTOSAEMIA/ DUARTE (D/G) CASES

S Mohamed, K Reddy, S Yap, C O'Neill, Y Rogers, PD Mayne, EP Treacy
National Centre for Inherited Metabolic Disorders and The National Newborn Screening Laboratory, Children's University Hospital, Temple Street, Dublin 1, Ireland

Introduction: Duarte galactosaemia (D/G) cases have approximately 25% activity of the enzyme galactose-1-phosphate uridyl transferase (GALT). Controversy remains as to the necessity for long-term dietary treatment. **Objectives**: To determine the long-term outcome of D/G cases and review the effect of diet. **Methods**: A retrospective study was performed on cases ascertained by the Irish newborn screening programme (1972–2003) and family screening. Initial and follow up blood levels of Gal-1-P and urinary galactitol were reviewed, with RBC GALT activity, mutation analysis and clinical/dietary information. **Results**: Of 1.7 million newborn screened, 14 D/G cases were ascertained, 4 cases by family studies. The median age at follow up was 10.5 years (*r*: 2–24). Median Gal-1-P levels at detection was 60 μmol/Lpc (*r*: 0–1196, *n*: 0–30), median galactitol: 7 mmol/mol creat (*r*: 0–800, *n*: 0–5). At follow up, median Gal-1-P levels was 3 μmol/Lpc (*r*: 0–10), galactitol: 5 mmol/mol creat (*r*: 0–5). Median GALT activity was 5 μmolsubc/H/gHb (*r*: 3.9–10, 18–24). Of 12/18 cases tested, the genotype was Q188R/N314D. Dietary intervention varied from strict galactose restriction from birth to follow up (5 cases), partial restriction (11 cases) and no dietary intervention (2 cases). Neurodevelopment and eye evaluations were within normal limits in all patients. **Conclusions**: We did not identify any significant complications in our study cohort in cases on and off diet. These data question the necessity of long-term dietary intervention.

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SUPPRESSION OF ENDOGENOUS GALACTOSE SYNTHESIS

HH Huidekoper[1], AM Bosch[1], SN van der Crabben[2], RJA Wanders[1,3], HP Sauerwein[2], MT Ackermans[3], FA Wijburg[1]
[1]Department of Pediatrics, [2]Department of Internal Medicine, [3]Department of Clinical Chemistry, Academic Medical Centre, Amsterdam, The Netherlands

Introduction: Most patients with classical galactosemia develop long-term complications despite dietary treatment. This is most likely due to endogenous production of galactose. The regulatory mechanisms of this endogenous galactose synthesis are not known. Goal of our study was to determine if endogenous synthesis of galactose is suppressed by exogenous galactose supplementation. **Materials and Methods:** Two adult patients (age 21, 29) with classical galactosemia (homozygous for Q188R mutation) and two healthy control subjects (age 27, 35) were given a primed D-[1-^{13}C]galactose infusion in order to measure the rate of endogenous galactose synthesis (EGS in mg/kg/h). After baseline measurement of the EGS, the total amount of exogenous galactose supplementation was doubled by unlabeled galactose infusion. The EGS during steady-state without (EGS_1) and with (EGS_2) unlabeled galactose infusion were compared. **Results and Conclusions:** No conclusive differences between EGS_1 and EGS_2 were measured in both patients and control subjects (patient 1: $EGS_1 = 0.44$, $EGS_2 = 0.43$; patient 2: $EGS_1 = 0.41$, $EGS_2 = 0.34$; control 1: $EGS_1 = 0.09$, $EGS_2 = 0.06$; control 2: $EGS_1 = 0.07$, $EGS_2 = 0.09$). In patient 1 we measured $EGS_1 = 0.51$ and $EGS_2 = 0.54$ when total exogenous galactose was quadrupled. We conclude that the rate of endogenous galactose synthesis is not influenced by exogenous galactose in patients with classical galactosemia. Based on these results a major relaxation of the dietary restrictions in classical galactosemia seems not warranted.

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MUTATIONAL SPECTRUM OF CLASSICAL GALACTOSEMIA IN THE NETHERLANDS

AM Bosch[1], L Ijlst[2], W Oostheim[2], J Mulders[2], HD Bakker[1], RJ Wanders[1,2], HR Waterham[2]
[1]Department of Pediatrics, [2]Laboratory Genetic Metabolic Diseases, Academic Medical Centre, Amsterdam, The Netherlands

Introduction: Classical galactosemia is caused by a deficiency of the enzyme galactose-1-phosphate uridyltransferase due to mutations in the encoding GALT gene. The incidence in The Netherlands is 1:33 000 with 6 new patients each year. We present here the mutational spectrum of classical galactosemia in The Netherlands based on our data generated in 10 years of molecular analysis for this defect.

Methods: In the past 10 years we analyzed the GALT gene of 122 Dutch patients, who were clinically and biochemically diagnosed with classical galactosemia. To this end, we sequenced all exons plus flanking intron sequences of the GALT gene using genomic DNA of the patients.

Results and Conclusion: We found a similar frequency of the common p.Q188R mutation in the Dutch cohort as reported for other Western European countries. In total we identified 23 different mutations, including 8 mutations that had not been reported previously. These novel mutations include 5 missense mutations (p.R51Q, p.S135W, p.K229N, p.Q252H and p.X380C), 1 frame shift mutation (c.410dupT), 1 splice site mutation (c.821-2A>G), and 1 large deletion encompassing at least exon 1–11. The number of novel mutations is surprisingly high, taken the fact that already over 180 different mutations in the GALT gene had been reported.

398-P

FASTING HYPOGLYCAEMIA AND MILD LIVER STEATOSIS DUE TO GLYCOGEN SYNTHASE DEFICIENCY ('GSD TYPE 0')

J Taybert, A Kepka, M Pronicki
Children's Memorial Health Institute, Warsaw, Poland

Deficiency of glycogen synthase is reported as a very rare inborn error of metabolism. True frequency of this usually mild disease is unknown. The aim of the report is to show a diagnostic approach, which led to final diagnosis of this rare metabolic disorder directly after the first episode of fasting hypoglycaemia. A son of unconsanguineous parents was without symptoms to the end of 7 when morning episode of preprandial loss of counsciousness occurred. Serum glucose level was 34 mg/dl. Mildly enlarged normoechogenic liver was seen on ultrasound. Tendency to low glucose level (35 mg/dl) after 7–10 h fasting, slight elevation of transaminases and uric acid (7.8 mg/dl), low serum free carnitine (29 μmol/L, total 54 μmol/L) were found. Preprandial lactate concentration was at high normal range (17.5 mg/dl). Intravenous glucose load showed marked increase of lactate (up to 37.8 mg/dl at 60 min) with signs of decreased tolerance of glucose. Remarkable ketosis without increase of lactate level was found at fasting test. With suspicion of glycogen storage disease type VI or IX liver biopsy was performed. Mild steatosis and no glycogen storage were found. There were normal activities of glucose-6-phosphatase, debrancher and phosphorylase complex. Liver glycogen content was decreased (16/6 mg/g, control value 20 50 mg/g). Measurement of glycogen synthase activity in liver tissue discovered decrease to $< 5\%$ of control value. This confirmed clinical suspicion of GSD0 ('glycogen storage disease type 0').

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PRELIMINARY DATA ON A NEW STARCH FOR GSD I

Bhattacharya K[1], Mundy H[1], Eaton S[1], Tester RF[2], Lee PJ[1]
[1]*Charles Dent Metabolic Unit and Institute of Child Health, London;* [2]*Starch Research Unit, Glasgow Caledonian University, UK*

Uncooked cornstarch (UCS) has been used to treat Glycogen Storage Disease I (GSD I) for the past 2 decades, but concerns exist about its efficacy. A physically modified cornstarch (WMHM20) has been developed. This modified cornstarch is theoretically more amenable to digestion than UCS. A randomized double-blind cross-over study was performed to test the hypothesis that the new starch has a longer duration of action.

Method: 5 patients with GSD I (3 male; aged 21 35 years) were admitted on 2 occasions at least 7 days apart for a 120 g starch load. Both starches were given, but were randomly allocated to the different days. Baseline blood glucose (BG), lactate, non esterified fatty acids and insulin were performed and measured hourly until the patient became symptomatic, BG < 3.0 mmol/L or the test had lasted for 10 hours. Breath CO_2 enrichment and hydrogen were measured after each load.

Results: The median duration of action for UCS was 8 h and for WMHM20 was 10 h. The mean blood lactate ranged from 3.1 to 11.1 mmol/L for UCS and from 2.4 to 9.42 mmol/L for WMHM20, with mean BG and lactate at the end of the test 3.0 and 7.56 mmol/L for UCS, 3.4 and 8.21 mmol/L for WMHM20, respectively. The hydrogen breath test showed more malabsorption of UCS than WMHM20 with mean hydrogen excretion difference being 6 ppm greater with UCS.

Conclusion: These early data suggest that WMHM20 starch may be more efficacious than currently used UCS, but these studies need expanding in more patients.

400-P

EFFICACY OF ACE-INHIBITORS THERAPY ON RENAL DISEASE IN GLYCOGEN STORAGE DISEASE TYPE I (GSDI): A MULTICENTRE RETROSPECTIVE ITALIAN STUDY

Melis D[1], Parenti G[1], Gatti R[2], Della Casa R[1], Internicola M[1], Majo F[1], Parini R[3], Riva E[4], Burlina A[5], Dionisi Vici C[6], Papadia R[7], Zammarchi E[8], Andria G[1]
[1]Department of Pediatrics, Federico II University, Naples; [2] Gaslini Hospital, Genoa; [3]S. Gerardo Hospital, Monza; [4] S. Paolo Hospital, Milan; [5]University of Padoa, [6]Bambino Gesù Hospital, Roma, [7]University of Bari, [8]University of Florence

Aim of the current study was to evaluate the efficacy of ACE-inhibitors (ACE-inh) on GSDI related nephropathy. Ninety-five patients, 71 GSDIa and 24 GSDIb, median age 14.5 (range 0.16–42) were enrolled. A retrospective study was conducted in order to investigate the prevalence of hyperfiltration, microalbuminuria and proteinuria at different ages and to study, during a ten years follow-up, the evolution of these parameters in patients who started and in patients who did not start ACE-inh treatment. The prevalence of hyperfiltration, microalbuminuria and proteinuria at the latest follow-up was 30.1%, 30.2% and 28.8%, respectively. Retrospective study showed that hyperfiltration, microalbuminuria and proteinuria were detected at a median age of 11 (range 4–25), 11.1 (range 5–24) and 15 years (range 6–36), respectively. A significant and progressive reduction of hyperfiltration was detected in patients who started ACE-inh. A significant difference between patients who started and patients who did not start the treatment was detected only after 3 years of treatment. In conclusion the results of the present study show that ACE-inh have a significant effect on hyperfiltration but are not effective on reducing proteinuria in GSDI patients.

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RENAL FUNCTION IN GSD I: NATURAL COURSE AND POSSIBLE RENOPRESERVATIVE EFFECTS OF ACE INHIBITION

DHJ Martens[1], JP Rake[1], GJ Navis[2], FJ van Spronsen[1], CML van Dael[3], GPA Smit[1]
[1]Department Metabolic Diseases, [2]Department Nephrology, [3]Department Pediatrics Nephrology, University Hospital Groningen, The Netherlands

In adult GSD I patients renal failure is a major complication. In this study we analyzed the course of kidney function and possible renoprotective effects of angiotensin converting enzyme inhibition (ACEi) in 37 GSD I patients. 205 glomerular filtration rate (GFR) measurements, performed with iothalamate and corrected for body surface area, were studied retrospectively. 122/205 were measured during ACEi treatment. Presence of hyper- and hypofiltration was assessed, defined as >145 and <90 ml/min/L,73 m^2, respectively. In GSD I patients with vs without ACEi prevalence of hyperfiltration was: 0–5 yrs 7/10 (70%), 5–10 yrs 11/16 (69%) vs 4/5 (80%), 10–15 yrs 9/11 (82%) vs 6/11 (55%), 15–20 yrs 11/14 (79%) vs 7/16 (44%, $p = 0.056$), 20–30 yrs 1/6 (17%) vs 4/12 (33%). Albuminuria (>2.5 mg/mmol creatinine) occurred in 23/37 patients (62%), independent of ACEi. 19/23 were >10 yrs of age (83%). Two patients >30 yrs showed hypofiltration, both were on ACEi. Thus, hyperfiltration is common in GSD I, a peak occurs between age 10–20. By treatment with ACEi the prevalence of hyperfiltration at this age is lower. After age of 20, less hyperfiltration is observed and hypofiltration occurs in some patients. These data suggest a biphasic pattern of renal damage in GSD I, hyperfiltration preceding progressive renal damage, resembling the pattern in diabetic nephropathy. Possibly ACEi can postpone further decline of GFR. However, the studied group of patients is relatively young and hypofiltration was only seen in 2 older patients. It seems worthwile to study this further by means of prospective controlled studies.

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GLYCOGEN STORAGE DISEASE TYPE IA IN AN ADULT PRESENTING WITH GOUTY ARTHRITIS

FS Ezgü[1], L Tümer[1], B Dalgiç[1], M Gündüz[1], A Hasanoğlu[1], R Günaydin[2], YS Shin[3]
[1]Gazi University Faculty of Medicine, Department of Pediatrics, Ankara, Turkey; [2]SSK Tepecik Hospital, Department of Physical Therapy and Rehabilitation, Yzmir, Turkey; [3]Munich Children's Hospital, Department of Pediatric Metabolism, Munich, Germany

Glucose-6-phosphatase deficiency is a well-known cause of hyperuricemia. Acute gout as the presenting manifestation of glycogen storage disease type Ia has been reported in a few patients. A 26-year-old male patient with gouty arthritis was referred to our department to rule out an underlying inborn error. He had frequent attacks of hypoglycemia in his childhood attenuated after puberty. The liver was palpated 3 cm and he had multiple swellings on auricles, metacarpophalengeal and metatarsophalengeal joints consistent with tophi. Serum alanine aminotransferase was 58 IU, aspartate aminotransferase 48 IU, uric acid 22 mg/dl and blood lactic acid 5 mmol/L. Serum biotinidase activity was found to be elevated. Liver biopsy showed PAS (+) storage material as well as fibrosis. The enzymatic examination of liver biopsy specimen revealed a low activity of glucose-6-phosphatase. The patient was begun allopurinol, angiotensine converting enzyme inhibitor and a diet consisted of frequent meals including corn starch. The hypoglycemic attacks as well as the pain in the extremities has been significantly decreased. Acute gout in an adolescent with liver enlargement and high blood levels of uric acid and cholesterol should always alert a physician to rule out glycogen storage disease.

403-P

DECREASED LEVELS OF VON WILLEBRAND FACTOR ANTIGEN (vWF:Ag) IN PATIENTS WITH GLYCOGEN STORAGE DISEASE TYPE I

C Mühlhausen[1], R Schneppenheim[2], U Budde[3], K Ullrich[1], R Santer[1]
Departments of [1]Pediatrics and [2]Pediatric Hematology and Oncology, University Children's Hospital Hamburg, and [2]Professor Arndt and Partners Laboratory, Hamburg, Germany

Objective: Despite highly increased blood lipids, patients with glycogen storage disease type I (GSDI) do not develop premature vascular complications. In addition to protective antioxidative factors, this could be due to changes in coagulation factors. GSDI patients have long been shown to have an increased bleeding tendency. A prolonged bleeding time and a response to DDAVP has already been reported in the 1980s. We therefore characterized corpuscular and plasmatic coagulation factors in GSDI patients.

Methods: Coagulation tests (including vWF:Ag (Elisa), collagen binding activity, and multimer analysis) were performed in 11 GSDI patients, single cases of other GSD types, and both in healthy and hyperlipidemic controls.

Results: More than 50% of GSDI patients showed abnormal results for vWF:Ag with low normal or mildly reduced collagen binding activity, presence of all multimers and a normal triplet structure of single oligomers ('mild von Willebrand syndrome 1'). Similar results were found in other types of GSDs but not in hyperlipidemic controls arguing against a methodologic problem.

Conclusions: A secondary reduction of vWF:Ag can be found in a high proportion of GSD patients. The underlying metabolic mechanism and a potential role in the protection from vascular complication still needs to be evaluated.

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GENOTYPE-PHENOTYPE CORRELATION IN GLYCOGEN STORAGE DISEASE (GSD) TYPE Ib: A RETROSPECTIVE ITALIAN STUDY AND A REVIEW OF THE LITERATURE

Melis D[1], Fulceri R[2], Parenti G[1], Della Casa R[1] Majo F[1], Internicola M[1], Marcolongo P[2], Gatti R[3], Parini R[4], Riva E[5], Zammarchi E[6], Andria G[1], Benedetti A[2]
[1]Department of Pediatrics, Federico II University, Naples; [3] Gaslini Hospital, Genoa; [4]S. Gerardo Hospital, Monza; [5] S. Paolo Hospital, Milan; [6]University of Florence, [2]Department of Pathology, University of Siena

We studied the genotype/phenotype correlation in a cohort of GSD Ib patients. Twenty-six Italian patients, 13 females, age range: 4.3–28.4 yrs, mean:15 $\pm$ 6.9 yrs, were enrolled. All patients were characterized by molecular analysis of glucose 6-phosphate translocase gene. The presence of neutropenia and of systemic complications was correlated to the mutations detected in each patient and in the ones described in the literature. No mutation was detected in three patients. This could suggest that a second protein plays a role in the phosphate microsomal transport. This hypothesis is supported by the detection of phosphate transport across the membrane of microsomes isolated from livers of GSDIb patients. Combining our results with the data of the literature we observed that mutations were determined in 226 (93.3%) of 242 independent alleles. The 400X mutation was the most prevalent. No correlation was found between individual mutation and the presence of neutropenia and/or systemic complications. These results suggest that different genes and proteins modulate neutrophil differentiation, maturation and apoptosis. A mutation-mutation interaction can not be ruled out in compound heterozygotes.

405-P

RESTING ENERGY EXPENDITURE IN PATIENTS AFFECTED BY GLYCOGEN STORAGE DISEASE TYPE I AND III

Giacchero R, Bonza M, Paci S, Torcoletti M, Fiori L, Giovannini M
Department of Pediatrics, San Paolo Hospital, University of Milan, Italy

Introduction: Glycogen storage diseases (GSD) are inborn errors of glycogen metabolism. Final height in patients affected by GSD diseases is usually lower than expected with respect to the genetic target (height of mother + height of father/2 $\pm$ 6.5 cm). Aim of the study: To evaluate resting energy expenditure (REE), by means of indirect calorimetry, among our GSD patients. **Subjects**: Nine patients (5 M and 4 F), 6 affected by GSD type I and 3 by GSD type III, with mean age 19.5 years (range 15–24 years) and 9 healthy controls (mean age: 20.6 years) were enrolled in the study. Mean weight of our patients was 67.38 kg (range 54–103 kg) and mean height was 163 cm (range 144–180 cm). The mean Body Mass Index (BMI) was 25 kg/m^2 (range 23.3–31.8 kg/m^2). **Methods**: REE was measured by indirect calorimetry (DELTATRAC MD1 METABOLIC MONITOR; DATEX INSTRUMENTATION, Helsinky, Finland). Results were compared to those of healthy controls, as well as with data derived from application of the equation of Harris-Benedict. **Results**: basal metabolism, assessed by indirect calorimetry, resulted significantly higher (1696.67 $\pm$ 122.17 vs 1297.78 $\pm$ 64.60 kcal/24 h; p = 0.005) in patients affected by glycogen storage disease, when compared to healthy subjects. **Conclusions**: our data show an increased requirement of energy for basal metabolism in patients affected by GSD, which might explain reduced growth parameters in these patients.A ccorrect evaluation of REE in GSD is important for an adequate dietary management.

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SEVERE GLYCOGEN STORAGE DISEASE TYPE IIIa IN THREE SIBLINGS: LACK OF RESPONSE TO DIETARY INTERVENTIONS

S Mohamed, U Hendroff, M O'Regan, C O'Neill, EP Treacy
National Centre for Inherited Metabolic Disorders, Children's University Hospital, Dublin

Introduction: Deficiency of glycogen debranching enzyme deficiency (AGL) causes Glycogen Storage Disease (GSD) type III. In GSD IIIa the enzyme is deficient in both liver and muscle. The symptoms include varying degrees of hypoglycaemia, hepatomegaly and skeletal and cardiac myopathies. We present a family of three siblings with GSD IIIa with complete absence of AGL activity in leukocytes and severe liver and muscle involvement. The progression of their disease is not halted by aggressive dietary manipulations. **Objectives**: To assess varying dietary regimes on the clinical course of three siblings with GSD IIIa. **Methods**: We studied three siblings (a) male age 6 years, (b) female age 4 years and (c) female age 2 years. We reviewed retrospectively the clinical notes, laboratory results including blood creatine kinase (CK), AST and dietary interventions (over an 18 month period). **Results**: All three siblings manifested growth retardation, hepatomegaly, and cardiomyopathy. On a dietary regime of high protein (average 4.5 g/kg/d) with high CHO (average 15 g/kg/d), the median CK levels (*n*: 20–155 U/L) were in (a) 3845 U/L (*r*: 1585–7948), (b) 1903 U/L (*r*: 119–3651) and (c) 757 U/L (*r*: 457–1576). On a regime of increased protein (average 6.5 g/kg/d) and increased CHO (average 15.5 g/kg/d), the median CK levels were in (a) 3139 U/L (*r*: 1447–6463), (b) 2479 U/L (*r*: 636–4549) and (c) 494 U/L (*r*: 292–918). There was no significant difference in results with the different interventions. The 3 patients have significant hyperinsulinism.

Conclusions: This case series illustrates the persisting challenges of satisfactory treatment of severe presentations of GSD III.

407-P

BONE MINERAL DENSITY IN GLYCOGEN STORAGE DISEASE TYPE III

[1]Mundy HR, [2]Fewtrell M, [2]Williams J, [1]Lee PJ
[1]Charles Dent Metabolic Unit, National Hospital for Neurology and Neurosurgery; [2]Institute of Child Health, London, UK

Reduction in bone mineral density (BMD) is a well known complication of glycogen storage disease type I (GSD I) but little attention has been given to bone mineralization in GSD III. This study reports the results of BMD measurements (total body, lumbar spine, total hip) by dual energy X-ray absorptiometry, tibial and ulnar speed of sound ultrasonography (SOS), and urinary and serum bone turnover markers in patients with GSD III and their age and sex matched normal controls.

Results: 15 patients (9 male, 6 female) aged 11–31 years completed the study. Twelve had GSD IIIa (muscle and liver) and 3 had GSD IIIb (liver only). Patients had markedly reduced bone density compared to the control group ($p<0.05$). Patient median z-scores (interquartile range): whole body –1.5 (–2.5 to –0.6), lumbar spine –2.2 (–2.8 to –1.4) and total hip –1.1 (–1.7 to –1.1). Reduction in BMD was not explained by differences in height or weight between patient and control groups. GSD IIIa patients had significantly lower ($p<0.05$) BMD than GSDIIIb patients in all areas studied. Patients had normal SOS measurements for both radius and ulna.

Conclusions: Bone mineral density is markedly reduced in GSD IIIa patients. The severity is comparable to that found in GSD I. There may be a similar final common pathway for this reduction stemming from reduction in muscle strength found in the two disorders.

408-P

MUSCLE PHOSPHOFRUCTOKINSE DEFICIENCY WITH NEONATAL SEIZURES: REPORT OF A CASE

ZN Al-Hassnan[1], M Al Budhaim[2], B Lach[3], H Aldhalaan[2]
[1]Department of Medical Genetics, [2]Department of Neurosciences, [3]Department of Pathology and Laboratory Medicine, King Faisal Specialist Hospital and Research Centre, PO BOX 3354, Riyadh 11211, Saudi Arabia

Muscle phosphofructokiase deficiency (PFK) is known to cause childhood-onset exercise intolerance, muscle cramps, and myoglobinuria. Rarely, PFK deficiency presents in infancy with fatal congenital myopathy and arthrogryposis. Here we report a 20-month old boy who presented on the 3rd day of life with intractable seizures that required several anticonvulsants to control. Prenatal and delivery history was unremarkable. His parents were first cousins. Two sisters died in infancy with hypotonia, developmental delay, and seizures of unclear etiology. On follow up, he has had hypotonia and mild developmental delay. However, he continues to gain developmental milestones, and his seizures are now well controlled on carbamazepine. Investigations including serum electrolytes, CK, tandem MS, urine GC/MS, lactate, VLCFA, phytanic acid, biotinidase level, CSF analysis for cells, glucose, protein, and amino acids, were all normal. Brain MRI at 5 months of age showed uncharacteristic hypomyelination. Brain MRS was nonspecific. Muscle biopsy showed fibers with subsacrolemmal vacuoles containing PAS positive glycogen. Histochemistry did not reveal myophosphorylase deficiency. Excessive subsacrolemmal accumulation of free glycogen was noted in EM exam. Phosphorylase kinase activity was normal. Muscle PFK was deficient with a level of 0.2 unit (N: 25.12 ± 10.3 U) confirming the diagnosis of glycogen storage disease (GSD) type VII. This case expands the phenotype of muscle PFK deficiency to include early onset neonatal seizures. It is also unique in its relatively milder course. We suggest considering GSD VII in the differential diagnosis of neonatal seizures.

409-P

NATURAL HISTORY AND TREATMENT OF FRUCTOSE 1,6-DIPHOSPHATASE DEFICIENCY IN THE NETHERLANDS

G Visser[1], HD Bakker[2], JBC de Klerk[3], JAM Smeitink[4], GPA Smit[5], FA Wijburg[2]
[1]Utrecht, [2]Amsterdam, [3]Rotterdam, [4]Nijmegen, [5]Groningen, The Netherlands

A retrospective case study was performed to study the natural history and treatment of patients with fructose 1,6-diphosphatase deficiency (FDPD). Patients were identified from hospital records of 6 metabolic centres in the Netherlands. All known patients in the participating centres born after 1980 were included. 14 Patients were identified, 4 ♂ 10 ♀, aged 0.5–15 yr (median 7 yr) of whom 2 are deceased and 1 is mentally retarded. Diagnosis was confirmed by enzyme assay in leukocytes in 13 patients and in liver in 1 patient. The estimated incidence of FDPD in the Netherlands is 1:350 000. Almost 50% of the patients present in the neonatal period, the remaining patients present before the age of 4 year. Two patients presented with convulsions, 2 with icterus, 8 with sepsis like presentation and 2 were sibs. Most commonly found are hypoglycaemia, lactate acidosis, and hyper ketosis, but not obligate. Not reported before are the facts that half of the patients have hepatomegaly during acute episodes and also raised liver enzymes. At present dietary advises show great diversity, with no clear beneficial effect of one diet. The fact that 2 patients died during an acute episode, stresses the need for an adequate emergency diet. Tolerance to fasting and dietary fructose seems to increase with aging, although great differences in the course of FDPD are reported. In older patients, obesity is a point of concern. Of the 4 patients aged >11 year, 3 show a weight-to-length ratio $>>+2$ SD.

410-O

NEW INHERITED DEFECTS IN THE PENTOSE PHOSPHATE PATHWAY

NM Verhoeven[1], JHJ Huck[1,2], B Roos[1], EA Struys[1], GS Salomons[1], MS van der Knaap[2], C Jakobs[1]

Departments of [1]Clinical Chemistry and [2]Child Neurology, VU University Medical Center, Amsterdam, The Netherlands

In recent years, we investigated abnormalities in urinary polyol profiles, which led to the discovery of two new inborn errors in the pentose phosphate pathway: transaldolase deficiency and ribose-5-phosphate isomerase deficiency.

Transaldolase deficiency was found in two patients. The first patient had liver problems in the neonatal period. Her intellectual development has been normal. She is now 14 years old and suffers from liver cirrhosis. The second patient was a neonate who died from severe hepatopathy and cardiomyopathy at the age of 18 days. Both patients presented with elevated concentrations of arabitol, ribitol and erythritol in urine. Transaldolase enzyme activity was undetectable. In the 2 patients, different mutations were found in the gene encoding transaldolase.

The second defect in the pentose phosphate pathway was a deficiency of ribose-5-phosphate isomerase. This defect, unlike transaldolase deficiency, presents with neurological problems. The affected boy, now 20 years of age, has been suffering from progressive leucoencephalopathy. In brain, arabitol and ribitol were elevated, as demonstrated by proton MRS. Also in urine, plasma and CSF, elevations of these polyols were found, with a strong brain:CSF:plasma gradient. Ribose-5-phosphate isomerase was deficient in lymphoblasts from the patient. Two pathogenic mutations in the RPI gene were found.

The discovery of two defects in pentose and polyol metabolism indicates that a new area of inborn errors has been encountered.

411-P

COMBINED ENZYME ASSAY FOR THE DETECTION OF DEFECTS IN THE PENTOSE PHOSPHATE PATHWAY

MMC Wamelink[1], JHJ Huck[1], B Roos[1], DEC Smith[1], EA Struys[1], MS van der Knaap[2], NM Verhoeven[1], C Jakobs[1]

Metabolic Unit, Departments of Clinical Chemistry[1] and Child Neurology[2], VU University Medical Center, Amsterdam, The Netherlands

Recently, we described two new inborn errors in the pentose phosphate pathway (PPP): transaldolase deficiency and ribose-5-phosphate isomerase deficiency. In both defects, urinary polyol abnormalities were observed. In the past years, we have been screening patients for potential defects in pentose/polyol metabolism, which led to the finding of additional patients with abnormalities in urinary polyol profiles. In order to investigate possible enzyme deficiencies in the PPP in these patients, we developed a method to analyse the reversible part of the pathway.

We incubated homogenates of fibroblasts or lymphoblasts with 6-phosphogluconate or ribose-5-phosphate for 2 hr at 37°C and 50 µl samples were taken at 0, 30 and 120 min. After adding $^{13}C_6$-glucose-6-phosphate as internal standard the incubation was terminated by adding perchloric acid. We measured the following formed sugar-phosphates: glyceraldehyde-3-phosphate, erythrose-4-phosphate, dihydroxyaceton-phosphate, ribose-5-phosphate, ribulose-5-phosphate, xylulose-5-phosphate, fructose-6-phosphate, glucose-6-phosphate and sedoheptulose-7-phosphate by LC-MS/MS.

Our method enables simultaneous analysis of the enzymes 6-phosphogluconate dehydrogenase, ribose-5-phosphate isomerase, ribose-5-phosphate epimerase, transaldolase, transketolase, triose-phosphate isomerase and glucose phosphate isomerase. The applicability of these methods have been demonstrated for transaldolase deficiency and ribose-5-phosphate isomerase deficiency.

412-P

GLYCEROL KINASE DEFICENCY – CLINICAL SYMPTOMS WHEN GLYCEROL IS AN IMPORTANT GLUCONEOGENETIC SUBSTRATE

Hellerud C, Lindstedt S
Department of Clinical Chemistry and Transfusion Medicine, Sahlgrenska University Hospital, Göteborg University, Gothenburg, Sweden

Glycerol kinase deficiency (GKD, MIM 307030) is an X-linked recessive inborn error of metabolism occurring isolated or in an Xp contiguous gene syndrome with adrenal hypoplasia and/or Duchenne muscular dystrophy. Children with isolated GKD have hypoglycaemic symptoms with or without low blood glucose concentration and pronounced ketonemia in conjunction with infections or after physical exercise. Discrepancy between severity of symptoms and concurrent infection is of diagnostic value. Adult individuals with GKD have no symptoms. The first individual (a 10-year-old boy) with symptoms from isolated GKD was described in 1983. We followed him and a similar case into adulthood (Acta Paediatr. 2004, In press). Controlled fasting provocations and exercise tests were performed in childhood and repeated at adult age. We suggest that the greater importance of glycerol as a gluconeogenetic substrate in children than in adults, explains the episodes of hypoglycaemic symptoms in the young GKD patient. With frequent carbohydrate-rich meals, carbohydrates with a low glycemic index, food or glucose when symptoms arise, extra food before and after physical activity, the prognosis is good. It is important to identify individuals at risk. Our studies have shown the need for a genetic analysis to distinguish between carriers and non-carriers, as the level of glycerol in plasma and urine or activity of GK can not discriminate. We have established methods to identify GKD as part of the Xp contiguous gene syndrome and an mRNA analysis enabling us to identify the genetic aberration.

413-A

GENOTYPE/PHENOTYPE CORRELATION IN HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA: AN UNUSUAL NEONATAL PRESENTATION

Cvitanovic-Sojat L, Jurcic Z, Gjergja R, Sojat T
Department of Pediatrics, UH "Sestre milosrdnice" Zagreb, Croatia

Objective: Aim of the study is to evaluate the clinical expression and to investigate the genetic basis of the familial hypercholesterolemia (FH), a lipid inherited disorder predisposing to coronary heart disease. **Patients**: Proband was born by cesarean section because of abnormal fetal presentation, had a normal psychomotor development and was admitted to the hospital at the age of 11.5 years because of learning problems and syncope. His mother was diagnosed to have FH at the age of 11 years and is taking statins irregularly. Mother's father died at the young age of heart failure and his 2 brothers and sister from stroke. **Results**: Proband's MRI of the brain showed an old infarct of left middle cerebral artery. EEG, fundi of eyes and evoked potentials were normal. Echocardiography showed thickness of the interventricular septum. Laboratory tests: cholesterol 11.4 and LDL-C 9.4 mmol/L, ApoB 219 and Lp(a) 60 mg/dl, Apo E genotype is e3/3. Endocrinological disorders, renal or liver diseases were excluded. Proband's brother, sister and mother are ApoE heterozygous e3/4, associated with FH in mother and sister. **Conclusion**: This family suggest that FH onset could be present since neonatal age and the selective screening is necessary in affected families. In the same family with FH different ApoE phenotypes are observed. The ApoE e3 allele is a more frequent in FH patients, it remains to proof his correlation with stroke. Appropriate diet and statins during childhood might be important for normalizing risk factors for atherosclerosis in children with FH.

414-O

SOURCES OF FETAL CHOLESTEROL AND DESMOSTEROL

GS Tint[1], Hongwei Yu[2], Guorong Xu[1], Shailish Patel[2]
[1]*VA Medical Center, East Orange, NJ and UMDNJ-NJ Medical School, Newark, NJ;* [2]*Medical University of South Carolina, Charleston, SC, USA*

To determine the sources of fetal sterols we measured sterol concentrations by GC/MS in liver, lung and brain from wild type and *Dhcr7*$^{-/-}$ mice. *Dhcr7*$^{-/-}$ mice are unable to convert 7-dehydrocholesterol to cholesterol (Ch) so that all Ch found in a *Dhcr7*$^{-/-}$ fetus must have come from its mother while virtually all of the accumulated 7DHC had to have been synthesized locally by the fetus. In controls, liver and lung total sterol concentrations were little changed from ED 11.5 onward except that lung seemed to add a burst of Ch immediately before birth. In contrast, in brain sterol levels increased steadily from 1.8 ± 0.4 mg/g in the ED 11.5 fetus to 4.4 ± 0.5 mg/g in neonates. We found that 32°110% of sterols in the 13.5 day-old fetal liver were synthesized by the fetus and that this fraction had increased to $61 \pm 2\%$ at birth. Data for lung was quite similar to that from liver. The brains of these animals synthesized $48 \pm 13\%$, $71 \pm 7\%$ and $87 \pm 3\%$, respectively, of their own sterols at ED 11.5, ED 13.5 and ED 21.5 (birth) demonstrating very early formation of the blood-brain barrier. In both *Dhcr7*$^{-/-}$ and +/+ mice Δ24 unsaturated sterols accounted for 2–5% of total sterols in the ED 11.5 brain, increased to 16–20% of total sterols in the newborn but were only 0.1–0.2% of liver and lung total sterols. This result implies that Dhcr24 activity should be reduced in brain. Using real time semi-quantitative RT-PCR we, in fact, found *Dhcr24* expression in fetal brain to be about half of that in liver. Because *Rest* expression is reported to be markedly elevated in the fetal mouse brain, we hypothesize that reduced *Dhcr24* transcription in brain is a result of the suppressive effect of Rest binding to one or more of the 3 occurrences of the neuron-restrictive silencer element (NSRE) in the *Dhcr24* promoter.

415-P

DNA ANALYSIS OF PATIENTS WITH SMITH-LEMLI-OPITZ SYNDROME FROM THE CZECH REPUBLIC AND SLOVAKIA

[1]L Kozak, [1]I Drapalova, [2]D Prochazkova, [3]V Juttnerova, [4]V Bzdúch, [5]J Zeman
[1]*Center of Molecular Biology and Gene Therapy,* [2]*1st Paediatrics Clinic, University Hospital Brno, Czech Republic;* [3]*Department of Medical Genetics, University Hospital Hradec Králové;* [4]*Department of Paediatrics, University Hospital Bratislava;* [5]*Department of Paediatrics, 1st Faculty of Medicine, Prague*

Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive metabolic disorder caused by mutations in the DHCR7 gene. The incidence of SLOS is estimated around 1:10 000 to 1:40 000 in our population. A rapid PCR/RFLP technique and sequencing was used to detect mutations in the DHCR7 gene. Thirty families with at least one clinically and biochemically characterized patient were examined. Fifty-nine from 60 mutant alleles were characterized (one unknown mutant allele comes from one unavailable parent). In total, 10 various mutations were found. Three most frequent mutations W151X (23/60 alleles), V326L (18/60 alleles) and IVS8-1G>C (5/60 alleles) account for 76.7% of all mutant alleles. The other mutations were less frequent. From genotype-phenotype correlation follows that the severe phenotype is associated with homozygosity or compound heterozygosity for the mutations W151X and IVS8-1G>C. These patients usually die over the next few days after birth. The mildest phenotype is usually associated with homozygosity for the mutation V326L. In addition, we detected two fetuses with SLOS via DNA analysis of amniocytes in previously unaffected families. They were suspected from the prenatal screening of pregnant women for the Down's syndrome in second trimester (abnormal level of human chorionic gonadotropin (hCG)).

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416-P

IN VITRO ^{14}C ACETATE LOADING OF HUMAN FIBROBLASTS AS A SENSITIVE METHOD TO IDENTIFY SMITH-LEMLI-OPITZ PATIENTS WITH A MILD BIOCHEMICAL PHENOTYPE

D Haas[1], J Morgenthaler[1], L Neumann[2], S Armbrust[3], J Zschocke[4], J Okun[1], G Hoffmann[1]
[1]University Children's Hospital, Heidelberg [2]Institute of Human Genetics, Charité, Humboldt University, Berlin, [3]University Children's Hospital, Greifswald, [4]Institute of Human Genetics, University Heidelberg, Germany

Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive malformation syndrome caused by a deficiency of 7-dehydrocholesterol reductase (DHCR7). The resulting accumulation of 7- and 8-dehydrocholesterol (7- and 8-DHC) is usually identified by gas chromatography mass spectrometry (GCMS) analysis of plasma sterols. However, mild forms may be left undiagnosed. Even GCMS analysis of fibroblasts cultured in lipid-depleted medium, which is generally accepted as the method of choice for further investigation of borderline plasma results may fail to reveal the biochemical defect (Am J Med Genet. 2003;122A:24–9). We describe an *in vitro* loading test which clearly identifies the abnormal metabolites even in a biochemical mild variant. Fibroblasts are cultivated in lipid-depleted medium for 24 h and incubated with ^{14}C acetate. Sterols are separated on a silver nitrate TLC plate and quantified by phosphoimaging. In a girl with classical features of SLOS and compound heterozygosity for the known mutation W151X and a novel mutation I178F in the *DHCR7* gene GCMS analysis of plasma sterols showed a 7-DHC level in the upper normal range and only a slight elevation of 8-DHC. The ^{14}C acetate loading test of fibroblasts revealed a distinct elevation of 7-DHC and reduced cholesterol compared to controls.

417-O

X-LINKED DOMINANT CONRADI-HUNERMANN SYNDROME DUE TO SINGLE GENE MOCAICISM IN A MALE PATIENT

HR Waterham[1,2], J Koster[1,2], MCE Jansweijer[1], JH Sillevis Smitt[3], RJA Wanders[1,2], M Duran[2], RCM Hennekam[1]
Departments of [1]Pediatrics, [2]Clinical Chemistry, [3]Dermatology, Academic Medical Center, Amsterdam, The Netherlands

Conradi-Hünermann or Happle syndrome, also known as X-linked dominant chondrodysplasia punctata 2 (CDPX2; MIM 302960), is characterized by a bilateral, asymmetric expression of various skeletal and skin abnormalities, including chondrodysplasia punctata (epiphysic stippling), shortening of long bones and ichtyosis. The disorder is caused by a deficiency of the sterol Δ^8-Δ^7 isomerase due to mutations in the *EBP* gene on chromosome Xp11.22-23. As a consequence of this enzyme deficiency, patients have elevated plasma levels of cholesta-8(9)-en-3β-ol but usually normal levels of cholesterol. Since the disorder is recognized almost exclusively in females it has been assumed to be lethal in males. We report a 24-old male patient initially diagnosed with CDPX2 on the basis of his clinical presentation. The patient is severely retarded, both mentally and developmentally, and has a variety of skeletal (polydactyly, dwarfism, scoliosis etc.) and skin abnormalities. Sterol analysis in plasma and cultured skin fibroblasts revealed elevated levels of cholesta-8(9)-en-3β-ol. Surprisingly, mutation analysis of the *EBP* gene revealed apparent heterozygosity for a 429delG mutation although the patient's karyotype was 46,XY. The different ratios of mutated versus wild-type allele in lymphocyte and fibroblast DNA indicate that the patient is mosaic for a single mutation in the *EBP* gene.

418-P

METHOD FOR THE DETERMINATION OF GUANIDINOACETATE METHYLTRANSFERASE ACTIVITY BY ESI/MS-MS

Cl Carducci, Ca Carducci, C Artiola, S Santagata, L Ellul, V Leuzzi, I Antonozzi
University of Rome 'La Sapienza' – Italy

Guanidinoactetate methyltransferase (GAMT) catalyses the biosynthesis of creatine (Cr) by the transfer of a methyl group from S-adenosylmethionine to guanidinoacetate (GAA). GAMT deficiency is a recently discovered inborn error of creatine metabolism characterized by depletion of Cr in brain and accumulation of GAA in physiological fluids. The determination of GAMT activity is an important step for the diagnosis confirmation. We developed a sensitive and specific method to assay GAMT activity in human lymphoblasts by FIA/ESI/MS-MS. The lysed cells were incubated with substrates (without S-adenosylmethionine for the blank), filtered, diluted with methanol/H_2O solution containing d_3-creatine and Cr concentration was assayed by SRM after the formation of butyl esters. The amount of produced Cr was calculated by subtraction of the amount of Cr found in the blank. The method was applied to the determination of GAMT activity in samples obtained by 5 normal subjects and 2 patients affected by GAMT deficiency. The range of GAMT activity in controls was 0.13–0.42 nmol creatine/h/mg. Patients enzyme activity was not detectable, as denoted by the lack of any difference in Cr concentration between blank and lysed cells incubated with substrates (patient 1: 6.74 ± 0.10 vs 6.77 ± 0.10 μmol/L; patient 2: 3.44 ± 0.02 vs 3.45 ± 0.04 μmol/L; number of determinations for each sample: 4). The FIA/ESI/MS-MS method is fast (analysis time: 1.5 min), reliable, sensitive and precise and it does not require the use of radioactive compounds.

419-P

CREATINE AND GUANIDINOACETATE: DIAGNOSTIC MARKERS FOR INBORN ERRORS IN CREATINE BIOSYNTHESIS AND TRANSPORT

L Almeida[1], NM Verhoeven[1], B Roos[1], C Valongo[2], ML Cardoso[2], L Vilarinho[2], GS Salomons[1], C Jakobs[1]
[1]*Department of Clinical Chemistry, Metabolic Unit, VUMC, Amsterdam, The Netherlands;* [2]*Unidade de Biologia Clínica, IGM, Porto, Portugal*

Primary creatine deficiency syndromes are a new group of disorders caused by defects in biosynthesis (AGAT deficiency, MIM 602360, and GAMT deficiency, MIM 601240) or transport of creatine (SLC6A8 deficiency, MIM 300036). Creatine (Cr) and guanidinoacetate (GAA) levels in plasma, urine and CSF are informative for biochemical diagnosis of GAMT (and possibly also AGAT) deficiency. The diagnostic approach for SLC6A8 deficiency is still not clear, however increased levels of urinary Cr have been reported. Several analytical techniques have been described for the determination of both GAA and Cr in relatively small control populations. However, no extensive studies for the determination of reference values have been performed.

In this study we determined the reference values of GAA and Cr in body fluids in a Dutch control population ($n = 225$) of age 0 to 90 years by stable isotope dilution GC-MS. Both compounds were analyzed in a single analysis. Random urine was used since variation of the compounds during the day was not significant. Furthermore, 8 GAMT-deficient patients and 8 SLC6A8-deficient patients were investigated. Reference values were age-dependent and no differences with gender were observed. Increased levels of GAA are present in urine, plasma and CSF of GAMT-deficient patients. The SLC6A8-deficient patients all show increased Cr/Crn ratio in urine demonstrating the importance of the Cr/Crn ratio as a reliable marker of the SLC6A8 deficiency.

420-P

FIVE NEW PORTUGUESE PATIENTS WITH GUANIDINOACETATE METHYLTRANSFERASE DEFICIENCY: BIOCHEMICAL, ENZYMATIC AND MOLECULAR DATA

Vilarinho L[1], Valongo C[1], Cardoso ML[1], Quelhas D[1], Verhoeven NM[2], Almeida LA[2], Garcia P[3], Diogo L[3], Salomons GS[2], Jakobs C[2]
[1]In Instituto de Genética Médica, Porto, [2]VU Medical Centre Amsterdam, [3]Hospital Pediátrico Coimbra

Guanidinoacetate methyltransferase (GAMT) deficiency (McKusick 601240) is an autosomal recessive disease of creatine biosynthesis. We present clinical, biochemical and molecular findings of five Portuguese patients (3 apparently unrelated families) not yet reported.

Patients	1	2	3	4	5
Diagnosis age	19 years	21 years	20 years	16 years	15 years
Clinical data	Epilepsy, psychomotor delay; myopathy	Epilepsy, psychomotor delay, myopathy	Epilepsy, autism psychomotor delay, myopathy	Epilepsy, autism psychomotor and speech delay	Speech and developmental delay, myopathy
Biochemical analysis	GAA - 546 Creatine - 462	GAA - 423 Creatine - 337	GAA - 406 Creatine - 366	GAA - 827 Creatine - 456	GAA - 1230 Creatine - 78
GAMT activity	5.6	ND	ND	5.2	UI
Molecular study	c.59G>C	c.59G>C	c.59G>C	c.59G>C	c.59G>C

Reference values: GAA 18–130 μmol/mmol creatinine, Creatine 142–5952 μmol/L
GAMT 60–243 pmol/h*mg protein. ND – not detectable, UI – under investigation

DNA sequence analysis revealed a missense mutation c.59G>C; p.W20S in exon 1 of the GAMT gene. This mutation has, so far, been exclusively identified in portuguese patients.

421-P

A STUDY OF AGAT AND GAMT GENE POLYMORPHISMS IN ITALIAN POPULATION

Ca Carducci, R Battini, C Artiola, T Giovanniello, A Voli, V Leuzzi, I Antonozzi
Università degli studi di Roma, 'La Sapienza' and IRCCS 'Stella Maris' Pisa, Italy

We previously reported a study of the large pedigree of the unique family with AGAT (arg-gly amidinotransferase) deficiency until now reported. When the probands' and relative's study of GAMT (gunidinoacetate methyltransferase) and AGAT genes was performed (Battini et al. Mol Genet Metab. 2002) we identified two missense mutations that did not segregate with the disease. One was located on exon 6 of GAMT gene (T209M) and the other on exon 3 of AGAT gene (Q110H). In order to elucidate the role of these missense mutations in the phenotypic expression of the disease, we performed a study of 120 alleles to evaluate the presence of AGAT and GAMT gene polymorphisms (variations respect wild type sequence >1%) in normal population. For the study we used anonymous neonatal screening dried blood spots coming from neonatal screening laboratories of center-southern and northern Italy. We used an exon scanning method (DGGE) (including also the exon-intron boundaries) and confirmed the sequence variations by direct sequencing analysis. We identified 3 sequence variations in AGAT gene: Q110H (g.8977A>T), with an allelic frequency of 26%, L418L (g.16336C>T), with an allelic frequency of 46%, IVS-27G>A (g.12220G>A) with an allelic frequency of 0.83%. In GAMT gene the frequency of sequence variation T209M (g.4024 C>T) turned out to be 8.3%. Taking into consideration the allele frequencies, the sequence variations in GAMT (T209M) and in AGAT (Q110H) gene observed in AGAT family, should be considered polymorphisms.

422-P

AGAT DEFICIENCY: IS THERE A SPECIFIC BIOCHEMICAL PATTERN OF THIS DISORDER?

MG Alessandri[1], L Celati[1], R Battini[1], G Cioni[1,2]
[1]*Department of Developmental Neuroscience, IRCCS Stella Maris,* [2]*Division of Child Neurology and Psychiatry, University of Pisa (Italy)*

AGAT (arginine: glycine amidinotransferase) deficiency is a disorder of creatine (Cr) metabolism, due to the absent activity of this enzyme that catalyses the first reaction in Cr biosynthesis. The product of this reaction is guanidinoacetic acid (GAA), that is subsequently methylated by guanidinoacetate-methyltransferase (GAMT) to form Cr. The impaired activity of AGAT brings to reduced or absent levels of GAA and Cr in cellular and extracellular compartments and creatinine (Crn) excretion. Low Crn-Cr and high GAA levels in plasma, urine and CSF are considered useful markers to screen GAMT disorder, while in AGAT deficiency are still some controversial data on the role of biochemical assays. It is probably due to the few patients until now discovered and to the high sensitivity of analytical methods required to correctly measure the very low levels of Cr and GAA in body fluids.

To point out the value of biochemical investigations to identify AGAT deficiency we have: (1) developed two analytical techniques with HPLC/UV and GC/MS to carefully measure GAA and Cr; and (2) analyzed plasma and urine samples of our three AGAT patients, their relatives and a newborn from the same Italian family. Our results show specific biochemical patterns of GAA and Cr in body fluids of homozygous and heterozygous subjects, and indicate that these simple chromatographic techniques could be powerful tools to recognize AGAT patients in screening programs.

423-O

AGAT DEFICIENCY IN A NEWBORN: A CHALLENGE TO PREVENT THE CLINICAL SYMPTOMS

R Battini[1], MG Alessandri[1], V Leuzzi[3], F Moro[1], M Tosetti[1], MC Bianchi[4], G Cioni [1,2]
[1]*Department of Developmental Neuroscience, IRCCS Stella Maris,* [2]*Division of Child Neurology and Psychiatry, University of Pisa,* [3]*Department of Child Neurology and Psychiatry, University La Sapienza, Rome,* [4]*Neurorad Unit, S. Chiara Hospital, Pisa (Italy)*

Arginine: glycine amidinotransferase (AGAT) deficiency is an autosomal recessive inborn error of creatine (Cr) biosynthesis, characterized by mental retardation, severe language impairment and behavioral disorders. We describe a male neonate, coming from the same Italian family as the two index cases (Bianchi et al. 2000), who was suspected as having AGAT deficiency at birth on the base of biochemical assessment of Cr and guanidinoacetate (GAA) concentrations in plasma (p) and urine (u), which were all extremely low (pCr: 16.22 μmol/L; uCr: 24.58 μmol/L; pGAA: 0.13 μmol/L; uGAA: 0.54 μmol/L). The diagnosis was confirmed by genetic analysis, that disclosed the same homozygous mutation at nt position 9093 converting a tryptophan (TGG) to a stop codon (TAG) at residue 149 (W149X), already reported in its affected relatives (Battini et al. 2002). At the age of three weeks brain magnetic resonance imaging was normal while Proton Spectroscopy (^{1}H-MRS) showed the almost complete absence of cerebral Cr peak at 3.05 ppm. In order to prevent the clinical consequences of brain depletion, we tried to supply Cr to the newborn throughout breastfeeding, providing the mother with oral Cr monohydrate supplementation (3–9 g/day) for three months. Although the increase of Cr levels in the breast milk, no modification of brain Cr was shown in the patients at the 3 month ^{1}H-MRS examination. At the last check (4 months of age) his growth and psychomotor development were normal. With the weaning, we started to supplement the diet of the child with Cr monohydrate.

424-A

AGAT ACTIVITY ASSAY IN HUMAN CELLS BY GC/MS: A NEW METHOD WITH UNLABELED SUBSTRATES

MG. Alessandri[1], L Celati[1], R Battini[1], G Cioni[1,2]
[1]*Department of Developmental Neuroscience, IRCCS Stella Maris,* [2]*Division of Child Neurology and Psychiatry, University of Pisa (Italy)*

AGAT (L-arginine-glycine amidinotransferase) catalyses the first reaction in creatine (Cr) biosynthesis, transferring an amidino group from arginine to glycine to form guanidoacetic acid (GAA), that is then methylated by GAMT to yield Cr. Only two diagnostic enzymatic assays have been reported until now to measure AGAT activity in human peripheral blood cells, both using radioactive substrates, due to the low activity of the enzyme in these cells [1, 2]. We describe a new method for AGAT activity assay in lymphocytes and lymphoblasts with unlabeled substrates using a GC coupled with a quadrupole detector. Briefly the cells have been incubated in a reaction mixture containing glycine, as substrate, and arginine, as amidino donor, at pH 7.5. The reaction was stopped by PCA 1 N after 4 hours of incubation at 37°C. The end-product of the reaction (GAA) was separated and quantified with an Agilent GC/MS, set in EI-SIM mode. In the control samples AGAT activity ranged 0.243–0.425 and 0.950–1.470 nmol/mg/h for lymphocytes and lymphoblasts, respectively. Enzyme activity in AGAT patients was below the detection limit in both cell types; in heterozygous subjects it was lower than in controls but still well detectable. Our data are in agreement with previous results obtained by using more expensive and sophisticated techniques. In conclusion, we have developed a new simple and fast method to measure AGAT activity in cells, easily reproducible in laboratories not equipped for radioactive assays.

[1] Item et al. Am J Hum Genet. 2001;69:1127
[2] Verhoeven et al. Clin Chem. 2003;49:803

425-O

GUANIDINOACETATE AND CREATINE/CREATININE LEVELS IN CONTROLS AND PATIENTS WITH UREA CYCLE DEFECTS

A Arias[1,2], J García-Villoria[1], A Ribes[1]
[1]*Instituto de Bioquímica Clínica, Corporació Sanitària Clinic, Barcelona, Spain* [2]*Centro de Desarrollo Infantil, MECD, Mérida, Venezuela*

We have established an analytical methodology for the evaluation of creatine metabolism that was based on the method of Hunneman et al 1997 and Struys et al 1998. Briefly, 100 µl of urine or plasma was mixed with of $NaHCO_3$, hexafluoroacetylacetamide and toluene 50:50:600 µl, 80°C 2 h. The sample was derivatized with 150 µl of BSTFA, 60°C 30 min; d_3-creatine and $^{13}C_2$-guanidinoacetate were used as internal standards. Quantitative analysis was performed in a GC/MS in the SIM mode. Guanidinoacetate concentration and creatine/creatinine ratio in controls decrease as age increases, but no significant differences were found in plasma. Because arginine is a substrate for creatine synthesis and because of creatine has a protective effect on axonal development, we decided to investigate creatine metabolism in patients with urea cycle defects, as one consequence of these defects (except for arginase deficiency) is a decreased level of arginine. We studied 15 patients and found that before treatment GAA concentration both in urine and plasma was very low, mean: 2 mmol/mol creat, range: 0.5–9, controls: 73 (21–124) and mean: 0.77 µmol/L, range: 0.03–0.87, controls: 1.7 (0.7–2.5), while after arginine or citrulline substitution GAA normalised. These results suggest that the GAA concentration is a parameter to consider in the follow-up of patients with urea cycle defects and arginine should be supplemented in sufficient amounts, as the brain seems to be impermeable to creatine influx, but not to its precursor, arginine.

426-P

CREATINE AND HHH SYNDROME

S Boenzi, C Rizzo, F Deodato, FM Santorelli, C Dionisi-Vici
Ospedale Pediatrico Bambino Gesù, Roma

Ornithine δ-aminotransferase deficiency (OAT), hyperornithinemia with gyrate atrophy of the choroid and retina, causes secondary creatine (CR) deficiency. In order to evaluate if other forms of hyperornithinemia could cause CR deficiency, we measured by LC-MS-MS plasma CR and guanidinoacetate (GAA) concentrations in 4 patients with HHH syndrome, a recessive inherited defect of mitochondrial ornithine transport caused by mutations of ORNT1 gene.

In one case, we observed marked reduction of CR levels (12.5 ± 0.9 μmol/L, controls 23–94), increased G (4.7 ± 0.6 μmol/L, controls 0.3–3.0), and elevated GAA/CR ratio (0.402 ± 0.02, controls <0.10), that normalise under CR supplementation (3 g/day). Clinically, the patient reported improvement of motor abilities and exercise intolerance with CR treatment. The 3 remaining patients showed normal CR and GAA levels.

Although limited to a single observation, these results seems to indicate the possible interaction between the pathway synthesis and ornithine metabolism. Differing from OAT, were CR deficiency results from inhibition of the mitochondrial membrane enzyme amidinotrasferase, based on our results showing reduced CR and elevated GAA, in HHH syndrome it could be hypothesised a selective inhibition of guanidinacetate methyltransferase by accumulated cytosolic ornithine.

427-O

HIGH PREVALENCE OF SLC6A8 DEFICIENCY, A NOVEL X-LINKED MENTAL RETARDATION (XLMR) SYNDROME

EH Rosenberg[1], LS Almeida[1], T Kleefstra[3], RS deGrauw[1], HG Yntema[3], N Bahi[3], C Moraine[3], HH Ropers[3], JP Fryns[3], TJ DeGrauw[2], C Jakobs[1], GS Salomons[1]
[1]Department of Clinical Chemistry, VU University Medical Ccenter, Amsterdam, [2]Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA, [3]European XLMR Consortium

Recently, a novel XLMR syndrome due to creatine deficiency in the brain, SLC6A8 deficiency (MIM 300036) was identified. The clinical presentation of affected males is MR, expressive speech and language delay, epilepsy, and autistic behaviour. In approximately 50% of the female carriers, learning disabilities of varying degrees have been noted. Affected males have a reduction of the creatine signal in the proton magnetic resonance spectroscopy (H-MRS) of brain, increased creatine/creatinine (Cr/Crn) excretion in urine and have impaired Cr-uptake in cultured fibroblasts.We have studied the prevalence of SLC6A8 mutations in a panel of 288 nonsyndromic X-linked mental retardation male patients archived by the European XLMR consortium. The full-length ORF and splice sites of the SLC6A8 gene were investigated by DNA sequence analysis. Four missense mutations, 1 single amino acid deletion and 1 nonsense mutation were identified in a total of 288 XLMR patients, showing a prevalence of at least 2.1%. Our data indicate that the frequency of SLC6A8 mutations in the XLMR population is close to that of CGG expansions in FMR1 responsible for the fragile X syndrome.

SLC6A8 sequence analysis, Cr/Crn measurement in urine and H-MRS of brain appear all to be valuable diagnostic tests for the evaluation of SLC6A8 deficiency, and should be considered for all males with mental retardation of unknown cause.

428-O

MAGNETIC RESONANCE SPECTROSCOPY IN THE VALIDATION OF A MOUSE MODEL OF GUANIDINOACETATE METHYLTRANSFERASE DEFICIENCY

HE Kan[1], WK Renema[1], D Isbrandt[2], A Heerschap[1]
[1]*Department of Radiology, University Medical Center Nijmegen, the Netherlands;* [2]*Center for Molecular Neurobiology, University of Hamburg, Germany*

Introduction: In the past decade, several patients suffering from guanidinoacetate methyltransferase (GAMT) deficiency and therefore lacking creatine (Cr) were described. Magnetic resonance spectroscopy (MRS) has played a decisive role in the diagnosis and the elucidation of the pathophysiology of this disease. In the present study, GAMT deficiency was studied in a knockout mouse model by *in vivo* ^{31}P and ^{1}H MRS. **Methods**: Hindleg skeletal muscle and brain of GAMT$^{-/-}$ mice and WT littermates were studied in a 7 T magnet. MR spectra were acquired during resting conditions and during ischemia and saturation transfer (ST) (skeletal muscle only). **Results**: In GAMT$^{-/-}$ skeletal muscle and brain, ^{1}H MR spectra showed a strongly reduced Cr content while ^{31}P MR spectra showed a severe reduction in phosphocreatine (PCr) along with a new signal which was assigned to phosphorylated guanidinoacetate (PGua). During ischemia in skeletal muscle, PGua was metabolically active and decreased at a similar rate as PCr in WT animals. ST measurements showed a strong reduction in flux from ATP to PGua compared to PCr. **Conclusion and Discussion**: These results show that Cr biosynthesis is blocked in GAMT$^{-/-}$ mice and that its immediate precursor Gua is metabolically active, despite lower enzyme kinetics. Overall, the ^{31}P and ^{1}H MR spectra of GAMT$^{-/-}$ mice are similar to that of human GAMT deficiency indicating that the model can be used for in depth study of the disorder in humans.

429-O

DIAGNOSIS OF DOPA-RESPONSIVE DYSTONIA (DRD): CSF, PHE LOADING, ENZYME ACTIVITY, OR DNA TESTING?

N Blau[1], JLK Van Hove[2], R Saunders-Pullman[3]
[1]*Division of Clinical Chemistry and Biochemistry, University Children's Hospital, Zurich, Switzerland;* [2]*Department of Pediatrics, University of Colorado Health Sciences Center, Denver, CO, USA;* [3]*Department of Neurology, Beth Israel Medical Center, New York, NY, USA*

Autosomal dominant DRD is a result of mutations in the GTP cyclohydrolase I (GTPCH) gene (*GCH1*). Because most *GCH1* mutations are unique and up to 40% of patients with DRD do not have identifiable mutations in the coding exons, clinical feature and response to L-dopa have remained the first diagnostic criteria for DRD.

Phe-loading has been proposed as a diagnostic test for DRD, however only some patients reach a four hour Phe/Tyr ratio of greater than 7.5 (cut-off), suggesting that additional parameters must be set to avoid missing the diagnosis of DRD, including the need for the plasma phenylalanine to reach a minimum level >600 μmol/L in order for the test to be valid. In cases where this minimum plasma phenylalanine level is not reached, tetrahydrobiopterin measurement is necessary.

CSF neopterin and biopterin are highly specific marker for DRD and both are decreased in manifesting and non-manifesting gene carriers. Together with CSF investigations, GTPCH activity in cytokine stimulated fibroblasts seems to be the gold standard laboratory test for the final diagnosis of DRD. GTPCH activity was found to be significantly reduced in a number of DRD patients with and without mutations in the *GCH1* gene.

430-P

SEVERE DEFICIENCY OF DOPAMINE AND SEROTONIN WITH NORMAL TETRAHYDROBIOPTERIN METABOLISM: RESPONSE TO TREATMENT

DL Renaud, K Hyland
Division of Child and Adolescent Neurology, Mayo Clinic, Rochester, MN, USA

An 18-year-old young woman presented for evaluation of severe global developmental delay. Her developmental age was approximately 2 years of age with limited single words and the ability to perform simple activities of daily living. Involuntary movements of the trunk, face and hands were noted. On examination, she had masked facies and a flat affect. Her gait was stooped and bradykinetic. Increased tone with hyper-reflexia and clonus was present in the lower extremities. Extensive genetic and metabolic investigations failed to reveal an etiology for her developmental delay. The presence of bradykinesia, involuntary movements and abnormal tone suggested the possibility of a disorder of neurotransmitter metabolism. The measurement of cerebrospinal neurotransmitters revealed a markedly decreased HVA, a metabolite of dopamine and also a markedly decreased 5-HIAA, a metabolite of serotonin. Concentrations of tetrahydrobiopterin and neopterin were normal thus ruling out a disorder of tetrahydrobiopterin metabolism as the cause of the severe dopamine and serotonin deficiency. Tyrosine, a precursor of dopamine synthesis, was present in the cerebrospinal fluid. The etiology of this neurotransmitter disorder is therefore unclear. Initial treatment with low dose Sinemet resulted in noticeable clinical improvement. The patient was animated and happy. Her gait was less stooped and she was able to walk more quickly. The involuntary movements were less frequent and an improvement in tone was noted. Most notably, the patient gained motor and language skills for the first time in many years. She had multiple new single words and could answer simple questions appropriately.

431-P

SEVERE FAMILIAL TYROSINE HYDROXYLASE DEFICIENCY: CLINICAL, BIOCHEMICAL AND MOLECULAR GENETICS DIAGNOSIS. TREATMENT RESPONSE

M Pineda[1], R Artuch[1], A Ormazabal[1], A Aracil[1], A Romstad[2], P Hougaard[2], F Güttler[2], L Birk Møller[2]
[1]*Hospital Sant Joan de Déu, Barcelona, Spain;* [2]*The John F. Kennedy Institute, Glostrup, Denmark*

Our aim was to present a new case of autosomal recessive TH deficiency and evolution.

Case: At 5 months of age, the girl showed severe hypotonia, drooling, glosoptosis, eyelid ptosis, irritability, and no spontaneous movements with marked delay of motor milestones. At 10 months oculogyric crises appeared. At 18 months she had severe truncal hypotonia with ptosis, sweating hypokinesia, sialorrea. At 3 years showed a more severe psychomotor delay anf fed by gastrostomy

Results: Concentrations of neurotransmitter metabolites and pterins in CSF showed a profound HVA deficiency (15 nmol/L: r.v: 334–570), whereas metabolites related to serotonin and pterins were within the reference ranges. Two new mutations were detected at the TH gene: 982C > T (Arg328Trp) located in exon 9 and 1196C > T (Thr399Met) in exon 11. In a 2° pregnancy, a female foetus compound heterozygote for the two mutations and also a 3rd pregnancy was affected.

Treatment with L-dopa and carbidopa made oculogyric crises disappear. One year after very slow increment of doses a clear improvement in neurological symptoms are evident. The slow increase (0.5 mg/kg/day in one of the doses/weekly) showed good tolerance, when we increased quicker or add Pyridoxine severe hyperkinesia and ballism appeared. She tolerates 9 mg/kg/day in 5 divided doses and has reached normal CSF values of HVA (330 nmol/L). (VIDEO images show evolution)

432-P

PUTATIVE TRYPTOPHAN HYDROXYLASE DEFICIENCY AND DYSFUNCTION OF THE HYPOTHALAMO-HYPOPHYSIAL AXIS

J de Vries[1,2], H Vles[3], M Rubio-Gozalbo[1,4], W Gerver[5], J Weber[3], N Abeling[6], L Spaapen[1], P Menheere[2], A van Gennip[1]

[1]Department of Biochem. Genetics; [2]Department of Clinical Chemistry; [3]Department of Pediatric Neurology; [4]Department of Pediatrics; [5]Pediatric Endocrinology, Academic Hospital Maastricht; [6]Department of Genetic Metabolic Diseases, Academic Medical Centre, University of Amsterdam

Cases: After an uneventful history, our patient developed at the age of 1.5 years a progressively abnormal behavior, apathy, lethargy, weight increase, increased transpiration, hypothermia, increased need of sleep and dysarthry with an abnormal gait, indicating a hypothalaam syndrome.

Objective: Is an inborn error of metabolism involved in the hypothalaam syndrome?

Methods: Analyses of hormones and neurotransmitter-catabolites in CSF, serum and urine.

Results: The levels of the gonadotrophins LH and FSH in serum were increased; TSH concentration was elevated but fT4 was normal. The levels of 5-HIAA and 5-OH-tryptophan in CSF were decreased with normal levels of HVA and MHPG. The urinary excretion of 5-HIAA was normal. This suggests a deficiency of cerebral tryptophan hydroxylase. It might diminish the cerebral production of serotonin and, subsequently, of melatonin, because serotonin is the precursor metabolite for melatonin. Indeed, serial measurements of melatonin in saliva showed consistently low melatonin concentrations and a disturbed circadian rhythm.

Conclusion: A putative deficiency of cerebral tryptophan hydroxylase seems to disturb serotoninergic pathways and leads to a dysfunction of the hypothalamo-hypophysial axis.

433-P

LOW HVA IN CSF IN A PATIENT WITH SEVERE ENCEPHALOPATHY MIMICKING BIOGENIC AMINES DEFICIENCY

García-Cazorla A, Artuch R, Ormazábal A, Blau N[1], Moller L[2], Fernández-Alvarez E, Campistol J

Neurology Service, Hospital Sant Joan de Déu, Barcelona; [1]Kinderspital Zürich, Switzerland; [2]The John F. Kennedy Institute, Denmark

Objective: To present a patient with persistently low HVA in CSF and a severe encephalopathy mimicking biogenic amines deficiency. **Case report**: A 5-month-old girl was referred for psychomotor regression, bilateral ptosis, inexpressive face, trunkal hypotonia, increased limb tone, hypersalivation, and high amplitude tremor. **Results** of intermediary metabolism and extensive neurologic exams (MRI, EEG, EMG) were normal. Respiratory chain in muscle showed slight non-specific global deficiency. Blood mitochondrial DNA was normal. CSF results (nmol/L):

AGE	3OMD	MHPG	5HT	5HIIA	HVA	HVA/5HIIA	Neopt	Biopt
8 months		54	9.8	306	188↓	0.61	15	9.5↓
15 months	26	20	6	314	100↓	0.32	14	34

TH and GTPCH genes were normal. L-Dopa initially impaired the neurological signs, but its later gradual introduction has produced a very slight improvement. However, at 20 months of life, she presents a severe encephalopathy and mechanic ventilation is needed. **Discussion**: Low HVA in CSF is found in TH, GTPCH deficiencies and in some brain insults such as hypoxia. In our case the clinical expression is very severe, HVA is not as low as in TH deficiency, and genetic studies are normal. In spite of the very suggestive clinical picture we hypothesise that this finding could be a secondary decrease of HVA.

434-O

DYSTONIA IN INBORN ERRORS OF METABOLISM

Campistol J, Fernández-Álvarez E, García Cazorla A, Vilaseca MA[2]
Neurology and [1]Metabolic Unit, Hospital Sant Joan de Déu, Barcelona, Spain

Introduction: Movement disorders including chorea, athetosis, dystonia, myoclonus, and tremor are common symptoms in childhood, due to different aethiologies (infection, drugs, tumours, neurodegenerative and metabolic diseases), in general, related to basal ganglia dysfunction.

Objective: To review the metabolic diseases diagnosed in our hospital in which dystonia was one of the main symptoms.

Material and Method: In a data base of 372 pediatric patients with inborn errors of metabolism diagnosed from 1974 to 2003 in our hospital, we reviewed the clinical data of the patients who complain of dystonia as one of the main clinical symptoms. We selected 45 patients with metabolic diseases who fulfilled the criteria.

Results: Glutaric aciduria type I (15 patients), Leigh disease (6), other mitochondrial diseases (5), homocystinuria (3), Niemann-Pick C (3), Wilson disease (3), panthotenate-kinase deficiency (3) and GM2 (2), brain creatine defects (2), Lesch-Nyhan (1), X-ALD (1) and MSUD (1).

Conclusion: Inborn errors of metabolism which can present with dystonia as one of the main symptoms are common, 12% in our series of metabolic diseases. Pathophysiology is not well known and more than one factor seems to be implicated. Glutaric aciduria type I and Leigh disease are the more common diseases presenting with dystonia. A diagnostic protocol for dystonia of metabolic origin may be very useful for clinical practice. Early diagnosis, symptomatic and etiologic treatment can improve prognosis, genetic counselling and prenatal diagnosis.

435-P

REDUCED CSF 5-METHYLTETRAHYDROFOLATE IN A GIRL WITH AN AICARDI-GOUTIÈRES-LIKE DISORDER

M Rasmussen[1], EA Kvittingen[2]
Departments of [1]Pediatrics and [2]Clinical Chemistry, Rikshospitalet, 0027 Oslo, Norway

Recently, very high neopterin and biopterin levels and lowered 5-methyltetrahydrofolate in spinal fluid were reported in a variant of Aicardi-Goutières syndrome (AGS). We now present reduced levels of spinal fluid 5-methyltetrahydrofolate in a girl who fullfills several, but not all the criteria for AGS. The patient, presently 6 years old, is severely retarded, spastic and has developed microcephaly and epilepsy. Cerebral imaging has shown calcifications of basal ganglia and other areas of the brain, and there is a pronounced substance loss, especially of white matter. Lymphocytosis or increased level of interferon-α in the spinal fluid was, however, not found. Intrauterine infection was not detected. In urine, specific disorders of amino acid or organic acid metabolism were not disclosed. Screening for peroxisomal disorders was normal as were enzyme analyses performed in leukocytes for some lysosomal diseases. In her younger sister, who showed similar signs and symptoms, the level of interferon-α was slightly increased in one of two spinal fluid samples. This sister had intracranial bleedings, was shunted for hydrocephalus and died at nine months of age. In the present girl, a single analysis of Ery-folate was low, but there were no other indications of systemic folate deficiency (no anaemia, Hcy 5 μmol/L). In three spinal fluid samples the level of 5-methyltetrahydrofolate was much reduced before a normalization following substitution with folinic acid/folic acid. These findings strengthen the need to focus on folic acid metabolism in AGS-like disorders.

436-O

CNS FOLATE METABOLITES IN RETT SYNDROME – A MULTICENTRE STUDY

B Ben-Zeev, VT Ramaekers, Y Anikster, M Ben-Akun, L Gabis, N Blau
Safra Pediatric Hospital, Sheba Medical Center, Ramat-Gan, Israel

Background: Preliminary CSF study of Rett patients with and without mutations suggested reduced folate transport. Based on this study folinic acid supplementation in Rett patients was raised. **Objective**: Measuring CSF folate metabolites and biogenic amines in a larger group of Rett patients in order to clarify this issue. **Methods**: The study included 16 patients from five centres in Europe and Israel; 14 with classical Rett, one early epileptic form and one male with Rett like features. Six harbored a coding sequence mutation, 2 with absent MeCP2 in lymphoblast line and 8 without mutation. All went through lumbar puncture in morning hours. CSF was immediately frozen and shipped on dry ice to the same laboratory. 5MTHF, biogenic amines and pterins were measured. **Results**: In 7 classical Rett patients (4 with and 3 without mutation) 5MTHF was in lower normal range for age, 1 mutation negative classical case and a male with Rett like features had significantly low 5-MTHF:43.1, 29.6 nmol/L(N-63-111) respectively. In these 2 patients folinic acid was recently introduced. Two mutated classical Rett patients showed low 5-HIAA. 2 had relatively low neopterin. **Conclusions**: The very low CSF 5-MTHF values shown in the only preliminary study published so far were not reproduced in an extended study. Our results are not related to patients' origin, age, weight and mutation existence. In almost 50% of patients 5-MTHF was in the lower range of normal and one classical case had significantly low levels suggesting secondary involvement of folate metabolism in Rett syndrome. Although folinic acid should not be routinely given consideration of a treatment trial based on CSF 5-MTHF levels individually is suggested. The very low level of 5-MTHF in a mutation negative male with Rett like features brings up the role of CNS folate metabolites in producing a phenocopy of Rett syndrome.

437-P

DIMETHYL SULFONE IN HUMAN CEREBROSPINAL FLUID AND BLOOD PLASMA

U Engelke[1], A Tangerman[1], M Willemsen[1], D Moskau[2], S Loss[2], S Mudd[3], R Wevers[1]
[1]University Medical Centre Nijmegen, Nijmegen, The Netherlands; [2]Bruker BioSpin AG, Faellanden, Switzerland, [3]National Institute of Mental Health, Bethesda, USA

Introduction: A prerequisite for the analysis of body fluid with NMR spectroscopy lies in the correct assignments of resonances. An unidentified singlet resonance at 3.14 ppm was frequently observed in the ^{1}H-NMR spectra of cerebrospinal fluid and plasma samples.

Methods: NMR spectroscopy on a standard 500 MHz spectrometer and a 500 MHz spectrometer equipped with a triple resonance cryogenic NMR probe was used to identify and quantify this peak.

Results: Using the increased sensitivity of CryoProbe technology, the ^{1}H-^{13}C signal could be assigned as dimethyl sulfone ($DMSO_2$) in an HSQC spectrum.

In plasma and CSF, the concentration of $DMSO_2$ ranged between 0 and 25 μmol/L. We also measured the concentration of $DMSO_2$ in plasma from patients with methionine adenosyltransferase I/III (MAT I/III) deficiency. In three out of four cases from severely affected patients, the $DMSO_2$ concentration was 2 times higher than the upper reference range limit.

Conclusions: The six equivalent protons of $DMSO_2$ cause the singlet resonance at 3.14 ppm. $DMSO_2$ is a regular metabolite in cerebrospinal fluid and plasma, which occurs in low micromolar concentration. $DMSO_2$ may occur as product of endogenous metabolism. A putative metabolic pathway is proposed.

438-P

LONG-TERM FOLLOW-UP OF A CHILD WITH HYPOACETYLASPARTIA

Burlina AP, Schmitt B, Wevers RA, Engelke U, Burlina AB, Boltshauser E
Department of Neuroscience and Pediatrics, University of Padua, I; Department of Neurology, University Children's Hospital, Zurich, CH; Laboratory of Pediatrics and Neurology, University Medical Center, Nijmegen, Netherlands

Martin et al. (Ann Neurol. 2001) reported a 3-year-old boy with no signals of N-acetylaspartate (NAA) and N-acetylaspartylglutamate (NAAG) in brain proton magnetic resonance spectroscopy (^{1}H-MRS). Here we report the clinical 5 years follow-up of the patient and the biochemical findings regarding NAA and its derivative NAAG. **Clinical findings**: At 8 years, his neurological examination showed truncal ataxia, no expressive speech, behaviour abnormalities, and mental retardation. He can walk unaided only for short distances and he appears ataxic. He started to have generalised seizure at 5 years with 9 episodes of status epilepticus in the following 24 months. **Biochemical findings**: CSF and urine for NAA/NAAG were analysed by ^{1}H-MRS and capillary electrophoresis (CE), which is more sensitive than ^{1}H-MRS. ^{1}H-MRS analysis of CSF and urine was unremarkable. CE in the patient's CSF did not detect NAA and NAAG, but NAA is usually undetectable also in CSF control samples, while NAAG is generally present in micromolar range. Interestingly, NAA and NAAG in the patient's urine were in the normal range, which could depend from a peripheral source of NAAG. **Conclusion**: Because of absence of NAA and NAAG in the brain and CSF of our patient a deficiency of L-aspartate N-acetyltransferase (ANAT), the enzyme, that synthesizes NAA from aspartate and acetate, could be considered. Furthermore, a block of ANAT could be responsible of a failure of NAAG synthesis, as a metabolic consequence.

439-P

RAPID DETECTION OF LARGE GENOMIC DELETIONS BY MULTIPLEX LIGATION DEPENDENT PROBE AMPLIFICATION (MLPA): IMPROVED MOLECULAR DIAGNOSIS OF CANAVAN DISEASE

GS Salomons[1], A Errami[2,3], SJM vanDooren[1], PS Darmin[1], JP Schouten[3], G Pals[2], JJP Gille[2], ON Elpeleg[4], C Jakobs[1]
Departments of Clinical Chemistry[1], Metabolic Unit, VU Medical Center, Clinical Genetics and Human Genetics[2], VU University Medical Center, Amsterdam, MRC-Holland[3] Amsterdam, The Netherlands, The Metabolic Disease Unit[4], Shaare-Zedek Medical Center, Jerusalem, Israel

Canavan disease is an autosomal recessive disorder caused by aspartoacylase (ASPA) deficiency, which currently is diagnosed by the detection of increased levels of N-acetyl-L-aspartic acid in body fluids followed by cDNA sequence analysis. However, these methods did not solve all cases, and especially for those cases prenatal diagnosis remained cumbersome. We therefore set up genomic based DNA sequence analysis and a novel method (MLPA) to rapidly detect heterozygous and homozygous exon deletions or duplications in the ASPA gene. MLPA is highly specific and allows the quantitative amplification for all ASPA exons in one reaction. To detect a specific sequence, two adjacent hemi-probes are necessary which after hybridisation, ligation, and amplification can be separated by capillary electrophoresis. By implementing these methods, we unraveled both mutant alleles in 22 out of 22 unrelated non-Jewish patients. Ten novel mutations, including 3 large genomic deletions were detected. Such deletions had escaped detection using conventional diagnostic techniques and have not been reported before. The combination of these molecular techniques has been successfully applied for eight prenatal diagnoses.

440-P

STABILITY OF HOMOCYSTEINE IN DRIED BLOOD SPOTS

A Barton, A Bowron, J Scott, D Stansbie
Department of Clinical Biochemistry, Bristol Royal Infirmary, Bristol, BS2 8HW, UK

Homocystinuria is a treatable inborn error of metabolism for which neonatal screening is not routinely performed. Screening by measurement of methionine in dried blood spots (DBS) misses at least 20% of cases. Measurement of homocysteine (hcy) in DBS may be a viable alternative, but homocysteine is unstable in whole blood and its stability in DBS is unknown.

The aim of the study was to examine the stability of homocysteine in DBS at room temperature over a 24 hour period, then for up to 28 days.

Blood collected into K-EDTA was spotted on to Guthrie cards and dried. The remaining samples were centrifuged and the plasma stored at –20°C. Hcy was eluted from the DBS in cysteamine (internal standard), and measured by HPLC with SBDF derivatisation. DBS hcy was measured at 1 (baseline), 4, and 24 h after sampling for the first part of the study ($n = 29$), and at 1 h (baseline) and on day 1, 2, 3, 4, 7, 14 and 28 for the second part ($n = 27$). Results were compared with the baseline and with plasma hcy concentrations in the original samples.

DBS hcy showed good correlation with plasma values ($r = 0.936$). There was no difference between hcy at baseline (8.9 ± 3.2) (mean $\pm$ SD) and 24 h (8.3 ± 3.7 μmol/L) $p = 0.26$, t-test. There was a small but consistent fall in hcy from baseline (14.5 ± 4.7) to 28 days (13.2 ± 4.7) $p < 0.001$.

Dried blood spot homocysteine is stable for 24 h and shows a consistent fall of $< 10\%$ over 28 days, therefore it is eminently suitable for the purposes of neonatal screening.

441-A

EVALUATION OF TWO BRAZILIAN PATIENTS WITH HOMOCYSTINURIA BEFORE AND AFTER UTILIZATION OF MEDICAL FOOD

Frangipani BJ, Oliveira RB, Micheletti C, Kyosen SO, Mendes CSC, Rand MH, Galdieri LC, D'Almeida V, Martins AM
Centro de Referência em Erros Inatos Metabolismo- Unifesp-São Paulo, Brazil

Introduction: Homocystinuria due to cystathione β synthase (CBS) deficiency is the most common disorder of sulfur amino acid metabolism. Some patients respond to pyridoxine and others donÆt. In patients who do not respond to pyridoxine, a low methionine diet must be introduced, and supplemented with a methionine-free medical food. In addition folate and betaine are given orally. **Objective**: To present the clinical response of pyridoxine no responsiveness homocystinurics patients before and after introduction of medical food. **Materials and Methods**: Anthropometrics and laboratorial evaluation of two nonresponsiveness patients on a methione restrict diet without amino acid medical food because they could not afford it in the beginning of the treatment. After more than 15 years of life they started a utilization of medical food with government support. **Results**: We observed that an average Hcy plasma levels in the first phase of treatment was 174.02 μmol/L (patient 1) and 151 μmol/L (patient2) and their body mass index (BMI) were 12, 34 kg/m^2 ($<$p3) and 14.3 kg/m^2 ($<$p3) respectively. After 2 months of the medical food introduction we observed decreasing of Hcy plasma levels to 6.4 μmol/L and 4.6 μmol/L respectively. Their (BMI) increased to 15.2 kg/m^2 and 17.3 kg/m^2 resulting in adequate Hcy plasma levels and improvement in their nutritional status. **Conclusion**: The nonresponsiveness patients who do not use medical food have higher hcy plasma levels and insufficient weight gain. The treatment must not be based only on a methione restrict diet, without amino acid medical food, because the results will not be satisfactory.

442-P

HOMOCYSTEINE-LOWERING EFFECT OF ORAL BETAINE – PHARMACOKINETIC AND PHARMACODYNAMIC (PK/PD) MODELLING

Balkenhol ND, Laryea MD, Hafner D, Wendel U, Schwahn BC
University Children's Hospital and Institute of Clinical Pharmacology, Düsseldorf, Germany

Aims: Our study aims to optimize the betaine dosage regiment in patients with homocystinuria and to reveal the parameters determining the extent of the homocysteine (tHcy)-lowering effect by PK/PD-modelling. **Methods**: We evaluated 13 patients with hyperhomocysteinemia due to CBS- or MTHFR deficiency receiving a single dose or repeated doses of oral betaine in varying intervalls and dosage. A two compartment indirect response model that simultaneously fits betaine, dimethylglycine and tHcy has been developed. **Results**: The major determinat of the Hcy-lowering effect of betaine is the initial tHcy (tHcy t_0) concentration and the duration of betaine treatment. The tHcy t_0 values correlated significantly with the minimal measured tHcy– (0.89) and the change in tHcy-concentration ΔHcy (0.67). The maximal achievable effect (E_{MAX}) increased with tHcy t_0 level while the initial betaine values negatively correlated with tHcy t_0. The rates of metabolism Cl_M and distribution Cl_D as well as the total clearance (Cl) were higher in patients than in controls and in both these parameters decreased significantly under steady-state (SS) conditions. Also the betaine concentration at half maximal lowering effect (EC_{50}) is elevated in terms of SS conditions. The initial betaine and DMG values were significantly lower in the group of untreated patients than in treated or controls. **Conclusions**: High plasma concentrations of tHcy activate the betaine:homocysteine methyltransferase and lead to hepatic betaine depletion. In addition Cl_D seems to be enhanced by high tHcy but decreased after repeated doses as sign of saturation.

443-P

HYPERHOMOCYSTEINEMIA IN CHILDREN AND ADOLESCENTS TREATED WITH ANTICONVULSANTS: EFFECT OF FOLATE SUPPLEMENTATION

Huemer M, Ausserer B, Huemer C, Schlachter K, Tscharre A, Simma B
LKH Feldkirch, Department of Pediatrics, Carinagasse 47, 6800 Feldkirch, Austria

Objective: Elevated plasma total homocysteine (tHcy) concentrations are associated with premature cardiovascular disease. Anticonvulsive treatment may interfere with tHcy metabolism and result in hyperhomocysteinemia. In this study, tHcy in children and adolescents treated with anticonvulsants is evaluated. In patients with hyperhomocysteinemia, the effect of a 12 weeks folate supplementation versus placebo is tested.

Methods: Prospective, randomised, double-blind trial including 123 patients (2–18 yrs) treated with anticonvulsants. THcy, folate and vitamin B_{12} were measured at baseline. Patients with tHcy >10.5 μmol/L were randomised to receive daily folate (1 mg/d) or placebo for 12 weeks. After 12 weeks, tHcy, folate and vitamin B_{12} were evaluated.

Results: In 104 patients, tHcy concentrations were <10.5 μmol/L (mean 6.77 μmol/L). Mean folate was 10.14 ng/ml. 19 patients had tHcy concentrations >10.5 μmol/L. 10/19 patients (mean tHcy 13.1 μmol/L, folate 5.9 ng/ml) received 1 mg/d folate, 9/19 patients (mean tHcy 13.1 μmol/L, folate 6.3 ng/ml) received placebo. After 12 weeks, the folate group had significantly ($p<0.01$) lower tHcy (7.3 μmol/L) and higher folate (13.9 ng/ml) concentrations than the placebo group (tHcy 12.18 μmol/L, folate 4.9 ng/ml).

Conclusion: Hyperhomocysteinemia is present in 18.3% of 123 pediatric patients receiving anticonvulsants. Folate supplementation with 1 mg/d results in a significant decrease of tHcy.

444-P

CEREBRAL DYSFUNCTION IN CLASSICAL HOMOCYSTINURIA: FIRST MANIFESTATIONS AND IMPROVEMENT UNDER THERAPY

Eyskens FJM
PCMA, Queen Paola Children's Hospital, University Hospital, Antwerp, Belgium

We present Turkish twin brothers. They were hospitalised at the age of 3 years with osteopenia and bone deformities. The amino acid pattern in urine showed a huge excretion of homocystine (2917 μmol/mmol creat) and methionine (3200 μmol/mmol creat). The diagnosis of classical homocystinuria due to a deficiency of cystathionine β-synthase was made, confirmed by molecular genetic analysis: they are homozygous for a new acceptor splice site mutation in intron 7 of the CBS gene. They are both mentally retarded (total IQ 45) and have a slight dislocation of the optic lenses. One of the brothers manifested episodes of epilepsia that proved to result from transient ischemic attacks. The EEG was transiently disturbed in different areas of the brain. In contrast to his brother he could not pronounce words neither in Flemish nor in Turkish. MRI of the brain could not reveal any abnormality. SPECT-scans showed a region of hypoperfusion that correlated on PET scan with a disturbed neuronal metabolism in the temporoparietal lobes of the dominant hemisphere. Under treatment with betaine (3 g/ day) the plasma total homocysteine levels dropped to 50–70 μmol/L. The transient ischemic attacks disappeared; after 2 years of treatment the mute boy mastered the power of articulated speech; a control PET scan at that moment gave normal result

Conclusion: PET scan reveals cerebral dysfunction in a patient with classical homocystinuria which is partly a result of perfusion disturbances and otherwise due to direct toxic effects on the neuronal cells, and results clinically in a disturbed speech and language development with improvement under treatment.

445-O

COPPER BINDING TO HOMOCYSTEINE IS RESPONSIBLE FOR COX DEFICIENCY IN HOMOCYSTEINE TREATED PC12 CELLS

M Linnebank, E Jarre, S Vielhaber[1], H Lutz, E Struys[2], C Jakobs[2], T Klockgether, W Kunz[3], U Wüllner
Department Neurology, Bonn, Germany; [1]Department Neurology, Magdeburg, Germany; [2]Department Clinical Chemistry, Amsterdam, The Netherlands; [3]Department Epileptology, Bonn, Germany

Hyperhomocysteinemia has been associated with vascular disease and neurodegeneration. We investigated the mechanism of homocysteine (HCys) toxicity in dopaminergic rat pheochromocytoma (PC12) cells. HCys dose dependently induced apoptotic cell death and specifically impaired mitochondrial electron transport chain activity. HCys incubation did not increase reactive oxygen species. The activity of cytochrome C oxidase (COX) was markedly reduced to 7.7% at LD_{50} whereas complex I activity remained unchanged. We subsequently showed that HCys effectively binds copper and pre-incubation of PC12 cells with copper prior to HCys prevented both the decrease of COX activity and cell death. This study suggests that binding of copper and concomitant inhibition of COX appear to be critical mechanisms of HCys (neuro)toxicity.

446-P

THE REGULATORY SUBUNIT OF GLUTAMATE-CYSTEINE LIGASE (GCLM) IS UP-REGULATED IN HYPERHOMOCYSTEINEMIC RATS: IMPLICATIONS FOR REDOX STATUS?

SG Heil[1], LAJ Kluijtmans[1], AS de Vriese[2], HJ Blom[1]
[1]*Laboratory of Pediatrics and Neurology, University Medical Center Nijmegen, The Netherlands,* [2]*Renal Unit, University Hospital Gent, Belgium*

Background: Hyperhomocysteinemia (HHcy) is associated with impaired endothelium-dependent vasodilatation. Despite a variety of mechanisms proposed to underlie this association, it is still not known whether homocysteine itself is proatherogenic or whether homocysteine levels are rather a reflection of a disturbed metabolism. We have applied microarray analysis to study the mechanism involved in hyperhomocysteinemia. **Methods and Results**: Wistar rats were fed a methionine enriched/low B vitamin diet to induce HHcy (n = 8) or standard rodent chow (n = 8). RNA was isolated from aorta and was amplified using T7-based RNA amplification. Gene-expression profiling using oligonucleotide arrays revealed, among other genes, a 1.7-fold up-regulation of the regulatory subunit of glutamate-cysteine ligase (GCLM) in aorta of HHcy rats. This up regulation was confirmed by real-time quantitative PCR analysis. GCL is the rate-limiting enzyme of glutathione synthesis. HPLC analysis of cysteine and glutathione in different tissues demonstrated that cysteine levels were lowered in liver ($p < 0.01$) and tended to be decreased in kidney and heart ($p \leqslant 0.10$). Redox status of glutathione (oxidized glutathione/total glutathione) tended to be decreased in kidney (p = 0.06). Total serum cysteine levels were decreased (p = 0.02) and total serum glutathione levels were increased (p <0.01) in HHcy versus control rats. **Conclusion**: GCLM mRNA expression is up-regulated in HHcy rats, which is in concordance with decreased cysteine levels and increased oxidized glutathione levels in tissues. We hypothesize that the pathophysiological mechanism related to HHcy involves perturbations of glutathione homeostasis due to increased oxidative stress.

447-P

PYRIDOXINE (B_6) RESPONSIVE PHENOTYPE ASSOCIATED WITH CYSTATHIONINE β-SYNTHASE (CβS) 919G→A (G307S) GENOTYPE

Yap S[1], Chew HB[1], Kluijtmans LAJ[2]
[1]*National Center for Inherited Metabolic Disorders, Children's University Hospital, Temple Street, Dublin, Ireland and* [2]*Laboratory of Pediatrics and Neurology, University Center Nijmegen, The Netherlands*

The common 'Celtic' mutation 919G→A (G307S) for homocystinuria (HCU) due to CβS deficiency, has been classically associated with a B_6 nonresponsive phenotype. We report the novel association of in-vivo B_6 responsiveness with the 919G→A CβS genotype in two siblings aged 31 and 35 years old. Investigated for ectopia lentis and was diagnosed at age 25 and 28 yrs with HCU. The diagnostic tHcy were 267 and 211 μmol/L with methionine of 100 and 91 μmol/L. CβS enzyme activity (nmol/hr/mg; control 2.27–18.2 in –PLP and 3.46–22.3 in +PLP) in cultured fibroblast were 0 (–PLP) and 1.1 (+PLP) for Sib 1 and 0 (–PLP and +PLP) for Sib 2. They were compound heterozygous for 919G→A and 502G→A (V168M) CβS genotype. Treatment included pyridoxine 100 mg bd, folic acid 5 mg daily and vitamin B_{12} 4 μg bd. Their dietary protein intake was high at 119 g/day with a caloric intake of 3000 kCal/day were maintained. tHcy responded within a week of treatment. Over a 5 yr period, the mean (range) tHcy was 25.8 (4–59; n = 28) and 38.4 (10–67; n = 27) μmol/L respectively for Sib 1 and 2. This report highlights the importance of a systematic trial of B_6 for newly diagnosed cases of HCU to ascertain *in-vivo* B_6 responsiveness, despite no fibroblast enzyme activity with pyridoxine and the presence of 919G→A (G307S) CβS genotype which until now has been described with the pyridoxine nonresponsive phenotype.

448-A

CYSTATHIONINE β-SYNTHETASE DEFICIENCY AND INSULIN DEPENDENT DIABETES

Martins E, Lima L, Barbot C, Vilarinho L
Hospital Maria Pia, Porto, Instituto de Genética Médica Jacinto de Magalhães, Porto, Portugal

Homocystinuria due to cystathionine β-synthetase (CBS) deficiency is inherited as autosomal recessive trait and it is the most prevalent inborn error of methionine metabolism. The major clinical findings include ectopia lentis, skeletal abnormalities, mental retardation, arteriosclerosis and thrombosis. The involvement of other organs like liver or pancreas is also found in some patients.

We report the case of a 16-year-old girl Caucasian who presented severe headaches and seizures, due to venous thrombosis of the left lateral venous sinus. The diagnosis of classical homocystinuria was made based on biochemical parameters (severe homocystinemia and homocystinuria, hypermethioninemia and decreased plasma cysteine levels) and was confirmed by DNA mutation analyses (homozygosity for T191M mutation on CBS gene). Further to B_6-nonresponsive homocystinuria, this patient also had a diabetes mellitus, previously diagnosed when she was 11 years old and treated with insulin. Since the treatment based on protein intake restriction, betaine citrate, folic acid and B_{12} was started, insulin needs gradually decreased to very low doses.

Pancreatic involvement was previously described in CBS (pancreatitis, hyperinsulinism or abnormal glucose tolerance deficiency) however, as far as we know, a diabetic presentation as first manifestation of homocystinuria.

449-O

DEFECTIVE REGULATION OF CBS BY ADOMET: A 'COMMON' OBSERVATION IN CLASSICAL HOMOCYSTINURIA

LAJ Kluijtmans[1], AAM Versleijen[1], GHJ Boers[2], LJM Spaapen[3], HMA van Lith-Zanders[1], FJM Trijbels[1], HJ Blom[1]
[1]Laboratory of Pediatrics and Neurology, and [2]Department of Internal Medicine, UMC St. Radboud, Nijmegen; [3]Department of Biochemical Genetics, Laboratory for Metabolic Disorders, UM Maastricht, The Netherlands

Homocystinuria due to cystathionine beta-synthase (CBS) deficiency is an inborn error of methionine metabolism, and is inherited as an autosomal recessive trait. Its clinical manifestations include ectopia lentis, mental retardation, skeletal abnormalities, and premature arteriosclerosis and thrombosis. S-adenosylmethionine (AdoMet) is an important allosteric regulator of the homocysteine flux through either the transsulfuration or remethylation pathway; it stimulates CBS activity about 2.5–5 fold, and decreases the formation of 5-methyltetrahydrofolate by inhibition of methylenetetrahydrofolate reductase (MTHFR). Recently, we identified a D444N mutation in the regulatry domain of CBS that interfered in the regulation by AdoMet (Kluijtmans et al. J Clin Invest. 1996;98:285–9). We have now studied the AdoMet regulation of CBS activity in cultured fibroblasts of more than 65 patients, and identified an additional 13 patients with a decreased, but not deficient, CBS activity, that was non- or less-responsive to AdoMet (mean $\pm$ SD: 1.03 ± 0.46 fold stimulation). This shows that a dysfunctional regulation of CBS activity by AdoMet is a relatively common observation in homocystinuria patients that may contribute to the homocystinuric phenotype. Further studies are in progress to elucidate the mutations in the CBS gene responsible for this impaired AdoMet regulation. This may lead to (1) the identification of the binding site of AdoMet to CBS, and (2) the mechanism by which AdoMet regulates CBS activity.

450-P

INTRACELLULAR *S*-ADENOSYLHOMOCYSTEINE CONCENTRATION AFFECTS GENOMIC DNA METHYLATION PATTERNS IN HUVEC

R Castro[1], I Rivera[1], C Martins[1], EA Struys[2], EEW Jansen[2], HJ Blom[3], C Jakobs[2], I Tavares de Almeida[1]

[1]*Centro de Patogénese Molecular, FFUL, Portugal;* [2]*Department Clinical Chemistry, VU University Medical Center Amsterdam, The Netherlands;* [3]*Department Pediatrics, University Hospital Nijmegen, The Netherlands*

Increased levels of intracellular *S*-adenosylhomocysteine (AdoHcy) have been associated with reduced DNA methylation patterns. This is one possible mechanism involved in the genesis of hyperhomocysteinemia related endothelial dysfunction. The present study was undertaken to evaluate the effect of AdoHcy on genomic global DNA methylation status in human umbilical vein endothelial cells (HUVEC). Intracellular accumulation of AdoHcy was induced by adenosine-2,3-dialdehyde (ADA). Increasing concentrations of ADA (0.5, 2 and 5 μmol/L) were tested and un-supplemented medium incubations were used as controls. Cytosolic and nuclear fractions were obtained from trypsinized cells, after 72 h of incubation. Cytosolic total homocysteine (tHcy), *S*-adenosylmethionine (AdoMet) and AdoHcy were measured by specific HPLC or LC-MS/MS methods, respectively. Genomic DNA was obtained from the nuclear fraction and global DNA methylation status was evaluated by the cytosine extension assay. ADA culture medium did not show cytotoxic effect. A strong significant negative correlation ($r = 0.84$; $p < 0.0001$) was observed between intracellular AdoHcy levels and HUVEC DNA methylation status. These findings strongly point to the importance of intracellular AdoHcy concentration as a pivotal biomarker of endothelial cells genomic DNA methylation status. Whether these observations are related to the *in vivo* Hcy induced endothelial dysfunction remains to be established.

451-P

ENDOTHELIAL CONNEXIN 40 mRNA EXPRESSION IS DECREASED IN RENAL ARTERIOLES OF HYPERHOMOCYSTEINEMIC RATS: RELATION TO EDHF-MEDIATED VASODILATATION?

SG Heil[1], AS de Vriese[2], LAJ Kluijtmans[1], BJM van der Rijt-Pisa[1], FJM Trijbels[1], HJ Blom[1]

[1]*Laboratory of Pediatrics and Neurology, University Medical Center Nijmegen, The Netherlands;* [2]*Renal Unit, University Hospital Gent, Belgium*

Background: Hyperhomocysteinemia is associated with an impaired endothelium-dependent vasodilatation, but it is still not clear whether homocysteine itself or another metabolite of the methionine cycle is pathogenic. Recently, we have demonstrated that the endothelium-derived hyperpolarizing factor (EDHF)-mediated renal vasodilatory response is disturbed in hyperhomocysteinemic rats. Myo-endothelial gap-junctions are considered to be involved in the signal transmission of EDHF. Blocking of one the structural subunits of these gap-junctions, Connexin 40, led to a decreased EDHF-mediated vasodilatation, indicating that Connexin 40 is essential. In this study, we studied Connexin 40 (Cx40) and Connexin 43 mRNA levels and s-adenosylmethionine (AdoMet) and s-adenosylhomocysteine (AdoHcy) levels in kidneys of hyperhomocysteinemic and control rats. **Methods and Results**: Rats were fed a diet enriched in methionine with low levels of folate, vitamin B_6 and vitamin B_{12} ($n = 8$) to induce hyperhomocysteinemia or were fed standard rodent chow ($n = 8$). Application of lasermicrodissection and real-time quantitative PCR demonstrated a down-regulation of Cx40 mRNA levels by 1.7-fold in endothelial cells of hyperhomocysteinemic rats ($p = 0.13$). The AdoMet:AdoHcy ratio was significantly decreased in kidneys of hyperhomocysteinemic rats ($p < 0.05$), and AdoMet and AdoHcy levels were slightly elevated ($p = 0.06$ and $p = 0.14$, respectively). Interestingly, Cx40 mRNA levels tended to be correlated with AdoHcy levels ($R_s = -0.46$, $p = 0.10$). **Conclusion**: The results from this small study show that endothelial Cx40 mRNA levels are down regulated in renal arterioles of hyperhomocysteinemic rats. Our findings suggest that not homocysteine but unbalanced methylation may underlie the association of elevated homocysteine and impaired renal EDHF-mediated vasodilatation.

452-O

MISFOLDING OF CYSTATHIONINE BETA-SYNTHASE MUTANTS

V Kožich, M Janošík, J Sokolová
Institute of Inherited Metabolic Diseases, Charles University First Faculty of Medicine, Ke Karlovu 2, Prague, Czech Republic

Misfolding of mutant enzymes has been proposed as a common mechanism in several inborn errors of metabolism (Gregersen et al. J Inher Metab Dis. 2001;24:189–212) and possibly also in cystathionine beta-synthase deficiency (Janošík et al. Am J Hum Genet. 2001;68:1506–13).

In the present study we aim at providing more evidence on conformational changes in a larger panel of 25 mutants, which are localized in different domains of the CBS enzyme. The mutants contained in the pHCS3 vector are being expressed in *E. coli.* Conformation of mutants is monitored by non-denaturing electrophoresis followed by western blotting, by staining for heme and also indirectly by assessing their catalytic activity. So far, mutants G85R, A114V, A155T, E176K, I278T, and del exon12 have been expressed showing aggregation with absence of tetramers, which are the predominant form of wild type CBS. The analyzed mutants also lack heme and with the exception of A114V exhibit activities below 5% of the wild type CBS enzyme.

The model mutants are also being co-expressed with GroES/EL and DnaK bacterial chaperones to test whether their aggregation may be reversed by preventing misfolding. Co-expression of mutants A114V, E176K and del exon 12 with GroES/EL indeed resulted in a substantial increase of catalytic activity to 90, 40 and 10% of wild type control, and to formation of some tetramer, while there was only small effect on the three remaining mutants. These ongoing experiments demonstrate that CBS mutants aggregate upon expression in *E. coli* and that this aggregation may be in part reversed by molecular chaperones. In summary, these data support the view that homocystinuria is another example of conformational disorders among IEMs.

453-P

CONTRIBUTION OF GENETIC FACTORS TO HYPERHOMOCYSTEINEMIA

Blom HJ[1], Gellekink H[1,2], Kluijtmans LAJ[1], Vermeulen HHM[2,3], den Heijer M[2,3]
Laboratory of Paediatrics and Neurology[1], University Medical Center Nijmegen; Department of Endocrinology[2] and Epidemiology and Biostatistics3, University Medical Center Nijmegen, The Netherlands

Introduction: Increased plasma homocysteine (tHcy) concentration is an independent and graded risk factor for various pathologies, including cardiovascular disease. In family studies it was estimated that the heritability of homocysteine is approximately 40%. Thusfar, the MTHFR 677TT genotype is the most important genetic determinant of tHcy, while other polymorphisms in genes encoding key enzymes in homocysteine metabolism have no or only minor effects. **Objective**: Over the last decade, our research group examined twenty genetic variants in genes involved in homocysteine metabolism and assessed their effects on tHcy in the general population (n = 500). The goal of this study is to quantify the independent effects of these polymorphisms in order to predict the amount of variation in tHcy concentration that can be attributed to known genetic variations. **Methods**: Using analysis of variance, we studied individual effects of the polymorphisms on tHcy. These include polymorphisms in MTHFR, MS, CBS, AHCY, TCII, TYMS, RFC-1, BHMT, SHMT, MTRR, eNOS and GCPII. **Results**: The mean tHcy concentration in our study population was 10.5 μmol/L. The genetic contribution to the variance in tHcy was approximately 5% and this effect was mainly explained by the MTHFR C677T polymorphism. BHMT 595GA, TCII 67GG, CBS 844ins68 and GCPII 1561TT genotypes have also an effect but are less frequent. **Conclusions**: The results indicate that ~5% of the variance in tHcy concentrations can be explained by the individual contributions of the polymorphisms examined so far. This means that a significant proportion of the heritability of tHcy is still unaccounted for which warrants the search for additional genetic determinants of elevated tHcy concentrations.

454-P

A COMMON 80 G>A POLYMORPHISM IN THE REDUCED FOLATE CARRIER IN RELATION TO HOMOCYSTEINE AND RECURRENT VENOUS THROMBOSIS RISK

Blom HJ[1], Gellekink H[1,2], Kluijtmans LAJ[1], den Heijer M[2,3]
Laboratory of Paediatrics and Neurology[1], University Medical Center Nijmegen; Department of Endocrinology[2] and Epidemiology and Biostatistics[3], University Medical Center Nijmegen, The Netherlands

Introduction: Folates play a key role as cosubstrates for the remethylation of homocysteine. Elevated total plasma homocysteine (tHcy) has been identified as a graded and independent risk factor for arterial vascular disease and venous thrombosis. A common 80 G>A (H27R) polymorphism in the reduced folate carrier (RFC-1) has recently been identified, but the data is not conclusive as to whether this polymorphism is a determinant of total plasma homocysteine (tHcy) or not. **Objective**: In this study we assessed whether the 80 G>A polymorphism in the RFC-1 alters tHcy and disease risk in a group of recurrent venous thrombosis patients and population-based controls. The A allele diminished a HhaI restriction site allowing for RFLP analysis. **Results**: The genotype distribution for GG, GA and AA was 36.8, 48.5, 14.7% and 37.6, 47.4, 15.0% in controls and cases, respectively. tHcy in the control group was highest in 80GG individuals (10.7 μmol/L) and decreased in GA (–5.2% [95% CI -11.9 to 2.0]) and AA (–8.1% [95% CI -17.2 to 2.0]) individuals; adjustment for the MTHFR 677C>T polymorphism did not change these estimates, suggesting an independent effect for the RFC-1 polymorphism on tHcy. No effect of the polymorphism on tHcy was observed in the cases. The AA genotype did not increase the risk of recurrent venous thrombosis (OR 1.0 [95% CI 0.6 to 1.7]) compared to the wild type allele at this locus. **Conclusions**: The RFC-1 80 G>A polymorphism may affect tHcy levels but does not increase the risk of recurrent venous thrombosis.

455-P

THE METHIONINE SYNTHASE REDUCTASE 66A>G POLYMORPHISM INCREASES MATERNAL NEURAL TUBE DEFECT RISK

HJ Blom[1], IJM van der Linden[1], LA Afman[1], H Gellekink[1,2], LAJ Kluijtmans[1], M den Heijer[2,3]
[1]Laboratory of Paediatrics and Neurology, [2]Department of Endocrinology and the [3]Department of Epidemiology and Biostatistics, University Medical Center Nijmegen, The Netherlands

The MTRR 66A>G polymorphism has been associated with neural tube defect (NTD) risk, however results are conflicting and sparse. In the present study, we determined the influence of the MTRR 66A>G polymorphism on NTD risk for 121 mothers of a NTD affected child compared to 292 female controls and for 109 spina bifida (SB) patients compared to 234 paediatric controls. Possible associations between the MTRR 66A>G polymorphism and plasma tHcy, folate, vitamin B_{12} and methylmalonic acid (MMA) levels and other SNPs were also studied.

In mothers, the MTRR 66GG genotype increased NTD risk 2.2 times (95%CI 1.4–3.5). This risk became even more pronounced in combination with elevated plasma tHcy levels (OR 3.0, 95%CI 1.4–6.8), the MTR 2756AG/GG genotype (OR 3.0, 95%CI 1.5–6.0) and the MTHFR 677TT genotype (OR 4.0, 95%CI 1.3–12.5). Furthermore, data from a recently performed pilot study suggest a strong interaction between elevated maternal plasma methylmalonic acid (MMA) levels and the MTRR 66GG genotype in NTD risk (OR 4.6, 95%CI 1.4–15.1).

In SB patients on the other hand, the MTRR 66GG genotype did not affect NTD risk significantly and rather exerted a protective effect (OR 0.6, 95%CI 0.4–1.1). This rather protective effect of the MTRR 66GG genotype on NTD risk disappeared in combination with decreased plasma folate levels (OR 1.5, 95%CI 0.6–3.9) and increased plasma tHcy levels (OR 1.8, 95%CI 0.4–4.6). No other associations or interactions could be demonstrated.

These data suggest that the MTRR 66GG genotype is a maternal risk factor for NTDs.

456-P

SATISFACTORY OUTCOME IN AN EPILEPTIC ENCEPHALOPATHY CAUSED BY METHIONINE SYNTHASE REDUCTASE DEFICIENCY (cblE)

B Merinero[1], MA Márquez[2], C Pérez-Cerdá[1], P Sanz[1], M Castro[1], MJ García[1], A Rincón, B Pérez[1], LR Desviat[1], M Ugarte[1]
[1]CEDEM, UAM, Madrid; [2]Servicio Pediatría, Hospital Ciudad Real, Spain

Hyperhomocysteinemia and hypomethioninemia in the absence of methylmalonic aciduria are major biochemical findings of defects in the homocysteine remethylation pathway. Here we report a female, born to related parents, who presented with failure to thrive at 3.5 months. At seven months of age she was investigated due to psychic regression, microcephaly, abnormal EEG, cortical atrophy in brain MRI. Important hyperhomocysteinemia (171 μmol/L; normal <10) with hypomethionemia (8 μmol/L; normal >15) and normal organic acid excretion were found. The reduced [^{14}C]-MTHF incorporation rate (17% of controls) found in patient's fibroblasts grown in both basal and OHCbl supplemented medium discarded a MTHFR defect. Cell fusion studies with cblE and cblG tester cell lines showed no complementation with cblE cells, confirming the diagnosis of methionine synthase reductase deficiency. Molecular analysis of the MTRR gene revealed the change V56M in homozygous fashion. Administration of i.m. hydroxycobalamin (OHCbl), folinic acid and betaine resulted in a dramatic clinical and biochemical improvement. She is now 21 months old and presents a psychomotor development according to age. In contrast to other previous reported cblE patients the progressive neurological picture was more relevant than the megaloblastic anemia for the investigation of an inborn error of metabolism.

457-P

METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) DEFICIENCY: MUTATIONS AND FUNCTIONAL ABNORMALITIES

Suormala T[1], Koch HG[2], Rummel T[2], Häberle J[2], Fowler B[1]
University Children's Hospitals, 1Basel (UKBB), Switzerland and [2]Münster, Germany

MTHFR catalyzes the NADPH dependent conversion of 5,10-methylenetetrahydrofolate (methyleneTHF) to 5-methylTHF, requires FAD as cofactor and is allosterically regulated by S-adenosylmethionine (AdoMet). MTHFR deficiency causes one type of homocystinuria with widely different clinical presentation, which shows some relation to residual fibroblast enzyme activity, as well as to the genotype. We identified mutations (sequencing of cDNA and/or of the 11 exons including flanking intronic regions using gDNA), and studied their functional consequences (kinetic parameters) in fibroblasts of 19 patients with residual MTHFR activity (2.6%–25% of mean control). The Km for methyleneTHF was normal in all patients. The Km for NADPH was clearly elevated (3.1–8.1 times mean control) in 12 and normal to 2.2 times mean control in 7 patients. Ki for AdoMet inhibition was clearly elevated (>8 times mean control) in 9, slightly to moderately elevated (1.8–3.8 times) in 8 and reduced (0.24 and 0.27 times) in 2 patients. All patients with elevated Km for NADPH also exhibited abnormal AdoMet inhibition. MTHFR activity showed FAD responsiveness in 3 patients (activity +FAD was 2.3–5.9 times that –FAD; controls and 16 patients 1.0–1.3 times). 21 mutations (13 novel) were detected including 2 small deletions, 3 splicing, 13 missense and 3 nonsense mutations. All patients with splicing mutations or a deletion also have a NADPH Km defect. Two missense mutations, 482G>A (known) and 596C>T (novel), seem to be related to *in vitro* FAD responsiveness. Particular mutations in the MTHFR gene can cause decreased affinity for NADPH, often associated with disturbed AdoMet interaction. Control Ki values in cell extracts are similar to tissue levels of AdoMet and point to physiological importance of AdoMet inhibition of MTHFR in the regulation of homocysteine metabolism. These findings may have implications for treatment.

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SEVERE METHYLENETETRAHYDROFOLATE REDUCTASE DEFICIENCY: RESPONSE TO THERAPY IN FOUR PATIENTS

G Huner Gokcay, M Demirkol, T Baykal, F Demir, [1]B Fowler
Children's Hospital, Nutrition and Metabolism Department, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey, [1]University Children's Hospital, Basel, Switzerland

Methylenetetrahydrofolate reductase (MTHFR) deficiency is an inborn error of folate metabolism. Severe MTHFR deficiency is associated with hyperhomocysteinemia, homocystinuria and hypomethioninemia. The main clinical findings are severe developmental delay, marked hypotonia, seizures, microcephaly, apnea and coma. Most patients with the severe infantile form are reported to show a rapid deterioration leading to death usually within 1 year. We report four patients with severe MTHFR deficiency, confirmed by enzyme analysis in cultured fibroblasts, from four families. The median age of the patients is 14 months (range 7–22 months), median age at initial symptoms 40 days (range 2–90 days) and follow up period 8 months (range 5–19 months). The clinical findings at onset were marked developmental delay (n = 4), microcephaly (n = 4) seizures (n = 2) and sepsis-like findings (n = 2). Prominent cranial MRI findings consisted of cerebral, cerebellar atrophy and hypomyelination. Neuronal migration abnormality as pachygyria and lissencephaly were observed in one patient. Treatment consisted of methionine, betaine, B_{12}, B_6 and folate. Low median methionine level at diagnosis (3.8 mmol/L normal 9–42) was elevated (116.8 mmol/L normal 9–42) with therapy but elevated median homocysteine level (167 μmol/L normal 0-12) at diagnosis stayed above the normal range (65 μmol/L normal 0–12). In all of the cases microcephaly resolved with therapy. Improvement in developmental milestones, evaluated with Denver Test II, was observed in a short period. In severe MTHFR deficiency early diagnosis and treatment has a positive impact on neurological development and prognosis.

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HOMOCYSTEINE, FOLATE, VITAMIN B_{12} AND METHYLMALONIC ACID LEVELS IN SPINA BIFIDA PATIENTS

HJ Blom[1], IJM van der Linden[1], IM van Beynum[2], M den Heijer[3,4]
[1]Laboratory of Paediatrics and Neurology, [2]Department of Paediatrics, [3]Department of Endocrinology and the [4]Department of Epidemiology and Biostatistics, University Medical Center Nijmegen, The Netherlands

In women, low folate and elevated homocysteine levels are associated with increased NTD risk. Little is known about these metabolites in NTD patients. In the present study, we have examined the influence of total homocysteine (tHcy), folate, vitamin B_{12} and methylmalonic acid (MMA) levels in plasma on NTD risk in 109 spina bifida (SB) patients and 234 paediatric controls.

Geometric mean plasma tHcy levels in SB patients (mean: 9.31 μmol/L, 95%CI: 8.62–10.05) were 47.6% (35.9% to 60.3%) higher than those in the paediatric controls (mean: 6.24 μmol/L, 95%CI: 5.93–6.56). Geometric mean plasma folate levels in SB patients (mean: 11.57 nmol/L, 95%CI: 10.50–12.76) were 40.5% (47.0% to 33.4%) lower than those in the control group (mean: 19.14 nmol/L, 95%CI: 17.90–20.49). SB patients had 20.9% (28.9%–11.9%) lower geometric mean plasma vitamin B_{12} levels (mean: 282 pmol/L, 95%CI: 255–311) compared to the controls (mean: 349 pmol/L, 95%CI: 326–374). Mean plasma MMA levels did not differ between SB patients and paediatric controls (mean: 0.18 μmol/L, 95%CI: 0.16–0.19). Plasma tHcy levels above the 50th, 60th, 70th, 80th and 90th percentile were associated with a 1.8 to 3.6 fold increase in NTD risk. Folate concentrations below the 50th, 40th, 30th, 20th and 10th percentile, increased NTD risk from 3.4 to 6.9 times. Both plasma vitamin B_{12} and plasma MMA levels did not increase the risk of NTDs.

To our knowledge, this study is the first to describe plasma tHcy, folate, vitamin B_{12} and MMA levels in SB patients and paediatric controls. Our results indicate that besides the maternal folate-homocysteine metabolism, the fetal folate-homocysteine metabolism plays an important role in the aetiology of NTDs.

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Cb1A TYPE OF METHYLMALONIC ACIDAEMIA: MOLECULAR GENETIC AND ENZYMOLOGICAL CHARACTERISATION

Coelho D[1], Baumgartner MR[1,2], Suormala T[1], Fowler B[1]
[1]*Metabolic Unit, University Children's Hospital, Basel, Switzerland;* [2]*Division of Metabolism and Molecular Pediatrics, University Children's Hospital, Zurich, Switzerland*

Isolated methylmalonic acidaemia (MMA) is caused by deficiency of methylmalonyl-CoA mutase itself (mut°, mut⁻) or by defective synthesis of its coenzyme, adenosylcobalamin (cblA, cblB complementation groups). These defects present with similar clinical abnormalities, but severity and response to vitamin B_{12} treatment is variable. The genes for the cblA (MMAA, Dobson et al. Proc. Natl. Acad. Sci. 2002;99:15554; function of coded protein unclear) and cblB have been recently identified. We report enzymatic and molecular genetic studies in fibroblasts of 5 cblA patients. The cblA defect was confirmed by the following findings: propionate incorporation was low in cells grown in normal medium with a clear response to hydroxocobalamin. Holomutase was low but total mutase was normal, and cobalamin adenosyltransferase activity was normal. Complementation analysis confirmed that each cell line belonged to the cblA mutant class. Mutation analysis was performed by direct sequencing of RT-PCR products amplified from fibroblast RNA followed by confirmation of identified mutations on genomic DNA. Mutations were identified in each of the 10 alleles, 4 in a homozygous state, and included 3 novel missense mutations, 1 novel and 1 known nonsense mutation and a known 4 bp deletion. These findings confirm mutations in the *MMAA* gene as the cause of the cblA form of MMA. Comprehensive characterisation of MMA patients as described here will provide insight into possible genotype-phenotype correlation, can be important for prognosis and may provide the rationale for treatment.

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CLINICAL HETEROGENEITY IN DEFICIENT COBALAMIN C/D PATIENTS – TWO CASE REPORTS

Estanqueiro P[1],Garcia P[1], Ramos L[2], Almeida M[1], Tavares de Almeida I[3], Diogo L[1]
[1]*Unidade de Doenças Metabólicas,* [2]*Departamento de Genética Médica Hospital Pediátrico de Coimbra, Portugal*

Cobalamin (cbl) C/D deficiency is a rare disorder of intracellular utilization of cbl. Cbl C is the most frequent defect. The early onset form is usually diagnosed in the first year of life and the patients show progressive neurologic deterioration and many develop multisystem pathology. On the late onset form, there are non specific neurological findings and macrocytic anaemia occurs only on one third. Homocystinuria and methylmalonic acidaemia are the biochemical hallmarks of this disease. Precise diagnosis requires tests in cultured fibroblasts. Treatment with twice weekly hidroxycobalamin and daily oral betaine decreases the elevated metabolites level, but prognosis is still poor. We present two patients with cobalamin C/D deficiency diagnosed in 2002. The first is an adult woman with non progressive mental delay. Methylmalonic acidúria and high blood and urine homocystine, without vit B_{12} deficiency, was disclosed. The second case is a boy; IUGR was detected in uterus and confirmed after birth. Generalized hypotonia since birth led to severe feeding difficulties by the end of the first week, followed by respiratory apnoeas needing mechanical ventilation. Methylmalonic acidúria and high homocystine on blood and urine was disclosed. Although biochemical parameters had good evolution under therapeutics, neurodevelopment (with severe retinopathy) and growth are very poor. This presentation illustrates the great variability of clinical severity of this disorder and the need to exclude it in apparently nonprogressive mental retardation, because of genetic counselling implications.

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COBALAMIN C/D – DEFECT COMPLICATED BY FATAL ACUTE PULMONARY HYPERTENSION IN TWO TODDLERS

B Schwahn[1], M Thiel[2], A Heusch[1], U Wendel[1]
[1]Clinic for General Pediatrics, University Children's Hospital Düsseldorf, [2]Children's Hospital, Florence Nightingale-Hospital, Düsseldorf, Germany

Pulmonary hypertension (PH) beyond the neonatal period is not recognised as typical complication in combined methylmalonic aciduria and homocystinuria (Cbl C/D – defect). We identified two patients, Patient 1 with a Cbl C and Patient 2 with a Cbl C/D – defect with neonatal presentation. Both had good metabolic control and satisfying somatic and psychomotor development during infancy under therapy with hydroxocobalamin and betaine. Patient 1 suffered from recurrent pneumonia until the age of 7 month. At the age of 18months he presented with pneumonia, associated with severe PH, and finally died at age 20 months due to cardiac failure. Patient 2 had a first pneumonia at age 13 months and developed severe arterial and pulmonary hypertension requiring mechanical ventilation. He eventually recovered completely until age 16 months. A second pneumonia at age 18 months again led to severe PH. He died at age of almost 20 months due to cardiac failure. Both families did not agree to post mortem investigation. In Patient 1 a pulmonary perfusion scintigraphy 2 weeks after onset of PH excluded pulmonary embolism. Patient 2 also suffered from a big left-atrial thrombus without evidence for arterial embolism. Cardiac function was well in both patients until their sudden death and we found no signs for microangiopathy such as hemolysis or thrombocytopenia. Just one case of PH in a related disorder (Cbl G) has been reported thus far. PH should be sought in respiratory distress in patients with cobalamin processing defects or hyperhomocysteinemia.

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COBALAMIN G DEFECT OF NEONATAL PRESENTATION

Durán G, Kattan J, Toso P, Escobar R
Pontificia Universidad Católica de Chile, Departamento de Pediatría, Santiago, Chile

Remethylation defects or methyonine sintetase deficiency (MS) are hereditary inborn errors of metabolism, characterized by hypomethyoninemia, hyperhomocysteinemia and methylmalonic aciduria. We report a patient with functional deficiency of MS or cobalamin G deficiency. **Case Report**: This is a 3year-old male. At birth developed laryngeal stridor because of mild laryngomalacia. At 18 days, he starts apneas, generalized seizures, severe hypotonia and lethargy. He became less responsive, with failure to thrive and hypertonia. He developed severe developmental delay and swallowing difficulties. At 3 months of age a mild urine increase of MMA was detected (10 ug/mg of creatinine) with normal blood vitamin B_{12} level, hypomethyoninemia (4 μmol/L) and hyperhomocysteinemia (113 μmol/L). Hyoxycobalamin (1 mg im/day) and L-carnitine (100 mg/k/day) was started without clinical or laboratory improvement (homocysteinemia 201 μmol/L). Central hypoventilation and unspecific pulmonary infiltrate was added to the clinical presentation and mechani ventilation was needed. At that time, triventricular hydrocephalus was diagnosed. Betaine (200 mg/k/day) and folic acid (5 mg/day) was added to the treatment, presenting conscience recovering 5 days later, followed with progressive clinical improvementnd concomitant with decrease of blood homocysteine level (47 μmol/L). The study on culture fibroblasts demonstrated a Cbl G defect. **Conclusion**: The appropiate therapy could improve the prognosis of this defect. Plasma homocysteine determination should be included in the study of all patients with neurologic deterioration and unknown ethiology.

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PROFICIENCY TESTING RESULTS OF DIFFERENT METHODS OF PYRIMIDINE ANALYSIS

C Schmidt[1], UF Engelke[2], RA Wevers[2], A Anninos[1], P Feyh[1], U Hofmann[3], M Schwab[3], GF Hoffmann[1]
[1]*University Children's Hospital, Heidelberg, Germany,* [2]*University Hospital Nijmegen, The Netherlands,* [3]*Dr M. Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany*

Objective: What is the diagnostic precision of different methods of pyrimidine analysis? We compared high pressure liquid chromatography (HPLC), gas chromatography mass spectrometry (GC-MS), nuclear magnetic resonance spectroscopy (NMR-S), gas chromatography tandem-mass spectrometry (GC-MS-MS) and liquid chromatography tandem-mass spectrometry (LC-MS-MS). **Method**: 4 urines (1 × dihydropyrimidine dehydrogenase (DPD) deficiency, 1 × dihydropyrimidinase (DHP) deficiency, 2 × controls) and 2 standard solutions (50 and 500 µmol/L) each with the native pH as well as acidified to a pH of 3 were analysed blinded by 2 HPLCs, GC-MS, NMR-S, GC-MS-MS and LC-MS-MS. **Results**: DPD deficiency was clearly recognised by all methods without large deviations. DHP deficiency was detected by NMR-S and HPLC analysis due to an increase of thymine. However, by HPLC analysis thymine excretion was normal in the native urine of the DHP patient. GC-MS, GC-MS-MS and LC-MS-MS showed clear elevations of DHU und DHT but concentrations were different. At physiologic concentrations uracil was the only metabolite detected by HPLC and NMR-S. The thymine concentrations measured in the standard solutions by both HPLCs were significantly too low. GC-MS, NMR-S-, GC-MS-MS- and LC-MS-MS analysis showed comparable amounts of pyrimidine metabolites. **Conclusion**: A MS-MS- or a NMR-S method should be used for routine diagnostics of pyrimidine degradation defects to prevent missing DHP- or β-alanine synthetase deficiency.

465-P

HPLC-ELECTROSPRAY-IONISATION TANDEM MASS SPECTROMETRY (ESI-MS-MS) OF PURINES AND OROTIC ACID FOR INHERITED METABOLIC DEFECTS

S Hartmann[1], D Kohlmüller[1], JG Okun[1], WL Nyhan[2], GF Hoffmann[1]
[1]*University Children's Hospital, Heidelberg, Germany, Division of Inherited Metabolic Diseases,* [2]*University of California, San Diego, Department of Pediatrics, Biochemical Genetics Laboratory*

Introduction: The defects of the purine metabolism show nonspecific spectrum of symptoms, including abnormalities of the central nervous system, kidneys and immune system. We present a simple HPLC-MS-MS method for the analysis of purines and orotic acid in urine.

Method: We investigated 13 metabolites in urine: adenine, 2,8-dihydroxyadenine, xanthine, hyoxanthine, uric acid, orotic acid, adenosine, inosine, guanosine, their deoxyderivatives and succinyladenosine. We used a reversed-phase-HPLC-ESI-MS-MS method. Sample preparation of the urine consists of filtration and dilution to 1 mmol/L creatinine. Internal standards which are labelled with stable isotopes are used for quantification. The metabolites are analysed by multiple reaction monitoring (MRM). The signal reliability of succinyladenosine was under further investigation.

Results: Total analysis time was 18 min. Recoveries were 86–108% in urine with added analytes. Intra-day-variance ($n = 12$) was 10% on average. Inter-day-variance ($n = 10$) was $\leqslant 15\%$. The analysis of pathological samples (HPRT-defect, XDH-defect, PNP-defect, ADSL-defect, APRT-defect) showed significant differences in the MS-MS-profiles as compared with normal urine samples.

Conclusion: The method developed allows fast, specific and reliable screening for defects in the purine metabolism. Nine different inherited metabolic defects can be detected.

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QUANTITATIVE DOSAGE OF THYMIDINE IN A MNGIE PATIENT IN URINE AND PLASMA USING HPLC-ESI-MS/MSG

G la Marca, S Malvagia, B Casetta[1], E Pasquini, MA Donati, E Zammarchi
Metabolic and Neuromuscolar Unit, A. Meyer Children's Hospital, Department of Pediatrics, University of Florence, Italy; [1]Applied Biosystems, Monza, Italy

Mithocondrial neurogastrointestinal encephalomyopathy (MNGIE) is an autosomal recessive disorders caused by thymidine phosphorylase defect. This enzyme catalyses conversion of thymidine to thymine and 2-deoxy-D-Ribose-1-phosphate. For this reason, MNGIE patients show increased levels of plasma and urine thymidine. Hemodialysis can reduce circulating plasma thymidine levels in some MNGIE patients. We developed a fast analytical method, based on HPLC-ESI-MS/MS (API4000 triple quadrupole), able to detect the complete spectrum of purine and pyrimidine metabolites in plasma and urine. We describe a 28-year-old female patient diagnosed as MNGIE patient 5 years before. Clinical manifestations were growth retardation, severe gastrointestinal dysmotility, peripheral neuropathy and leukodistrophy. Patient underwent hemodialysis and thymidine concentrations were controlled during treatment. Analytic method by MS/MS permits a no time-consuming monitoring in order to offer an appropriate therapy. In addition, this method enables to investigate for suspected purine and pyrimidine metabolic disorders both in plasma and urine.

467-O

CLINICAL, BIOCHEMICAL AND GENETIC FINDINGS OF THE FIRST THREE PATIENTS WITH A β-UREIDOPROPIONASE DEFICIENCY

ABP Van Kuilenburg[1], R Meinsma, E Beke, A Ribes, I Lorente, R Busch, A Van Cruchten, AEM Stroomer, H van Lenthe, W Kulik, B Assmann, GF Hoffmann, T Voit, RA Wevers, F Rutsch, AH Van Gennip
[1]Academic Medical Center, University of Amsterdam, Emma Children's Hospital and Department of Clinical Chemistry, The Netherlands

β-Ureidopropionase deficiency is a novel inborn error of the pyrimidine degradation pathway, affecting the cleavage of N-carbamyl-β-alanine and N-carbamyl-β-aminoisobutyric acid. In this study, we report for the first time, the elucidation of the genetic basis underlying a β-ureidopropionase deficiency in three patients presenting with neurological abnormalities and strongly elevated levels of N-carbamyl-β-alanine and N-carbamyl-β-aminoisobutyric acid in plasma, cerebrospinal fluid and urineAnalysis of the β-ureidopropionase gene (*UPB1*) of these patients revealed the presence of two splice-site mutations (IVS1-2A>G and IVS8-1G>A) and one missense mutation (A85E). Heterologous expression of the mutant enzyme in *Escherichia coli* showe that the A85E mutation resulted in a mutant β-ureidopropionase enzyme without residual activity. Our results demonstrate that the N-carbamyl-β-amino aciduria in these patients is due to a deficiency of β-ureidopropionase which is caused by mutations in the *UPB1* gene. Furthermore, an altered homeostasis of β-aminoisobutyric acid and/or increased oxidative stress might contribute to some of the clinical abnormalities encountered in the patients with a β-ureidopropionase deficiency.

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UREIDOPROPIONASE DEFICIENCY – A NEW CASE PRESENTING WITH FEBRILE STATUS EPILEPTICUS

B Assmann[1], A Van Kuilenburg[2], F Distelmaier[1], N Abeling[2], T Rosenbaum[1], E Mayatepek[1]
[1]*Department of General Pediatrics, University Children's Hospital, Düsseldorf, Germany, and*
[2]*Academic Medical Center, Amsterdam, The Netherlands*

Background: Ureidopropionase (E.C. 3.5.1.6) is the third enzyme in the catabolic pathway of uracil and thymine. Here we report the fith patient with an inborn error of this enyzme.

Case Report: The male patient is the offspring of non-consanguineous parents, development was normal. At the age of 4 months he was found cyanosed with his head covered by his blanket. At admission he was febrile, alert, without respiratory abnormalities but with a heart rate of about 240 per minute and elevated blood pressure. Electrolytes and glucose were normal but compensated metabolic acidosis and elevated lactate in blood and CSF was present. Two hours later he went into a status epilepticus which was particularly hard to overcome (generalised anesthesia necessary). No infectious agent could be detected (blood, CSF, urine, stool, gastric secretions). The child vomited once and had moderate diarrhea. Neither cranial CT scan nor ultrasound revealed cerebral edema. In due course CSF lactate normalised. In urine elevated dihydrothymine and dihydrouracil as well as N-carbamyl-β-alanine and N-carbamayl-β-amino-isobutyric acid concentrations were detected. Ureidopropionase deficiency was proven enzymatically in liver tissue.

Conclusion: Pyrimidine degradation defects should be included in the differential diagnosis of convulsions and status epilepticus and perhaps also in ALTE (acute life-threatening event).

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A PIVOTAL ROLE FOR β-AMINOISOBUTYRIC ACID IN DIHYDROPYRIMIDINE DEHYDROGENASE DEFICIENCY

ABP van Kuilenburg[1], AEM Stroomer[1], H van Lenthe[1], NGGM Abeling[1], AH van Gennip[2]
[1]*Academic Medical Center, 2Academic Hospital Maastricht, the Netherlands*

Dihydropyrimidine dehydrogenase catalyz the first step of the pyrimidine degradation pathway in which the pyrimidine bases uracil and thymine are catabolised to β-alanine and the R-enantiomer of β-aminoisobutyric acid, respectively. The S-enantiomer of β-aminoisobutyric acid is predominantly derived from the catabolism of valine. It has been suggested that an altered homeostasis of β-alanine underlies some of the clinical abnormalities encountered in patients with a DPD deficiency. In this study, we demonstrated that only a slightly decreased concentration of β-alanine was present in the urine and plasma whereas normal levels of β-alanine were present in the cerebrospinal fluid of patients with a DPD deficiency. Therefore, the metabolism of β-alanine-containing peptides, such as carnosine, may be important in the homeostasis of β-alanine in DPD patien The mean concentration of β-aminoisobutyric acid was approximately 2- to 3-fold lower in cerebrospinal fluid and urine of patients with a DPD deficiency. In contrast, strongly decreased levels (10-fold) of β-aminoisobutyric acid were present in plasma of DPD patients. Our results demonstrate that, under pathological conditions, the catabolism of valine can result in the production of significant amounts of β-AIB. Furthermore, significant crossover exists between the thymine and valine catabolic pathways.

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SUBDURAL HEMATOMA AS PRESENTING SYMPTOM OF DIHYDROPYRIMIDINE DEHYDROGENASE DEFICIENCY

K Mention[1], M Williams[1], V Valayannopoulos[1], N Bahi Buisson[1], I Desguerre[1], JM Saudubray[1], D Rabier[2], M Duran[3], P De Lonlay[1]
[1]*Department of Metabolic Disorders,* [2]*Biochemical Laboratory, Hôpital Necker, Paris, France;* [3]*Biochemical laboratory, Amsterdam, The Netherlands*

A 15-month-old boy, first child of consanguineous parents of Shrilanken origin was hospitalised on the neurosurgical ward with an epileptic insult which revealed a subdural hematoma, without necessity of neurosurgical intervention. After the acute period the clinical examination showed a facial dysmorphism, macrocrania (>2SD) and equilibrium difficulties, without (extra) pyramidal symptoms. A psychomotor developmental delay of 7 months was noted.

Analysis of urinary organic acids revealed a high level of uracil 22 (μmol/mmol creat) and thymine 65 (μmol/mmol creat). This profile is typical of a deficiency in dihydropyrimidine dehydrogenase. The diagnosis was confirmed in mononuclear cells by measuring the enzyme activity which was lower then 0.015 nmol/mg/h. After two months he presented with an unilateral infectious sacroileitis, necessitating 4 weeks of antibiotic treatment and 6 weeks of immobilisation. During hospitalisation for evaluation of his developmental delay a further 3 subdural hematomas were discovered by MRI investigations. No coagulation abnormalities could be found. Ophthalmic examinations showed no abnormalities. Is the deficiency in dihydropyrimidine dehydrogenase causative of subdural hematomas?

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AICA-RIBOSIDURIA: A NOVEL INBORN ERROR OF PURINE BIOSYNTHESIS CAUSED BY MUTATION OF *ATIC*

S Marie[1], B Héron[2], P Bitoun[3], T Timmerman[1], G Van den Berghe[1], M-F Vincent[1]
[1]*Laboratory of Physiological Chemistry, Christian de Duve Institute of Cellular Pathology and Cliniques universitaires Saint-Luc, Université catholique de Louvain, Brussels, Belgium.* [2]*Department of Paediatric Neurology, Hôpital Saint Vincent de Paul, Paris, France;* [3]*Department of Medical Genetics, Hôpital Jean Verdier, Bondy, France*

In a female infant with dysmorphic features, severe neurological defects and congenital blindness, a positive urinary Bratton-Marshall test led to identification of a massive excretion of 5-amino-4-imidazolecarboxamide(AICA)-riboside, the dephosphorylated counterpart of AICAR, an intermediate of *de novo* purine biosynthesis. AICAR and its di- and triphosphate accumulated in the patient's erythrocytes. Incubation of her fibroblasts with AICA-riboside led to accumulation of AICAR, not observed in controls, suggesting impairment of the final steps of purine biosynthesis, catalyzed by the bifunctional enzyme AICAR transformylase/IMP cyclohydrolase (ATIC). AICAR transformylase was profoundly deficient whereas IMP cyclohydrolase was 40% of normal. Sequencing of *ATIC* showed a K426R change in the transformylase region in one allele, and a frameshift in the other. Recombinant protein carrying mutation K426R completely lacks AICAR transformylase activity.

The discovery of this novel inborn error of purine synthesis reinforces the necessity to search for dephosphorilated intermediates of the *de novo* pathway in all cases of unexplained mental retardation and/or neurologic symptoms.

471a-A

INHIBITION OF BRAIN Na^+,K^+-ATPase ACTIVITY CAUSED BY HYPOXANTHINE IS PREVENTED BY GLUTATHIONE

Wyse ATS, Bavaresco CS, Franzon R, Matté C, Chiarani F, Wannmacher CMD, Wajner M
Departament of Biochemistry, ICBS, UFRGS, Porto Alegre, RS Brazil

Lesch-Nyhan is an inherited disease of purine metabolism caused by a severe deficiency of hypoxanthine-guanine phosphoribosyl-transferase activity. Affected patients present spasticity, mental retardation and self-mutilation, whose pathophysiology is still obscure. Hypoxanthine (Hpx) is the major metabolite accumulated in tissues of patients with Lesch-Nyhan. Na^+,K^+-ATPase, a membrane-embedded enzyme, is essential for cellular excitability and its activity is decreased in several neurological diseases. In the present study, we investigated the effect of Hpx on Na^+,K^+-ATPase activity in rat striatum. We also evaluated the effect of 1 mmol/L glutatione (GSH) and trolox (T, soluble vitamin E) alone or combined with Hpx on striatatal Na^+,K^+-ATPase activity. Synaptic membranes from striatum of 6-day-old Wistar rats were used for Na^+,K^+-ATPase determination. Results showed that 10 μmol/L Hpx significantly inhibited Na^+K^+-ATPase activity. GSH and trolox *per se* did not alter this enzyme activity. However, GSH, but not T, prevented the inhibitory effect provoked by Hpx on Na^+K^+-ATPase activity. Our findings suggest that inhibition of Na^+,K^+-ATPase activity could be mediated by oxidation of thiol groups on the enzyme. Considering the importance of Na^+,K^+-ATPase for normal brain development and functioning, it is tempting to speculate that inhibition of this critical enzyme may be one of the mechanisms involved in the neurological dysfunction observed in Lesch-Nyhan disease.

472-P

THE USE OF STABLE ISOTOPES FOR THE BIOCHEMICAL CHARACTERISATION OF METABOLIC KNOCK-OUT MOUSE MODELS

Bodamer OA[1], Stromberger C, Sunehag A, Haymond M
[1]*Department of General Paediatrics, University Children's Hospital Vienna, Austria and Children's Nutrition Research Center, Baylor College of Medicine, Houston, USA*

Mouse models are generated to mimic the phenotype of human disease and/or elucidate mammalian gene function. Knowledge of mouse physiology and metabolism is an essential pre-requisite for phenotypic evaluation of such models. We have developed and tested stable isotope techniques to assess glucose, glycerol, leucine and bicarbonate kinetics in 69 C57BL/6 wild-type mice of different ages (newborn, 10 days, 1 month) using either an oral bolus of [U-^{13}C] glucose, [2-^{13}C] glycerol or [1-^{13}C] leucine or a primed continuous infusion of [^{13}C]-sodium bicarbonate. Twenty-six newborn glycerol kinase knock-out mice (Gyk/–, +/– and +/+) were studied following an oral bolus of [2-^{13}C] glycerol and the oxidation rates compared with their respective genotypes in separate blinded experiments. Recovery of ^{13}C in breath CO_2 was significantly higher in Gyk+/+ mice, indicating higher glycerol oxidation compared to Gyk+/– ($p<0.03$) and Gyk–/– or /– mice ($p<0.0002$) at all time points. In newborn C57BL/6 mice average glucose oxidation was 8.36 mmol/g/min, average glycerol oxidation was 40.81 μmol/g/min and VCO_2 was 0.570 μmol/g/min. Stable isotope tracer techniques provide reproducible data about *in-vivo* metabolism in newborn mice and are particularly suited for phenotypic evaluation of 'metabolic' knock-out mice.

473-P

PROSPECTIVE DIETARY THERAPY IN A PATIENT WITH MOLYBDENUM COFACTOR DEFICIENCY (MoCoD)

Kurian MA[1], Randall T[2], Barnfield P[3], Turner C[4], RN Dalton[4], Fairbanks L[5], Champion MP[1]
[1]Department Paediatric Metabolic Medicine, [2]Department of Dietetics,[3]Paediatric Laboratory, [4]Wellchild Trust Research Laboratory, [5]Purine Research Laboratory, Guys' Hospital, London, UK

Molybdenum cofactor essential to the function of 3 enzymes: sulphite oxidase, xanthine oxidase and aldehyde oxidase. MoCoD causes intractable neonatal seizures. Neurotoxicity is thought to be related to sulphite accumulation from the breakdown of sulphur containing amino acids. Treatment is currently only symptomatic, but previous cases have shown biochemical and clinical improvnt when treated with methionine/ cystine restriction. We report a case of an antenatally diagnosed infant who received such a trial of dietary therapy from birth.

The female infant was born at term weighing 2.47 kg. The parents were consanguineous and had a previous severely affected child. MoCoD was diagnosed antenatally on chorionic villous sampling. Seizures were noted within 24 h of birth. A methionine and cystine restricted diet was started at birth; 1–1.7 g/k/day natural protein supplemented with Analog X methionine X cystine. Urinary sulphite was negative by day 14. Methionine and cystine were monitored aiming to maintain levels at the lower end of the normal range; median methionine 18 μmol/L (range 5–35 μmol/L) and median cystine 1 μmol/L (range 0–7 μmol/L). Adequate growth was maintained. Despite consistent sulphite reduction (median plasma sulphocysteine 23 μmol/L, range 9–37 μmol/L.), the diet failed to prevent intractable seizures, developmental regression and progressive features of cerebral atrophy on MRI brain scan. In our case, dietary treatment did not appear to prevent clinical deterioration in MoCoD, even when commenced from birth.

474-A

NEONATAL ISOLATED SULFITE OXIDASE DEFICIENCY IN ISRAEL

Y Anikster, C Hoffman, JL Johnson, J Zlotnik, N Brand, B Ben-Zeev
Safra Pediatric Hospital, Sheba Medical Center, Ramat-Gan, Israel

Isolated sulfite oxidase deficiency (SOD) is a rare autosomal recessive disease resulting from mutations in sulfite oxidase gene(SUOX). The clinical picture is of profound MR, intractable seizures and dislocated lens. Positive sulfite dipstick in fresh urine is highly suggestive but has to be differentiated from molybdenum cofactor deficiency. **Objective**: To analyze the clinical, neuroimaging and molecular findings in 3 Israeli patients with SOD. **Methods**: 3 infants (2 siblings) SOD patients presented as neonates with intractable seizures and hypotonia. Preliminary diagnosis was made by a positive urine sulfite stick that was negative in older sibling. MRI and MRS studies were performed in all. Genomic DNA was isolated and SUOX gene was sequenced. **Results**: All patients presented with intractable seizures, severe hypotonia followed by spasticity, profound MR and feeding problems. Lens dislocation was noticed at 8m in one. The older sibling died at 2y of age. Based on positive urine sulfite stick, and complimentary biochemical SOD was diagnosed. Gene sequencing revealed 2 novel mutations. The Arab-Muslim siblings harbored a 1414CtoT change causing nonsense R472X. First cousin parents were heterozygotes. The 3rd patient from Iamanite-Jewish origin carried a 926-927TG del. Unrelated parents were heterozygotes. On next pregnancy an heterozygote fetus was identified and pregnancy ended sucssesfully. On MRI done in first few weeks in 2 of patients severe white matter changes were noticed replaced by diffuse encephalomalacia in a few months. MRS showed elevated lactate peak, decreased NAA/Cr and Increased Cho/Cr. **Conclusions**: SOD is most probably panethnic. If clinically suspected urine sulfite stick should be repeated if returns negative. The destructive encephalomalcia and elevated lactate peak on MRI and MRS probably indicates an energy failure process. The 2 novel mutations detected both caused premature transcription termination probably explaining the severity of the clinical picture.

475-P

HYPEREKPLEXIA AND MOLYBDENUM COFACTOR DEFICIENCY IN ONE PATIENT WITHOUT GENETIC ALTERATIONS IN THE GLRA1, MOCS1, MOCS2 AND GEPHYRIN GENES

Arranz JA, Riudor E, Macaya A, Brunsó L[1], Fernández N, Raspall M, Cuenca E, Del Toro M, Roig M, Cormand B[2]

Unitat de Malalties Neurometabòliques and [1]Servei de Psiquiatria, Hosp. Maternoinfantil Vall d'Hebron, Barcelona; [2]Departament de Genètica. Universitat de Barcelona, Spain

Hereditary hyperekplexia is due in some cases to mutations in glycine receptor GLRA1 and GLRB subunit genes. Glycine and GABA receptors require gephyrin to correct postsynaptic localization that also catalyses the insertion of molybdenum in molybdopterin, the common cofactor to sulphite oxidase, aldehyde oxidase and xanthine dehydrogenase.

The patient showed hyperekplexia the 1st day of life. Ictal and interictal EEG and cranial ultrasonography were normal. A positive sulphite test with marked hypouricemia/-uria, brought us to a metabolic work: increase of sulphocystein [1] (579 μmol/g creat, nv: <100), taurine (676 mmol/mol creat, nv: 8–226) and xanthine (429 mmol/mol creat, nv: <68) were found. At 15 days, he showed an unexplained renal failure and in spite of hemo filtration and died at 28 days. Analysis of the GLRA1 [2] gene did not show genetic alterations. We focused our attention on gephyrin, due to its implication in both metabolic pathways. No mutations in the GPH gene were found after sequencing of the whole coding and intronic flanking regions. Finally we sequenced the MOCS1 and MOCS2 genes, which encode proteins responsible for the synthesis of the organic moiety of MoCo, but again no alterations were found. Gene expression levels and study of gephyrin isoforms are further points to be investigated.

[1] Dr. Stevens, Duke University
[2] Dr Ginjaar, Leyden

476-P

EXPERIENCES IN DIAGNOSIS OF PORPHYRIAS AMONG ADULT TURKISH POPULATION

Kurt I, Serdar M, Erbil K

Department of Clinical Chemistry, Gulhane Military Medical Academy and Medical Faculty, Ankara, Turkey

Objective: The porphyrias are a heterogenous group of inherited disorders of heme biosynthesis. A partial deficiency of one of seven enzymes in the pathway cause excess accumulation and excretion in porphyrins and/or porphyrin precursors. Diagnosis of these disorders relies on the laboratory investigations. We present 21 adult porphyria cases who had been missed by depending solely clinical evaluation and then diagnosed by laboratory investigation in the Porphyria Unit. **Methods**: Urine total porphyrin, plasma emission scanning and whole blood total porphyrin determination were carried out as first line analyses in patients suffering from skin lesions and/or sunlight sensitivity. An abnormality in any one of the screening tests is considered as positive, and the case was subjected to other analytical approaches in accordance with the diagnostic algorithm. **Results**: Twenty-one late cases were identified by laboratory finding during the last seven years. The diagnostic distribution of the cases were as follows: erytropoietic porphyria (n = 8), porphyria cutanea tarda (n = 6), variegate porphyria (n = 5), congenital erytropoietic porphyria (n = 2). **Discussion**: These findings suggest that particularly the cutaneous forms of porphyrias may have frequently been underdiagnosed due to relatively well tolerated initial signs by the patients and/or other numerous minor reasons, and the incidence of these disorders in Turkey may be more frequent than ever known.

477-P

EARLY TREATMENT WITH PAMIDRONATE IN OSTEOGENESIS IMPERFECTA INCREASED QUALITY OF LIFE

JS Marques[1], MV Real[1], R Guedes[1], RC Costa[2]
[1] Serviço de Pediatria do Centro Hospitalar Vila Nova de Gaia, Portugal; [2] Imagiologia Clínica Dr. Campos Cost a, Portugal

Osteogenesis imperfecta (OI) is a heritable disorder characterized by increased bone fragility. There are five types. Type III is the most severe form in children surviving the neonatal period with multiple fractures. A 2-month-old girl, first and only child of an unrelated healthy couple was diagnosed OI because of her phenotype, blue sclerae and bilateral femoral fractures. At 5 months old, pamidronate infusion was administered in cycles of 3 days and repeated every 6 weeks, according to Plotkin H protocol using this treatment in patients under the age of 3 years. After 26 months of treatment, only one time was recorded a fracture at 13 month of age. Bone deformity and dentinogenesis imperfecta were not detected and no side effect was found during the treatment. Urine calcium and phosphorus increased and bone density T – score showed – 5 , 3 (normal for age) compared to 9 , 5 before the treatment. The bone density (g/cm^2) increased from 0 , 000 before treatment to 0 , 468 after 26 months. Early treatment with pamidronate, increased the quality of life of affected patients.

478-P

A QUANTITATIVE LC-MS/MS ASSAY FOR PREGNENOLONE AND 17-HYDROXYPREGNENOLONE FOR A RARE FORM OF CAH

CD Cuthbert, RJ Singh
Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA

Background: Classical 3-β-hydroxysteroid dehydrogenase deficiency is a rare form of congenital adrenal hyperplasia (CAH), accounting for 1% to 10% of all CAH. Levels of pregnenolone and 17-hydroxypregenolone are useful markers of 3β-HSD deficiency. **Methods**: We describe a quantitative assay for the measurement of derivatized pregnenolone and 17-hydroxypregenolone using LC-MS/MS. Chromatographic separation was achieved using the Supelco Discovery C18 column and the API 4000 MS/MS spectrometer (Applied Biosystems/MDS SCIEX) was used to monitor the transitions of the derivatives of pregnenolone (m/z 450.0 to 125.2), 17-OH-pregnenolone (m/z 466.0 to 341.4), d_4-pregnenolone (m/z 454.2 to 129.2) and d3-17-OH-pregnenolone (m/z 469.4 to 344.4) in the multiple reaction monitoring mode. **Results**: The lower limit of quantitation using 1 ml serum was 1 ng/ml for both pregnenolone and 17-hydroxypregnenolone. A six-point calibration curve from1 to 100 ng/ml was used for quantitation and exhibited consistent linearity and reproducibility throughout this range. **Conclusions**: We have developed a fast and highly specific LC-MS/MS method for the determination of both pregnenolone and 17-hydroxy-pregnenolone in serum (analytical time: 3 min). Since the salt-wasting form of CAH is life-threatening, patients with this rare deficiency would significantly benefit from early recognition and immediate treatment.

479-A

ONE-MONTH-OLD BOY WITH CONVULSIONS CAUSED BY PRIMARY HYPOMAGNESEMIA

JS Marques[1], E Gaspar[2]
[1]Serviço de Pediatria do Centro Hospitalar Vila Nova de Gaia, Portugal; [2]Serviço de Pediatria do Hospital de São Pedro, Portugal

A 1-month-old boy was admitted in hospital because of several episodes of convulsions, characterised by rigth deviation of eyes and head, hyperextension of head and left leg movements. He is first and only child of unrelated healthy parents. He borned by normal delivery. Birth weight: 3140 g. Neonatal period without intercurrence. Laboratory findings showed hypomagnesemia –0.6 mg/dl (1.4–2.2) and hypocalcemia: 6.2 mg/dl (9–11). He started treatment with intravenous infusion of magnes sulfate and calcium gluconate. Infectious and metabolic screening, parathormone, phosphorus, blood gas and cerebral magnetic ressonance image were normal. After 12 h, convulsions stopped and 36 h later magnesium and calcium levels normalized. Since being discharged from hospital, he is being treated with high dose magnesium supplementation. At 1 year of age during an episode of upper respiratory tract infectious he had an episode of convulsion (magnesium: 0.86 mg/dl and calcium: 6.3 mg/dl) After increasing the dose no more episodes of convulsions were registered. Primary hypomagnesemia is caused by impaired absorption of magnesium.This was confirmed by 24 h stool determinations which showed total magnesium of 3.34 mg/day (24–170). The 24 h urine magnesium determination was in the limit low levels: 9.7 mg/L (12–120). Actually the patient is 4 years old with normal growth and psycomotor development. The oral magnesium supplementation must be life long.

480-P

AN UNUSUAL CASE OF HYPERAMMONAEMIA

SL Hogg[1], U Ramaswami[2], J Calvin[1]
[1]Biochemical Genetics Unit and [2]Department of Paediatrics, Addenbrooke's Hospital, Hills Road, Cambridge, UK

An 11-year-old male presented in a barely conscious state following a 12 h history of vomiting. His ammonia on admission was 156 μmol/L. His past medical history is complex and includes: an atrioventricular septum defect, reflux nephropathy, sensorineural hearing loss and mitral valve replacement. At 10 years he was diagnosed with attention deficit hyperactivity disorder (mental age 5 years). During this admission an EEG showed seizure activity and he was presumed to be in non-convulsive status, which was treated with lamotrigine and phenobarbitone. Infection screens were negative and he gradually improved over 7 days without further treatment. An inborn error of metabolism was suspected as the ammonia was persistently raised (57–156 μmol/L) with no evidence of liver disease. However extensive investigation did not reveal a urea cycle defect or organic acidopathy. An allopurinol loading test showed subnormal results suggesting inadequate allopurinol had reached the liver. Imaging revealed aberrant venous drainage of the gut. The extrahepatic portal vein was patent but there was a large vessel shunting blood along the lesser curvature of the stomach. It was not possible to demonstrate a patent portal vein within the liver. Presumably ammonia produced in the gastrointestinal tract is bypassing the liver, via the shunt, and entering the systemic circulation. It is not clear whether the portosystemic shunt is related to a previous portal vein thrombosis or whether it reflects a congenital abnormality. Whatever the underlying cause this is the most likely explanation of the persistent hyperammonaemia.

481-O

MUTATIONS IN THE *VPS33B* GENE, ENCODING A REGULATOR OF SNARE-DEPENDANT MEMBRANE FUSION, CAUSE ARC SYNDROME

P Gissen[1,2], CA Johnson[2], NV Morgan[2], PJ McKiernan[1], AS Knisely[3], RH Houwen[4], DA Kelly[2], ER Maher[1]

[1]*Section of Medical and Molecular Genetics, University of Birmingham,* [2]*The Liver Unit, Birmingham Children's Hospital,* [3]*Institute of Liver Studies, King's College Hospital, London, UK,* [4]*Wilhelmina Children's Hospital, Utrecht, the Netherlands*

Background: ARC syndrome (OMIM 208085) is an autosomal recessive disorder characterised by Arthrogryposis multiplex congenita, Renal dysfunction and neonatal Cholestasis with bile duct hypoplasia and low gamma glutamyl transpeptidase (GGT) activity. Platelet dysfunction is common. Affected infants fail to thrive and usually die in the first year of life. **Methods and Results**: We used autozygosity mapping to identify the *ARC* locus on 15q26.1. We then screened the candidate genes in the region by direct sequencing and identified 9 germline mutations in the *VPS33B* gene in 14 ARC kindreds. *VPS33B* encodes a homologue of the class C yeast vacuolar protein sorting *Vps33* gene that contains a Sec1-like domain important in the regulation of vesicle to target SNARE complex formation. Immunofluorescence showed that overexpression of VPS33B caused perinuclear clustering of late endosomes, identifying a role in mobilising these membrane-bound organelles. Immunostaining suggested disordered trafficking of the polarised integral membrane proteins. **Summary and Conclusions**: We identified mutations in *VPS33B* gene in patients with ARC syndrome. ARC is the first human disorder associated with mutations in a gene involved in regulation of the SNARE-mediated mechanism of membrane tether and fusion. Studies to elucidate the function of the *VPS33B* gene product are in progress.

482-O

THE MOLECULAR GENETICS OF PYRIDOXINE-RESISTANT PYRIDOXAL PHOSPHATE SENSITIVE SEIZURES

PB Mills[1], CE Beesley[1], R Surtees[1], P Scambler[1], RN Dalton, MP Champion, GF Hoffmann, J Zschocke, PT Clayton[1]

[1]*Institute of Child Health, University College London and Great Ormond Street Hospital for Children NHS Trust, London, WC1N 1EH, UK*

We have recently identified a group of babies who, within hours of birth, present with seizures which are resistant to anticonvulsants and pyridoxine and can cause death within weeks. Biochemical analyses suggested reduced levels of pyridoxal-phosphate in the brain and other tissues. Treatment of an affected infant with pyridoxal-phosphate was successful and resulted in cessation of fits within an hour. We suspected that the defect causing this condition was in the pyridoxine 5′-phosphate oxidase (PNPO) enzyme, which is responsible for the conversion of pyridoxol-phosphate to pyridoxal-phosphate, the only form of vitamin B_6 that can act as a co-factor for enzyme-catalysed reactions involving amino acids. Identification of PNPO in the human genome database enabled us to sequence all 7 exons and intron/exon boundaries in our patients. Homozygous mutations were found in all cases and include a splice site mutation at the canonical AG acceptor site of intron 3, missense mutations and a stop codon mutation. RT-PCR analysis of RNA extracted from fibroblasts of the proband with the splice site mutation confirmed that the mRNA had been aberrantly spliced, with skipping of exon 4. Pre-natal diagnosis was performed in two of the families. These are the first reported mutations in the PNPO gene and confirm the molecular basis of pyridoxine-resistant pyridoxal phosphate sensitive seizures.

483-P

PIPECOLIC ACID: MARKER IN PYRIDOXINE-DEPENDENT EPILEPSY

MAAP Willemsen[1], AMC Mavinkurve-Groothuis[1], RA Wevers[2], JJ Rotteveel[1], C Jakobs[3]
[1]Department of Pediatric Neurology and [2]Lab. of Pediatrics and Neurology, UMC Nijmegen, Nijmegen; [3]Department of Clinical Chemistry, VU UMC, Amsterdam, The Netherlands

Pyridoxine-dependent epilepsy (PDE) is characterised by neonatal seizures only responsive to pyridoxine (B_6). Biochemical/genetic backgrounds are unknown. Pipecolic acid (PA) elevations have been found in blood and CSF of three patients with PDE (B Plecko et al. Ann Neurol. 2000).

The boy, fourth child of non-consanguineous parents, was born at 35 wks of gestation with normal APGAR scores. One hour after birth seizures and respiratory failure occured. EEG showed a burst suppression pattern. Conventional anti-epileptics failed. On day 3, 50 mg iv pyridoxine immediately turned the EEG iso-electric. B_6 was continued orally resulting in slow improvement. In the first year spasticity and mental retardation became evident and obstructive hydrocephalus developed. Aged 6 years now, the patient is still on B_6 and shows moderate developmental delay, spastic dysarthria and gait, but no seizures. Extensive work-up in the first weeks of life revealed no conclusive abnormalities. PA, however, was repeatedly found in urine, but never quantified. Especially amino acids (incl. lysine and α-aminoadipic acid) and GABA in CSF were normal. Recently, we quantified PA in CSF (sample taken at age 3 wks), and fresh blood and urine samples. PA was highly increased in CSF (6.99; ref. 0.009–0.12 μmol/L), slightly elevated in plasma (5.25; ref. 0.54–2.46 μmol/L), and normal in urine (0.10; ref. 0.01–1.54 mmol/mol creatinine).

Although the relation between B_6 and PA is unclear, elevated PA levels might provide a diagnostic marker in PDE and should prevent patients from dangerous B_6 withdrawals.

484-P

A RELIABLE DIAGNOSTIC ASSAY FOR SJÖGREN-LARSSON SYNDROME BASED ON A DEFICIENCY IN PHYTOL DEGRADATION

DM van den Brink, JNI van Miert, RJA Wanders
Departments of Clinical Chemistry and Pediatrics, University of Amsterdam, Academic Medical Center, Emma Children's Hospital, Meibergdreef 9, 1105 AZ, Amsterdam, The Netherlands

Sjögren-Larsson Syndrome (SLS) is a metabolic disorder characterized by ichthyosis, mental retardation and spastic di- or tetraplegia. The underlying enzymatic defect of the disease is a deficiency in the microsomal fatty aldehyde dehydrogenase (FALDH), which converts fatty aldehydes into fatty acids. The diagnosis of SLS usually consists of measurements of FALDH activity in patients' fibroblasts using octadecanal as substrate and production of NADH as a measure of enzymatic activity. However, even in patients expected to be completely deficient in FALDH activity due to nonsense mutations in the *FALDH* gene, significant amounts of residual activity are measured, due to substrate overlap of other aldehyde dehydrogenases present in human cells. An assay without this interfering residual activity would therefore be very useful.

Recently, we found that FALDH is involved in the breakdown of phytol to phytanic acid; fibroblasts derived from SLS pare deficient in the production the intermediate phytenic acid. This prompted us to set up a novel assay in which fibroblast homogenates were incubated with phytol and FALDH activity was assessed by quantification of production of phytenic acid by GC-MS. In this way, FALDH activity could be measured in human fibroblasts, lymphoblasts and in chorion villi biopsies. No significant residual activity was present in material from patients, making this assay a reliable tool for the routine diagnosis of SLS as well as for prenatal diagnosis.

485-P

THE CRYSTALLINE RETINOPATHY IN SJOGREN-LARSSON SYNDROME: NEW INSIGHTS BY A NOVEL IMAGING TECHNIQUE

MAAP Willemsen[1], T Theelen[2], J Fuijckschot[1], JJ Rotteveel[1], JRM Cruysberg[2]
[1]Department of Pediatric Neurology and [2]Institute of Ophthalmology, University Medical Centre Nijmegen, Nijmegen, The Netherlands

Sjögren-Larsson syndrome (SLS) is a recessive disorder characterized by ichthyosis, spasticity, and mental retardation, due to fatty aldehyde dehydrogenase (FALDH) deficiency. FALDH catalyzes the oxidation of different medium-/long-chain fatty aldehydes (derived from alcohols). Their toxicity and storage are thought to cause SLS. The presence of distinctive crystals in the macular area of the retina is a cardinal, pathognomonic sign of SLS. Ophthalmoscopy and fundus photography show that the crystals are located in the innermost layers of the retina.

Optical coherence tomography (OCT) is a new technique, using coherent light, to gain high-resolution cross-sectional images of the retina comparable to histological sections. Two patients with SLS underwent ophthalmological examination and OCT. Ophthalmoscopy showed bilateral, glistening yellow-white crystalline deposits in the maculae of both patients. OCT revealed focal hyperreflectivities that were restricted to the level of the inner nuclear layer (INL) of the retina.

The retina consists of several layers of highly specialized neuronal and glial cells. The results from OCT in SLS strongly suggest that cells affected by FALDH deficiency are located in the INL, which is home for the Müller glial cells, bipolar cells, horizontal cells and amacrine cells. OCT as a non-invasive, rapidly applicable imaging technique appears to be useful to study retinal abnormalities of inborn errors of metabolism.

486-P

GAIT DISTURBANCES, CHOREOATHETOSIS, DYSARTRIA AND SEVERE AGRESSIVE BEHAVIOUR IN THE COURSE OF PANTHOTENATHE KINASE-ASSOCIATED NEURODEGENERATION

M Gıiewska[1], L Cyrylowski[2], P Nowacki[3], A Bieleninik[1], T Adamczyk[1], M Walczak[1]
[1]II Department of Children s Diseases, [2]Department of Radiology, [3]Department of Neurology, Pomeranian Medical University, Szczecin, Poland

Pantotenathe kinase associated neurodegeneration (PKAN)(OMIM 234200), previously known as Hallervorden-Spatz disease is a rare, autosomal recessive neuroaxonal dystrophy accompanied by symmetrical iron deposition in globus pallidus and substantia nigra. The main symptoms are gait disturbances, rigidity of all limbs, choreoathetosis, slowing of voluntary movements, mental deterioration, retinitis pigmentosa and/or optic atrophy. The course of the disease is severe and depends on the age of its onset; life span of affected children varies-some patients achieved the 3rd decade of life, the others die after 2–3 years of rapidly progressive defect. We report a case of 16 year-old boy, who as a child presented delay in psychomotor development, followed by gait impairment (walking on toes), with equinovarus deformations of the feet, dysartria with slow and obscure speech, increasing rigidity in the lower limbs and progressive mental retardation. On T2-weighted brain MRI the picture of 'eye of tiger' was found. Additionally, the patient exhibited difficult to control, repeated attacks of aggression toward his family members and schoolmates. This resulted in many everyday conflict situations. Although rare, PKAN should be always taken into the consideration, when progressive encephalopathy with symptoms of pyramidal-extrapyramidal syndrome is followed by aggressive behavior and typical changes on brain MRI.

487-P

MOLECULAR ANALYSIS OF PATIENTS WITH MACHADO-JOSEPH DISEASE FROM SOUTH BRAZIL

Pereira MLS, Carvalho TS, Giugliani R, Jardim LB
Medical Genetics Service, Hospital de Clínicas de Porto Alegre; Departments of Biochemistry, Internal Medicine and Genetics, Federal University of Rio Grande do Sul Porto Alegre, RS Brazil
E-mail: mlpereira@hcpa.ufrgs.br

Machado-Joseph Disease (MJD) is an autosomal dominant multisystem neurodegenerative disorder characterized mainly by cerebellar ataxia, spasticity, and peripheral neuropathy. Unstable CAG trinucleotide repeat expansion in the MJD gene on the long arm of chromosome 14 has been identified as the pathological mutation for MJD. The number of CAG repeats units in expanded alleles of the *MJD1* gene was reported to range from 61 to 84, whereas that in normal alleles ranges from 12 to 41. The aim of the present study was to establish a reliable protocol for identification of the CAG repeats to be tested in patients with clinical signs and symptoms of MJD and determine the frequency of MJD within a population with ataxia. Peripheral blood samples were submitted to DNA extraction. The region of interest was amplified by PCR using specific primers and product was analysed by electrophoresis in agarose gel in order to detect normal and/or expanded alleles, based on a non-isotopic protocol. We analysed 112 individuals and found 77 samples with an expanded allele. Our results also indicated that the CAG repeat number was inversely correlated with the age of onset, as previously reported. This protocol is now currently being used for the diagnosis of new cases as well as for presymptomatic diagnosis in selected individuals.

(Supporte by CNPq, FAPERGS, FIPE/HCPA, and PRONEX)

488-O

CALLOSAL DYSGENESIS IN INBORN ERRORS OF METABOLISM: A CASE OF PLASTICITY MISDIRECTED?

AN Prasad[1], K Bunzeluk[2], CR Greenberg[2], BN Chodirker[2], KG Magnus[3], C Prasad[2]
Departments of Pediatrics[1], Human Genetics and Biochemistry[2], and Radiology, University of Manitoba, Winnipeg, Canada[3]

Background and Methods: We examined the spectrum of CNS abnormalities in children with diagnosed inborn errors of metabolism (IEM). Systematic review of the metabolic clinic database, neuroimaging results, and postmortem findings enabled the collection of data from patients with both IEM and callosal dysgenesis (ACC). **Results**: Of 2000 patient records, there were 21 patients (11 males, and 10 females) identified with the following diagnoses; defects of the glycolytic pathway (2); Krebs cycle (1); electron transport chain (3); glycogen storage (1); urea cycle (1); fatty acid oxidation (1); glycine cleavage (1), lysine and tryptophan metabolism (2), lysosomal storage disorder (l), copper transport (1), cholesterol metabolism (3), and xanthine metabolism (4). Callosal abnormalities included; hypoplasia (12), complete agenesis (4) and partial agenesis (5). Additional CNS anomalies included diffuse ventriculomegaly (11), colpocephaly (7), increased extraxial CSF spaces (12), abnormalities of grey matter (9) including neuronal migration defects and porencephaly, white matter changes (12) and posterior fossa anomalies (10). **Conclusions**: The widespread structural abnormalities represent structural adaptation (plasticity) of the nervous system in the face of metabolic perturbation. The link between biochemical abnormalities and key neurobiological processes underlying cerebral morphogenesis is central to our understanding of 'misdirected plasticity'.

489-P

HYPOMYELINATION AND ATROPHY OF THE BASAL GANGLIA AND CEREBELLUM, A NEW SYNDROME: CASE REPORT

S Mercimek-Mahmutoglu, M van der Knaap, I Baric, V Konstantopoulou, D Prayer, S Stöckler-Ipsiroglu
Children's Hospital, Vienna University, Austria

Introduction: Hypomyelination and atrophy of the basal ganglia and cerebellum (H-ABC) syndrome is a newly described disease characterized by typical MRI changes. Clinical features include progressive pyramidal and extrapyramidal movement disorders, developmental retardation. The syndrome was described so far in 7 patients [1].

Case report: This new patient was diagnosed at age of 3 years with H-ABC syndrome due to characteristic MRI changes and clinical features. Oculogyric crises were particularly remarkable. Application of our recently developed myelination score [2] revealed progressive deviation of myelination from age related normal values (62% at age 8 months, and 30% at age 40 months).

Discussion: The H-ABC syndrome represents a new differential diagnosis in patients with cerebral hypomyelination and should be considered particularly in patients after exclusion of Pelizaeus Merzbacher disease.

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[2] Plecko B, Stockler Ipsiroglu S, Gruber S et al. Degree of hypomyelination and magnetic resonance spectroscopy findings in patients with Pelizaeus Merzbacher phenotype. Neuropediatrics. 2003;34:127–36.

490-O

CYSTIC LEUKOENCEPHALOPATHY WITHOUT MEGALENCEPHALY – CLINICAL AND MOLECULAR CHARACTERISATION

M Henneke[1], F Rüschendorf[2], H Thiele[2], P Nürnberg[2], J Gärtner[1]
[1]*Department of Pediatrics and Pediatric Neurology, Göttingen, Germany;* [2]*Gene Mapping Center, MDC, Berlin-Buch, Germany*

Leukodystrophies are inborn diseases affecting brain myelin leading to hypomyelination, demyelination and dysmyelination. The patients' clinical pictures are highly variable and range from a comparatively benign course to a severely progressive deterioration of motor and mental functions. In more than a third of all leukodystrophies seen on MRI the underlying molecular defect is unknown. We and other work groups established a clinical network ('LEUKONET UNKLAR') to accelerate the identification of new disease entities among currently unclassifiable leukodystrophies using clinical, neuroradiological, electrophysiological and genetic criteria. We identified at least six new disease entities and focused on one of them, the cystic leukoencephalopathy without megalencephaly.The disease is characterized by early onset of severe psychomotor impairment and non-progressive encephalopathy. MRI reveals bilateral anterior temporal lobe cysts and multifocal white matter lesions. So far, we have acquired a total of 15 patients. We performed a genome wide search for linkage in two informative consanguineous families with four affected children using about 10 000 SNPs and microsatellite markers and mapped a new gene locus to chromosome 6 (14 cM, highest multipoint LOD score Z_{max} = 5.2). Analyses of candidate genes in the identified region are in progress to determine the primary defect within this disease entity.

491-P

VANISHING WHITE MATTER DISEASE – A NEW LEUKOENCEPHALOPATHY: CASE REPORT OF 3 PATIENTS

S Mercimek-Mahmutoglu[1], M van der Knaap, O Ipsiroglu[1], G Scheper, V Konstantopoulou[1], S Stöckler-Ipsiroglu[1]
[1]*Children's Hospital, Vienna University, Austria*

Vanishing white matter (VWM) disease is an autosomal recessive leukoencephalopathy. On the basis of 3 patients, the clinical heterogenety of this disease is described.

Case report: ***Patient 1***: Until 18 months of age normal psychomotor development. After febrile infection, she developed an atactic dystonic movement disorder and brain MRI showed diffuse generalized leukodystrophy at the age of 2 years and diffuse CSF-like signal intensity at the age of 9 years. ***Patient 2*** (brother of patient 1): Mild motor developmental delay until 14 months of age. After febrile infection he experience a rapidly progressive course of disease with spasticity, dystonia and tetraparesis and died at the age of 21 months. T2 brain MRI showed diffuse high signal intensity in basal ganglia and brain stem. ***Patient 3***: Normal neurological development until age of 5 years. After a generalized seizure, brain MRI showed generalized leukodystrophy at age of 5 and additional cystic lesions isointense to CSF in the frontoparietal area at age of 9 years. Patient 1 is homozygous for (R269Q) mutation in the EIF2B5 gene, parents are heterozygous.

Conclusion: The patients fulfilled all diagnostic criteria of VWM disease, while the clinical picture and progress of the disease were different in 3 patients. Initially normal psychomotor development, onset of neurological deterioration after an infection or head trauma should rise suspicion of VWM.

492-P

SENSORY ATAXIA IS A TYPICAL FEATURE IN LBSL

T Linnankivi[1], N Lundbom[2], T Autti[2], A-M Häkkinen[3], H Koillinen[4], T Kuusi[2], T Lönnqvist[1], K Sainio[5], L Valanne[2], T Äärimaa[4], H Pihko[1]
Departments of [1]Pediatric Neurology, [2]Radiology, [3]Oncology and [5]Clinical Neurophysiology, University of Helsinki, Finland, [4]Department of Pediatric Neurology, University of Turku, Finland

Objective: A novel leukoencephalopathy with brainstem and spinal cord involvement and high brain lactate (LBSL) was defined in 2003. We describe five new patients with this entity. **Methods:** Our patients were from four nonconsanguineous families. Somatosensory evoked potentials, nerve conduction velocities and brain MRI were performed in all patients and spinal MRI and proton magnetic resonance spectroscopy (^{1}H-MRS) in four patients. **Results:** Ataxic gait was the most prominent symptom and manifested from 3 to 16 years of age. Dorsal column dysfunction and mild distal spasticity were evident by adolescence. One 13-year-old patient was asymptomatic. Extensive laboratory investigations were unrevealing. Neurophysiological studies suggested impaired conduction in the sensory tracts of the spinal cord. MRI showed signal abnormalities in the periventricular and deep white matter, in mesencephalic trigeminal tracts, in the cerebellar connections, pyramidal tracts and dorsal columns of the spinal cord. MRS showed decreased N-acetylaspartate and increased lactate in the white matter of all patients. In one patient choline was elevated. **Conclusions:** A slowly progressive sensory ataxia is a typical feature in this new disease. MRS favors a primary axonal degeneration.

Van der Knaap MS, van der Voorn P, Barkhof F, et al. A new leukoencephalopathy with brainstem and spinal cord involvement and high lactate. Ann Neurol. 2003;53:252–8

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LETHAL X-LINKED ENCEPHALOPATHY MAPPED TO XP22

I Giurgea, G Viot, R Gesny, F Roels[1], I Desguerre, L Hertz-Pannier, P De Lonlay
Department of Pediatrics and Pediatric Radiology Hôpital Necker-Enfants Malades, 75743 Paris Cedex 15; [1]Department of Human Anatomy Godshuizenlaan 4, B-9000 Gent, Belgium

We report a family with X-linked mental retardation (XLMR) in seven affected males over five generations. Affected males had a severe encephalopathy causing death in the first year of life. Carrier females do not have somatic anomalies or mental impairment. Molecular studies are performed for 2 affected and 6 unaffected males. Affected males had cholestasis, hepatomegaly with steatosis, myoclonic seizures, dystonia and abnormal turning of the eyes. MRI showed white matter and brain stem abnormalities. Investigations of urine (amino acid and organic acid levels, sulfites, oligosaccharidiuria, mucopolysaccharidiuria), cerebrospinal fluid (lactate, amino acids, neurotransmitters, glucose, creatine and guanidino-acetate), plasma (uricemia, very-long-chain fatty acids, phytanic, pipecolic acids, plasmalogen), visual evoked potentials, electroretinogram and respiratory chain enzyme activities in skeletal muscle and in liver biopsies were normal. Liver electronic microscopy revealed abnormal peroxysomes. An X wide linkage analysis allowed us to identify the closest recombination points between DXS8051 and DXS8027 markers in Xp22 with a Lod score of 2 at zero recombination. This 15 Mb region encompasses the Serine/Threonine Kinase gene (STK9) which accounts for mental retardation and West syndrome. This gene and four outer candidate genes could be excluded in this family by direct sequencing. To date, not similar clinical cases with severe encephalopathy and early death has been mapped to this region.