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JIMD – Journal of Inherited Metabolic Disease

JIMD

Aims and Scope

JIMD is the official journal of the Society for the Study of Inborn Errors of Metabolism, SSIEM. By enhancing communication between workers in the field throughout the world, *JIMD* aims to improve the management and understanding of inherited metabolic disorders. It publishes results of original research and new or important observations pertaining to any aspect of inherited metabolic disease. This includes clinical (medical, dental and veterinary), biochemical, genetic (including cytogenetic, molecular and population genetic), experimental (including cell biological), methodological, theoretical, epidemiological, ethical and counselling aspects. *JIMD* also reviews important new developments or controversial issues relating to metabolic disorders and publishes reviews and short reports arising from the Society's annual symposia. A distinction is made between peer-reviewed scientific material that is selected because of its significance for other professionals in the field, and non-peer-reviewed material that aims to be important, controversial, interesting or entertaining.

JIMD welcomes scientific contributions in the following categories:

- **Original articles:** Important manuscripts that may be expected to influence or change clinical or research practice with regard to inherited metabolic disorders. Original articles may include comprehensive studies on disease features in groups of patients, important novel information on a disease or relevant research findings.
- **Rapid communications:** Highly competitive and timely manuscripts; please discuss this with the editors: editor@jimd.org.
- **Reviews:** Concise summaries of metabolic pathways, specific disorders, methods, models, treatment options etc.
- **Metabolic dissertations:** Review articles on the topic of a doctoral thesis in the area of inherited metabolic disorders, published with photograph of the person who has graduated.
- **Short reports:** Short articles that are relevant to clinical or research practice and should be documented in the literature. Short Reports are published as abstract only, with the full text available via the internet; they are formatted in *JIMD* style by the authors.
- **Data reports:** Data tables or other systematic information for deposition on the internet. A summary of the information with up to two references will be published in print.
- **Images in Metabolic Medicine:** Illustrative colour pictures e.g. of clinical findings.
- **Correspondence:** Comments to previous articles or other relevant topics.

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For the non-peer-reviewed section, *JIMD* invites submission of **Editorials** (comments that place *JIMD* articles into a wider context, usually provided by reviewers of this article), **Observations and Opinions** (relevant or stimulating opinions or ideas that merit communication, unusual findings, instructive errors, important side effects etc.), **Fillers** (interesting stories or personal experiences) and **Book Reviews**. In addition, *JIMD* wishes to publish **Obituaries** of all recently deceased members of the SSIEM and other persons who were important in the area of inborn metabolic disease.

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Society for the Study of Inborn Errors of Metabolism

The SSIEM was founded in 1963 in the North of England and has developed into the largest international organisation concerned with all aspects of inherited metabolic diseases. The aim of the Society is to promote the exchange of ideas between professional workers in different disciplines who are interested in this group of disorders. This aim is pursued in scientific meetings and publications.

The Society holds an annual symposium concentrating on different topics each year with facilities for poster presentations. There is always a clinical aspect as well as a laboratory component. The meeting is organized so that there is ample time for informal discussion; this feature has allowed the formation of a network of contacts throughout the world. The international and multidisciplinary approach is also reflected in the *Journal of Inherited Metabolic Disease*.

If you are interested in joining the SSIEM then contact the Treasurer: Dr. Maureen Cleary, Metabolic Office, 7th floor, Southwood Building, Great Ormond Street NHS Trust, Great Ormond Street, London WC1N 3JH, UK. The subscription includes the 6 issues of the *Journal of Inherited Metabolic Disease*. The SSIEM web site is on <http://www.ssiem.org>.

001-P

A HIGH DIAGNOSTIC YIELD IN CHILDREN ATTENDING A METABOLIC CENTRE WITH DEVELOPMENTAL DELAY

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Objectives: IEMs are reported to be a rare cause (1–5%) of developmental delay, (Shevell et al., 2003). We sought to determine the yield of metabolic investigation in children referred for investigation of developmental delay to our service and to identify variables predictive of a diagnosis.
Methods: The case notes of children <16 years referred with developmental delay during the years 2004–2006 were reviewed for the history, examination findings, investigations and diagnoses.
Results: 106 cases, (64 male, 42 female), (age range 0–176 months) were identified. Referrals were from paediatricians (55.7%), paediatric neurologists (34%), GPs (2.8%) and others, (7.5%). Of the 106 cases, 40 (37.7%) had a proven metabolic diagnosis. Of the IEMs noted, mitochondrial respiratory chain disorder was the commonest diagnosis (20 definite and 19 probable cases). Variables associated with a higher diagnostic yield included referral from a neurologist, (47% yield vs 29%) in paediatrician group ($p<0.000005$), microcephaly ($p<0.0005$), abnormal eye examination ($p<0.00005$), and biochemical markers: elevated serum lactate ($p<0.00000005$), abnormal plasma amino acids ($p<0.0005$) and abnormal urine organic acid analysis ($p<0.00005$).
Conclusion: We conclude that while accounting for a referral bias in the total group by neurology referrals, the diagnostic yield of metabolic investigation in our study group is significantly higher than observed in other studies, probably reflecting the documented high prevalence of IEMs in Ireland and high referral rates. Appropriate testing and guidelines should reflect the specific population.

002-P

THE INVESTIGATION OF INHERITED METABOLIC DISEASE IN POST MORTEM SAMPLES

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Samples taken at post mortem offer the last opportunity for obtaining a diagnosis of IMD. Between 1989 and 2007 we have investigated 728 cases of unexplained/unexpected death in neonates, infants and children by fibroblast studies on skin biopsies obtained post mortem. Where possible we obtained urine for organic acid analysis and more recently we added the investigation of acylcarnitine analysis by MS/MS on DBS, bile and CSF samples. We have analysed 120 DBS, 60 bile and 30 CSF samples and established post mortem reference ranges for the full range of acylcarnitine species C0-C18. Using post mortem acylcarnitine analysis we detected and confirmed a case of each of the following: MCAD; MADD; beta-ketothiolase and GAI. Overall in the period between 1989 and 2007 we have diagnosed, or confirmed a suspected diagnosis, in cultured fibroblasts in 66 cases, mostly fatty acid oxidation disorders and respiratory chain defects: 11 MADD; 8 MCAD; 6 CPTII; 6 VLCAD; 6 LCHAD/TFP; 2 CAT; 1 CPTI; 1 PCD and 11 respiratory chain defects. Acylcarnitine analysis on DBS, bile and CSF and urine OA analysis with confirmation by fibroblast assay is an effective method of detecting and confirming IMD through autopsy and offers the opportunity for future prenatal diagnosis for the family.

003-P

METABOLIC AUTOPSY: THE VALUE OF A PROTOCOL IN POST MORTEM STUDIES OF CHILDREN WITH UNDIAGNOSED INBORN ERRORS OF METABOLISM

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Background: Inborn errors of metabolism (IEMs) comprise a group of disorders which has single gene defect causes a clinically significant block in a metabolic pathway resulting either in accumulation of substrate behind the block or deficiency of the product. An understanding of the broad clinical manifestations of IEMs provides the basis for knowing when to consider the diagnosis. However, besides all the clinical and biochemical investigation, many patients die without a definitive diagnosis.
Aims: To determine the utility of the metabolic autopsy in the investigation of pediatric patients with high index of suspicion of an IEM.
Material and Methods: This was a retrospective review of all metabolic autopsies performed at a large university hospital over a 2-year period. Premortem clinical diagnoses were correlated with autopsy findings and results of postmortem testing.
Results: Of the 13 metabolic autopsies performed, a metabolic disorder was diagnosed before death in one and after death by extensive studies initiated before death in four. In the remaining seven cases, postmortem samples were inadequate for subsequent enzymatic analysis in four, a nonmetabolic explanation for symptoms was identified in one, and no unifying diagnosis could be defined in two. The inborn errors of metabolism detected were Pompe disease, Niemann–Pick disease type C, LCHAD and Mucolipidosis type II.
Conclusions: In a significant percentage of cases (37%), the metabolic autopsy successfully identified an undiagnosed metabolic disease. Families were provided a plausible reason for the demise of their children and an opportunity to receive genetic counseling, and to seek prenatal diagnosis.

004-A

INBORN ERRORS OF METABOLISM (IEM) IN ADOLESCENCE AND ADULTHOOD. A NEW CHALLENGE FOR PHYSICIANS

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Introduction: Improvements in screening program and therapeutic interventions IEM have led to increasing number of children surviving through childhood into adolescence and adulthood. The type of transition from the children's services to adult sector it is a subject very discussed in specialized forums and publications at the moment because there is an urgent need for structures that guarantee appropriate treatment of this growing population
Method: In South Spain there actually are 50 PKU patients and 15 with others IEM (organic acidemias and urea cycle disorders mainly) with 14 to 44 years old. These individuals are often able to integrate into society, but many have complex, multisystem problems that require ongoing care. The pregnancy of women with this type of pathologies is a challenge for the own woman and the equipment that takes care of it. We have a woman 27 years old with propionic acidemia in her fifth month of pregnancy, normal evolution. Detailed therapeutic guidelines for these disorders are not available, and medical management must be tailored to the individual patient. Close interaction between biochemical and molecular laboratories, primary care physicians, psychologists and others specialist is necessary for an integrated care plan.
Results and Conclusions: After making a survey to our greater patients of 14 years we have reached the conclusion the best model is probably and outpatient service for all ages with a coordinator contributing and sharing access to dietary and laboratory services. Reference centre would be an effective means of acquiring the information on the adult follow-up.

005-P

BRAZILIAN INFORMATION SERVICE ON INBORN ERRORS OF METABOLISM (SIEM): RESULTS OF FIRST THOUSAND CASES

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SIEM is a tool-free service, pioneer in Brazil and South America, with a team specialized in IEM to help health professionals to diagnose, manage and treat suspected cases. These diseases are poorly recognized in Brazil. Fast diagnosis and management are crucial steps to allow a better prognosis for the affected patients. Between Oct. 2001 and March 2007, 1000 consultations were made. 76% of these cases were from South and Southern Brazil. In 46% of the cases the contact was made by pediatricians. In 65% of patients presented symptoms between 0 and 12 months of age. 84.5% of the professionals were looking for diagnostic support and early management guidance. 8% were only looking for more information on IEM and 7.5% were looking for support in the follow-up as the diagnosis had been already established. Of the total 1000 records, excluded the calls for information (80), 605 had their investigation concluded. We have established the diagnosis of IEM in 94 (15%) cases. On the remaining cases, 228 (38%) were non-metabolic, 170 (28%) inconclusive and 113 (19%) the follow up was lost. Classification of the IEM cases was: OA (24%); AA disorders (22%); LSDs (16%); defects on carbohydrate metabolism (10%); disorder on energy metabolism (10%); UCD (6%); peroxisomal diseases (5%); other (7%). We believe SIEM is an extremely important tool to increase awareness and to improve diagnosis and management of IEM in countries were such group of disorders is mostly unrecognized

(Acknowledge: Support/Milupa/SHS/Nutricia group).

006-P

PRE-IMPLANTATION GENETIC DIAGNOSIS: PREVENTIVE TOOL FOR NON-KETOTIC HYPERGLYCINAEMIA

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Introduction: Non-ketotic hyperglycinemia (NKH) is an autosomal recessive disorder characterized by a defect in glycine cleavage system. Patients present in the neonatal period with acute encephalopathy. No effective treatment is available. Up to 80% of NKH cases are caused by mutations in the P-protein. The *GLDC* gene spans at least 135 kb and consists of 25 exons.
Patient and methods: A family suffering from NKH caused by a deletion in the first 3 exons of the *GLDC* gene was enrolled in PGD program. Both parents are carriers for the mutation. Single leucocytes were separated for both parents and amplified by multiple displacement amplification for test optimization. Embryo biopsy was performed by laser assisting hatching followed by multiple displacement amplification and analysis of the blastomere DNA.
Result and discussion: IVF cycle produced 6 embryos, which were all successfully biopsied. Five out of the six embryos were found normal or carrier and one was homozygous for the deletion. Three embryos with good development after the embryo biopsy were transferred and a singleton pregnancy was obtained. A normal baby girl was delivered.
Conclusion: Up to our knowledge this is the first report showing the application of PGD as a preventive tool for NKH.

007-P

METABOLIC LEARNING CENTRE

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Project Metabolic Learning Centre is a two-year project aimed at lifelong learning and is supported by European social fund, state budget and budget of the capital of Prague.

The aim is to establish the Centre, learning and information centre of IMD and to create a pilot programme for lecturers who take part in education of IMD.

The target group is staff of the General Faculty Hospital and 1st School of Medicine of the Charles University in Prague, who participate in diagnostics, therapy, monitoring, genetic counselling, prenatal diagnostics, research and education of students, physicians, molecular geneticists and laboratory technicians in IMD.

Key activities include foundation and equipment of the Centre and four educational programmes: (1) Professional education in medical genetics including biochemical genetics – stress is laid on key principles of the subject and differential diagnostics on the clinical and laboratory level. (2) English language education – assistance towards the costs of learning English and of international language exams (PET, FCE and CAE). (3) Pedagogical-psychological education – to improve knowledge in professional education in adults. (4) Management education – to improve knowledge of personality evaluation, teamwork and time management.
Results of the project are lectures, Metabolic handbook 2006 and 2007, collection of papers, book Compendium of laboratory investigation of IMD, translation of the book J. Fernandez, J.-M. Saudubray, G. van den Berghe and J.H. Walter: Inborn Metabolic Diseases, Springer 2006, posters (main pathways and its disorders) and web pages www.udmp.cz with all texts.

Supported by the project Metabolic Learning Centre CZ.04.3.07/3.2.01.2/2048

008-P

SCREENING OF INHERITED METABOLIC DISORDERS BY TANDEM MASS SPECTROMETRY

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With the aim to prepare conditions for national-wide neonatal screening of inherited metabolic disorders (IMDs) in the Czech Republic by tandem mass spectrometry (MS/MS), we analysed 70 149 samples from newborns born between 2000 and 2006 and 22 901 samples from patients suspected from IMDs. Control group was 1130 blood spot samples from 118 patients with known IMDs.
Results: In the neonatal screening we detected 16 patients with IMDs: 10 × hyperphenylalaninemia (PKU/HPA), 3 × LCHAD deficiency, 1 × MCAD deficiency, 1 × 3-methycrotonyl-CoA carboxylase deficiency and 1 × methylmalonic acidemia. Incidence of IMDs in neonatal population in our catchment area is 1:4384. In the selective screening we detected 37 patients (10 × MCAD deficiency, 5 × PKU/HPA, 4 × multiple acyl-CoA dehydrogenase deficiency, 2 × glutaric aciduria type I, 3 × LCHAD deficiency, 4 × tyrosinemia type I, 2 × VLCAD deficiency, 2 × methylmalonic acidemia, 1 × propionic acidemia, 2 × CPT II deficiency, 1 × SCAD deficiency and 1 × β-ketothiolase deficiency). Profile of acylcarnitines was specific in all patients with known IMDs. Normal results were found in three samples of patients with SCAD, CPT II and LCHAD deficiency in well metabolically compensated states.
Conclusions: Routine neonatal screening by MS/MS is useful for diagnoses of selected IMDs. Early detection of IMDs enables appropriate medical treatment, close monitoring of metabolic status of a child and genetic counselling in families. We are prepared to start the national-wide neonatal screening program for 15 various IMDs by MS/MS in the Czech Republic.

The work was supported by the project VZ 64165 of Ministry of Health of Czech Republic.

009-P

EXPANDED NEWBORN SCREENING: LONG-TERM FOLLOW UP OF 63 PATIENTS SCREENED BY TANDEM MASS SPECTROMETRY

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There are quite different recommendations which genetic metabolic diseases should be screened. As a contribution to this discussion we report on the long term follow up of 63 patients (8 patients with amino acidemias except HPA, 12 patients with organic acidemias, 14 with urea cycle disorders, 29 with fatty acid oxidation disorders) found in the newborn screening program in Germany between June 1999 and March 2007. Documentation of clinical, biochemical and molecular genetic data were performed using our database www.ramedis.de. Observation time is up to 5 years of age. Two children died in spite of early treatment. 58 infants (92%) were detected by expanded newborn screening. Six newborns were symptomatic before results of screening tests were available. Twelve children showed metabolic changes where clinical relevance is not clear. In 49 children treatment was indicated, in 44 of them treatment was initiated on the basis of screening test results only. Thus, 44 out of 63 patients (69%) had direct profit from results of expanded newborn screening. In our opinion there is evidence that inclusion of these diseases in expanded newborn screening programs has a positive cost benefit especially in milder phenotypes which become clinically symptomatic in later age.

010-P

NEWBORN MASS SCREENING USING TANDEM MASS SPECTROMETRY: RESULTS OF VALIDATION AND COMPARISON OF TWO METHODS

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Introduction: Since April 2002 tandem mass spectrometry (MS/MS) was introduced in our neonatal mass screening program. We report our results of comparison between the derivatized and the non-derivatized assay procedures.
Methods: Blood was collected on S&S 903 filter paper between 3–5 days after birth. Acylcarnitines (AC) and amino acids (AA) concentrations (μmol/L whole blood) were determined against a mixture containing (d9)-carnitine, 11(d3)-AC stable isotopes (Cambridge Isotope Laboratories versus Neogram MS (d6C5-DC, d9C5) reagent kit, Perkin Elmer) and 10-12 AA stable isotopes (Cambridge Isotope Laboratories versus Neogram MS reagent kit, Perkin Elmer), using a Quattro Micro MS/MS, Waters. Data were interpreted using a multiple parameter profile testing system (Micromass).
Results: The recovery, the repeatability and reproducibility is comparable for the non-derivatized and the derivatized method assay procedure concerning the acylcarnitines analysis. We could not confirm the improved quantification of glutarylcarnitine using d6-C5-DC (Neogram non-derivatized assay) in our lab: C5-DC acylcarnitine remains difficult to measure and lots of false-positives are found.

For the amino acids the derivatized assay is superior while the recovery is excellent and the repeatability and reproducibility are good. The non-derivatized assay cannot be used for the detection and quantification of L-methionine.
Conclusions: (1) The non-derivatized method is suitable for screening of fatty acid oxidation defects, but less preferable for screening of amino acid disorders, with exception made for MSUD and PKU; (2) The non-derivatized method is faster and significantly simpler than the traditional derivatized method.

011-P

HIGH RISK SCREENING OF INBORN ERROR METABLOLISM USING TANDEM MASS SPECTROMETRY

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Tandem mass spectrometry (TMS) has been used for diagnostic work-up or high risk screening for fatty acid oxidation disorders (FAODs), some aminoacidopathies and organicacidopathies in other countries. Here we report 6 types of inborn errors of metabolism (IEM) diagnosed using MS/MS starting January 2005 until December 2006. Blood was collected from the heel prick of the high risk patients using a S&S 903 filter paper and dried for 4 h at room temperature. 3 mm punch was made and metabolites of acylcarnitine and amino acid were extracted from the blood using methanol mixed with deuterium-labeled internal standards. Derivatisation to the corresponding butyl derivatives were done and it was then injected in the MS/MS. About 1192 samples with the age range from day 1 until one year old were analyzed. Diagnoses were made based on the accumulation of diagnostic acylcarnitines and amino acids by TMS and abnormal compounds detected in urine organic acid analyses (UOAA) and plasma amino acids. Five patients with increased C3-acylcarnitine whom were confirmed cases of methylmalonic aciduria (MMA), one with maple syrup urine disease (MSUD), two with elevated citrulline and liver metabolites were confirmed cases of citrullinemia type 2, four with increased C5OH which were confirmed biotinidase deficiency, five with elevated C5-acylcarnitine were confirmed isovaleric acidemia (IVA) and one with elevated C4, C5 and C5DC was confirmed case of glutaric aciduria Type 2. The diagnostic rate is 1.5%.

012-P

EXPANDED NEWBORN SCREENING EXPERIENCE IN ISTANBUL

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Objective: To evaluate the impact of expanded newborn screening using tandem mass spectrometry (MS/MS) on the overall detection rate of inborn errors of metabolism (IEM) in Istanbul and to make recommendations for the future development of neonatal screening program in Turkey. **Methods**: 77,840 neonates were investigated for IEM by MS/MS between August 2005 and December 2006. The overall value of the screening program was estimated by complete ascertainment of all positive tests; definite assignment of all diagnoses including reconfirmation at 12 months. **Results**: During the study 93 diagnoses were confirmed, for an overall incidence of 1:837. Twenty-nine infants were identified with fatty acid oxidation disorders, 7 with organic acidaemias and 57 with aminoacidopathies. Identification of affected infants has allowed retrospective testing of other family members, resulting in an additional 15 diagnoses. Medium-chain acyl-CoA dehydrogenase deficiency (MCADD), 3-methylcrotonyl-CoA carboxylase deficiency and disorders of phenylalanine metabolism were the most common disorders detected. The high rate of C3-acylcarnitines allowed to determine an important nutritional problem of vitamin B12 deficiency at that population. **Conclusions**: To our knowledge the results of the study showed a very high rate of IEM detected by expanded newborn screening that was not mentioned before. The screening by MS/MS for IEM has approximately doubled the detection rate compared with that achieved by the conventional methods used in Istanbul. MCAD deficiency was detected ten times more as before.

Questions about the effectiveness and risks of expanded newborn screening need to be answered prior to its widespread acceptance as a state-mandated program.

013-A

A PILOT STUDY FOR NEONATAL METABOLIC SCREENING BY GC/MS – AN 11-YEAR STUDY IN NORTHERN KYUSHU, JAPAN

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Objective: To examine the usefulness and applicability of mass spectrometry, particularly the GC/MS method using urine, as a means of mass screening for neonatal metabolic disorders. **Methods**: The test was conducted on urine collected from 68,913 newborns in Northern Kyushu over 11 years from January 1996 to December 2006. Samples were prepared using a simplified urease-pretreatment and stable isotope dilution, and the urinary metabolites were simultaneously analyzed and chemically diagnosed by GC/MS.
Results: A total of 57 cases consisting of 16 cases of disease, including 10 cases of methylmalonic aciduria (MMA), were found in 68 913 newborns. The total incidence was 1 out of 1200 subjects, and particularly, the incidence of MMA was 1 out of 6900 subjects, which is the highest incidence of inherited metabolic disorders in Japan.
Conclusion: These results proved the sensitivity and diagnostic accuracy of the GC/MS method. Interestingly, it was found that MMA, is a very important disease to be screened for in Japan, due to its frequency. Moreover, a mild form of MMA, for which screening is considered most beneficial, has been shown to have a very high probability of being missed by the MS/MS method. These findings indicate that mass spectrometry is very useful as a new method for neonatal screening in Japan and the GC/MS method should therefore be implemented to screen for methylmalonic aciduria.

014-P

PILOT PROJECT ON NEONATAL SCREENING OF INBORN ERRORS OF METABOLISM (IEM) USING TANDEM MASS SPECTROMETRY IN MALAYSIA

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In Malaysia, neonatal screening program for IEM has not been established yet. A 2 years pilot project starting from September 2006 has been carried out to serve as a preliminary finding for future program. The aim of this study is to determine the normal reference ranges and cut off values for various analytes (amino acids and acylcarnitines) in dried blood spot and to identify the incidence and prevalence of IEM in Malaysia. The research is done with collaboration with 11 major hospitals in Malaysia. Blood spots were collected from the heel-prick of the neonates aged 24 h to 7 days, born in the participating hospitals and send to this laboratory. Screening of samples for amino acids and organic acids disorders and fatty acids oxidation defects by analysing acylcarnitines and amino acids levels were conducted by using TMS. Samples with abnormal results will be repeated and the patients will be recalled to confirm with follow-up testing: plasma amino acids and urine organic acid. From September 2006 till December 2006 about 13 793 samples have been received and analysed. 6 positives IEM cases were diagnosed. 2 babies with elevated C4 acylcarnitine, 3 babies with elevated leucine/isoleucine but 2 were confirmed having Maple syrup urine disease while the other has E3 deficiency. One baby has elevated C3 and found to have moderate elevation of methylmalonate in the urine. Incidence of IEM at this point is 1 in 2300 deliveries. Normal reference range for amino acids and acylcarnitines have been calculated from this study.

015-P

STABILITY OF ACYLCARNITINES AND FREE CARNITINE IN DRIED BLOOD SAMPLES – INFLUENCE ON ACCURACY OF QUANTITATION AND LIMITATIONS FOR RETROSPECTIVE ANALYSES

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Background: Whole blood dried on filter paper is the standard specimen for newborn screening. More recently the spectrum of analytes measured from dried blood spots has been expanded to acylcarnitine profiles for the detection of fatty acid oxidation disorders, carnitine cycle disorders, and organic acidurias. Dried blood specimens are also used for high risk screening and follow up. However, the accuracy of the quantitation of acylcarnitines and free carnitine in dried blood spots is controversially discussed due to the known instability of acylcarnitines.
Methods: Whole blood spiked with acylcarnitines was stored (1) at –180°C for 330 days and (2) at room temperature up to 1000 days, and measured repeatedly within the routine newborn screening. 3.2 mm spots of these samples were extracted with 150 µl of methanol. Free carnitine and acylcarnitines were measured by electrospray ionization-tandem mass spectrometry (ESI-MS/MS) after conversion to their corresponding butyl esters.
Results: At –180°C acylcarnitines are stable for at least 330 days. At room temperature acylcarnitines are hydrolyzed to free carnitine and the corresponding fatty acids. The velocity of the decay depends upon the chain length of the acylcarnitines. The decay obeys a first order kinetics ($y = e^{b*t}$). Shorter acylcarnitines hydrolyze quicker than long-chain acylcarnitines ($t_{1/2}$-palmitoylcarnitine: 1733 d, $t_{1/2}$-propionyl-carnitine: 224 d).
Conclusion: Retrospective quantitation of free carnitine and acylcarnitines on filtercards requires the utilization of correction factors and standardized storage within a strictly quality controlled laboratory setting.

016-P

PRIMARY CARNITINE DEFICIENCY IN ASYMPTOMATIC ADULT FEMALE DIAGNOSED BY LOW FREE CARNITINE IN THE NEWBORN

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Newborn screening (NBS) for IEM is active in Liguria region (North-Western Italy) since June 2005. We report the first Italian case of maternal IEM diagnosed by abnormalities found in the newborn at NBS.

At 3rd day of life, a newborn male showed a free carnitine (C0) concentration of 5.9 microM (lower cut-off: 17.7) and low long-chain AC profile. This results was confirmed on two further bloodspots (C0 4.9 and 4.5 at day 10 and 13 respectively). The breast fed baby showed no clinical nor biochemical abnormalities other than low C0 in plasma (3.1, controls: 4–29) and in urine (4.7, controls: 22–47 micromol/mmol creatinine). The mother, asymptomatic even during the pregnancy and delivery, was investigated as well and showed low long-chain ACs and plasma C0 (2.4) and normal urine C0 (13.7). Oral carnitine supplementation (100 mg/kg/day) in the newborn led to restoration of plasma C0 levels (72 after two weeks) without increase of urine C0, excluding a defect in carnitine uptake (CUD).

The same approach to the mother showed a slight increase of plasma C0 (34.7) but a significant raise of urine excretion to 221. These levels remained steadily up to one month.

Carnitine transport in cultured fibroblasts was 0.09 nmol/ml cell water/h (control 1.51) confirming a primary carnitine deficiency. The lady, still under carnitine supplementation, is clinically well. Molecular studies are in progress.

017-A

ONE CASE OF 3-METHYLCROTONYLGLYCINURIA FOUND IN NEONATAL METABOLIC SCREENING USING MASS SPECTROMETRY

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Mainly in Northern Kyushu, we performed neonatal screening for inherited metabolic disorders by the GC/MS method using neonatal urine from 1996, and using the MS/MS method with dried blood spots from 2004. During this period, we found a female infant with 3-methylcrotonylglycinuria who developed the disease along with a disturbance of consciousness and convulsions at the age of 1 year and 3 months and survived thanks to a prompt chemical diagnosis by GC/MS. A GC/MS urine analysis of the patient confirmed the presence of a large amount of 3-methylcrotonylglycine and 3-hydroxyisovaleric acid and was chemically diagnosed to have a 3-methylcrotonyl-CoA carboxylase (MCC) deficiency. In the MS/MS blood spot analysis submitted at the same time, increased C5-OH carnitine was observed and the results obtained strongly supported the chemical diagnosis by GC/MS. In the MS/MS analysis of reserved Guthrie blood spots obtained from the same patient on the 5th day after birth, increased C5-OH carnitine was observed, from which an increased urinary excretion of 3-methylcrotonylglycine and 3-hydroxyisovaleric acid could thus be inferred. From these results, it was considered that GC/MS screening can sufficiently detect early stage MCC deficiency in order to prevent the onset of this disease, and it was strongly suggested that there is a need to actively begin early neonatal screening using mass spectrometry in Japan.

018-P

GENETIC HETEROGENEITY AND DIVERSITY OF BIOCHEMICAL PHENOTYPES OF MEDIUM-CHAIN ACYL-CoA DEHYDROGENASE DEFICIENCY AS REVEALED BY POPULATION SCREENING

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Background: Medium-chain acyl-CoA dehydrogenase deficiency (MCADD) is the most common defect in fatty-acid-β-oxidation with variable clinical expression. The genotypes of individuals identified by newborn screening (NBS) differ from those identified clinically. The exact population spectrum of genotypic and phenotypic variants and validation data are missing. Aims of the study were to characterize a biochemical borderline population of NBS for MCADD, to estimate the false negative rate, and to provide a validation strategy as model for NBS-evaluation of conditions with unknown disease expression. **Methods**: Case-control cohort study utilizing retrospective genotyping of two *ACADM* gene variants (c.985A>G, c.199T>C) in 333 NBS samples with biochemical abnormalities >/=95th percentiles, judged unremarkable in NBS, 333 controls, 34 patients. Receiver-operated-characteristics (ROC) to discriminate *ACADM* variants. **Results**: The carrier frequency of both mutations correlated to increasing octanoylcarnitine concentrations. c.985A>G was identified more often in the study group (1:4.3 vs. 1:42) showing that carriers can display a borderline phenotype. ROC revealed that the octanoylcarnitine concentration and the ratio octanoylcarnitine/dodecanoylcarnitine were reliable markers to discriminate patients from carriers and to decrease the false positive rate. Three out of 470 247 newborns retrospectively turned out to be false negative. **Conclusions**: Since the course of milder variants cannot be evaluated by observational data, our validation strategy could serve as a model for assessing population heterogeneity of conditions with unknown clinical penetrance. Identification of false negatives confirmed the expected limitations to detect all variants. Nevertheless, MCADD-NBS can be accomplished with excellent performance due to a low recall rate and a high biochemical discriminatory power.

019-P

ENZYMATIC ANALYSIS OF MCAD, VLCAD, GLUTARYL-CoA DEHYDROGENASE (GCDH), AND ISOVALERYL-CoA DEHYDROGENASE (IVD) IN LYMPHOCYTES WITH IMPLICATIONS FOR NEONATAL SCREENING

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Background: Existing neonatal screening programs around the world have been expanded recently and now include a range of different IEMs. In the Netherlands PKU, CHT, and AGS were the only three disorders screened for until recently. Starting January 1, 2007, the following IEMs have been added: (1) MCADD; (2) VLCADD; (3) LCHAD/MTP deficiency; (4) biotinidase deficiency; (5) galactosemia; (6) glutaric aciduria type 1; (7) HMG-CoA lyase deficiency; (8) holocarboxylase synthase deficiency; (9) homocystinuria; (10) isovaleric academia; (11) maple syrup urine disease; (12) 3-methylcrotonyl-CoA carboxylase deficiency, and (13) tyrosinaemia type 1. A major problem with the expanded neonatal screening program is the occurrence of false-positives. Discrimination between true- and false-positives requires straightforward and unequivocal methods of detection. To achieve this goal we have decided to generate simplified procedures for the enzymatic analysis of all disorders included in the expanded Dutch neonatal screening program in lymphocytes. **Methods:** Lymphocytes from controls and established patients were isolated according to standard procedures and incubated in different reaction media with specific substrates for MCAD, VLCAD, GCDH and IVD dehydrogenase. **Results:** Using ferricenium hexafluorophosphate rather than ETF each of the four enzymes could be detected in lymphocytes. We have determined the intra- and interassay variation coefficients for each of the four enzymes and have established the feasibility of the methods by measuring activities in lymphocytes from established (i.e. genetically confirmed) patients with excellent results in all cases. **Conclusion:** Direct analysis of enzyme activities in lymphocytes, is an excellent method to discriminate between true- and false-positives.

020-P

RAPID SECOND TIER TEST FOR MEASUREMENT OF 3-OH-PROPIONIC AND METHYLMALONIC ACIDS ON DRIED BLOOD SPOTS: A NEW APPROACH FOR REDUCING FALSE-POSITIVE RATE FOR PROPIONYLCARNITINE DURING EXPANDED NEWBORN SCREENING BY LC-MS/MS

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The expanded newborn screening programs have increased the diagnosis of inborn errors of metabolism in the presymptomatic phase. However, it has also led to a significant increase in the number of false-positive results. This is costly for public health resources and causes anxiety for parents.

Since propionylcarnitine is one of the analytes most frequently responsible for false-positive results, we developed a rapid LC-MS/MS method that identifies free methylmalonic and 3-OH propionic acids in blood spots thus reducing false-positive rates due to C3 during expanded newborn screening programs. This method could also be suitable for diagnosing and routinely monitoring blood spots for methylmalonic aciduria and propionic acidemia.

Analytical method consists of chromatographic separation on a C6-Phenyl column of an extracted 3.2 mm dried blood spot and injection into triple quadrupole mass spectrometer equipped with a Turbo Ion Spray Ionization Source. No derivatization is required and total analysis time is 5 min per sample.

Of 124 infants recalled for abnormal C3, only five were truly affected, with a positive predictive value of 4%. If this method had been applied as a second tier test only the five true-positives would have been recalled for additional samples and the positive predictive value would have been 100%. Instead, 119 infants were recalled unnecessarily with false-positive results for C3.

021-P

MORE THAN ONE *GCDH* MUTATION CAUSES GLUTARIC ACIDURIA TYPE 1 (GA1) IN A CANADIAN ABORIGINAL GENETIC ISOLATE

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In 1991 we described a severe variant of GA1 in a remote aboriginal genetic isolate spanning northeastern Manitoba and northwestern Ontario. Patients are low excretors of glutaric acid (GA) and 3-hydroxyglutaric acid (3-OHGA), demonstrate significant residual enzyme activity and normal acylcarnitine profiles. All have been homozygous for a splicing mutation (*GCDH* IVS-1 +5 g>t) and carrier frequency is ~1 in 10. Between 1999–2006, 9 of 4300 newborns from the high-risk communities genotyped in Winnipeg, Manitoba were homozygous for the splicing mutation and confirmed to have GA1. In August 2006 province-wide newborn screening by tandem mass spectrometry (MS/MS) began in neighbouring Ontario. A newborn from a high-risk community with grossly elevated glutarylcarnitine (C5DC) was referred to Winnipeg, the closest metabolic centre and confirmed to have GA1 with highly elevated urinary GA and 3-OHGA. The duplicate specimen sent to Winnipeg at birth for *GCDH* IVS-1 +5 g>t genotyping had shown heterozygosity. This aboriginal baby was subsequently found to be a genetic compound with one paternal copy of *GCDH* IVS-1 +5 g>t and a 2nd novel maternally inherited frameshift mutation c.1209delG in exon 10. We then performed acylcarnitine analysis by MS/MS on newborn spots of 12 Ontario babies from this genetic isolate born in 2006, genotyped in Manitoba as carriers and reported as unaffected. These analyses were normal, in agreement with the genotyping results. We conclude that even in genetic isolates there can be molecular heterogeneity for rare recessive traits. Targeted DNA-based newborn screening must proceed cautiously even in genetic isolates.

022-P

EFFECTIVE AND AFFORDABLE 1ST TIER NEWBORN SCREENING (NBS) FOR TYROSINEMIA TYPE I (TYR-I)

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Background: TYR-I is a severe disorder causing early death if left untreated. NBS is problematic because tyrosine is a nonspecific marker for TYR-I and so far the determination of the diagnostic marker, succinylacetone (SUAC), required a separate 1st tier or only partially effective 2nd tier analysis based on tyrosine level. To overcome these problems we developed a new assay that simultaneously determines acylcarnitines (AC), amino acids (AA), and SUAC in dried blood spots by flow injection tandem mass spectrometry (MS/MS).
Methods: 3/16 inch DBS punches are extracted with 300 5L methanol containing AA and AC internal standards (IS). This extract is derivatized with butanol-HCl. Meanwhile, SUAC is extracted from the residual filter paper with 100 5L of a 15 mM hydrazine solution containing 13C5-SUAC as IS. The derivatized aliquots are then combined in acetonitrile for traditional MS/MS analysis of AC and AA now including additional MRM experiments for SUAC (m/z 155 to 137) and its IS (m/z 160 to 142). Analysis time is 2.5 min.
Results: SUAC was found to be elevated in the retrospective analysis of original NBS samples of two TYR-I patients (35 and 78 μmol/L) with Tyr levels of 70 and 114 μmol/L, respectively (abnormal >150) compared to 350 controls (SUAC mean: 1.08; 99th percentile: 2.08).
Conclusion: The inclusion of SUAC analysis into the routine analysis of AC and AA allows for rapid, cost effective NBS for TYR-I with no tangible risk of false negative results.

023-A

ALCAPTONURIA DIAGNOSIS IN EXPANDED NEWBORN SCREENING MS/MS USING DRIED BLOOD AND URINE SAMPLES

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Background: Alcaptonuria is a rare autosomal recessive disease in which homogentisinic acid (HA), an intermediary product in the metabolism of phenylalanine and tyrosine, cannot be further metabolized and is excreted in the urine. Galician's Newborn Screening (NS) Program includes blood and urine filter paper strips collected at the third day of life (SS-903); since 2000, ESI-MS/MS is performed to analyze aminoacids (AA) and acylcarnitines (AC) in blood. In 2003 we developed specific methods for screening dried urine samples to detect AA and AC in positive mode and acylglycines and organic acids in negative mode (Quim Clim 2004; 23 (5):282). We use these analysis as second level tests. **Methods**: Tandem mass spectrometry was carried out with an API 2000 (Sciex Applied Biosystems). Data were acquired and processed using Analyst 1.4 and Chemoview software. We used MRM experiments to detect AA and organic acids and precursor scans for AC and acylglycines. Quantification was based on isotope-labeled internal standards. **Case report:** Newborn female who showed, in NS analysis (age 48 h), moderate elevation of tyrosine: 2655 M (normal<236). Second level tests on dried urine sample at same age showed an important excretion of homogentisic acid: 7000 mmol/mol creatinine (normal<6). Alcaptonuria was confirmed at 18 days of life (HA:10642 mmol/mol creatinine). Until the moment (age, 6 months) the patient is asymptomatic and she receives vitamin C and moderate protein restriction as preventive treatment. **Conclusion**: In this case, the expanded NS by MS/MS combining dried blood and urine samples shows the usefulness for early diagnosis of alcaptonuria.

024-P

FIVE FAMILIES WITH HYPERMETHIONINEMIA ASSOCIATED WITH THE DOMINANTLY INHERITED METHIONINE ADENOSYLTRANSFERASE I/III FORM DEFICIENCY

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Methionine adenosyltransferase deficiency (MAT I/III deficiency, MIM 250850) is an inborn error of metabolism resulting in isolated hypermethioninemia. This enzyme (MAT, E.C 2.5.16) catalyses the biosynthesis of S-adenosylmethionine from methionine and ATP, and both forms of hepatic MAT (MAT I and III) are encoded by the *MAT1A* gene. In the majority of the cases, the MAT I/III deficiency is inherited as an autosomal recessive trait, although a dominant form has been reported in several families, associated with the R264H mutation.

In two years of expanded newborn screening, six cases were confirmed to have increased plasma methionine levels. In one of these cases, a cystathionine beta-synthase deficiency was confirmed by molecular analysis. In the other five cases the persistent observation of slightly increased plasma levels of methionine (70 to 170 μmol/l) and total homocysteine (11–13 μmol/l) lead to the molecular analysis of *MAT1A* gene. All five newborns revealed to be heterozygous for R264H mutation and no other molecular lesion could be found in this gene.

In two families, the presence of slightly increased levels of plasma methionine and total homocysteine was already confirmed in one of the parents, thus indicating the presence of a MAT I/III deficiency form with dominant inheritance. The other families are still under study.

The high frequency that R264H mutation seems to have in Portugal indicates the importance of the molecular studies in the Portuguese families with MAT I/III deficiency in order to identify the individuals with an increased risk factor for strokes.

025-P

EXTREME INCIDENCE OF HYPERMETHIONINEMIA ON A SINGLE NEONATAL INTENSIVE CARE UNIT: A PITFALL IN A NEWLY INTRODUCED NEONATAL SCREENING PROGRAM

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Background: From January 1, 2007 an expanded neonatal screening program has been initiated in the Netherlands, including homocystinuria with methionine as the primary marker. During the first 2 months hypermethioninemia was detected in 14 newborns that, after proper evaluation, were demonstrated not to have homocystinuria. Remarkably, all these children were admitted to a single neonatal intensive care unit (Academic Medical Center, Amsterdam (AMC-NICU)). **Aim**: To evaluate the underlying causes of hypermethioninemia in the AMC-NICU. **Methods**: The cohort of newborns with hypermethioninemia (group 1) was compared to the cohort of neonates with normal screening results admitted to the AMC-NICU in the same time period (group 2). In addition, enteral and parenteral nutrition protocols from all NICU's in The Netherlands were compared. **Results**: Mean birth weight and gestational age were significantly lower in group 1 than in group 2. All patients in group 1 received parenteral feeding (TPN) at the time of screening and a higher mean amino acid intake per kilogram body weight than patients receiving TPN in group 2. Also, the AMC-NICU uses a different amino acid mixture for TPN than the other Dutch NICU's, containing 2.5 times more methionine per ml. **Conclusion**: The extremely high incidence of hypermethioninemia on the AMC-NICU is explained by a combination of low birth weight, low gestational age, and high protein intake supplied by a specific parenteral amino acid mixture containing large amounts of methionine. In order to prevent this hypermethioninemia the protocol for TPN in the AMC NICU will be reconsidered.

026-P

EXTENTED NEONATAL SCREENING FOR HOMOCYSTINURIA IN THE QATARI POPULATION

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Total homocysteine (t-Hcy) is the direct biochemical parameter for classical homocystinuria. Cystathionine β-synthase (CBS) deficiency is a common inborn error of metabolism in Qatar with an estimated prevalence of 1:3000 newborns. We developed a reliable HPLC method with tandem mass spectrometry detection for extended neonatal screening to determine t-Hcy in dried blood spots (DBS). For mutation analysis in DBS, common mutations R336C and D234N in the *CBS* gene were investigated only in native Qatari. Both methods are suitable for processing a large number of samples.

We analyzed 10 679 newborns, 4559 of them of Qatari origin. Five neonates with homocystinuria were identified showing highly elevated t-Hcy concentrations in DBS whilst methionine was elevated in two neonates only. As far as it is known, no patient with CBS deficiency has been missed by this method. Four children were homozygous for the common mutation R336C. Sequence analysis in the fourth patient revealed homozygosity for mutation G347S, not previously observed in the Qatari population. In conclusion, metabolic screening of t-HCY in DBS is a promising method for the identification of newborns with CBS deficiency.

027-P

CYSTATHIONINE β-SYNTHASE DEFICIENCY: DIAGNOSIS AND EARLY MANAGEMENT OF PATIENTS DETECTED BY TANDEM MASS SPECTROMETRY NEWBORN SCREENING

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Background: Tandem mass spectrometry screening is now universal in Australia. About 1.5 million babies have been screened to April 2007. For cystathionine β-synthase deficiency (CBS) there are no reports of pyridoxine-responsive patients detected by newborn methionine screening, worldwide.
Methods: The cut off level for methionine was 53–67 μmol/L in the 5 programmes. Using data from one state's cohort of 53 patients with CBS, we would expect 16–17 cases in our cohort, 8–9 being pyridoxine non-responsive.
Results: We detected six babies with CBS deficiency. Initial dried blood spot methionine levels were 64, 77, 81, 120, 167, and 190 μmol/L on days 2–4. Confirmatory plasma levels were: free homocystine 10–69, total homocysteine 110–653, methionine 137–934 μmol/L on days 13 to 24. All were B6 non-responsive on formal challenge. Treatment began at 16-31 days. For 4 patients biochemical control was rapid, with free homocystine levels below 10 μmol/L by age 35 days. The two patients with the highest newborn methionine levels were difficult to control initially. All patients had additional folate and a low dose of pyridoxine. In two, betaine and vitamin B12 were started early. Total homocysteine levels in all patients remained > 100 μmol/L for a prolonged period, even when free homocysteine levels were < 3 μmol/L.
Conclusions: Newborn screening by tandem mass spectrometry does not detect pyridoxine-responsive CBS and some pyridoxine non-responsive patients could be missed using methionine as sole indicator. Initial confirmation depends on measurement of plasma homocyst(e)ine and methionine, both significantly elevated. Dietary restriction rapidly controls plasma free homocysteine.

028-P

HYPOCITRULLINEMIA IN EXPANDED NEWBORN SCREENING REVEALS AN OTC DEFICIENCY

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Background: Low blood citrulline levels is a marker for defects in the first two steps of urea cycle, ornithine transcarbamylase (OTC) and carbamoyl phosphate synthetase I, and for some mitochondrial disorders (NARP and MELAS syndromes). However, these inborn errors are not currently included in conventional newborn screening programs.
Methods: Expanded newborn screening using liquid chromatography tandem mass spectrometry (LC-MS/MS) on dried blood spot (DBS) began in Tuscany in 2001.
Results: Screening results showed low citrulline levels (2.4 μmol/L, n.v. 3–25 μmol/L) in a male newborn. At first recall, the baby presented feeding difficulties, failure to thrive, tremors and irritability. Because of the clinical picture, a second test was performed on DBS and plasma samples. Hypocitrullinemia was confirmed on the plasma sample (4 μmol/L, n.v. 9-33 μmol/L), whereas on DBS citrulline levels were just above the lower range (3.7 μmol/L, n.v. 3–25 μmol/L). Ammonia, glutamine, transaminases and orotic acid were normal. Family history was positive for hypertransaminasemia in patient's sister and maternal aunt. Molecular analysis of the patient's *OTC* gene identified the hemizygous c.794G>T (p.Trp265Leu) mutation, previously reported in mild OTC patients. His mother, sister and aunt resulted heterozygous for this mutation.
Conclusions: To our knowledge, this is the first case of OTC deficiency identified through expanded newborn screening. We would like to underline that in cases of hypocitrullinemia the clinical picture and family history became important to justify other investigations as molecular analysis, even if citrulline is normal in the second DBS.

029-P

HYPERARGININEMIA: A NEW CASE IDENTIFIED BY EXTENDED NEONATAL SCREENING

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Background: Hyperargininemia (OMIM 207800) is an autosomal recessive disorder caused by a defect in the arginase I enzyme (EC 3.5.3.1.). Unlike other urea cycle disorders, this condition is not generally associated with a hyperammonemic encephalopathy in the neonatal period or in early infancy. Patients with hyperargininemia manifest a neurological syndrome that consists of cognitive deficits, epilepsy, and progressive spastic diplegia.

A favourable outcome can be achieved if dietary treatment and alternative pathway therapy are instituted early in the disease course.

Early diagnosis of this disorder through newborn screening programs may lead to a better outcome.

Methods: Accumulation of arginine, the biochemical hallmark of this disorder was measured by tandem mass spectrometry (MS/MS).

Results: Among 150 000 newborns screened in our laboratory a case of hyperargininemia was identified. The newborn screening sample taken at 5th day had an arginine level of 50.3 μmol/L (controls: 2–32) and at confirmation 57.4 μmol/L and 120.1 micromoles/L values were found at 18th day and 31th day of life, respectively. Until this time the orotic acid and organic acid profile were normal.

The guanidine compounds derived directly from the arginine in the patient as well as the molecular data (homozygous for T253I) confirmed the suspected diagnosis of hyperargininemia.

Conclusion: Further research is necessary to determine the sensitivity of MS/MS to detect hyperargininemia due to slight values detected at screening time.

030-P

NEWBORN SCREENING AND DIAGNOSTICS OF GALACTOSEMIA TYPE I IN RUSSIA

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A newborn screening program for the early detection of classic galactosemia was started in Russia in 2006. 350 000 newborns have been screened for five last months. The galactose-1-phosphate uridyl transferase (GALT) activity was measured and DNA analysis, based on PCR with subsequent restriction analysis, was performed for 43 newborns with increased level of galactose. The determination of GALT enzyme activity was done by the modified quantitative Beutler test. Mean GALT enzyme level was 1.18 + 0.89 U/gHg in patients and 3.37 + 1.14 U/gHg in carriers (normal 7.94 + 2.33). Five cases of classic galactosemia, three cases of heterozygotes for K285N, ten cases of compounds Q188R/D314N, seven cases of heterozygotes for D314N and three cases of homozygotes for D314N were identified. 15 from 43 newborns had normal GALT activity and had not the Q188R, K285N, D314N mutations. 23 patients with classic galactosemia clinically diagnosed previously and three patients detected by newborn screening were analyzed for the mutations in the *GALT* gene. DNA studies were carried out by automated DNA sequencing of all the 11 exons and the exon-intron boundaries of the *GALT* gene. 50 from 52 mutant alleles were detected. The prevalent mutations were Q188R (57.7%) and K285N (15.4%). The other mutations were IVS3nt-2a>c (3.8%) E352Q (3.8%), L358P (3.8%), F95L, M142K, L264P, Y286X, W316X, R333W (1.9% each). Four novel mutations Y286X, W316X, E352Q and L358P were found.

The detection of *GALT* gene mutations in newborns from Russia should focus first on N314D, Q188R, K285N.

031-P

EXTRACTION OF 17-HYDROXYPROGESTERONE (17-OHP) FROM DRIED BLOOD SPOTS WITH DIETHYLETHER PRIOR TO STANDARD ELISA-TEST SIGNIFICANTLY REDUCES THE FALSE POSITIVE RATE IN NEWBORN SCREENING FOR CONGENITAL ADRENAL HYPERPLASIA (CAH)

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Background: While the sensitivity of newborn screening for the classical (salt wasting) form of CAH is good, the positive predictive value is poor due to the high false positive rate of the immunological assay for 17-OHP. Cross-reactivity with steroid-sulfates is one of the main causes for false positive results. Several approaches have been described to improve CAH screening like adjusting cut-off levels to gestational age or birth weight or second-tier molecular genetic analysis.

Methods: 17-OHP was extracted with diethyl ether from dried blood samples in order to separate 17-OHP from polar steroids (like steroid sulfates). The dried ether extracts of calibrators, controls, and patient samples were redissolved and measured with the 17-OHP test kit (Wallac).

Results: In a retrospective study, 358 normal, 234 false positive, and 200 samples of confirmed cases with CAH were analysed. 17-OHP values were significantly lower after extraction: Normal samples: 16.9 nmol/L vs. 3.1 nmol/L; FP: 99.4 nmol/L vs. 26.6 nmol/L; CAH: 265 nmol/L vs. 225 nmol/L. With the application of a cut-off value of 12.3 nmol/L (mean + 3 SD of the normal values), 124 of the false positives turned out to be normal. All confirmed cases of CAH were also positive after ether extraction.

Conclusions: The false positive rate of CAH screening can be reduced by about 50% by ether extraction without loosing sensitivity.

032-P

NEWBORN SCREENING FOR OLIGOSACCHARIDOSES

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Oligosaccharidoses are lysosomal storage diseases with a low prevalence considered individually, however, when considered as a group, the combined prevalence is substantially higher. Effectiveness of treatment of some LSDs lies on the early detection through a newborn screening program. We developed an screening method for total urine oligosaccharides quantification based on the formation of a blue-green compound in presence of anthrone. We use urine samples impregnated on paper (Whatman 903) received from the Galicia Newborn Screening Program. We have performed the procedure described by Dische (1955) adapted to microtiter plate. A solution of anthrone (1%w/v) in 95% H_2SO_4 produces a blue-green color when added 100 microliters of reagent to 25 microliters of paper eluate (obtained by eluting with 300 microliters of water, 4 discs of 6 mm of diameter) in a 96 microtiter plate. Glucose range from 5 to 80 mg/dl. After measuring 2200 samples, the percentile 99 was 800 mg glucose/dl and 70 mg glucose/mg creatinine. The standard regresion was y = 0.0164x+0.41 (we did a calibration curve for each run). LOD = 0.1524 mg/dl and LOQ = 16.29 mg/dl both referred to glucose. We compared results obtained by this method with previous analyses of the same samples so we saw it is a sensible, simple, simple, reproducible method and suitable for oligosaccharides quantification in neonatal screening.

033-P

GUANIDINOACETATE MEASUREMENT IN NEWBORN SCREENING SAMPLES

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Background: Guanidinoacetate methyltransferase deficiency (GAMT; OMIM 601240) belongs to the group of primary creatine deficiency disorders. Clinical features include early onset severe epilepsy, progressive psychomotor delay, movement disorders, pallidal lesions on MRI, autism and severe mental retardation. Reports on GAMT treatment support the hypothesis that an early treatment can improve the prognosis.

The increasing number of GAMT patients diagnosed, along with the availability of effective treatment, makes this one of the interesting diseases to be included in Newborn Screening (NBS) Programs. Guanidinoacetate (GAA) is considered a reliable marker for GAMT deficiency, although no information is available about values presented by patients during first days of life.

Objectives: In order to evaluate variability of GAA values in Portuguese newborn population and the feasibility of this screening, we set up GAA measurement in blood spots by MS/MS, including it in our routine NBS protocol for aminoacids and acylcarnitines. We also tested samples from four older patients.

Results and **Conclusions**: We tested 3534 neonates and the average of GAA was 1.19 micromoles/L, with a standard deviation of 0.62. The adult patients presented GAA values of 20.59 micromoles/L, 26.54 micromoles/L, 18.66 micromoles/L and 19.33 micromoles/L.

The inclusion of GAA measurement in routine MS/MS analysis allows its easy introduction in newborn screening programs. Although, more studies are needed in order to evaluate the sensitivity of GAA in NBS of GAMT deficiency.

034-P

NEWBORN SCREENING FOR POMPE DISEASE USING MS/MS

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Background: With the advent of novel treatment modalities in lysosomal storage diseases (LSD) such as bone marrow transplantation and/or enyzme replacement therapies, newborn screening for LSD has become a focus point. From a technological perspective high-throughput newborn screening for LSD may be feasible using different analytical approaches. Among these, screening by tandem-mass spectrometry using unique, specific substrates and internal standards seems to be the most promising method as enzyme activities can be readily measured in dry blood spots from neonatal filter cards. In particular newborns with Pompe Disease are amenable to early enzyme replacement therapy and would potentially benefit the most from newborn screening.

Methods: Routine neonatal screening filter cards were punched into 96 well plates and extracted with methanol. Unique Pompe specific substrate and internal standard were added to the solution and the plates incubated overnight at 37°C. Following liquid/liquid and solid phase extraction steps 10 µl of solution were injected into the MS/MS. The formation of product and internal standard were monitored using MRM.

Results: GAA activity in 1560 dry blood spot samples: 15.50 ± 7.86 µmol/l/h; median 14.06; GAA activity in 3 adult Pompe disease: mean 0.37 µmol/l/h (0.16–0.93); median 0.19.

Conclusion: Although newborn screening for Pompe disease using MS/MS may be technically feasible, additional pilot studies have to demonstrate its validity, sensitivity, specificity and the potential to multiplex with additional LSD. In addition, strategies for confirmatory testing, treatment, follow-up care and scientific evaluation have to be defined and agreed upon at an international level.

035-P

ELUCIDATION OF THE ORIGIN OF L48S *PAH* MUTATION IN SERBIAN POPULATION

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In Serbian population, 19 phenylalanine hydroxylase gene (*PAH*) mutations were identified. The most frequent one, L48S (21%), was reported in many populations with low relative frequency (<13%). The prominence of L48S detected in Serbia, suggests the influence of either founder effect and genetic drift or its autochthonous origin.

Twelve patients, compound heterozygotes (L48S/other mutation), and one homozygote for L48S were analyzed by PCR-RFLP. Since L48S was associated with just four haplotypes (3, 4, 16 and 28) in the populations studied so far (www.pahdb.mcgill.ca), we focused on EcoRI and XmnI polymorphisms. Expected heterozygosities for these two sites have been calculated. Also, *PAH* gene intron 5 DNA fragment of patient homozygous for L48S mutation was sequenced.

We found that all patients were heterozygous ($\pm$) for EcoRI restriction site. Heterozigosity of EcoRI site in patient homozygote for L48S was confirmed by DNA sequencing. For XmnI restriction site, there were 4 homozygotes (+/+) and 9 heterozygotes ($\pm$). Calculated expected heterozygosities for EcoRI and XmnI sites were 0.5 and 0.46 respectively.

Based on high heterozygosity for the analysed polymorphic sites, we conclude that more than one haplotype is associated with L48S mutation in Serbian population. The unique haplotype associated with L48S would suggest its independent (Serbian) origin. Therefore, our results exclude the posibility that L48S mutation originates from Serbia and suggest that it was imported from populations with different genetic backgrounds.

Family study of all patients with L48S mutation will provide full and final information about haplotypes in Serbian population and definitely elucidate its origin.

036-P

INVESTIGATION OF PHENOTYPE–GENOTYPE CORRELATIONS FOR PATIENTS WITH PHENYKETONURIA IN LATVIA

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Phenylketonuria, the most severe form of hyperphenylalaninemia (HPA), is caused by deficient activity of the hepatic enzyme phenylalanine hydroxylase (PAH) which results from mutation at the *PAH* locus on chromosome 12q23.1.

For diagnostic and therapeutic purposes is used the following phenotype classification: 'classic', 'moderate' and 'mild' PKU and 'mild hyperphenylalaninemia'.

The prevalence of the R408W mutation among Latvian PKU patients determines the severe clinical form for the disease. The R408W mutation (c.1222C>T), a C to T transition in exon 12 of the *PAH* gene, results in the substitution of tryptophan for arginine at amino-acid residue 408 and is a null mutation associated with <0.3% of normal enzyme activity. In Latvian population the R408W mutation is associated with RFLP haplotype 2, the VNTR-3 allele, and the 238bp STR allele with frequency 77% from all mutant chromosomes.

R408W-2.3 exhibits a west-to-east cline of relative frequency reaching its maximum in the Balto-Slavic region. Almost 56% of patients are homozygous for the R408W, 40% are compound heterozygous and 3.3% have no R408W mutation. According to the pre-treatment Phe blood level and Phe tolerance, 90.2% (55/61) patients are classified as having a classical PKU phenotype, 4.9% (3/61) patients – moderate, and 4.9% (3/61) patients – mild. Patients with moderate and mild PKU phenotype are compaund heterozygotes, five of them have R408W in one allele and G272X, A104D and E178G in other allele, two patients have an unidentified other allele and one patient has no R408W mutation. Final results are in progress.

037-P

HYPERPHENYLALANINEMIAS IN TUNISIA

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Background and aims: Based on the high rate of consanguinity (32%) in Tunisia and the fact that newborn screening is not yet installed, the aim of this retrospective study was to estimate the frequency of hyperphenylalaninemia (HPA) including phenylketonuria (PKU) and persistent HPA in Tunisia.
Methods: From 1987 to 2006 and following clinical orientation diagnosis, 11 461 patients with signs and symptoms suggestive of inborn errors of metabolism were investigated. Ion-exchange chromatography of plasma and urine free amino acids was performed on an amino acids analyzer. Urinary organic acid profile was determined by gas chromatography/mass spectrometry. Blood phenylalanine levels were determined by fluorometric method. Measurement of biopterin in urine or blood and dihydropterin reductase (DHPR) activity in blood was carried out in the laboratory of Pr JL Dhondt (Lomme), France.
Results: A total of 471 abnormal cases were diagnosed. The number of HPA was 154 patients (32.6%), belonging to109 families. Patients were divided into 145 cases with PKU (94.1%) and 9 (5.8%) cases with DHPR deficiency. The sex ratio was 0.83. The age ranged from 1 day to 31 years, with most cases being diagnosed between 2 and 6 years. Consanguinity was observed in 66.2% of the parents and 44.8% have had familial cases previous. Using the Hardy-Weinberg formula, the incidence was estimated to be 1/7454 for HPA.
Conclusion: HPA seems to be highly frequent in Tunisia. PKU is the major form suggesting that the establishment of systematic neonatal screening is urgent.

038-A

PROFILE OF AMINOACIDOPATHIES OTHER THAN PHENYLKETONURIA AND ORGANIC ACIDURIA IN TUNISIA

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Background/objectives: The aim of this retrospective study was to determine the profile of aminoacidopathies (AAs) other than phenylketonuria (PKU) and organic acidurias (OAs) following clinical diagnosis, and to estimate their incidence in Tunisia.
Materials and methods: During the period 1987 to 2006, the laboratory achieved 11 461 analysis for patients with signs and symptoms suggestive of AA and AO. Patients originating from all areas of the country were aged from 1 day to 20 years. Ion-exchange chromatography of plasma free aminoacids was performed on amino acids analyzer. Urinary organic acids profile was determined by gas chromatography-mass spectrometry.
Results: After excluding PKU, 327 patients (2.85%) were diagnosed with 28 different AAs and OAs. These were divided into 173 cases of AAs (54.5%) and 144 cases of OAs (45.5%). The most frequent AAs were maple syrup urine disease (MSUD) (30.2%) and tyrosinemia type I (HTI) (24.5%). The most frequent OAs were methylmalonic aciduria (MMA) (32.6%); propionic aciduria (PA) (16.7%) and L-2-hydroxyglutaric aciduria (L-2-OHG) (11.8%). Using the Hardy–Weinberg formula, the incidence was estimated to be 1/14836 for MSUD, 1/15974 for MMA and 1/16863 for HTI.
Conclusion: AAs and OAs seem to be highly frequent in Tunisia. After PKU, MSUD is the major AA, suggesting that the establishment of systematic neonatal screening is urgent for these two diseases in our country. A prenatal diagnostic approach should available for the other inherited diseases.

039-A

SCREENING FOR IVS10 MUTATION IN EGYPTIAN PATIENTS WITH PHENYLKETONURIA

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Background: Phenylketonuria (PKU) can be caused by more than 512 mutations. In Egypt it is the most prevalent inborn error of metabolism. Identifying the most common mutations and VNTR marker is a prerequisite for developing a limited screening panel for rapid identification, thus saving time and cost.
Methods: One hundred and twenty unrelated diagnosed patients with PKU were screened for IVS10nt546 mutation and VNTR polymorphism patterns. The detection of mutation was based on a rapid and easy technique, PCR amplification and restriction enzyme assay. The VNTR patterns were pursued by specific PCR amplification.
Results: Screening identified 17.5% of alleles. The IVS10nt546 mutation was found in 42/240 alleles. The exclusive association of a VNTR repeat unit 7 with the IVS10 mutation was observed. However, further identification of the other mutations is recommended in order to target a wider range of specific mutations for rapid screening in Egypt.
Conclusion: In this Egyptian study the IVS10 nt546 was found to be a relatively common mutation, so screening for the repeat unit 7 and the IVS10nt546 mutation would be the first step for limited molecular screening.

040-P

CHARACTERIZATION OF 8 kb DELETION IN THE PHENYLALANINE HYDROXYLASE (*PAH*) GENE

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Background: Phenylketonuria (PKU) is an autosomal recessive disorder caused by mutations in the gene for phenylalanine hydroxylase (*PAH*). More than 500 different mutations have been identified at *PAH* locus up to date. Only sporadic cases of single or multiple exon and promoter deletions in the *PAH* gene have been described so far. Mutation analysis using exon by exon screening may fail to detect the mutant allele in the case of large intragenic deletions avoiding primer annealing in the deleted area. To date, there are no reports with a detailed characterization of the large deletion breakpoint in the exon 6 of the *PAH* gene.
Methods: Multiplex ligation-dependent probe amplification (MLPA) was used for detection of larger DNA alteration in the *PAH* gene. Long-range PCR (LR-PCR), restriction analysis, sequencing and bioinformatic analysis has enabled characterization of one partial *PAH* deletion and has elucidated the mechanism of its origin.
Results: MLPA analysis was performed in one PKU family from Romania where one mutation was missing. Deletion of exon 6 of *PAH* gene was detected in the proband. To determine the area of the deletion breakpoint, purified aberrant long-range PCR products were digested with various restriction endonucleases. Fine mapping of the deletion breakpoint was then achieved by using direct sequencing. Breakpoint analysis of the deletion showed loss of 8kb including the entire exon 6.
Conclusion: We conclude that MLPA is a convenient, rapid and reliable method for detection of intragenic deletions in the *PAH* gene.

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041-O

MUTATIONS IN THE *PAH* GENE LEAD TO PROTEIN MISFOLDING WITH DISTINCT ALTERATIONS IN OLIGOMERIZATION, ENZYME KINETICS, AND PROTEIN STABILITY

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Background: The clinical phenotype of tetrahydrobiopterin (BH_4) responsive phenylalanine hydroxylase (PAH) deficiency has recently been associated to an increasing number of *PAH* mutations. Although evidence exists for disturbed oligomerization and accelerated degradation of some variant PAH, the molecular pathophysiology of both, PAH deficiency and BH_4 responsive PAH deficiency, is still unclear. In this study, we aimed to analyze whether protein misfolding contributes to the pathogenesis of PAH deficiency. **Methods**: We characterized the molecular phenotype of wildtype and ten variant PAH proteins identified in patients with BH_4-responsiveness on the basis of enzyme kinetics, protein folding, and thermodynamic stability. The putative impact of local side-chain replacements on the protein conformation was analyzed using a composite 3D model of the PAH tetramer. **Results**: Considerable residual enzyme activity was found for most mutations. However, specific alterations of other kinetic parameters reflecting altered enzyme regulation were observed. The oligomeric state of variant PAH varied from disturbed assembly of functional tetramers to formation of aggregates. Assessment of PAH stability against proteolytic cleavage, thermal inactivation, and thermal denaturation revealed protein destabilization due to partial protein unfolding. 3D structural analyses showed that missense mutations can affect networks of side-chain interactions inducing global conformational changes of the PAH protein. **Conclusions**: Our results substantiate the hypothesis of PAH deficiency being a protein misfolding disorder. We provide several lines of experimental evidence that the molecular phenotype does not only arise from disturbance of local interactions but also from global structural changes due to disruption of functional networks of side-chain interactions.

042-P

FOLDING RESCUE OF MILD PKU MUTATIONS BY CHEMICAL CHAPERONES

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Phenylketonuria (PKU; OMIM 261600) is caused by mutations in the gene codifying for the homotetrameric protein phenylalanine hydroxylase (hPAH; EC 1.14.16.1). *PAH* gene mutations lead to the synthesis of hPAH proteins presenting a decreased enzymatic activity and/or stability. We have shown that the I65T, R261Q and V388M hPAH mutant proteins, increased its residual enzyme activity (up to 3.3-fold) when produced in the presence of 1% glycerol. The aim of our work was to evaluate the structural effects on hPAH and address the molecular mechanism underlying the chemical chaperones stabilizing action.

The recombinant hPAH mutant proteins were produced in a prokaryotic expression system, in the absence and in the presence of 1% glycerol. The enzymatic activity and oligomerization profile of the purified proteins were determined. The tetrameric and dimeric forms were isolated and characterized regarding its thermal stability and structural characteristics using spectroscopic techniques (far-UV CD and Trp emission).

The intracellular presence of glycerol shifted the oligomeric equilibrium towards the tetrameric (R261Q and V388M) or dimeric (I65T) forms. In respect to controls expressed in the absence of glycerol, the studied variants exhibited higher secondary structure content, thermal transitions with higher cooperativity and a reduction in the relative percentage of aggregated species.

These results are compatible with intracellular hPAH folding rescue resulting from stabilization of intermediate and native states by glycerol. Understanding the molecular mechanisms of the stabilization of misfolded proteins is essential to the development of therapeutic strategies to control conformational disorders.

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043-P

MODIFICATION OF PHENYLALANINE HYDROXYLASE BY SITE-DIRECTED MUTAGENESIS: PRODUCTION OF CHIMERICAL PROTEINS WITH HIGHER STABILITY

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One of the strategies proposed to stabilize a protein functional state is to genetically engineer it, in order to alter some of the major energetic factors that drive and/or oppose folding into the native structure. With this objective several approaches have been developed directed for a better core packing, surface ion pairing, surface loop stabilization, etc.

The aim of our work was to produce a chimerical phenylalanine hydroxylase protein (hPAH; EC 1.14.16.1) presenting higher enzyme activity and/or stability. Human PAH is of particular interest in Human Health since its impairment results in phenylketonuria (PKU; OMIM 261600) the most frequent disorder of amino acid metabolism.

Human *PAH* cDNA site directed mutagenesis was performed in order to exchange the superficial hPAH charge (mutations D145K, D151K, E181K and E360K) or introduce residues less susceptible to oxidation (mutations C29D, C29S, C284S and C445S). Using a prokaryotic expression system the chimerical hPAH proteins were produced, purified and characterized regarding: (1) the expression level; (2) enzymatic activity; (3) oligomerization profile and (4) thermal stability. The C.S mutants presented the higher enzyme activity increase (up to 3.5 ×). A shift in the oligomeric profile towards the dimeric state was detected for C284S and C445S.

The obtained results demonstrate that it is possible to engineer mutant hPAH proteins presenting a higher enzyme activity. These data may contribute to a better understanding of the biological function and structural properties of hPAH and to the development of new approaches to PKU treatment.

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044-P

PRE-DIAGNOSIS WEIGHT INCREASE AND PHENYLALANINE LEVELS IN PKU PATIENTS

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Aim: To compare weight increase rate in 63 PKU patients (20 with a biochemical phenotype requiring diet-therapy and 43 with a milder biochemical phenotype not requiring diet therapy) at entry for diagnostic confirmation (median 20 days of life) on the basis of the early type of feeding (breastfeeding, BF, or formula feeding, FF).
Methods: Plasma concentrations of phenylalanine (Phe) were analysed by ion exchange gas-chromatography. Weight at birth and at entry for diagnosis were determined with standard methods. The rate of weight (wt) increase was calculated on the basis of the expression [(wt at entry – wt at birth)/days of life]. The relative wt gain was calculated as [(wt at entry-wt at birth)/wt at birth]. Statistics: non parametric tests.
Results: Both the rate of wt increase and the relative wt gain were lower ($p = 0.05$ and $p < 0.001$, respectively) in PKU BF patients with a biochemical phenotype requiring diet-therapy (associated with higher plasmatic Phe level) compared to PKU BF patient whose biochemical phenotype did not require diet.
Conclusion: The lower wt gain in PKU BF patients requiring dietary intervention might be explained by the low protein supply of human milk, coupled with the limited ability to manage Phe for tyrosine synthesis. Further investigations are needed to understand the long term effects of this pattern, even if early BF seems to have a neuroprotective role.

045-P

COBALAMIN STATUS IN PKU PATIENTS, HOW TO MEASURE?

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Background: PKU patients are at risk for nutritional deficiencies. Natural protein intake is limited and low in quality. The main source of vitB12 is the protein substitute. It is hard to determine vitB12 status as serum vitB12 concentrations reflect both the active and inactive form. MMA and Hcy are considered reliable parameters for vitB12 status in healthy individuals (Monsen, Clin Chem 2003;49:2067–75).
Objective: The aim of this study was to relate serum vitB12 to MMA and Hcy in PKU patients.
Methods: VitB12, MMA and Hcy concentrations of 60 PKU patients (age range 1–37 years) were correlated (Spearman Rank). Age and metabolic control were used as confounding variables.
Results: Over 50% of patients showed a decreased concentration of serum vitB12. MMA was negatively related to serum vitB12 for the age group 1–10 years ($r = -0.561$, $p < 0.001$), and 11–20 years ($r = -0.535$, $p < 0.05$), but not at adult age. Plasma Hcy concentration showed a strong negative relation with serum vitB12 concentration at any age. Four patients showed elevated MMA and one high Hcy concentration despite normal vitB12 concentrations.
Conclusions: VitB12 status should be investigated in PKU patients as deficiencies easily arise. VitB12 concentrations within the reference range do not automatically imply a sufficient vitB12 status. MMA and Hcy measurements ought to be included in routine laboratory investigations. Presumably, deficiencies of intracellular vitB12 are the consequence of intake. In addition, inadequate absorption should be considered.

046-P

NUTRITIONAL INDICES AND POLYUNSATURED FATTY ACIDS IN PKU PATIENTS AT 20 DAYS OF LIFE

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Aim: To evaluate the fatty acid (FA) plasma levels and nutritional parameters in 58 PKU patients at entry for diagnostic confirmation (median 20 days of life).
Methods: Measurements included plasma concentrations of amino acids (AA), fatty acid (FA) profiles and nutritional indices (albumin, urea, total protein and transferrin). Plasma concentrations of AA were analysed by ion exchange chromatography. FA were measured with high resolution capillary gas-chromatography and expressed as weight%. Nutritional parameters were analysed by standard immune-enzymatic method. On the basis of the phenotypic classification 15 patients were then posed on diet-therapy, group 1, and 43 remained on a free-diet, group 2. All of them were further sub-divided into two subgroups, breastfed (BF, 1a and 2a) and formula-fed (FF, 1b and 2b).
Results: The BF group showed a reduction in concentrations of parental essential fatty acids including linoleic acid (1a: 19.8 ± 2.5 and 2a:17.0 ± 1.5 vs 1b:22.1 ± 3.3 and 2b:24.9 ± 2.8; $p < 0.0001$) and alpha-linolenic acid (1a: 0.2 ± 0.1 and 2a:0.1 ± 0.1 vs 1b:0.4 ± 0.3 and 2b:0.3 ± 0.2, $p = 0.001$) and an increase of long-chain derivatives, arachidonic acid (1a:8.3 ± 1.4 and 2a:8.9 ± 1.7 vs 1b:5.3 ± 2.3 and 2b:6.2 ± 1.7, $p < 0.0001$) and docosahexanoic acid (1a:2.1 ± 0.6 and 2a:17.0 ± 1.5 vs 1b,22.1 ± 3.3 and 2b:24.9 ± 2.8, $p < 0.0001$). FA differences were not found on the basis of the phenotypic classification. No statistically significant differences were found among the four subgroups as far as the investigated nutritional parameters.
Conclusion: The LCPUFA status of PKU patients at 20 days of life seems to be influenced by the early type of feeding while the PKU phenotype, previously hypothesized at the possible origin of a deranged FA status (Infante et al, Mol Genet Metab 2001; 72:185–98), seems to be ininfluent.

047-P

VISUAL FUNCTION ABNORMALITIES IN PKU PATIENTS, LACK OF CORRELATION WITH DEFICIENT PLASMA DOCOSAHEXAENOIC ACID

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Background: Phenylketonuric (PKU) patients showed low concentrations of long chain polyunsaturated fatty acids (LCPUFA) secondary to a natural protein-restricted diet. LCPUFA play an important role in postnatal development of visual function as structural components of retina membrane phospholipids. Our objective is to describe visual abnormalities in PKU patients with dietary control and search for a possible relationship between them and plasma concentrations of LCPUFA. **Methods**: Transversal study in 35 classic PKU-treated patients (15 males, 20 females, mean age 23.4 years (range: 13.0–40.7). Visual function was evaluated by brain MRI (SE-T2 sequences and coronal STIR), visual evoked potentials and oftalmologic exam. Serum lipids, phenylalanine (Phe), carnitine and LCPUFA were quantified. **Results**: Hypersignal in chiasma, nerve or optic radiations in brain MRI was observed in 16/27 and periventricular whitte matter hyperintensity in 14/27. Low latencies in PEV were detected in 16/35 (moderate 11.9% and severe 8.5%) and visual field reduction in 4/30. No significant correlation among LCPUFA or Phe levels and visual alterations were found. Periventricular white matter alterations were only associated with higher plasma Phe levels. Concerning LCPUFA, only docosahexaenoic acid (DHA) levels were significantly lower in patients compared with controls. **Conclusions**: PKU patients treated with a protein-restricted diet showed alterations in visual function, MRI changes in visual pathways and significant lower levels of DHA. However, no correlation between visual function abnormalities and plasma DHA have been observed. Further studies with broader samples and quantification of LCPUFA in erythrocyte membranes may give us more information.

048-P

AMINO ACID STATUS 6 HOURS AFTER THE ASSUMPTION OF TWO AMINOACID PREPARATIONS IN PKU PATIENTS ON DIET-THERAPY

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Background: Phenylketonuria is an inborn error of phenylalanine metabolism whose treatment is based on a dietary Phe restriction including a Phe-free amino acid formula supplemented with minerals, trace elements and vitamins. Early dietary treatment prevents neurological impairment and mental retardation. If the dietary compliance is suboptimal some patients may develop a delay in growth besides nutritional deficiencies.
Objective: To evaluate the nutritional parameters and the amino acid profile during a 6 h intake of two different amino acid preparations, the one supplemented with minerals and vitamins (preparation 1) and the other made up by microgranules of slowly absorbed amino acids (preparation 2).
Methods: Thirty-two PKU patients on diet therapy (19 females, 13 males, aged 15.6 ± 7 years) were randomised to preparation 1 or 2 administered once or more times per day, respectively. Nutritional plasma parameters (albumin, total protein, prealbumin and transferrin) were analysed at baseline. The amino acid profile was monitored at 0, 2, 4 and 6 h after the assumption of the preparation.
Results: Thirty-two patients were followed randomised to preparation 1 ($n = 18$) and 2 ($n = 14$), respectively. No statistically significant differences were found between the two groups as far as nutritional parameters. The amino acid profile at 2, 4 and 6 h after intake shows higher plasmatic levels of some essential amino acids (threonine, isoleucine, leucine, tyrosine, lysine and hystidine) with preparation 2, particularly in patients assuming the mixture at least twice per day.
Conclusions: A slowly-released amino acid mixture could have important competitive functions in regulating phe absorption by influencing amino acid kinetics.

049-P

WHAT HAPPENS WITH A LARGE BOLUS OF AMINO ACIDS IN PKU PATIENTS?

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Background/**Objective**: Compliance to dietary prescriptions is important in treatment of amino acid IEMs. Division of the amino acid mixture (AAM) over three or more portions per day is recommended, but in daily practise patients take the AAM in one portion. In order to give evidence-based advise for the distribution of the AAM over the day, the utilization was studied in well controlled PKU patients and controls, receiving the AAM (PKU 3, Milupa, Germany) in one bolus. **Methods**: 4 adult PKU patients and 2 healthy adults were studied. They consumed a bolus (0.8*1.2 g/kg), representing 100% of daily prescribed AAM. Stable isotope kinetics of ^{13}C-valine and $NaH^{13}CO_3$ were used to study whole-body protein metabolism before and after intake of AAM.

Results:

Time (min)		Infuse rate (μmol/kg/h)	Rate of appearance of Valine (μmol/kg/h) Controls	PKU	Valine oxidation rate (μmol/kg/h) Controls	PKU
Fasting	t<0	7.36 (0.12)	97 (10)	110 (13)	23 (4)	23 (3)
Post prandial-	15	10.71 (0.82)	136 (24)	165 (23)	22 (10)	25 (5)
	60	18.79 (0.90)	240 (6)	267 (22)	35 (3)	33 (4)
	120	15.24 (0.53)	193 (26)	217 (35)	55 (9)	55 (9)
	180	11.17 (0.51)	158 (18)	154 (22)	68 (11)	69 (10)
	270	7.70 (0.21)	104 (10)	110 (17)	71 (8)	67 (12)

Thirty-eight and 32% of the bolus was oxidised at t = 270 min in controls and PKU's, respectively. Maximum estimated protein synthesis rate (uncorrected for time delays) was reached 60 min after bolus ingestion. **Conclusions**: Synthesis rate seems influenced by plasma concentration. Oxidation starts rather slowly but contributes largely to disappearance. Amino acid metabolism is similar in PKU patients and healthy controls. The effect of distributing the AAM-bolus will be investigated.

050-P

EXPERIENCE WITH LONG TERM USE OF LNAA IN TREATMENT OF PKU

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Previous loading of short term studies with Large Neutral Amino Acids (LNAA) in PKU patients resulted in decrease of blood phenylalanine (Phe) levels. The long term safety, efficacy and acceptability of LNAA tablets (NeoPhe) have not been evaluated. In this study, four patients, three female and one male, ages 25 to 38 years, were given NeoPhe tablets, 0.5 g/kg/day in three divided doses to be taken with meals. The patients were not on medical foods, for more than 10 years previously. Their blood Phe prior to taking NeoPhe had a mean value of 1507 μmol/L. Blood Phe was determined two weeks after entering the study and once a month for a period of 12 months. The mean blood phe level declined for each of the subjects during the study period: 642 μmol/L, 707 μmol/L, 899 μmol/L and 869 μmol/L. The mean change from pre- to post-NeoPhe was significant (paired t-test: p = 0.002). Patients reached levels within NIH recommendations. Patients were monitored for weight in case, LNAA was used for protein synthesis. None of the patients gained or lost any weight beyond minor fluctuation of $\pm$0.2kg. The acceptability of the pills was monitored with review on every visit and there were no complains regarding the number of pills or of abdominal discomfort, nausea or changes in bowel habits. All patients asked to continue taking NeoPhe tablets because they were happy with their blood phe levels and indicated they felt 'more focused' at work. Future studies should include larger number of patients and neuropsychological tests need to be added.

051-P

THE EXPERIENCE WITH LARGE NEUTRAL AMINO ACIDS IN UKRAINE

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Kharkiv Specialized Medical Genetic Centre (KhSMGC) took part in collaborative study with Texas University (Prof. R. Matalon) on using of new formulas (Large Neutral Amino Acids (LNAA)) for PKU patients in Ukraine. LNAA have been used with the purpose to decrease the influx on phenylalanine (Phe) to the brain.

The mixture of LNAA (NeoPhe) was obtained from Prekulab, Korsor, Denmark. Patients with PKU were observed at KSMGC. NeoPhe was given 1 pill/kg/day in 3 doses. For biochemical control of Phe level we used fluorometric assay and TLC; for confirming diagnostics – HPLC; PCR – for mutation analysis. Patients were instructed to continue their diet as they did before the trial. Baseline was determined before and after taking NeoPhe.

Clinical approbation of NeoPhe in five PKU patients at the age of 11–22 years (mean 15.2) was performed. There were revealed such genotypes: R408W/R252W, R408W/R408W, R408W/R408W, R261X/R408W. The mean Phe level before NeoPhe administration was 1143.2 μmol/L; after taking NeoPhe – 739.3 μmol/L. We have detected the decreased mean Phe blood level by 35.3%.

Our data indicate that the inhibition of transport of Phe may occur in the GI tract using LNAA. The decrease of blood Phe levels in PKU patients taking LNAA was reported in Ukraine at the first time. It is possible if natural protein is limited (less Phe), more effective competition of LNAA with Phe might occur, that leads to lower blood Phe concentrations.

052-P

THE BLOOD/BRAIN BARRIER IN NEONATES: ^{1}H-MR SPECTROSCOPY SHOWS LOW PROTECTION AGAINST HIGH PHENYLALANINE

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Background: ^{1}H-MRS has been used to determine the blood/brain ratio for Phe in adults PKU patients. While the exact value is still debated, there is consensus that brain Phe is 3–4 times lower than in blood. The most crucial time for outcome is early childhood but data on blood/brain ratio for Phe in newborns are not available.
Objective: To determine the blood/brain ratio for Phe in neonates with PKU.
Methods: All spectra were recorded on a 1.5 TMR scanner. So far 2 neonates with PKU were investigated: Patient A, 43 weeks gestational age (GA), at 9 days; Patient B 36 weeks GA at 9 and 14 days. Controls: 2 healthy neonates 43 and 44 weeks GA. Adults: 6 PKU patients (23 ± 8 y old) and 6 healthy subjects.
Results: ^{1}H-MRS spectra were all of good quality. The blood/brain ratio is strikingly higher for neonates (0.52–1.2) than adults (0.29 ± 0.04). After treatment Phe droped to normal in parallel with blood levels in patient B, such that their ratio (1.2) is ill-defined.
Discussion: This study shows that the blood–brain barrier does not provide the same protection against high Phe for newborns as it does for the adults. At identical blood Phe levels newborn PKU patients' brain is exposed to much higher Phe level than adults. This underlines the importance of strictest dietary control in young age.

053-P

PKU – IS THERE A GROUP RISK? INVESTIGATION OF 126 EARLY TREATED PATIENTS

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Phenylketonuria (PKU) studies show that we need to look beyond the global intellectual quotient (IQ) to have a comprehensive perspective of this population. The heterogeneity of PKU population in terms of the degree of hyperphenylalaninemia seems to be an important factor determining the effects of a poor dietetic control. Our aim was to characterize our population of PKU patients in terms of their intellectual, neuropsychological and socio-emotional development, trying to find and characterize any risk factors.

We studied 126 patients aged 1–25 years. We analysed their global IQ, WISC-III subscale IQ, subtest profile and school performance. Self-control, self-esteem and family stress were also studied. Statistical analysis were performed taking in account all these variables and others directly related to the disease itself, namely the screening values and quality of dietetic control.

The results showed significant negative correlations between global IQ values and the annual median of phenylalanine (Phe) values in almost all age groups till the age of 18 years. The WISC-III subscales IQ also showed significant negative correlations with the screening Phe values and the current levels of Phe. The same occurs in specific WISC-III subtests, cognitive self-control, some aspects of self-esteem and domains of school performance. We identified a risk group, the individuals with diagnosis of classical PKU and poor long-term dietetic control.

We conclude that those PKU patients will need special supervision concerning their regular follow-up. Caution is needed concerning diet liberalisation after the age of 12 years, specially in the classical PKU group.

054-P

PKU AND THE INFLUENCE OF PHENYLALANINE LEVEL IN REACTION TIME PARADIGMS: A METAANALYSIS

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Background: A metaanalysis was conducted to quantify effects of phenylalanine levels from PKU patients in reaction time paradigms.
Methods: We identified 21 studies with computer based reaction time measurements published from 1986 to 2007. Three fixed factors were defined: (1) categories of tasks; (2) Age (<=13, >13 and <=18, >18 years); (3) Phenylalanine levels as a covariate. To model effect sizes, a generalised least square (GLS) approach with task, age and phenylalanine level as fixed factors was applied. GLS models deals with heteroscedasticity and correlated within-group errors to be expected in the data.
Results: There is a clear asymmetry of phenylalanine levels between age groups with no studies of adults with phenylalanine levels <750 μmol/L, and only one study with children above a phenylalanine level of 750 μmol/L. Only two studies after the year 1998 have examined adults. Studies examining adolescents are in the minority. Phenylalanine levels are interacting with age. For children and adolescents, increasing phenylalanine levels lead to greater effects between controls and PKU patients, whereas for adults the difference between controls and PKU is stable at ~0.5 standard deviation.
Conclusion: PKU clearly effect performance in reaction time tasks for children, adolescents, and adults. There is a need to study adults with phenylalanine levels below 750 μmol/L in order to support current recommendations for treatment.

055-P

IMPROVEMENTS IN NEUROPSYCHOMETRIC OUTCOME WHEN RE-INTRODUCING DIET IN ADULTHOOD IN PHENYLKETONURIA (PKU)

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Background: There remains much debate regarding the benefits of phenylalanine-restricted diet in adults with PKU, although most guidelines recommend lifelong therapy. Few data about long-term impact of re-introducing diet in adulthood exist. We describe the effects of re-starting diet therapy 16 years after discontinuation.
Case: A 31-year-old male re-presented concerned about poor concentration and short-term memory. He was also irritable and depressed. Born in 1968, PKU was diagnosed at 2 months. He went onto restricted diet for 15 years. He attended normal schools, leaving at 16 years. He has worked in the catering service all his adult life, but continues to live with his parents.
Results: At 31 years, WAIS-R assessment showed verbal IQ 74 and performance IQ 64. Diet was commenced with 12 g natural protein intake and 140 g XP Maxamum/day. Over a 6 year period phenylalanine was well controlled: mean 597 μmol/L (range: 30–1427 μmol/L). Body weight fluctuated from 79–90kg. He was re-assessed 6 months, 3.5 years and 6.5 years after starting PKU diet. Verbal IQ increased to 78, 79 and 81 respectively; performance IQ to 68, 76 and 77. He subsequently moved into independent accommodation and became 'employee of the month.'
Conclusions: The infrequent assessments over a long time period make the observed improvements unlikely to be practice effects. Verbal IQ improved by 9.5% and performance IQ by 20.3% associated with clear quality of life benefits. Careful evaluation of neuropsychometric outcome is useful when considering recommencing diet in adults with PKU.

056-P

A RANDOMISED CONTROL TRIAL OF DIET IN ADULTS WITH PREVIOUSLY UNTREATED PHENYLKETONURIA (PKU)

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Background: Anecdotal reports suggest phenylanine-restricted diet can improve quality of life in never-treated adults with PKU. We tested this in a randomised placebo-controlled trial – the first of diet in untreated PKU.
Method: 150 adults with previously untreated PKU were identified by a UK-wide postal survey. Of these 36 participated in a double-blind randomised crossover trial over 60 weeks – 8 weeks baseline followed by two 24 week trial diets (low and normal phenylalanine diets) with a 4 week washout period between them. Blood phenyalalanine, behaviour charts and videos to assess behaviour were monitored throughout.
Results: Of 36 subjects (aged 23–62 years, mean 49 years,) only 17 completed all 60 weeks. Three withdrew during the second trial diet, to follow a low phenylalanine diet, due to behaviour deterioration following dramatic improvement on the first diet. Three withdrew during the second diet as carers felt unable to continue. The remaining 13 were withdrawn in the baseline or first diet due to dislike of the supplements or blood test refusal. For those that completed the trial mean (±SD) phenylalanine concentrations (μmol/L) during the baseline period were 1582±206, on placebo 1474±267, on active diet 572±139 and washout 1520±234. Behavioural assessments are being analysed.
Conclusions: Low phenylalanine diet is difficult to institute in this group of individuals for a variety of reasons. This trial has emphasised the problems with management of these patients, but is essential to properly assess whether there are beneficial effects of low phenylalanine diet in adults with previously never-treated PKU.

057-P

DECREASED BONE DENSITY IN ADULT PATIENTS WITH PHENYLKETONURIA (PKU)

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Introduction: During childhood, phenylketonuria (PKU) is treated by protein restriction and amino acid supplementation. Target phenylalanine levels are age dependent with a lower goal in early childhood. Previously, affected individuals were allowed to go off diet during adolescence. Recently, however, many clinicians recommended a special diet and possibly amino acid supplementation throughout life. To investigate the consequences of long lasting diet and amino acid supplementation, bone metabolism was investigated in adult subjects with PKU.
Methods: Patients with PKU presenting in the adult metabolic clinic were clinically examined, their diet was assessed, and they were subjected to various laboratory tests and Dual-energy X-Ray-Absorption (DEXA).
Results: 28 subjects (age 18–45) were examined. Compared to normal population, the bone density was significantly reduced in patients with PKU. The lumbar spine (L1-L4) median T-score was –1.14; for left femur, T-score was –1.02. Age corrected bone densities (Z-scores) were also significantly decreased (median Z-score for lumbar spine: –0.88; for left femur –1.1). No correlation of bone density to plasma amino acids, vitamin levels, bone specific alkaline phosphatase, parathyroid hormone, calcium and phosphate was found. However, there was a slight trend towards higher bone mass in patients with a lenient diet at the time of presentation.
Conclusion: Although nowadays subjects with PKU can reach adulthood in good health, attention to the patient's outcome after long term diet has to be paid. Early changes of diet or supplementation may be necessary to prevent severe long term consequences.

058-P

CORRELATIONS BETWEEN LUMBAL BONE MINERAL DENSITY (BMD) AND BIOCHEMICAL MARKERS IN PATIENTS WITH PHENYLKETONURIA

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Reduced BMD has been observed in PKU. The pathophysiology remains obscure.
Objective: To investigate correlations between lumbal BMD, phenylalanine concentrations, markers of bone metabolism (Ca, P, Mg and vitamin D) and markers of vitamin B12 metabolism (vitamin B12, methylmalonic acid, homocysteine) in PKU. **Materials and methods**: BMD was examined in 53 PKU patients aged 4–38 years using DXA-scans of the lumbal spine. Subjects were divided in three age groups: 0–10 years ($n = 6$), 10–20 years ($n = 22$) and >20 years ($n = 25$). Mean individual phenylalanine concentrations over the year prior to DXA-scan was calculated. Other biochemical markers were usually taken at BMD-measurement.
Results: Z-scores were reduced in age groups 10–20 years (-0.91 ± 1.172, $p = 0.002$) and >20 years (-0.64 ± 0.877, $p = 0.001$), without a significant longitudinal course. No significant correlation between BMD and phenylalanine, bone metabolism markers or vitB12 metabolism markers was found.
Conclusion: Reduced BMD in PKU is not correlated to phenylalanine, deficiency of trace elements, or vitamin B12 metabolism.

059-P

EVALUATION OF BONE MASS DENSITY AND BODY COMPOSITION MEASURED WITH DEXA IN PATIENT AFFECTED BY HYPERPHENILALANINEMIA

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Background and Aims: Measurement of body composition have evident value in evaluating growing children, and Dual-Energy X-ray Absorptiometry (DEXA) is a tool that provides accurate measurements of whole-body bone mineral content, lean body mass and fat mass. The evaluation of these parameters may be useful in patients affected by pathologies that need diet therapy as hyperphenilalaninemic (HPA) patients.
Methods: DEXA was performed with Hologic Densitometer in 24 HPA patients (12 on diet therapy and 12 on free diet, comparable for age, sex and BMI), followed by our Metabolic Diseases Unit. We considered for each patient, bone mass content (BMC), bone mass density (BMD), lean body mass (LBM) and fat mass (FM). Statistics: Mann–Whitney test was performed to compare data obtained from the two groups. Descriptive data: mean values and Z-score referred to general population.
Results: We did not find statistically significant differences between the two groups according to the considered parameters (BMC:1044 g vs 1045 $p = 0.843$; BMD 0.85 g/cm^2 vs 0.82 $p = 0.347$; LBM 25 354 g vs 26 753 $p = 0.932$; FM 10 326 g vs 10 635 $p = 0.977$). Considering all the 24 patients we have found a low mean z-score according to BMD (–1.56).
Conclusions: From these preliminary data we can say that diet therapy doesn't influence body composition in HPA patients. This study seems to confirm low Mineral Density in HPA subjects, but not only diet influences these parameters as previously reported. Further studies are needed to clarify which variables are involved in determining BMD, focusing on genetics or mineral absorption.

060-P

CRITERIA FOR DEFINING BH_4-RESPONSIVENESS IN PKU

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Aim of the study was to determine the prevalence and identify subjects with phenylketonuria (PKU; phenylalanine hydroxylase deficiency) responsive to 6R-tetrahydrobiopterin (BH_4) and to establish selection criteria for potential treatment with BH_4. We analyzed blood phenylalanine levels from 557 newborns and children with various degrees of PKU (blood phenylalanine 301–4743 μmol/L) challenged with BH_4 (20 mg/kg body weight) 8 and 24 h after BH_4 administration. The two modalities were compared for phenylalanine reduction (% of responsiveness). The overall prevalence of BH_4-responsiveness within patients with PKU for the blood phenylalanine reduction of 20, 30, 40, and 50% was 48, 38, 31, and 24%, respectively, using the 8 h modus, and 55, 46, 41, and 33%, respectively, using the 24 h modus. Using the 30% cut-off, BH_4-responsiveness was similar regardless of the two modalities (8 of 24 h) in patients with MHP (79–83% responders), mild PKU (49–60% responders), and classical PKU (7–10% responders).These data confirms that BH_4-responsiveness is more prevalent than initially assumed, particularly in patients with MHP and mild PKU. Depending on the severity of hyperphenylalaninemia (mild or classical PKU), selection criteria for the potential treatment with BH_4 may range between 20 and 40% of blood phenylalanine reduction after 24 h.

061-O

BIOPKUdb AND MOLECULAR GENETICS OF BH_4-RESPONSIVE PHENYLKETONURIA

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Tetrahydrobiopterin (BH_4)-responsive hyperphenylalaninemia has been recently described as a variant of PAH deficiency caused by specific mutations in the PAH gene. It has been suggested that BH_4-responsiveness may be predicted from the corresponding genotypes. Data from BH_4 loading tests indicated an incidence of BH_4-responsiveness of $>40\%$ in the general PKU population and $>80\%$ in mild PKU patients. The current project entailed genotype analysis of 315 BH_4-responsive patients tabulated in the BIOPKUdb database and comparison with the data from the PAHdb locus-specific knowledgebase, as well as with previously published PAH mutations for several European countries, Northern China, and South Korea. We identified 60 mutations, presenting with a substantial residual PAH activity ($\sim 47\%$), presumed to be associated with BH_4-responsiveness. About 64% of these mutations are located in the catalytic domain, 15% in the regulatory domain, 13% in the tetramerization domain, and 8% are intronic. The three most common mutations found in $>5\%$ of BH_4-responsive patients are p.A403V, p.R261Q, and p.Y414C. Using the Hardy-Weinberg formula the predicted average frequency of BH_4-responsiveness in European populations was calculated to be 62% (range 18–95%, lowest in Baltic countries and highest in Spain), 57% in Northern China, and 55% for South Korea. The genotype-predicted prevalence of BH_4-responsiveness was higher than prevalence data obtained from BH_4 loading tests. Inconsistent results were observed for mutations p.L48S, p.I65T, p.R158Q, p.R261Q, p.Y414C, and IVS10-11G>A. Our data suggest that BH_4-responsiveness may be more common than assumed and to some extend may be predicted or excluded from the patient's genotype.

062-P

TETRAHYDROBIOPTERIN (BH_4) RESPONSE IN PKU PATIENTS CARRYING THE MUTATION Y414C

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PKU is caused by a deficiency of phenylalanine hydroxylase (PAH). PAH requires the cofactor tetrahydrobiopterin (BH_4).

The main therapy for PKU has been a diet low in phenylalanine for more than 50 years. In 1999 a Japanese group introduced BH_4 as an alternative treatment for patients with mild PKU. Specific mutations have been reported to respond to BH_4 treatment but it has been difficult to foresee the effect in individual patients.

Methods: We studied the effect of oral BH_4 load (Schricks Lab), 20 mg/kg, in 18 children and adult patients carrying either of the mutations Y414C, R241H, R408W, P281L or R158W; previously known to respond to BH_4 (14 with Y414C, 4 with the remaining mutations). The diet was modified to achieve morning PA values $>$ 400 µM/L. The study was carried out during 2 days, the first day at home as a control, and the second day in the hospital when BH_4 was given before breakfast. All meals were prepared in double portions for day 1 and 2. Capillary blood spots were sampled for 24 h. Phenylalanine and tyrosine concentrations were analysed with MS-MS.

Results: All patients with Y414C, in homozygous or compound heterozygous form, responded to BH_4 ($>30\%$ reduction in 8 h). However, patients with R241H, R408W, P281L or R158W did not respond.

Conclusion: In this study all patients with Y414C responded to BH_4. No patients with other mutations, previously reported to be responsive to BH_4, showed a reduction in phenylalanine in contradiction to previously reported results.

063-P

'RESPONSIVENESS' AND UNRESPONSIVENESS TO BH_4 OF PAH DEFICIENCY

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Background: BH_4-responsiveness of many patients suffering from PKU has been extensively reported in the last years, but not definitely demonstrated. With this aim, we compared the outcome of Phe and BH_4 loading tests performed at different ages in patients affected by different forms of PAH deficiency.

Methods: Seven patients (3–22 years) were enrolled in this study and, on the basis of their biochemical phenotype and genotype, assigned to the severe (2), mild (3), and benign (2) class of PAH deficiency. All causal mutations had been previously reported as BH_4-responsive. All these patients had been submitted in the first month of life to a traditional combined Phe and BH_4 loading test. Presently, after normalization of plasma Phe levels, three quantitative loading tests were applied to each patient: a simple loading with Phe (100 mg/kg), a combined loading with Phe (100 mg/kg) followed after 3 h by BH_4 (20 mg/kg) administration, and a combined loading with BH_4 (20 mg/kg) followed after 3 h by Phe (100 mg/kg) administration. During the tests a protein-free, normocaloric diet was administrated.

Results: In the neonatal period, patients affected by mild or benign PAH deficiency showed a clearance of plasma Phe meeting the criteria of BH_4-responsiveness, though coherent with their Phe tolerance. At the present age, however, the comparison between the different tests showed an identical course of plasma Phe and Tyr concentration, irrespective of BH_4 administration.

Conclusion: BH_4 administration in PKU patients resulted ineffective on Phe and Tyr metabolism, questioning the existence of BH_4-responsive PKU.

064-P

RESPONSE TO ORAL SINGLE DOSE TETRAHYDROBIOPTERIN IN PATIENTS WITH PHENYLKETONURIA IN RELATION TO THE METABOLIC PHENOTYPE DEFINED BY PROTEIN LOADING

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Background: Tetrahydrobiopterin (BH_4) responsiveness is a common trait in patients with mild forms of phenylalanine hydroxylase (PAH)-deficient hyperphenylalaninemia (HPA). There is ongoing discussion whether BH_4 could be an alternative treatment for some patients with HPA.

Methods: 17 adult patients with PAH-deficient HPA were ascribed to one of three phenotypic groups according to their phenylalanine levels 72 h after a standardised protein loading test with 180 mg Phe/kg bw/d (group 1, mild PKU: 10–17 mg/dl (600–1020 µmol/L), group 2 moderate PKU 17.1–26 mg/dl (1021–1560 µmol/L), group 3 classical PKU $>$26 mg/dl ($>$1560 µmol/L)). The effect of BH_4 in single doses of 10, 20 and 30 mg/kg bw on Phe blood levels was evaluated.

Results: Mean Phe decrease was 31.3% (+ 11.8%) in group 1, 11.8% (+ 9.6%) in group 2 and 15.0% (+ 11.1%) in group 3 ($p = 0.003$). Effect of BH_4 on Phe levels was not dose dependent in the three phenotypic groups ($p = 0.241$). Levels of B+P increased significantly with increasing BH_4 doses ($p < 0.0001$). Maximum B+P levels were reached 4 h after application of BH_4 in all patients. There was no correlation between B+P levels and Phe level decrease ($p = 0.69$).

Conclusion: The effect of BH_4 on Phe levels in patients with HPA is not dose dependent, but differs between the phenotypic groups. There is no correlation between B+P levels and BH_4 responsiveness.

065-P

PKU 006: THE EFFECT OF SAPROPTERIN DIHYDROCHLORIDE (TETRAHYDROBIOPTERIN OR 6R-BH_4) TREATMENT ON PHENYLALANINE (PHE) TOLERANCE IN CHILDREN WITH PHENYLKETONURIA CONTROLLED ON A PHE-RESTRICTED DIET

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Background/Objectives: Sapropterin dihydrochloride (sapropterin) can lower blood phenylalanine (Phe) levels in phenylketonuria (PKU). We investigated whether sapropterin can also increase Phe tolerance in children with PKU. **Methods**: This double-blind, placebo-controlled Phase 3 study enrolled children (4–12 years old) with PKU on a Phe-restricted diet. Part 1 identified children whose Phe levels were reduced by an 8-day course of sapropterin dihydrochloride (20 mg/kg/day). Children with a 30% reduction in blood Phe (responders) and blood Phe level <300 µmol/L at day 8 were randomized 3:1 to receive sapropterin (20 mg/kg/day) or placebo for 10 weeks (Part 2). Phe supplements to the baseline diet were prescribed beginning at Week 3 and adjusted according to Phe levels. Primary endpoint was the change in Phe tolerance between Weeks 0 and 10, while maintaining blood Phe level <360 µmol/L. **Results**: Of 89 children evaluated, 50 (56%) met criteria for Part 2 and 45 were treated with sapropterin (n = 33) or placebo (n = 12). At Week 3, blood Phe levels were significantly reduced from Week 0 (275.7 ± 135.2 µmol/L) by 148.5 ± 134.2 µmol/L ($p < 0.001$) in the sapropterin group. At week 10, sapropterin significantly increased the amount of Phe that could be supplemented as compared to baseline (20.9 ± 15.4 mg/kg/day vs 2.9 ± 4.0 mg/kg/day; $p < 0.001$). Sapropterin increased Phe tolerance from 16.8 ± 7.6 to 43.8 ± 24.6 mg/kg/day vs 16.3 ± 8.4 to 23.5 ± 12.6 for placebo. Treatment was generally well tolerated. **Conclusions**: Sapropterin increased Phe tolerance in children with PKU on a Phe-restricted diet and may allow an increase in dieatary Phe intake in this population.

066-P

SAFETY AND EFFICACY OF A 22-WEEK TREATMENT WITH SAPROPTERIN DIHYDROCHLORIDE (TETRAHYDROBIOPTERIN OR 6R-BH_4) IN PHENYLKETONURIA (PKU)

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Background/Objectives: Sapropterin dihydrochloride can decrease phenylalanine (Phe) levels in PKU patients. We studied the long-term safety and efficacy of three doses of sapropterin dihydrochloride in PKU patients. **Methods**: This open-label phase 3 study enrolled 80 PKU patients 8 years who had participated in a randomized trial of sapropterin dihydrochloride (PKU-003). In Part 1, subjects received consecutive 2 week courses of sapropterin dihydrochloride 5, 20 and 10 mg/kg/day (forced-dose titration), followed by 10 mg/kg/day for 4 weeks. In Part 2, subjects received 12 weeks of sapropterin dihydrochloride at a fixed-dose of 5, 10 or 20 mg/kg/day based on Part 1 blood Phe levels. **Results**: Dose-dependent reductions in blood Phe levels were observed in the 6-week forced-dose titration period. Mean (SD) blood Phe levels decreased from 844 ± 398 µmol/L at week 0 to 645 ± 393 µmol/L at week 10. This reduction was maintained through the final 12 weeks of the study (652 ± 382 µmol/L). Sixty-eight (85%) subjects had at least one adverse event (AE). All AEs, except one, were mild or moderate in severity. The investigators judged 32% of AEs as possibly or probably related to sapropterin dihydrochloride. Most frequently reported AEs were nasopharyngitis, upper respiratory tract infections, headache and migraine, vomiting, and diarrhoea. There was no apparent relationship between dose and incidence, frequency or type of AE's. No patient discontinued treatment because of an AE. **Conclusions**: Sapropterin dihydrochloride, 5–20 mg/kg/day was generally safe and caused a dose-related decrease in blood Phe that was maintained over a 22 week.

067-P

NUTRITIONAL MANAGEMENT CONCERNS IN 3 PHENYLKETONURIA (PKU) PATIENTS ON BH_4

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Background: Three PKU patients who took part in a BH_4 trial in 2004 have continued $BH_4$with ongoing good results. LD now 9 years (genotype p.R408W/p.L48S), CR 6 years (p.I65T/p.S273F) and JL 4 years (IVS10-3C > T/p.L48S) were diagnosed by newborn screening. All 3 children had one allele previously associated with classical PKU. LD and JL had one allele previously identified as likely to be BH_4 responsive (p.L48S). CR had an allele of uncertain BH_4 responsiveness (p.S273F).
Objective: To describe the differing nutritional concerns in the management of 3 children taking BH_4.
Results: LD's and CR's BH_4 responsiveness was identified in our initial study, whilst JL was found to be BH_4 responsive as consequence of a BH_4 load test. Since BH_4 treatment, average phenylalanine levels have remained within target range (100–350 µmol phe/L) for all children. With a BH_4 dose of 10–20 mg/kg/day, CR and JL were able to stop amino acid (AA) supplements and move to a nutritionally adequate omnivorous diet. Initially calcium was the vulnerable nutrient for CR; later, her dairy intake met calcium needs. LD has been able to significantly increase protein intake but still avoids animal protein, limits dairy products, requires some AA supplement to maintain phenylalanine levels in range, and takes calcium and multivitamin supplements to meet recommended micronutrient requirements.
Conclusion: The variability of BH_4 responsiveness allows some to revert to a normal diet while others still need AA supplements, mild protein restriction and mineral and vitamin supplements.

068-P

DIETARY MANAGEMENT OF BH_4 RESPOSIVE PKU

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Aim: To describe the dietary protein intake in BH_4 responsive patients with PKU.
Introduction: A BH_4 load (20 mg/kg) has been given to all newly diagnosed infants with PKU in our service since 2001 to assess responsiveness prior to commencement of dietary therapy. In older PKU patients, a loading dose of Phe (100 mg/kg) is given prior to BH_4 load to ensure meaningful blood Phe levels.
Results: Six patients (5 infants, 1 child) have shown responsiveness to BH_4. For infants diagnosed through ENBS the rate of responsiveness was 1 in 5. Median reduction in Phe level from baseline following BH_4 administration was 64.5% (range 28–76). 2 patients currently require no protein restriction and consume a diet with up to 4 g/kg/day of natural protein. 4 need some protein restriction, median Phe intake of 51 mg/kg/day (range 44–110), significantly greater than median 'predicted' tolerance of 20mg/kg/day for classical PKU. Four are supplemented with Phe free amino acid formula for nutritional adequacy. Protein restriction was required mostly after 6–12 months of age when blood Phe levels increased after introduction of solid foods and growth rate declined. Median dose of BH_4 is 10 mg/kg/day (range 4.8–17).
Conclusion: In PKU patients who respond to BH_4, Phe tolerance is significantly higher than theoretical predicted tolerance. Advantages include breastfeeding exclusively for the first 6 months of life and a more relaxed diet in childhood, which may improve quality of life. However, dietary protein restriction may still be required to maintain acceptable Phe control, and should be introduced early for practical reasons.

069-P

IS THERE A RISK TO OVERESTIMATE A POSITIVE BH_4 LOADING RESPONSE PERFORMED IN THE NEONATAL PERIOD?

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Background/Methods: A BH_4 loading test was performed in a 9-day-old boy with PKU, later genetically verified (Y414C, R210Q).
Results: The result was excellent, FA values declining from 1270 μmol/L to 340 μmol/L after 8 h (> 70% reduction) with full breast feeding. BH_4 therapy, 10 mg/kg/day was started. During the first 6 months the FA levels were kept well within the therapeutic range. At 4 months of age, solid food was introduced and the FA levels eventually increased. At 7 months of age the BH_4 dose was adjusted to correspond to 10 mg/kg/day. This only marginally improved the FA concentrations. Despite a further increase of BH_4 to 15 mg/kg/day at the age of 9 months, the boy continued to have too high FA levels, necessitating the introduction of protein restricted diet.

The boy has had a normal growth velocity apart from the first 6 weeks of life when his weight gain was above average.
Conclusions: A newborn child is catabolic during the first days of life, gradually changing to an anabolic state and increasing growth velocity in infancy. Hence, the result from a BH_4 loading test in the neonatal period may be overestimated as it coincides with the increase in growth. The prognostic information to the parents may thus be overoptimistic. The relatively low protein content in breast milk compared to solid foods may maintain a good metabolic control during the first months of life. However, when the child gets older protein restriction may be necessary

070-P

THE SCREENING FOR TETRAHYDROBIOPTERIN METABOLIC DISORDERS AND RELATED GENE ANALYSIS AMONG THE PATIENTS WITH MOTOR DISTURBANCE AND MENTAL RETARDATION

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Objective: To get the incidence of various enzyme deficiency in tetrahydrobiopterin (BH_4) metabolism among the patients with motor disturbance and mental retardation and the analysis of related gene mutation.
Methods: One hundred patients (4 months–14 years) with unknown motor and mental retardation were referred to this study. All patients were performed by phenylalanine (Phe) (100 mg/kg) or combined with BH_4 (20 mg/kg) loading test, the analysis of urinary pterin and dihydropteridine reductase (DHPR) activity. Some patients suspected as dopa-responsive dystonia (DRD) were treated with dopa for diagnosis. The mutation analysis of GTP cyclohydrolase 1 gene (*GCH1*) and 6-pyruvoyl tetrahydropterin synthase gene (*PTS*) were done for the parents with DRD and PTPSD.
Results: Seventy of 100 patients had normal basic blood Phe levels, 16 of 70 cases were received the treatment of dopa 50–300 mg/d, 6 (6%) were diagnosed as DRD. The other thirty patients had hyperphenylalaninemia(HPA) (Phe 1022 ± 290 mmol/L). 8 (8%) patients were diagnosed as PTPS deficiency. Their Phe concentrations remarkably decreased after BH_4 loading, and urinary biopterin percentage were $1.2 \pm 1.0\%$. Twenty-two (22%) HPA patients were diagnosed as phenylalanine hydroxylase (PAH) deficiency. The mutations IVS5 +3insT of *GCH1* gene were found in 2 patients with DRD and the 7 kind of PTPS mutations (166G>A, 259C>T, 286G>A, 155A>G, 430G>C, 276T>A, 393A>C) were found in 8 patients with PTPSD.
Conclusions: Some patients with unknown motor disturbance and mental retardation may suffer from BH_4 metabolism related diseases. Theses patients are necessary to be screened for such kind of diseases in order to receiving the appropriate treatment.

071-P

A NEW PRESENTATION OF 6-PYRUVOYL TETRAHYDROPTERIN SYNTHASE (PTPS) DEFICIENCY – DOPA RESPONSIVE DYSTONIA (DRD)

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PTPS deficiency typically presents asymptomatically via newborn screening or with progressive mental and physical retardation with extra-pyramidal signs, epilepsy and lighter pigmentation. In Hong Kong, there is no mass newborn screening for inborn error of metabolism like hyperphenylalanaemia. We report a patient with PTPS deficiency who presented with DRD. She was the first child of a non-consanguineous Chinese couple with uneventful perinatal period. There is no relevant family history. Since 9 months of age, she started to have episodic tremor with eye starring. This happened few times per month. She was treated as epilepsy but the condition did not improve with anticonvulsants. In early childhood and was noted to have dysarthria with dystonia and rigidity of both the upper limbs. There was diurnal fluctuation. She has normal intelligence. L-dopa was started and was kept at a dose of 5 mg/kg/day. All her symptoms resolved. She is now 16 years old. Cerebrospinal fluid (CSF) for neurotransmitter assays was done after stopping L-dopa for 5 days. She then developed generalized dystonia and parkinsonism with swallowing difficulty. Her baseline phenylalanine was 179 μmol/L (normal 30–90). CSF revealed a low biopterin, homovanillic acid, 5-hydroxyindoleacetic acid level and a high neopterin level. The pattern was compatible with PTPS deficiency. This was confirmed with genetic mutation study. We therefore recommend screening plasma phenylalanine level and CSF neurotransmitter assays for all patients with DRD.

072-P

LONG-TERM FOLLOW-UP OF TAIWAN CHINESE PATIENTS WHO RECEIVED EARLY TREATMENT FOR 6-PYRUVOYL-TETRAHYDROPTERIN SYNTHASE DEFICIENCY

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Background: The reports of the outcomes of patients who received early treatment for 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency, particularly over long periods of observation, remain scarce. Quite a few PTPS patients, even though detected by newborn screening and given early treatment still had unsatisfactory outcomes. In Taiwan, the prevalence of PTPS deficiency ($\approx 1/100\,000$) is considerably higher than in Caucasian populations. This provides us with more opportunities to observe and treat this form of illness within a single medical center.
Methods: We reviewed the IQ outcomes of all of our PTPS deficiency patients who were found by the newborn screening and received an early treatment. The possible factors related to outcomes such as genotypes, peak level of phenylalanine and the levels of urinary pterin at diagnosis, birth body weight, and the timing and dosages of administered BH_4 and neurotransmitters were also analyzed in this study.
Results: All of our patients achieved a normal IQ, even though we just based treatment dosage on clinical response and adverse effects of the neurotransmitters, without monitoring the levels of the CSF neurotransmitter metabolites during the administration of neurotransmitters. The average intelligence quotient (IQ) score of our PTPS patients is 97 ± 10, which is much better than other previous reports. In this study we also found genotype, birth body weight and treatment starting age are related to the IQ outcomes.
Conclusions: An effective newborn screening referral system and an early adequate therapy can still achieve a normal IQ outcome in PTPS patients, even if the prenatal brain impairment has existed.

073-P

HIGH INCIDENCE OF DHPR DEFICIENCY IN SOUTH ITALY: REPORT OF THREE PATIENTS WITH THE SAME MUTATION (L14P)

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Deficiency of dihydropteridine reductase (DHPR) causes a variant form of phenylketonuria associated with a devastating neurological disease. Hyperphenylalaninaemias (HPA) with BH_4 deficiency are about 3% of all HPA. We describe three patients from Calabria, a southern region of Italy, affected by DHPR caused by same mutation. We used serum prolactin levels as a marker for optimal dosage of hydroxylated precursors in long-term treatment monitoring. All patients were children of unrelated parents DHPR diagnosis were made by BH_4 oral loading test (20 mg/kg) and the measurement of DHPR activity in erythrocytes. None of patients showed neurological signs before the beginning of pharmacological treatment. In the case 1 the annual median dosage of L-dopa was of 5.19 mg/kg/day. Other two patients showed increase of serum levels of prolactin that required adjustments of L-dopa independently of the body weight. Actually, case 2 needs L-dopa at the dosage of 6.0 mg/kg/day and case 3 needs L-dopa at the dosage of 5.8 mg/kg/day. The outcome of three patients is until now very favourable. Molecular analysis on *QDPR* gene on these three patients showed the mutation pL14P in homozygosity on exon 1. This mutation has been found in Mediterranean populations and a founder effect has been hypothesized. Thus our molecular data seem to confirm this hypothesis and could explain high incidence of DHPR deficiency in our region. In conclusion we found an high incidence of DHPR deficiency in our region and we show that the prolactin could be a good indicator of optimal dosage of neurotransmitter precursors.

074-P

DIAGNOSIS OF TETRAHYDROBIOPTERIN DEFICIENCY USING FILTER PAPER BLOOD SPOTS: A 2 YEARS EXPERIENCE

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A method for the measurement of pterins (total neopterin and biopterin) in filter paper blood spots was introduced as an alternative for the screening of BH_4 deficiencies in urine. This new method enables measurement of pterins, DHPR activity, and amino acids (Phe and Tyr) from a single filter paper specimen. During the last two years we analyzed more than 800 urine specimens from patients with hyperphenylalaninemia and in 214 of them pterins were measured in dried blood spots in parallel. During this period we diagnosed 9 patients with PTPS deficiency, 16 patients with DHPR deficiency, and 1 patient with GTPCH deficiency. In patient with the GTPCH deficiency and in all patients with PTPS deficiency, both urine and dried blood pterins pattern were diagnostic. Only in some patients with DHPR deficiency urinary and dried blood pterins were suggestive and diagnosis was done by the enzyme assay. Following reference values were established for pterins in dried blood spots (median; 5–95 perc.): neopterin = 1.37 (0.34–4.72) nmol/g Hb; biopterin = 0.86 (0.20–3.48) nmol/g Hb; %biopterin = 38.1 (14.2–72.2). PTPS deficiency; neopterin 4.87 (2.32–7.94) nmol/g Hb; biopterin = 0 nmol/g Hb; %biopterin = 0. DHPR deficiency: neopterin = 1.00 (0.32–3.42) nmol/g Hb; biopterin = 0.81 (0.26–3.16) nmol/g Hb; %biopterin = 38.6 (18.5–79.8). GTPCH deficiency: neopterin = 0.06 nmol/g Hb; biopterin = 0 nmol/g Hb; %biopterin = 0. Our data confirm the usefulness of dried blood spots for the diagnosis of BH_4 deficiencies; however DHPR activity measurement remains an essential test.

075-P

VARIATION OF MATERNAL BLOOD PHENYLALANINE IS A PREDICTOR OF OFFSPRING OUTCOME FOLLOWING PREGNANCY IN TREATED PKU

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Background/Objectives: Developmental delay in the offspring of women with PKU can be prevented by maintaining maternal blood phenylalanine (Phe) within a target range (100–250 μmol/L in UK). Our objectives were to analyse offspring outcome following PKU pregnancy managed in a single centre to identify prognostic factors.
Methods: We compared birth weight (BW) and occipito-frontal circumference (OFC) and neurodevelopment (DQ/IQ) scores at 1, 4, 8 and 14 years to Phe concentrations during pregnancy in infants born to PKU mothers attending our centre between 1977 and 2005.
Results: Our study included 105 children born to 67 PKU mothers. Mean ($\pm$SD) BW and OFC (Z-scores) did not differ significantly between pre- and post-conception groups (0.22 ± 1.02 vs -0.25 ± 0.97 and; 0.42 ± 1.24 vs -0.96 ± 1.19 respectively). 1 year DQ and 8 year IQ were higher when diet started before conception (107 ± 13.8 vs 99.3 ± 13.3, $p = 0.014$ and 110.6 ± 14.8 vs 91.2 ± 23.9, $p = 0.005$); 4 year IQ was not. Maternal Phe concentrations correlated negatively with DQ/IQ scores and variations (SD) in all maternal blood Phe correlated negatively with 4, 8 and 14 year IQ ($r = -0.385, -0.433, -0.712$ and $p = 0.002, 0.008, 0.031$ respectively), even when levels were consistently within the target range.
Conclusions: Phe restricted diet should start before conception. Keeping maternal blood Phe within the target range predicts good outcome, but variations even within that range should be avoided.

076-P

FIVE YEAR POSTNATAL GROWTH OF OFFSPRING OF WOMEN WITH PHENYLKETONURIA (PKU) IN THE MATERNAL PKU COLLABORATIVE STUDY (MPKUCS)

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There were 576 pregnancies with 414 live births in the MPKUCS. Postnatal growth data at 5 years were available for 148 offspring of PKU women and 26 non-PKU controls. Subjects were grouped according to their blood phenylalanine (Phe) concentrations prior to or before 10 weeks gestation, group 1 ($n = 32$) 120–360 μmol/L; group 2 ($n = 39$) 360–600 μmol/L; and group 3 ($n = 77$) >600 μmol/L. This report compares change in z-scores of height, head circumference (HC) and weight from birth to 5 years between offspring of PKU women, relative to control offspring. None of the PKU groups differed significantly from controls on change in height z-scores. Mean HC z-scores of offspring from women in group 1 improved during the 5 years of follow up (–0.30 +0.88 to 0.50+1.53. The mean HC z-scores of offspring of women in groups 2 and 3 declined, group 2 from –0.65+0.87 to –0.97+1.49, and group 3 from –1.46+1.08 at birth to –2.09+1.57 at 5 years. In 5 years, catch-up growth did not occur in height and HC of offspring of women in groups 2 and 3. Change in weight z-scores at 5 years differed significantly among the groups, with group 1 increasing (0.74) significantly more than controls ($p < 0.02$). Head circumference seems to be a sensitive parameter for high concentration of blood Phe during pregnancy. The data suggest that blood Phe should be <360 μmol/L before and throughout pregnancy. By 5 years, HC did not improve if blood Phe concentrations were >360 μmol/L after 10 weeks gestation.

077-P

MATERNAL PKU IN DENMARK

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Background/Objectives: In 1979 the first child of a woman with phenylketonuria, PKU, in dietetic treatment was born in Denmark. Since then more Danish women with PKU have given birth to healthy children. Some of these pregnancies have earlier been reported in a Nordic Maternal Study, but this is the first complete survey of the Danish maternal patient group.
Patient group: Our maternal patient group includes 31 women with hyperphenylalaniemia (14 classic, 4 moderate, 9 mild, 4 mild hyperphenylalaninemia, MHP), today 23 to 58 years. Twenty-four were diagnosed before one month of age; seven were diagnosed in the age 5 months to 32 years. Our present guidelines: S-phe 120–300 μmol/L (420 before year 2000) and weekly blood samples.
Results: Since 1974, 58 children in 55 pregnancies have been born. In 43 pregnancies (13 classic, 3 moderate, 9 mild, 4 MHP) the dietetic control was excellent or good (median S-phe 146–387 μmol/L). In 6 pregnancies (2 classic, 1 mild) it was suboptimal or poor (median S-phe 417–862 μmol/L). Four women with undiagnosed PKU (1 classic, 3 moderate) gave birth to 6 children (estimated S-phe 900–1450 μmol/L). In the last group one child died of a heart defect as a newborn, five are microcephalic and mentally retarded. Head circumference, weight and length at birth correlate negatively to S-phe during pregnancy. Most of the children have not been followed regularly afterwards, but are reported by parents to develop normally. Psychological testing are planned.
Conclusions: The dietetic compliance is good in Danish women with PKU.

078-P

MATERNAL PKU AND HETEROZYGOUS WOMEN FOR HYPERPHENYLALANINEMIA (HPA): WHAT IS THE OUTCOME FOR THE OFFSPRING?

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Objective: To evaluate the relationship between the condition of heterozygosis for HPA and pregnant and perinatal pathologic conditions.
Patients and Methods: One hundred and fifty-three newborns of heterozygous women for HPA condition (74 females, 79 males) were compared to one hundred and fifty-three newborns of healthy women. We evaluated auxologic parameters (weight, length and head circumference), gestational age, APGAR score, neonatal problems and obstetrician anamnesis of the mother (number of children, pregnancy course and abortivity recurrence). In heterozygous women for HPA condition we also evaluate the Phe/Tyr ratio which is usually over than 1,08.
Results: The abortivity and gestational complications in healthy women compared to the heterozygous ones were more frequent in the second group ($p = 0.000$ and $p = 0.04$ respectively); the comparison between the two groups shows that newborns of heterozygous women for HPA condition are shorter ($p < 0.0001$, SD 2.3 vs 2.4); Furthermore, an inverse correlation was found between Phe/Tyr ratio and gestational age ($p = 0.01$).
Conclusions: The results obtained suggest that more studies are necessary to investigate the pregnant complications and the fetal outcome related to the condition of heterozygosis for HPA in pregnant women. In conclusion, we suggest the necessity to evaluate the dietetic intake in heterozygous women for HPA in order to consider the eventuality of a nutritional intervention.

079-P

PHENYLALANINE TOLERANCE IN THREE PHENYLKETONURIC WOMEN PREGNANT WITH FETUSES OF DIFFERENT GENETIC STATUS

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Recommendations for managing pregnancy in women with PKU include continuous dietary control with blood phenylalanine concentrations between 60 and 240 μmol/L starting before conception, irrespective of the fetal genetic PKU status. While the type of maternal PKU mutations will influence metabolic control, the effect of the fetal genetic PKU status on maternal metabolic control during pregnancy is not well understood. **Methods**. We followed three pregnancies of women with classical PKU by dietary protocols allowing the calculation of phenylalanine intake, by phenylalanine blood levels, and by standard obstetrical care. Patients #1 (R408W/I65T) and #2 carried a heterozygous (not PKU-affected) fetus, while #3 (R408W/R261Q) carried a PKU-affected fetus (R408W/R408W). **Results**. The expected dynamic increase in phenylalanine tolerance during the course of pregnancy differed remarkably in the three women. In patients #1 and #2 phenylalanine intake rose steadily from 400 to 1600 mg/day while phenylalanine blood levels remained in the desired range. Gain of body weight was 14.0 and 17.6 kg, respectively. In patient #3 the phenylalanine tolerance rose only to 600 mg/day, and phenylalanine blood levels were above the desired range on several occasions. Caloric intake was therefore encouraged which led to a weight gain of 19.0 kg. All pregnancies were otherwise normal and produced infants with normal birth weight and head circumference. **Conclusion**. The different phenylalanine tolerance in the pregnancies with PKU-affected and non-affected fetuses suggests that the genetic and metabolic status of the fetus influences maternal metabolic control. Pregnancies with affected fetuses may be more difficult to manage.

080-P

MATERNAL PHENYLKETONURIA AND TETRAHYDROBIOPTERIN

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In the final report of the International Collaborative Maternal PKU Study (2002) the rate of microcephaly was reported as 24% and congenital heart (CHD) disease was reported in 7% of the offspring despite the best efforts of professionals who provided the care of the women. Microcephaly and CHD occurred chiefly in poorly treated women with PKU with blood phenylalanine (phe) concentrations >360 μmol/L. With the use of tetrahydrobiopterin (BH_4) blood phe concentrations have shown a reduction as much as 60% in persons with mild to moderate hyperphenylalaninemia and in 10–20% of persons with classic PKU. It is clear that BH_4 would be an important addition to our present therapy, especially during pregnancy.
Methods: After obtaining Food and Drug Administration (FDA) approval, two pregnant women with PKU were treated during 3 pregnancies with BH_4 and the phe-restricted diet.
Results: The head circumference in all three offspring measured 35.5 to 36 cm with normal outcome despite elevated maternal blood phe concentrations before conception and during the first trimester of pregnancy. All three offspring exhibit normal development.
Conclusion: BH_4 and dietary restriction of phe appear to be quite important in improving fetal outcome of maternal PKU pregnancies. Obviously, more data are required to prove this hypothesis.

081-P

24 HOUR BLOOD PHENYLALANINE VARIABILITY IN MATERNAL PKU

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In maternal PKU, blood phenylalanine concentrations are kept within low and narrow ranges (i.e. 100–250 μmol/L). Routine blood samples are usually collected in a morning; when blood phenylalanine levels are at their highest. However, blood phenylalanine concentrations may vary widely in 24 h but there is no published data in pregnancy.
Aim: To establish 24 h variability in blood phenylalanine concentrations in maternal PKU.
Methods: Skin puncture blood tests were taken by women or their partners every 4 h for 24 h (i.e. 6 blood samples in 24 h) monthly in 12 PKU pregnancies. In total, 55 twenty-four hour blood phenylalanine profiles were collected (median 5 per pregnancy; range 1–8). They were analysed by HPLC. All patients were on a low phenylalanine diet, and at the beginning of pregnancy they were taking between 3 to 6 g/daily (median 4 g) natural protein from phenylalanine exchanges. Protein substitute was divided into 3 doses and spread evenly over 16 h.
Results: Median change in 24 h blood phenylalanine profile was 80 μmol/L; range 40–410). 39% of all blood phenylalanine concentrations were less than 90 μmol/L ($n = 126$); with only 7% ($n = 24$) less than 40 μmol/L. 12% of ($n = 40$) samples were over 250 μmol/L.
Conclusions: Maintaining blood phenylalanine levels between 100–250 μmol/l in pregnancy is likely to lead to low phenylalanine concentrations for almost 40% of the day. This may affect fetal growth.

082-P

EDUCATION OF PKU WOMEN IN RELATION TO MATERNAL PKU DAMAGE PREVENTION. IS IT EFFECTIVE?

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Prevention of maternal PKU damage is now an important issue of medical services dealing with phenylketonuria. The population of normally functioning PKU adult females systematically increases. However about 50% of pregnancies in healthy women is unplanned.

The aim of the study was to asses the influence of our educational system on sensible maternity in PKU. In 2000 educational program MPKU was introduced in our department and has been continued annually. MPKU meetings are still being performed by the team of specialists (gynecologist, dietitian, psychologist, geneticist, pediatrician). Annually participates about 40 young PKU females with their families.
Patients: 12 pregnancies in 9 women aged 17–34 years (average 24.7 years) were analyzed. All the women participated in our educational program. Eight prepared to pregnancy were on restrictive diet 3 months before the conception. Two came in 3rd week of pregnancy. The phe levels normalized in 1–2 weeks. Average phe and tyrosine levels controlled during pregnancies were within recommended ranges. In offspring no malformations typical for MPKU were found. Psychological tests performed every year revealed normal DQ in all children.
Conclusion. Presented data suggests that MPKU education results in planned pregnancy with good offspring outcome. Subsequent systematic educational work is necessary for next PKU young women.

083-P

PREGNANCY OUTCOMES IN MATERNAL PHENYLKETONURIA – A RETROSPECTIVE ACCOUNT OF EXPERIENCES IN NORTHERN IRELAND

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Background: Metabolic control and clinical outcomes of babies born to Northern Irish women with phenylketonuria (PKU) were investigated. Main objectives were to determine if recommended phenylalanine levels (100–250 μmol/L) were achieved, and to compare control and outcome in planned and unplanned pregnancies.
Methods: All women who delivered January 2000 to March 2007 were included. Information collated included: average phenylalanine levels, pre-pregnancy and during each trimester; average protein exchanges; maternal genetics; baby's health and birth weight; information from developmental/cognitive assessments.
Results: Fourteen women delivered 21 babies during this time, 76% were planned pregnancies. Average phenylalanine in the unplanned group of 819 μmol/L, 513 μmol/L, 346 μmol/L and 309 μmol/L for pre-pregnancy, first, second and third trimesters respectively, showed optimal control was not achieved despite fewer exchanges. Optimal control was achieved within the planned group. One infant within the planned group was born with a cerebral hernia despite good metabolic control throughout the pregnancy. No congenital abnormalities were reported within the unplanned group, although one child born had a low birth weight (2.27 kg) and another had a head circumference below average. Developmental/cognitive assessment indicated attention/concentration problems in three infants, from both planned and unplanned pregnancies.
Conclusion: Despite repeated education, a significant number of PKU pregnancies remain unplanned. It is difficult to achieve optimal metabolic control during pregnancy in this group, which is associated with more 'classic' mutations requiring protein restriction compared to the planned pregnancy group. Maternal PKU carries a risk of congenital abnormality even when optimal control is achieved.

084-P

PHENYLALANINE (PHE) CONTROL IN PATIENTS WITH PHENYLKETONURIA (PKU) CONSUMING A NOVEL METABOLIC MEDICAL FOOD (Add Ins)

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Background: Amino acid-based medical food products are effective in nutrition management of phenylketonuria (PKU), however, long term compliance can be poor. A flavourless novel medical food (Add Ins) was developed, and contains free amino acids, excluding phenylalanine (Phe), encapsulated in a lipid coating. Add-Ins is designed to be incorporated into low protein Phe-free foods.
Objectives: Primary outcome was quantitation of plasma amino acid concentrations and protein status indices; secondary outcome variables included product acceptability and serum lipids.
Methods: Ten patients with PKU replaced at least 1/3 of their medical food requirements with Add Ins for 28 days. Baseline, 2 and 4 week data were analyzed.
Results: Patients (18 ± 8 years) were prescribed on average 2 sachets (1 sachet contains 10 g protein equivalents) daily of Add Ins. Mean baseline plasma Phe concentration (505 ± 408 μmol/L) did not statistically differ at 28 days (582 ± 332 μmol/L). Plasma tyrosine (14 and 28 days), protein status indices and serum lipid concentrations (28 day) were not statistically different from baseline and were in normal reference ranges. Patient comments included 'it's simple and easy', 'food was the taste, rather than the product', and 'no detectable odour, which is good'.
Conclusion: Add Ins is safe and effective in supporting normal nutrition status indices as part of a Phe-restricted diet and may help to 'normalise' diet regimen. Add Ins may help compliance as an alternative flavourless and flexible medical food compared to traditional powders or liquids.

*Known as Phlexy-10 Add Ins in USA.

085-P

ON LINE ORDERING OF AMINO ACID SUPPLEMENTS

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Background: To make it convenient for patients with PKU, The Kennedy Institute (KI) introduced on-line ordering of amino acid (AA) supplements in 2003. KI is the national PKU centre for Denmark, Greenland and the Faeroe Islands and PKU treatment includes children, adults and late-diagnosed patients. The companies providing AA supplements in Denmark were willing to provide the on-line service. The procedure is as follows: The patient has a personal prescription from the doctor at the KI. Every patient has an individual log-on and is only allowed to order three months supply of the prescribed product. The on-line order will show up in the intranet at KI and be approved by a person in the PKU-centre, who will then forward it to the company, supplying the product. The company will send e-mail in return to KI, confirming the order and informing about date of direct home delivery to the patient. The PKU-centre can monitor the ordering and observe if the patient doesn't order sufficient amount of AA supplement and furthermore control the AA budget.
Results: A survey was conducted including 258 patients, 112 patients returning the questionnaire. The questions and answers (number of patients) were: 'Using on-line ordering regularly?' (50), 'Not using the on-line system?' (11), 'Will rather call and obtain personal contact?' (18), 'Doesn't work for me?' (8) and 'Other issues, like no access to the internet?' (25).
Conclusion: On-line ordering of AA supplement has improved delivery to patients with PKU at KI and has decreased the waste of products and the over all cost.

086-A

CLASSICAL PHENYLKETONURIA AND ANTLEY–BIXLER SYNDROME IN ONE SAUDI BOY

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Introduction: Phenylketonuria (PKU) is an uncommon metabolic disease in the Arab world. Antley–Bixler Syndrome (ABS) is a rare genetic disorder described by Antley and Bixler in 1975.
Case report: MH was born at 38 weeks by spontaneous vaginal delivery. Parents are not consanguineous. The patient developed neonatal apnea required mechanical ventilation. Routine newborn metabolic screening revealed phenylalanine level of 1285 μmol/L (38–137) consistent with classical PKU. MH responded well to phenylalanine free diet and the level remains within an acceptable range. DNA study confirmed that MH carries a homozygous known mutation, R261 in exon 7. It is leading to a codon stop.

Clinical examination revealed microcephaly, craniocynstosis, brachycephaly, bossing, depressed nasal bridge, bilateral proptosis, and low set rotated ears. These dysmorphic features are consistent with ABS. CT of maxillofacial area revealed marked deviation of the nasal septum with no choanal atresia. Chromosomes and MRI brain were normal. MH developed recurrent apnea, epilepsy, eustachian tube dysfunction and hypotonia. Intellectually was normal.
Conclusion: We report the co-existence of two rare genetic disorders in one boy.

087-P

WHICH AMINO ACIDS ARE REQUIRED FOR SCREENING AND MONITORING INBORN ERRORS OF METABOLISM?

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Background: Developing evidence-based guidelines for laboratory investigations requires an understanding of the analytical performance of different methods which can be obtained from external quality assurance (EQA) data. Assessment of performance in the ERNDIM amino acids (AAs) scheme is complex as there is data on 29 analytes. In order to focus efforts on the most important AAs, an expert opinion was sought on which are required for screening or monitoring inborn errors of metabolism (IEMs).
Methods: A questionnaire was sent to 21 Metabolic Biochemistry Network (MetBioNet) and associated laboratories from the UK and Ireland asking whether the plasma AAs in the ERNDIM scheme are always, sometimes or rarely/never useful for either screening or monitoring IEMs.
Results: 26 completed questionnaires were received from 19 laboratories. $>90\%$ stated that the following are always or sometimes useful for screening or monitoring: alanine, arginine, citrulline, glutamine, glycine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, tyrosine and valine. $>50\%$ stated that the following were rarely/never useful for screening: alpha-aminobutyrate, asparagine, aspartate, hydroxyproline, 1-methylhistidine, and sarcosine. $>50\%$ regarded the following additional AAs as rarely/never useful for monitoring: cystathionine, histidine, proline, taurine, serine and sulphocysteine. Results were equivocal on 9 further AAs used for screening and 4 for monitoring.
Conclusion: Comparison of analytical techniques with EQA performance can be based on a limited range of useful AAs as determined by questionnaire of experts on the field of AA analysis.

088-P

QUANTITATIVE ANALYSIS OF UNDERIVATISED AMINO ACIDS IN BODYFLUIDS USING ION-PAIRING ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY ESI-MS/MS

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Background: The quantitative or semi-quantitative analysis of aminoacids in physiological fluids using classical amino acid analysers is a time-consuming process allowing only a few samples to be analysed per day. We have developed and validated the quantitative determination of 52 amino acids in plasma and urine using ion-pairing ultra-performance liquid chromatography ESI-MS/MS
Methods: The instrumentation used was a Micromass Quattro Premier quadrupole mass spectrometer equipped with a Waters Acquity Ultra Performance Liquid Chromotograph(Waters, Milford MA, USA). Liquid chromatography was performed using a 2.1 W 100 mm Acquity UPLC BEH C18 column with 1.7 5m particles. Urine and plasma samples were deproteinated by acid precipitation and amino acids were eluted using a gradient system containing tridecafluoroheptanoic acid as the ion pairing-agent. Amino acids were detected using specific MRM transitions.
Results: All 52 amino acids for which the assay was validated were separated and detected in 13 min, the run-to-run time being 35 min. Linear calibration curves were observed for all amino acids in plasma and urine. Typical recovery was $100\% \pm 10\%$, typical intra- and inter run variations were $<10\%$ in both plasma and urine.
Conclusions: We have developed a rapid method to quantify a large number amino acids in plasma and urine samples. The validation results were satisfactory.

089-P

DRUG INTERFERENCE IN AMINO ACID ANALYSIS

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Many drugs, particularly antibiotics, have ninhydrin positive metabolites. These can cause confusion in the interpretation of amino acid chromatography. Despite requests for drugs to be identified on laboratory request forms this information is frequently lacking. It is therefore useful to be able to catalogue these interferences where possible. It is also helpful to be able to characterise the interferences by two separate chromatography systems. We have characterised the interference of a small group of drugs by 2 dimensional thin layer chromatography (TLC) on cellulose plates and automated ion exchange chromatography using a Biochrom 30 analyser (AAA). The following list describes approx position of metabolite spots or peaks:

Antibiotics
Amoxicillin TLC; beta ala; ileu/leu/phe AAA; gly-val; tyr
Ampicillin TLC; ileu/leu/phe AAA; homocys-lys
Cephradine TLC; ileu/leu/phe AAA; OH lys

Anticonvulsant
Vigabatrin TLC; beta ala; AAA; above BAIBA alpha amino adipipic; OH lys

Analgesic
Paracetamol TLC; ala AAA; phe

Images of the chromatograms have been mounted on the website of the MetBioNet www.metbio.net. We would like to invite contributions of other examples to add to this database in order to create an open access resource.

090-P

UPLC-MS/MS ANALYSIS OF SUCCINYLACETONE IN DRIED BLOOD SPOTS FOR THE DIAGNOSIS OF HEPATORENAL TYROSINEMIA

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Background: Recently, chemical derivatization techniques has made possible the analysis of trace levels of succinylacetone (SA) in dried blood spot (DBS) specimens by MS/MS and LC-MS/MS. In this work, we describe the improvements made on our LC-MS/MS assay of SA as a dansylhydrazone (Al-Dirbashi et al. J Chromatogr B. 2006;831:274) that was achieved by implementing the use of UPLC, a relatively recent technology that enormously reduced the turnaround time.
Methods: SA was extracted from DBS specimens and derivatized according to the published procedure. The UPLC-MS/MS system consisted of an ACQUITY Ultra Performance LC system (Waters) interfaced with a Quattro Premier XE triple quadruple mass spectrometer. Separations were performed on a Waters ACQUITY UPLC BEH C18 column (2.1×50 mm, 1.7 micron) using 80% acetonitrile containing 2 mmol/L ammonium acetate at 0.4 ml/min as a mobile phase.
Results: SA was eluted at 0.6 min with a total run time of 1 min. The calibration curve was linear up to 100 μmol/L with a quantification (S/N = 10) and a detection limit (S/N = 3) of 0.67 and 0.2 μmol/L, respectively. Within-day (n = 13) and between-day (n = 10) variations at concentrations ranging between 1 and 100 micromol/L were less than 10%. SA in DBS specimens from controls was up to 0.63 μmol/L (n = 152) whereas it ranged between 5.2–26 μmol/L in patients (n = 11).
Conclusions: We describe a 1-min assay for the quantitative analysis of SA using UPLC-MS/MS. The assay enables a definitive diagnosis of hepatorenal tyrosinemia from the same DBS sample received for newborn screening.

091-P

RELATIONSHIP BETWEEN PHENYLALANINEMIA AND NEUROPSYCHOLOGICAL OUTCOME OF NTBC-TREATED PATIENTS WITH TYROSINEMIA I

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2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexadione (NTBC), a new drug used in the treatment of tyrosinemia type 1 (OMIM 276700) since 1992, improved considerably the prognosis of the disease. The long-term neuropsychological outcome of NTBC-treated patients with tyrosinemia type 1 is still unclear. Learning difficulties and cognitive deficits have been found in patients on psychological testing. The etiology of these deficits is still unknown.

We report the neuropsychological outcome of 10 NTBC-treated patients (5 girls and 5 boys, aged 4–13 years) with tyrosinemia type 1 followed in Belgium. The treatment (NTBC and tyrosine restricted diet) was started between the neonatal period and the age of 6 years (mean: 12 months). IQ was above 100 in two patients, between 80 and 100 in eight and below 80 in one. Mean concentrations of phenylalanine, tyrosine and methionine were calculated for each year, and compared with the neuropsychological outcome (IQ). Tyrosinemia was increased (mean: 478 μmol/L; range: 111–834 μmol/L) but no relationship was observed with the IQ. Methioninemia was not correlated with neuropsychological outcome. The 3 patients with the lowest IQ had, precociously, the lowest phenylalanine concentrations (<40 μmol/L during the first 24 months of treatment). The child with the highest IQ had normal phenylalaninemia.

Our results suggests that low phenylalaninemia during the first years, more than increased tyrosinemia, could play a role in the neuropsychological evolution of NTBC-treated patients with tyrosinemia I. Further investigations are needed to confirm this first observation.

092-P

HEREDITARY TYROSINEMIA TYPE 1: IMPAIRMENT OF DNA REPAIR BY CHARACTERISTIC METABOLITES

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Background: Tyrosinemia type1 (HT1) is a clinically heterogeneous autosomal recessive disorder in which the fumarylacetoacetate hydrolase enzyme (FAH, E.C. 3.7.1.2) is defective. Characteristic of the chronic form is the mutation reversion in the liver and the eventual development of cellular hepatocarcinoma. Although p-hydroxyphenylpyruvic acid (pHPPA) is used as one of the diagnostic markers of this disease, it was suggested that it is unlikely to be involved in the pathophysiology of HT1.
Objective: The aim of this study was to investigate the effect of pHPPA on DNA damage and repair in liver cells.
Results: Experiments showed that pHPPA caused significant DNA damage and challenging the DNA repair capacity of metabolite-treated cells with H2O2, revealed a marked impairment in the DNA repair capability of these cells.
Conclusion: We suggest that the main effect of pHPPA is the long-term impairment of the DNA repair machinery in addition to the direct DNA damaging effect. This, and the proven genotoxic effect of other characteristic HT1 metabolites, i.e. FAA and MAA, is responsible for the development of cellular hepatocarcinoma in HT1. To study the combinatorial effect of these metabolites on DNA repair and mutation reversion, the ideal would be to mimic the *in vivo* situation prevailing in HT1. To accomplish this, a knock-down model via RNAi technology seems to be the option to follow.

093-P

THREE NOVEL MUTATIONS IN THE FUMARYLACETOACETASE GENE CAUSING TYROSINEMIA TYPE 1

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Background: Hereditary tyrosinemia type I (HT1) is a autosomal recessive disorder caused by deficiency of fumarylacetoacetase (FAH), the last enzyme of tyrosine degradation. The *FAH* gene consists of 14 exons. According to the Human Gene Mutation Database (HGMD. Cardiff University 2007) 42 different mutations have been reported, of which two are small deletions.
Methods: 24 Norwegian HT1 patients have been genotyped the last two decades, 8 of them recently. DNA from leukocytes or fibroblasts was sequenced, including all 14 exons and the exon-intron boundaries. Mutations were confirmed by PCR amplification and enzyme digestion and were checked in 100 normal controls to exclude polymorphisms.
Results: Three novel, small deletions in the *FAH* gene were found; F205L (615 delT), G248G (743delG) and Q279R (835delC). All three lead to frame shift and an early stop-codon. One patient was compound heterozygous for F205L and the missense mutation G337S. Two patients were compound heterozygous for Q279R and G337S. G248G was found in five patients. Two were homozygous, 2 were compound heterozygous with the splice site mutation IVS12+5(g>a), and one patient was compound heterozygous with the nonsense mutation W262X.
Conclusions: The *FAH* sequence from 47 of 48 allels from Norwegian HT1 patients are now characterised. There are 9 known mutations in the Norwegian HT1-population. IVS12+5(g>a) (38%), G337S (29%), G248G (15%), Q279R, A134D (4%), F205L, P342L, W262X, E357X (2%). Most patients have a chronic phenotype and no clear cut genotype/phenotype correlation is found. One patient, homozygous for G248G, recently presented with an acute form of HT1.

094-P

TYROSINEMIA TYPE I – MOLECULAR CHARACTERIZATION OF 10 PORTUGUESE PATIENTS

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Background: Tyrosinemia type I (OMIM +276700) is an autosomal recessive disease with an approximate incidence of 1:100 000 worldwide, caused by a deficiency in fumarylacetoacetate hydrolase (FAH), the last enzyme of the catabolic pathway of tyrosine. The severity of the clinical symptoms and the presentation age are highly variable. The human *FAH* gene is localized in the region 15q23-q25 contains 14 exons, and it spans approximately 35 kilobases of DNA.
Patients and Methods: The entire coding sequence of the *FAH* gene and at least 40 bp of the intron-exon boundaries were amplified by PCR, and sequenced using Big Dye Terminator Cycle Sequencing Ready Reaction kit. Ten patients with Tyrosinemia Type I (aged from 18 months to 15 years old) and a couple with a deceased child with biochemichal diagnosis of this disease were characterized at molecular level.
Results: Five of these patients harboured the c.554-1G>T mutation, the most common in the Mediterranean area, four of them are homozygous for this mutation. Furthermore, we have identified four more already described mutations: c.193+1G>T, c.1065G>A, c.1269.1272delTTinsCA, c.1063 +5G>A and the novel c.607+1G>A that disrupts a splice donor site at intron 7.
Conclusion: With this study we were able to identify the molecular background of tyrosinemia type I in Portuguese population. We also developed a strategy for an easy and rapid molecular characterization of the suspected cases of this disease detected by MS/MS analysis of blood spot in the expanded newborn screening in our country.

095-P

IMPROVEMENTS IN METABOLIC CONTROL WITH A CHANGE OF AMINO ACID SUPPLEMENT IN A PATIENT WITH TYROSINAEMIA TYPE II

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Background: Tyrosinaemia type II is an aminoacidopathy characterised by corneal erosions, palmar and plantar hyperkeratoses and, sometimes, learning difficulties. Dietary management consists of a low protein diet combined with a tyrosine and phenylalanine-free amino acid supplement.
Case: The patient, a 23-year-old female, had been diagnosed at the age of seven. Her metabolic control had never been good, despite the placement of a gastrostomy in childhood for overnight feeds and for the administration of her amino acid supplements. She also had a left sided cataract and ulcerative colitis, which was currently well controlled.
Results: Her metabolic control had been very poor with tyrosine levels ranging from 872 to 1369 μmol/L. She was not using her gastrostomy and wished to have it removed. She was unable to tolerate taking her XPhen XTyr Maxamum orally. In an attempt to improve her metabolic control, her supplement was changed to TYR Express, 4 sachets daily. She found this supplement much more palatable and mean tyrosine levels fell from 1086 μmol/L to 383 μmol/L (range 260–497). It has been possible to increase the natural protein in her diet. She has now been referred for removal of her gastrostomy.
Conclusions: As in other aminoacidopathies, the advent of newer, more palatable amino acid supplements can dramatically improve metabolic control and allow natural dietary protein to be increased leading to significant benefits in quality of life for patients with tyrsoinaemia type II.

096-P

PITFALLS IN THE DIETARY MANAGEMENT OF INBORN ERRORS OF METABOLISM; AN EXACERBATION OF TYROSINAEMIA TYPE II DURING THE TRANSITION OF CARE

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Background: Tyrosinaemia type II is an aminoacidopathy characterised by corneal erosions, palmar and plantar hyperkeratoses and, sometimes, learning difficulties. Dietary management consists of a low protein diet combined with a tyrosine and phenylalanine-free amino acid supplement.
Case: Our patient was diagnosed with tyrosinaemia type II aged 1. She was commenced on dietary treatment at diagnosis and, apart from mild learning difficulties, remained well. At 16, she was transferred to the adult metabolic clinic. At the same time, her amino acid supplement was changed to a newly available, easier to use preparation. Four months later she developed severe eye pain with typical, painful lesions of her hands and feet. Despite restriction of all natural protein and increasing her TYR express supplements to 5 times a day, her tyrosine levels continued to rise, peaking at 2097 μmol/L. After careful questioning, it emerged that when her supplement had been changed, her primary care physician had prescribed MSUD express instead of TYR express. Once the correct supplement was re-introduced, tyrosine levels fell to 904 μmol/L within two days and had dropped to 369 μmol/L by day 7. Her symptoms improved rapidly accompanied by gradual resolution of the hyperkeratoses. Dietary protein was re-introduced.
Conclusions: The transition of care represents a vulnerable period for patients. It is critically important to ensure that communication between all the various professionals involved in their management is maintained. Direct examination of dietary supplements and their use, is sometimes necessary.

097-P

MOLECULAR CHARACTERIZATION OF MAPLE SYRUP URINE DISEASE PORTUGUESE PATIENTS

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Background: Maple syrup urine disease (MSUD) is an autosomal recessive disorder, caused by the defective function of the branched chain alpha-ketoacid dehydrogenase complex (BCKD). BCKD is a mitochondrial complex, encoded by four nuclear genes (*BCKDHA*, *BCKDHB*, *DBT* and *DLD*), involved in the metabolism of branched-chain amino acids (BCAAs). When BCKD activity is impaired both BCAAs and the respective branched-chain alpha keto acids accumulate in body fluids, with severe clinical consequences. Many MSUD causing mutations were described, but the MSUD mutational spectrum has not been previously assessed in Portugal. **Objective**: In this study we present the molecular characterization of 30 Portuguese MSUD patients. **Methods**: Direct sequencing of the whole coding regions of *BCKDHA* (E1a), *BCKDHB* (E1b) and *DBT* (E2) was performed in order to identify the disease causing mutations. **Results and Conclusions**: We were able to characterize 24 patients and in 4 other cases one of the mutations was identified. The most common alteration was a C deletion in *BCKDHA* (R40GfsX23), already reported in the Spanish population. Interestingly, it was found in homozygosity in 11 patients all belonging to a gypsy community. Another mutation (D302A) was identified in this gene, as well as a new substitution (Y413H). In E1b, 4 alterations were identified – P200X; I214K; Q267X and R285X – all of them previously reported. In E2 gene, 4 patients have a newly described CT deletion (Y122LfsX2), and 4 other mutations were identified – K313N (already reported); D390G; L398P and P411Q. Concerning the remaining patients, search for molecular defects is still being performed.

098-P

MOLECULAR GENETICS OF MAPLE SYRUP URINE DISEASE (MSUD) IN TURKISH PATIENTS

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Background: In MSUD disease causing mutations can affect the *BCKDAH*, *BCKDHB* or the *DBT* genes encoding for the E1a, E1b and E2 subunits of the branched-chain 2-keto acid dehydrogenase (BCKDH) complex.
Methods: During the last years mutation analyses were performed in 34 Turkish patients with various forms of MSUD (29 classical, 5 variants) and their families by studying the exonic coding sequences of all three genes (*BCKDHA* with nine, *BCKDHB* and *DBT* with eleven exons each) derived from peripheral blood leucocytes. 14 patients were migrants living in Germany, Austria and The Netherlands, all with a consanguineous background.
Results and Conclusions: In 28 patients we identified mutations in two alleles, in one patient in only one allele and in 5 patients we could not identify any disease causing mutation. In total, we found 25 different mutations. In 28% of cases (total 57 alleles) the disease causing mutations were located in the *BCKDHA*, in 49% in the *BCKDHB* and in 23% in the *DBT* gene. In all except two patients the mutations occurred homozygously. One mutation was found three times, four mutations were found twice. The families came from 15 different Turkish cities/areas scattered over Turkey except for South-East Anatolia. In the collective studied we did not find a cluster for a single mutation.

099-A

OXIDATIVE STRESS IN MAPLE SYRUP URINE DISEASE: ANTIOXIDANT ENZYME ACTIVITIES AND SELENIUM LEVELS

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Background: Maple Syrup Urine Disease (MSUD) is caused by an inherited deficiency of the branched-chain α-keto acid dehydrogenase complex leading to accumulation of the branched-chain amino acids (BCAA) leucine, isoleucine and valine and their corresponding branched-chain alpha-keto acids. Therapy for this disease is based on a natural protein restricted diet with low BCAA and supplemented with a semi-synthetic formula of essential amino acids, vitamins and minerals (MSUD 1 or 2 – Milupa^{R}), without selenium. This treatment minimizes the accumulation of the toxic metabolites and contributes to the survival of affected individuals.
Methods: In the present study we evaluated the activities of antioxidant enzymes glutathione peroxidase, catalase and superoxide dismutase in erythrocytes from MSUD patients under treatment, and the levels of selenium, an important antioxidant element, in plasma from MSUD patients at diagnosis and under treatment.
Results: It was observed that there is a significant decrease in selenium levels at diagnosis, as well as during treatment in MSUD patients and that glutathione peroxidase erythrocytes activity is importantly reduced in treated patients, suggesting that oxidative stress occurs in MSUD.
Conclusions: It has been proposed that these biochemical findings could be secondary to dietary therapy and probably selenium supplementation may be useful to MSUD patients.

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100-P

THE INFLUENCE OF CONCURRENT LEUCINE BLOOD LEVELS ON ATTENTION PROCESSES IN ADULT MSUD PATIENTS

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Background: MSUD is a condition resulting in a lifelong risk for chronic and acute mental impairment. We investigated the acute effect of concurrent leucine blood levels on sustained attention in a repeated measurement design. These data could possibly contribute to the specification of target leucine blood concentrations for treatment.
Methods: Measures of sustained attention in a binary choice reaction time paradigm for 6 adult patients with MSUD (median 22.6 years) were compared with those of 7 healthy age-matched controls (median 24.6 years). Patients were tested between 2 and 7 times under different leucine blood levels (median 673 μmol/L, range 72–1643 μmol/L). Dependent variables were speed, stability of sustained attention and sensitivity to differentiate a signal from noise in the theory of signal detection (d′).
Results: Overall, patients were slower than controls. Speed of performance reduced significantly by 760 ms/100 μmol/L leucine blood level ($p < 0.04$), and stability deteriorated by 100 ms/100 μmol/L leucine blood level ($p < 0.001$). Sensitivity d' decreased significantly by 0.1/100 μmol/l leucine ($p < .009$). Starting with leucine blood levels above 600 μmol/L d′ was below the range of healthy controls. Correlation between d' and speed of performance was $r = -0.29$ ($p = 0.15$).
Conclusion: Obviously the effect of increasing leucine levels overrides a possible positive effect of test repetition, thereby demonstrating a clear dose-dependent influence of leucine blood levels on sustained attention.

101-P

ESSENTIAL FATTY ACID STATUS IN MAPLE SYRUP URINE DISEASE: TRIAL OF SUPPLEMENTATION

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Background: We previously documented essential fatty acid (EFA) deficiency in our patients with classical maple syrup urine disease (MSUD) who followed the UK dietary recommendations (1% En C18:2 n-6 and 0.2% En C18:3 n-3). We sought to supplement EFAs in this cohort according to the EU Population Reference Intakes (PRIs), (2% En C18:2 n-6 and 0.5% C18:3, n-3).
Methods: 10 subjects with MSUD (age range 6–28 years) were supplemented with either walnut oil, rapeseed oil, 'Solagen' or 'Mapleflex' to meet the EU PRI guidelines. Fatty acid profiles in erythrocytes were analysed at 18 and 52 weeks after supplementation.
Results: Arachadonic acid (AA) and eicosapentanoic acid (EPA) levels were noted to be within the reference range at the baseline and interval testing. Docosahexanoic acid (DHA) levels at the baseline measurement ranged from 2.6–12.4 pmol/10^6; (mean 9.05), (reference range: 15.2–37.6 pmol/10^6). DHA levels at 18 weeks ranged from 4.3–12.4 pmol/10^6; (mean 8.7) and at 1 year from 5.8–14.8 pmol/10^6; (mean = 10.9). The percentage increase in DHA levels over 1 year ranged from 11–123% (mean 37.5%).
Conclusion: There was no significant increase in DHA levels in patients on supplementation over the year period, all patients remained deficient. The palatability of the supplements was also problematic. We suggest that increased doses of EFAs could be incorporated into synthetic protein substitutes, or alternatively, supplementation with direct sources of EFAs rather than the precursors may improve EFA status over a shorter period.

102-P

PSEUDO-GLUTAMINE DEFICIENCY IN INFANT LIVER DISEASE

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Background: The γ-glutamyltransferase (γ-GT, EC 2.3.2.2) is a membrane-bound enzyme with highest expression in the liver and biliary tract. It is an early laboratory marker for liver cell damage and cholestasis. Moreover, increased γ-GT activity can cause a decrease in the serum glutamine concentration due to deamidation of glutamine. This phenomenon has been described in adult patients with liver disease but not yet in infancy.
Methods: During the metabolic work-up of an infant with liver disease (serum level of γ-GT 4287 U/L, normal $<$230 U/L) low levels of serum glutamine (268 μmol/L, normal 300–800) led to further investigations. Measurements of amino acids in serum by cation exchange chromatography were performed at different time intervals.
Results: There was a striking time dependent decrease of the serum glutamine concentration from 756 μmol/L to 268 μmol/L after 48 h. At the same time serum glutamate concentrations increased from 139 μmol/L to 567 μmol/L (normal 70–200).
Conclusions: Increased *in vitro* γ-GT activity can lead to severely decreased levels of serum glutamine unless amino acids are measured immediately after sampling. Therefore, physicians should tell the laboratory accompanying elevations especially of the γ-GT.

103-P

PROLIDASE DEFICIENCY WITH SEVERE NEUROLOGICAL MANIFESTATIONS: HOMOZYGOSITY FOR A NOVEL INTRONIC MUTATION

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Background: Prolidase deficiency (OMIM 170100) is a rare autosomal recessive condition caused by mutations in the prolidase gene (*PEPD*) located at 19q12–q13.11. This cytosolic enzyme is ubiquitously expressed and catalyzes hydrolysis of di- and tri-peptides with a C-terminal proline or hydroxyproline. Its deficiency results in iminodipeptiduria, skin lesions, susceptibility to pyogenic infections, and varying degrees of dysmorphisms and mental retardation. Seizures and profound neurological impairment have not previously been associated with this condition. **Methods and Results:** We describe a male infant, product of a consanguineous union, who presented at birth with significant hypotonia, feeding difficulties, myoclonic jerks and facial dysmorphism. Urine amino acids TLC analysis showed iminopeptiduria and glycylproline accumulation, suggesting a Prolidase Deficiency diagnosis. At 3 months of age he was developmentally delayed and started seizures. At 6 months of age, he has intractable seizures, profound delay and cerebral atrophy on MRI. Extensive investigations to identify a second disease responsible for the neurological symptoms have been negative. *PEPD* gene analysis revealed homozygosity for a novel intronic splice-site mutation, IVS11 +1G$>$A: both parents were found to carry this mutation in heterozygous state. Prolidase enzyme assay in the proband's leukocytes showed activity at 1% of control values: enzyme assay on the mother's leukocytes showed 50% of control values. **Conclusion**: We postulate that the severe neurological manifestations are caused by Prolidase Deficiency, and in this case, are attributable to homozygosity for this intronic mutation. Urine amino acids TLC should be part of the routine workup of a child with developmental delay or seizures.

104-P

IDENTIFICATION AND BIOCHEMICAL CHARACTERIZATION OF NOVEL MUTATIONS IN PATIENTS WITH 3-PGDH DEFICIENCY

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3-Phosphoglycerate dehydrogenase (3-PGDH) deficiency is a rare inborn error in the biosynthesis of the amino acid L-serine, characterized by congenital microcephaly, psychomotor retardation and intractable seizures. The biochemical abnormalities associated with this disorder are low concentrations of L-serine, D-serine and glycine in cerebrospinal fluid. Two missense mutations (V425M and V490M) have been identified in *PHGDH*, the gene encoding 3-PGDH, but it is currently unclear how these mutations affect enzyme function. Here, we set out to identify and biochemically characterize novel disease-causing mutations in *PHGDH*. Sequence analysis of *PHGDH* yielded four previously undescribed mutations in three patients with 3-PGDH deficiency; one frameshift mutation, and three missense mutations (R135W, V261M, A373T). None of the five known missense mutations lead to reduced expression of the enzymes, and pulse-chase experiments demonstrated that protein stability and degradation rates were unaffected. Enzyme activity analysis in patient's cultured fibroblasts revealed a significant, but incomplete, reduction in maximal activities. In contrast, when 3-PGDH was transiently overexpressed in HEK293T cells, the A373T, V425M and V490M mutations resulted in almost undetectable enzyme activities. Molecular modeling of the R135W and V261M mutations onto the partial crystal structure of 3-PGDH, predicted that these mutations effect substrate binding. This prediction was confirmed by the results of kinetic measurements in fibroblasts and transiently transfected HEK293T cells, which revealed a markedly decreased Vmax and an increase in Km values. Taken together, these data suggest that missense mutations associated with 3-PGDH deficiency either primarily affect substrate binding or result in very low residual enzymatic activity.

105-A

CHRONIC ADMINISTRATION OF METHIONINE INCREASES LIPID PEROXIDATION AND DECREASES ANTIOXIDANT DEFENSES IN LIVER OF RATS

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Tissue accumulation of methionine has been encountered in various inherited disorders, including methionine adenosyltransferase deficiency. Affected patients present neurological and hepatic damage. Since very little is known about the mechanisms underlying the liver damage present in this disorder and oxidative stress has been associated with the pathogenesis of hepatic injury, the objective of the present study was to investigate the effect of chronic administration of methionine on various parameters of oxidative stress, namely chemiluminescence, total radical-antioxidant potential (TRAP), total antioxidant reactivity (TAR), sulfhydryl content and carbonyl content, as well as on the activities of the antioxidant enzymes catalase, superoxide dismutase and glutathione peroxidase in liver of hypermethioninemic rats. Animals were treated daily with two subcutaneous injections of methionine (1.34–2.68 mmol/g of body weight) or saline from the 6th to the 28th day of life and were sacrificed 12 h after the last injection.
Results showed that methionine significantly increased chemiluminescence, decreased TRAP and catalase activity. In contrast, TAR, thiol, carbonyl contents, superoxide dismutase and glutathione peroxidase activities were not affected by hypermethioninemia. These findings suggest that the induction of oxidative stress caused by chronic administration of methionine may be related to the liver damage observed in methionine adenosyltransferase deficiency.

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106-P

NON-DETECTABLE URINARY SERINE BY TLC SCREENING IN A GROUP OF CHILDREN WITH SEIZURES

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Objective: L-Ser deficiency is a rare inborn error caused by defects in L-Ser biosynthesis: low serum and CSF Ser levels are associated with congenital microcephaly, PMR, and seizures. Low plasma Ser has also been found as a side effect of valproate therapy, and in patients with fibromyalgia. Reduced urinary Ser reportedly accompanies chronic fatigue syndrome; plasma D-Ser is decreased in patients with schizophrenia.
Methods: During a long-term experience of IEM screening we occasionally met a curious Ser abnormality: while all controls and patients had Ser in urine, in some children it was repeatedly below the detection limit of 2-d-micro-scale TLC on cellulose; since plasma Ser and other amino acids did not seem to be changed, they were not quantified. Similarity of the associated symptoms (always seizures, often hypotonia and/or mental retardation) has finally drawn our attention. Such cases have been recently followed, and TLC results supplemented with CZE and HPLC quantification.
Results: Contrary to normal plasma concentrations, lower urinary Ser levels, namely those of D-Ser were demonstrated; CSF was not available. Concentrations of Gly, Thr, Ser(P) and other amino acids, saccharides, and organic acids were normal. Gastrointestinal or renal problems have not been found; effect of therapy is not probable.
Conclusion: Unclear involvement of insufficient magnesium or protein intake versus a metabolic defect (Ser racemase, D-amino acid oxidase, or Ser transport) is speculated.

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107-P

BIRTH PREVALENCE OF HOMOCYSTINURIA IN CENTRAL EUROPE: FREQUENCY AND PATHOGENICITY OF MUTATION c.1105C>T (p.R369C) IN THE CYSTATHIONINE BETA-SYNTHASE GENE

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Objectives: Although homocystinuria due to cystathionine beta-synthase (CBS) deficiency is considered a rare disease molecular epidemiological studies in several European countries suggested that homocystinuria may be as common as phenylketonuria or MCAD deficiency. In this study we determined the prevalence of a common mutation c.1105C>T in Central Europe and we examined pathogenicity of the corresponding mutant CBS enzyme p.R369C.
Methods: The c.1105 C>T transition was analyzed by PCR-RFLP in 600 anonymous Czech newborn blood samples and pathogenicity of the p.R369C mutant was tested by determining its catalytic activity and ability to form tetramers in two expression systems.
Results: The c.1105 C>T mutation was detected at six of 1200 control Czech chromosomes resulting in the expected birth prevalence of homozygotes for this mutation 1:40 000 (95% CI 1:8000–1:295 000). In bacterial system the mutant p.R369C polypeptide severely misfolds and exhibits only 2% activity of the wild type enzyme. However, in the mammalian expression system employing Chinese hamster ovary cells, 31% of the p.R369C mutant enzyme present in cell extracts formed tetramers and yielded 42% specific activity relative to the wild type enzyme.
Conclusions: The mutation c.1105 C>T is present in high frequency also in the Czech Republic, where the calculates prevalence of homocystinuria due to c.833T>C and c.1105C>T mutations reaches unexpectedly high value of 1:15 500. Although pathogenicity of the p.R369C mutations is uncertain, our expression data show that it moderately impairs folding and activity of the enzyme predicting a rather mild clinical phenotype in homozygotes or compound heterozygotes carrying c.1105 C>T.

108-P

HOMOCYSTEINEMIA AMONG YOUNG CHILDREN IN MALAYSIA

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Besides dietary low vitamin B6, vitamin B12 or folate levels, elevated total homocysteine levels may be caused by cobalamine defects or by a genetic cystathionine-b synthase deficiency. For the quantitative determination of total homocysteine in plasma we used BIO-RAD homocysteine reagent kit by HPLC with precolumn derivatization with SBD-F and fluorescence detection. The normal range in plasma is 5.5–17 μmol/L in our study. Plasma level between 17 and 30 μmol/L are usually caused by diet-related vitamin deficiency. Plasma levels between 30 and 100 μmol/L after methionine load indicate the possibility of a heterozygous homocysteinemia, while plasma levels of more than 100 μmol/L usually are attributable to a homozygous homocystinuria. From the year 2004 to March 2007, we have analysed 255 samples received from all over Malaysia to rule out homocysteinemia. We found 5 cases with total homocysteine values of more than 100 μmol/L. They were presented with global developmental delay, learning difficulties, visions blurring and were found to have bilateral lens dislocation. Among them were 3 siblings aged between 7 to 15 years old from non-consanguineous marriage. One male patient from consanguineous parents also presented with recurrent abdominal pain. They were subsequently treated with low methionine diet (Hominex), pyridoxine and folate and Vit B12. We also found 2 patients with homocysteine level between 30 to 100 μmol/L, low methionine and elevated urinary methylmalonic acids and normal vitamin B12 level where provisional diagnosis of diagnosed cobalamin disorders was made. DNA mutational analysis for CBS and the cobalamin gene is under way.

109-P

DIAGNOSIS AND FOLLOW UP OF MOROCCAN PATIENTS WITH HOMOCYSTINURIA: EXPERIENCE OF BIOCHEMISTRY LABORATORY OF CHILDREN'S HOSPITAL OF RABAT

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Homocystinuria due to cystathionine beta synthase deficiency is an IEM of sulfur-containing amino acids that results in ectopia lentis, mental retardation, osteoporosis with bone deformities, thromboembolism and psychiatric disturbances. The major biochemical findings include high plasma homocysteine and methionine concentrations and low plasma cystein concentration. Two major phenotypes of the disease are equally common, a milder pyridoxine-responsive form, and a more severe non-responsive type.

In this study, we report our laboratory's experience in diagnosis and surveillance of patients affected by homocystinuria. Selected screening was realized by thin layer chromatography of amino acids associated to the nitroprusside test. Diagnosis confirmation was obtained by plasma amino acids determination (ion exchange chromatography) and/or by immunoenzymatic assay of homocysteine.

Since 1996, 22 cases of homocystinuria were diagnosed representing the second amino-acid disease screened in our service, just behind PKU. Mean age patients was of 10 years. Moreover, most frequent clinical signs were ectopia lentis (17 cases), vascular disease with life threatening thromboembolism (7 cases), skeletal deformities or osteoporosis (8 cases) and mental retardation (17 cases). High levels of homocysteine were shown either in plasma and urine associated to elevated plasma methionine suggesting a defect in cystathionine beta synthase. Therapeutic measures including vitamins B6 and B12, Folic acid and dietary management have concerned only 3 patients resulting in normalized plasma homocysteine level in one patient but no response was observed in the two others. These results are indicatives of the necessity of an urgent program of precocious diagnosis and efficient treatment of homocystinuria in our country.

110-A

HOMOCYSTEINE REDUCES ANTIOXIDANT DEFENSES IN PARIETAL CORTEX OF RATS *IN VIVO*

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Homocystinuria is an inborn error of metabolism biochemically characterized by tissue accumulation of homocysteine (Hcy) and clinically by vascular complications and neurological dysfunction. In the present study, we evaluated the effect of chronic hyperhomocysteinemia on total radical-trapping antioxidant potential (TRAP), total thiol content, and the antioxidant enzymes activities catalase (CAT) and superoxide dismutase (SOD) in parietal cortex of rats. Wistar rats received daily subcutaneous injections of Hcy (42–80 mg/kg) from the 6th to the 28th day of life. Control rats received the same volume of saline solution. 12 h after the last injection the rats were sacrificed and parietal cortex was dissected. Our results showed that Hcy administration reduced TRAP (27%) and CAT activity (19%), whereas SOD and total thiol content were not altered. Our data suggest that Hcy reduces non-enzymatic and enzymatic antioxidant defenses in parietal cortex, suggesting an induction of oxidative stress. These findings could contribute to explain, at least in part, the neurological dysfuntion characteristic of homocystinuria.

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111-P

EFFECT OF EXTRACELLULAR HOMOCYSTEINE ON H19 EXPRESSION IN HUVEC

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The involvement of epigenetic mechanisms in homocysteine's (Hcy) vascular toxicity is an emergent hypothesis. In fact, evidence has been documented the Hcy ability, via accumulation of its precursor S-adenosylhomocysteine (AdoHcy), to affect DNA methylation status. Methylation is a major epigenetic feature of genomic DNA, which can lead to alterations in gene expression. The present study is aimed at evaluating the effect of Hcy, using cultured human endothelial cells, on the expression pattern of an imprinted gene (H19), which is regulated by methylation.

A heterozygous cell line for a suitable RsaI RFLP in H19 gene was selected and cells were incubated with increasing concentrations of Hcy (0, 25, 75 and 150 mM, $n = 9$). After 24 h, cells were trypsinized and RNA, DNA, and cytosolic fractions were obtained. AdoHcy cytosolic concentrations were measured by stable-isotope dilution tandem MS. Assessment of global DNA methylation was accomplished using the cytosine extension assay. Allelic expression was analysed by RFLP pattern of cDNA digested with RsaI and H19 expression was quantified by qRT-PCR.

Cells incubated under the studied conditions exhibited neither significantly increased intracellular AdoHcy levels nor significantly decreased DNA methylation patterns, when compared to the control. Accordingly, a monoallelic H19 expression pattern and similar levels of H19 mRNAs were always observed. The obtained results were most probably due to an insufficient intracellular AdoHcy accumulation, and do not exclude the Hcy ability to affect H19 expression. Future studies are warranted to assess whether epigenetic changes are involved in the pathogenesis of Hcy related vascular disease.

112-P

MANIPULATING THE HOMOCYSTEINE METABOLISM IN HUVEC: EFFECT ON ADMA PRODUCTION

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Recent observations suggest that asymmetric dimethylarginine (ADMA), an endogenous inhibitor of endothelial nitric oxide synthase, may play a role in endothelial dysfunction associated with hyperhomocysteinemia. The present study was aimed at evaluating the effect of disturbed homocysteine (Hcy) metabolism on ADMA levels. With this purpose, methotrexate (MTX), an antifolate, and adenosine-dialdehyde (ADA), an S-adenosylhomocysteine (AdoHcy) hydrolase inhibitor, were used to modulate Hcy metabolism in cultured human endothelial cells (HUVEC). Incubation studies were carried out during 48 h, either in the absence (controls) or in the presence of different concentrations of MTX (25, 50 and 100 nM) or ADA (2.5, 5.0 and 10.0 microM). All experiments ($n = 3$) were performed between the second and fourth passage, after 3 days in monolayer. ADA and MTX cytotoxicity were ruled out by the lactate dehydrogenase (LDH) release assay. Extracellular concentrations of either Hcy or ADMA were determined by HPLC. Intracellular AdoHcy concentrations were determined by stable-isotope dilution tandem MS.

Under standard conditions, HUVEC exported a constant amount of Hcy (1.45 ± 0.67 microM/24 h). In the presence of MTX, a trend towards increased Hcy export, decreased intracellular AdoHcy levels, and no changes in extracellular ADMA concentrations were observed. In the presence of ADA, a significant decrease in Hcy and ADMA exports as well as increased AdoHcy intracellular levels were observed, when compared to the controls. The results indicated that the methylation inhibitor, AdoHcy, modulates ADMA formation by endothelial cells. Further studies are necessary to clarify the interplay between Hcy, ADMA and endothelial dysfunction.

113-P

AN UNUSUAL CASE OF B6-RESPONSIVE CYSTATHIONINE BETA SYNTHASE (CBS) DEFICIENCY REVEALED BY ECLAMPSIA

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CBS deficiency is typically associated with Marfan phenotype, mental retardation, ectopia lentis, premature osteoporosis, and vascular events. B6-responsive CBS patients have milder phenotypes and are often misdiagnosed.

A 32-year-old female presenting a clinical and biological syndrome of eclampsia was hospitalized at 28 weeks of pregnancy. Foetal death in utero was diagnosed. The biological analysis showed normal values for all the thrombophilic factors but a severe hyperhomocysteinemia (482 µmol/L) associated with low levels of folates (3.9 nmol/L; N: 6.9–39) and B6 vitamin (5.6 nmol/L; N: 20–90); B12 vitamin was normal (178 pmol/L; N: 133–664); the patient was homozygous for the C677T (A223V) MTHFR mutation. Blood amino acids chromatography showed elevated methionine (54 µmol/L) and low cystine (10 µmol/L) in line with the diagnosis of CBS deficiency. Examination did not find any abnormal phenotype or any lens ectopia; there was no previous vascular event in the patient's history. After 1 month of treatment with B6vitamin and folates, the homocysteine value was normalized (11 µmol/L). In fibroblasts, the low CBS enzymatic activity was confirmed (0.25 Katal/kg protein; N: 2.2–4.4).

Conclusion: It should be advised that (1) such a severe hyperhomocysteinemia cannot be only explained by the association of kidney injury, B6 vitamin and folate deficiency and MTHFR C677T homozygoty, and (2) in eclampsia the diagnosis of B6-responsive CBS deficiency should be considered.

114-P

TOTAL HOMOCYSTEINE, B-VITAMINS AND GENETIC POLYMORPHISMS IN PATIENTS WITH CLASSICAL PHENYLKETONURIA

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Background: In classical phenylketonuria (PKU) patients, significantly higher as well as lower concentrations of total homocysteine (tHcy), vitamin B6, cobalamin (Cbl) and folate compared to controls have been observed. Genetic polymorphisms influencing folate and Hcy metabolism have not been investigated. **Methods**: Open clinical trial. In 37 treated patients with classical PKU tHcy and Cbl, folate, vitamin B6, genetic polymorphisms, intake of total and natural protein, and phenylalanine (Phe) concentrations were investigated. THcy, folate and Cbl concentrations were compared to measurements in 63 healthy age and sex matched controls. **Results**: THcy exceeded the 97th percentile in 27% of cases. Cbl and folate concentrations were significantly ($p < 0.001$) and tHcy tendentially ($p = 0.06$) higher in PKU patients compared to controls. No difference between Hardy–Weinberg expectations and observed prevalence of genetic polymorphisms (*MTHFR* 677C>T, *MTHFR* 1298A>C, *MTR* 2756A>G, *MTRR* 66A>G and *GCP2* 1561C>T) was present in the PKU patients. Different genotypes had no impact on tHcy, Cbl or folate concentrations. The normal age-dependent decline of folate and Cbl and increase in tHcy concentrations was not present in PKU patients and tHcy concentrations were not determined by folate and Cbl concentrations. Phe concentrations and protein ingestion had no impact on tHcy concentrations. **Conclusions**: THcy metabolism is altered in PKU patients: tHcy concentrations are elevated but in contrast to other populations this finding is not explained by genetic polymorphisms and/or vitamin deficiencies. Additionally, no correlation between tHcy and adherence to diet is present.

115-P

EFFICACY AND SAFETY OF ORAL BETAINE IN THE TREATMENT OF HOMOCYSTINURIA

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Aim: To review the current knowledge about biochemical and clinical efficacy and safety of betaine in the treatment of severe hyperhomocysteinemia.

Methods: An exhaustive literature search to collect individual data on homocystinuric patients' clinical and biochemical condition before and/or after betaine administration was done. Prevalence of cardiovascular symptoms under betaine was compared with historical data of untreated patients. The disappearance of more acute clinical symptoms in individual patients after introduction of betaine was examined. We evaluated adverse events under betaine which were collected as solicited reports or as part of the post-marketing surveillance (PMS) for betaine anhydrous (Cystadane) in Europe and in the US.

Results: Individual biochemical and clinical features were available in 140 patients. The median dose of betaine was 150 mg/kg*day and median treatment duration was 12 months. Homocysteine responded in >92% of patients. Betaine was associated with a decrease of neurological symptoms and of cardiovascular complications. Safety data were available in >170 patients from the literature and >1000 patients from PMS. Adverse events under betaine were rare and generally not serious. However, 4 cases of cerebral oedema could have been associated with betaine therapy. 7/9 successful pregnancies were reported in 7 women while taking betaine.

Conclusions: The available data do not allow correlating dosage and clinical efficacy. The pooling of data, as described above, clearly demonstrated the evidence for a biochemical (Level II) and a clinical (Level III) benefit of betaine supplementation in homocystinuria. Betaine therapy seems to be safe unless there is poor dietary control of hypermethioninemia.

116-P

RAPID AUTOMATED ANALYSIS OF TOTAL HOMOCYSTEINE AND METHIONINE IN PLASMA BY STABLE ISOTOPE DILUTION AND ELECTROSPRAY TANDEM MASS SPECTROMETRY

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Background: We developed a method based on ultra performance liquid chromatography tandem mass spectrometry for the simultaneous quantitation of total homocysteine (tHcy) and methionine (Met) in plasma. The objective of this method was to develop an automated high throughput method suitable for routine analysis of tHcy. In addition, we wanted to include Met as a tool to simultaneously cathegorize patients with disturbances in the remethylation cycle.

Methods: The reductant tris(2-carboxyethyl)phosphine (TCEP) was used instead of more conventional reductants. TCEP is more user friendly and is specific for disulphide bonds. Isotopically labeled internal standards were mixed with plasma followed by addition of TCEP in a 96-well format. Barcode reading and pipetting was performed by an AutoDelfia. The samples were then deproteinized by filtration into a new plate. The samples were injected on a C8 column connected to a mass spectrometer operating in MS/MS mode.

Results: The agreement was compared with reference methods (HPLC with fluorescence detection and ion exchange chromatography for Hcy and Met respectively). The correlation between the reference method and the proposed method showed an acceptable agreement for tHCY (slope = 0.87, $r^2 = 0.93$) and Met (slope = 0.93, $r^2 = 0.79$). The CV was 13% (range 10–38 mM) for tHCY and 10% (range 20–65 mM) for Met. The injection-to-injection time was 1.5 min.

Conclusions: The method has been in use for three years and allows rapid determination of tHcy and Met while maintaining high specificity and sensitivity. The method is suitable for routine laboratories as well as metabolic laboratories interested in metabolic investigations.

117-P

DOES MATERNAL ANTENATAL INTERVENTION AND TREATMENT FROM BIRTH IMPROVE THE OUTCOME OF METHIONINE SYNTHASE (cbl G) DEFICIENCY? A COMPARISON OF TWO SIBLINGS

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Methionine synthase (cbl G) deficiency (McKusick 250940) is a rare congenital metabolic disorder characterised by methylcobalamin deficiency, megaloblastic anaemia, severe developmental delay and homocystinuria in the absence of methylmalonic aciduria. We report and compare two siblings with this disorder. The index case, a 3-month-old boy, presented with severe failure to thrive and neurological regression (weight 2nd centile, head circumference <0.4th centile). He had macrocytic anaemia (MCV 103 fl (76-100)) with low B12 (137 ng/l (210–900)) and homocystinuria without increased methylmalonate excretion. Further investigation revealed total homocysteine 115 μmol/L (<14.3) and methionine 8 μmol/L (15–45). An inherited defect of B12 metabolism was suspected. Intramuscular injection of B12 was commenced with dramatic symptomatic improvement. Methionine synthase deficiency was confirmed by fibroblast studies. On B12, betaine and latterly folic acid, total homocysteine levels are maintained in the range 20–38 μmol/L and methionine 13–48 μmol/L. At the age of 6 he is well but has residual significant learning difficulties and hyperactivity. In contrast his affected sister, now 2 years old, has demonstrated normal growth and development. The defect was identified in utero by functional and complementation studies on cultured amniocytes. Maternal treatment with twice weekly B12 injections was commenced at 22 weeks gestation. B12 and betaine therapy were commenced at birth. Homocysteine and methionine levels are in the range 14–22 μmol/L and 16–39 μmol/L respectively. The contrasting clinical outcome of these two siblings suggests early intervention with antenatal therapy can significantly improve the morbidity associated with this disorder.

118-P

WHOLE BLOOD S-ADENOSYLHOMOCYSTEINE – A RELIABLE BIOMARKER OF S-ADENOSYLHOMOCYSTEINE HYDROLASE DEFICIENCY

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S-Adenosylhomocysteine hydrolase (AdoHcyase) deficiency is a recently reported inherited multisystemic disorder of sulfur amino acid metabolism with myopathy present at birth. The diagnosis of this disorder has so far been confirmed in five patients. Our experience with three patients indicated that early diagnosis and intoduction of methionine restriction diet and supplementation with phosphatidylcholine and creatine may improve clinical outcome. Elevated plasma S-adenosylhomocysteine (AdoHcy) seems to be a reliable biomarker of the disease.

Unfortunately, hypermethioninemia as a marker important also for neonatal screening, may be absent or nonsignificant. Unspecific laboratory changes include elevation of creatine kinase (up to 70× normal) and aminotransferases (up to 4× normal). In this study we checked if whole blood AdoHcy could be a useful biomarker of the disease. We measured AdoHcy and S-adenosylmethionine (AdoMet) concentration in perchloride acid-treated whole blood by HPLC with UV-detection (modification by Fux R. et al., 2005).

In three patients in the period of normal diet, AdoHcy was 6.5–24-fold higher than the reference range, previously not reported in children (n = 75). AdoMet/AdoHcy ratio was also significantly decreased, i.e. down to 18× normal. Therefore, AdoHcy and AdoMet/AdoHcy ratio in whole blood could be useful biomarkers for the diagnosis of AdoHcyase deficiency, particularly in laboratories without tandem mass spectrometry and sensitive HPLC detectors. This is additionally important since our past studies indicated that the disease might be largely underdiagnosed.

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119-P

GENETIC POLYMORPHISMS OF METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR), METHIONINE SYNTHASE REDUCTASE (MTRR), AND REDUCED FOLATE CARRIER-1 (RFC-1) IN A HIGH NEURAL TUBE DEFECT RISK POPULATION

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The frequency of polymorphisms associated with folate and homocysteine metabolism varies significantly among different ethnic groups. Population screening and genotyping may provide insight to the frequency of certain populations' illnesses. Ukrainian population displays NTD incidence that is over fourfold the expected frequency. We investigated the polymorphic frequency of methylenetetrahydrofolate reductase (MTHFR), methionine synthase reductase (MTRR), and reduced folate carrier-1 (RFC-1) mutations in 200 Ukrainians. Ukrainian polymorphic frequencies were analysed and compared to African American, Ashkenazi Jewish, Caucasian, and Hispanic populations. DNA was isolated from blood on discarded filter paper from routine newborn screenings in Ukraine. Gene fragments of *MTHFR*, *MTRR*, and y were amplified via PCR. We studied genotypes and allele frequencies of *MTHFR* C677T, A1298C, G1793A; *MTRR* A66G, and *RFC-1* G80A. Homozygosity for the *RFC-1* G80A allele (GG) in Ukrainians (38.42%) was significantly higher than 20.8% in African Americans (p = 0.002). The *RFC-1* G80A allele frequency (A) for Ukrainians (38.4%) was lower than for Ashkenazi Jews (p = 0.047) and African Americans (p <0.0001). Ukrainian population demonstrated an average allele frequency for the *MTHFR* C677T polymorphism. The *MTRR* A66G and *RFC-1* G80A polymorphisms were particularly high in this population, 57.0% and 60%, respectively. The *MTRR* A66G polymorphism allele frequency was highest in Ukrainians than in any other population currently reported aside from the anomalously high Russians. In addition to low folate status, single and compound homozygosity and heterozygosity for the *MTRR* A66G and *RFC-1* G80A polymorphisms may play a role in high incidence of NTDs in Ukrainians.

120-P

ASYMMETRIC DIMETHYLARGININE, HOMOCYSTEINE AND NITRIC OXIDE LEVELS IN HYPERCHOLESTEROLEMIC CHILDREN

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One of the leading cause of the health problems is mortality related with atherosclerotic heart diseases, worldwide and in our country.The clinical symptoms of atherosclerosis appear in adult period but the atherosclerotic process starts to affect in childhood. Therefore the risk factors which are diagnosed in childhood, can be the predictive indicators for cardiovascular risks in future.

In the presented study asymmetric dimethylarginine (ADMA), nitric oxide (NO) and homocysteine concentrations of hypercholesterolemic children were determined.The study is being performed on 47 hypercholesterolemic children ages between 5–15 and control group includes 20 healthy children. ADMA and homocysteine concentrations of the samples were determined with high performance liquid chromatography.

ADMA levels were significantly high in hypercholesterolemic children than in the control group (1.92 μmol/L, 1.52 μmol/L respectively, p <0.01) while there were no considerable difference in NO and homocysteine levels.

As a conclusion ADMA level can be the early leading indicator in hypercholesterolemic children with atherosclerotic risks.

121-P

DEVELOPMENTAL CHANGES IN THE L-ARGININE/NITRIC OXIDE-PATHWAY FROM INFANCY TO ADULTHOOD: ADMA LEVELS DECREASE WITH AGE

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Introduction: Asymmetric dimethylarginine (ADMA) has adverse effects on renal and cardiovascular function in adults. First steps to study its role in the paediatric population have been made. Compared to age-matched controls we found elevated ADMA levels in infants with citrullinaemia[1]. Reference data for healthy children in the age group from infancy to adulthood are lacking.
Aim: We investigated the status of metabolites and regulatory compounds of the L-arginine/NO pathway in 34 healthy children/juveniles.
Methods: ADMA in plasma and urine as well as L-arginine in plasma were determined by GC-MS-MS[2]. Nitrate and nitrite in plasma and urine were determined simultaneously by GC-MS[3].
Results: ADMA levels in plasma decreased with age (Pearson correlation: –0.619). This finding was paralleled by an increase of nitrate (p.c.: 0.471) and nitrite (p.c: 0.484) in urine with increasing age. No significant changes were found for nitrate, nitrite and L-arginine in plasma.
Conclusions: ADMA levels in childhood decrease with age. Reference data for metabolites of the L-arginine/NO pathway established for adults cannot be used for the paediatric population. The lower concentrations of nitrate and nitrite in the urine of very young children may indicate inhibition of NOS by elevated ADMA levels.

[1]Lücke T et al. Metabolism 2006;55(12):1599–603.
[2]Tsikas D et al. J Chromatogr B 2003; 798:87–99.
[3]Tsikas D et al. Anal Chem 2000;72:4064–4072.

122-P

A NOVEL APPROACH FOR ANALYZING ORGANIC ACIDS BASED ON DERIVATIZATION WITH A BENZOFURAZAN REAGENT AND UPLC-MS/MS; APPLICATION TO GLUTARIC ACIDEMIA TYPE 1

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Background: A large number of metabolic disease markers have poor ionization in electrospray ionization tandem mass spectrometry (ESI-MS/MS). To overcome this, we developed and evaluated labeling reagents carrying an ionizable moiety with high proton affinity and a lypophilic benzofurazan structure to improve the chromatographic behavior in the reversed phase mode. In this work, we report a new LC-MS/MS approach for the diagnosis of glutaric acidemia type 1 (GA1) and related disorders based on derivatization with DAABD-AE (4-[2-(N,N-(dimethylamino) ethylaminosulfonyl]-7-(2-aminoethylamino)-2,1,3-benzoxadiazole). **Methods:** Glutaric acid (GA), 3-hydroxyglutaric acid (3HGA) and related dicarboxylic acid markers in untreated urine (5 microliter) were derivatized with DAABD-AE for 45 min at 60°C. Separation was performed on an ACQUITY C18 column (2.1 × 50, 1.7 micron) within 5 min using a UPLC system interfaced with Quattro micro API triple quadrupole mass spectrometer. **Results**: Under optimized conditions, the product ion spectra yielded a major fragment at m/z 151 common to all derivatives and was assigned to the dimethylaminoethylaminosulfonyl moiety originated from DAABD-AE. Calibration curves of GA and 3HGA were linear up to 1 mmol/L in urine with detection limits (signal-to-noise ratio = 3) of 0.025 and 0.02 micromol/L, respectively. Intra-day (n = 11) and inter-day (n = 6) variations were less than 11.2%. The assay was successfully applied to control (n = 134) and GA1 patients' urine samples (n = 55). **Conclusion:** A derivatization approach to improve LC-MS/MS analysis of organic acids was introduced. The method was successfully applied for the diagnosis of GA1 and was applicable to glutaric acidemia type 2, ethylmalonic aciduria, HMG-CoA lyase deficiency and 3-methylglutaconic aciduria.

123-P

MEASUREMENT OF URINARY ACYLGLYCINES BY TANDEM MASS SPECTROMETRY

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Background: Acylglycines are the product of an important detoxification process in the liver reducing the accumulation of acyl-CoA esters. They are excreted in increased concentrations by patients with certain metabolic disorders, including fatty acid oxidation defects, and measurement of these urinary acylglycines is an important diagnostic tool. Analysis has historically been performed by gas chromatography-mass spectrometry, which is time consuming. We have developed a faster and more sensitive method for their analysis using tandem mass spectrometry (TMS) with stable isotope dilution. **Methods**: Analysis was carried out using a liquid chromatograph interfaced to a TMS system (Waters 2795/Quattro micro) with electrospray ionisation (ESI) operated in negative ion mode. Sample preparation involved diluting urine with acetonitrile/water (50:50) containing internal standard (13C2 hexanoylglycine). Multiple reaction monitoring mode was used to allow quantitative analysis of nine acylglycines: propionylglycine, tiglylglycine, 3-methylcrotonylglycine, isovalerylglycine, 2-methylbutyrylglycine, hexanoylglycine, octanoylglycine, 3-phenylpropionylglycine and suberylglycine. The possibility of separating the two pairs of isomers was examined and normal ranges established. Urine samples from a group of patients with known diagnoses and external control samples were analysed. **Results**: We were unable to separate one pair of isomers (isovalerylglycine, 2- methylbutyrylglycine) by mass difference. The other pair (tiglylglycine, 3-methylcrotonylglycine) could almost be separated (3-methycrotonylglycine interfered with tiglylglycine by 2% and tiglylglycine interfered with 3-methycrotonylglycine by 22%). All the affected cases excreted increased amounts of the expected acylglycines consistent with their diagnoses. **Conclusions**: The method works well as a rapid screening technique able to identify metabolic defects that produce increased acylglycines.

124-P

ROUTINE DETERMINATION OF OXALIC ACID IN PLASMA AND URINE USING LC-MSMS

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Background: During the last decade, LC-MSMS has become an important analytical tool for routine measurement of a variety of metabolites in many laboratories working with IEM. Oxalic acid (OX), one of the diagnostic markers of primary hyperoxaluria (PH), is eliminated from the body by renal excretion, but because of poor solubility of CaOX, deposition in the kidneys occurs. The laboratory findings in PH depend on the clinical stage of the disease: As long as the kidney function is sufficient, plasma oxalate can be normal or close to normal, while the urine level is largely increased. Later in the course of the disease, renal failure typically develops, causing a decrease in renal excretion combined with increased plasma level. As a result, quantification of oxalic acid in both plasma and urine is important.
Methods/results: We have developed and validated a fast and reliable LC-MSMS method for this purpose, using isotopically labelled oxalic acid as internal standard. The procedure involves automatic sample cleanup using solid phase extraction followed by derivatization. OX and its internal standard are separated from interfering compounds on LC and identified and quantified on MSMS using multiple reaction monitoring.
Conclusion: Our LC-MSMS method has shown to be applicable for routine determination of oxalic acid in both plasma and urine.

125-P

IMPACT OF SHORT AND MEDIUM CHAIN ORGANIC ACIDS, ACYLCARNITINES, AND ACYL-CoAs ON CENTRAL COMPONENTS OF MITOCHONDRIAL ENERGY METABOLISM

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Background: Accumulation of organic acids as well as their CoA and carnitine esters in tissues and body fluids is a common finding in organic acidurias and beta-oxidation defects. Pathomechanistic approaches for these disorders have been often focused on the effect of accumulating organic acids on mitochondrial energy metabolism and have sometimes revealed conflicting results. However, little was known about the pathophysiologic role of short and medium chain acyl-CoAs and acylcarnitines.
Methods: We studied the impact of short and medium chain mono- and dicarboxylic acids as well as their CoA and carnitine esters (C1-C8, DC1-DC5; up to 1 mM) on central components of mitochondrial energy metabolism, namely respiratory chain, pyruvate dehydrogenase complex (PDHc), and alpha-ketoglutarate dehydrogenase complex (KGDHc).
Results: The main finding of our study was the inhibition of PDHc and KGDHc by acyl-CoAs depending on their concentration, chain length, and number of carboxy groups, most likely via product feedback inhibition of the E2 subunits. The strongest inhibitory effect of pathophysiologically accumulating acyl-CoAs (1 mM approx. 70% inhibition) revealed glutaryl-CoA on KGDHc and propionyl-CoA on PDHC. The tested organic acids had no effect on any of the investigated enzyme complexes except for the known inhibition of succinate dehydrogenase by malonate. The corresponding acylcarnitines did not affect PDHc and respiratory chain but inhibited slightly KGDHc independent of their chain length.
Conclusions: In summary, product feedback inhibition of KGDHc and PDHc is a metabolic modulator that may induce damage to the cell when acyl-CoAs structural similar to the products accumulate pathophysiologically.

126-P

OUTCOME OF ORGANIC ACIDEMIA IN INDIAN CHILDREN

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Background: With a population over 1 billion and annual birth of about 28 million babies, the number of children born with inborn errors of metabolism is expected to be quite high. In absence of any formal newborn screening it is impossible to even guess the number of actual babies born alive with any IEM, especially organic acidemias. As regards organic acidemia, Muranjan et al. has shown an incidence of 32 out of 231 children (13%) with organic acidemia and overall mortality of 9.3%.
Methods and Results: Out of 1844 children investigated for IEM in last 7 years, we had 88 children with organic acidemia (4.77%) and a mortality of 37.5% (33/88). Amongst the 6 commonest OAs we had MMA 13 (14.77%), PA 11(12.5%), GA Type I –10 (11.36%), FDPase def. 9 (10.23%), biotinidase deficiency 8 (9.09%) and MSUD 7 (7.96%). Mortality rates were – MSUD (100%), GA Type II (100%), HMG CoA Lyase def. (100%) and propionic acidemia (55%). Neurological affection was 100% in GA Type I and quite high in MMA (53.85%).
Conclusion: Organic acidemias are common in Indian children. Unfortunately they are detected late, only after the symptoms have appeared. Most of the times, the diagnostic facilities are not available immediately and the cost of diet and medicines is beyond reach of most of the Indian patients. In such a situation the mortality is unacceptably high and those who survive have high rate of neurological affection and other complications.

127-P

OUTCOME OF ORGANIC ACIDEMIA: AN 8-YEAR EXPERIENCE IN MALAYSIA

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Organic acidemia represents a group of treatable inborn errors of metabolism. Early diagnosis and management affects their outcome. They can be detected by newborn screening and can be diagnosed by urine GC-MS. Over 8 years, we diagnosed 115 patients with various organic acidemia by GC-MS without newborn screening in Malaysia giving an estimated incidence of 1 in 35 000 live-births. There were 41 methylmalonic acidemia (MMA), 10 propionic acidemia (PA), 13 isovaleric acidemia (IVA), 17 glutaric acidemia type I (GAI), 12 with 3-hydroxyisobutyric acidemia, 6 biotinidase deficiency and 4 multiple carboxylase deficiency, 3 with 3-hydroxy-3 methylglutaric CoA lyase deficiency, 3 had beta-ketothiolase deficiency, 3 had malonic aciduria, 2 with 3-methylglutaconic acidemia, and one with 3-methylcrotonyl aciduria. 88 patients (77%) still survived on our follow-up management. 15 patients (13%) from distant hospitlas were diagnosed post-mortem. Good outcome with normal neurological status was achieved in HMG CoA lyase deficiency (100%), biotinidase deficiency (100%) and IVA (70%) despite crises. PA and 3-hydroxyisobutyric acidemia patients had moderate outcome, 50% being normal or mildly delayed. 17 patients with MMA (42%) passed away and 14 of them in early neonatal period. 10 out of 23 (43%) MMA patients who had dialysis survived. Only 11(26%) had good outcome and half of them were B12 responsive or late variant. 15 out of 17 patients (88%) with GA I were diagnosed late and neurologically devastated. All 3 malonic acidemia patients passed away due to dilated cardiomyopathy. These data provide useful information for strategic planning of newborn screening in the country.

128-P

MALONIC ACIDURIA – CLINCIAL COURSE AFTER DIAGNOSIS IN NEWBORN SCREENING

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Background: Malonic aciduria (deficiency of malonyl-CoA decarboxylase) is said to exist in two forms, a neonatal and late-onset form. The clinical features are variable and comprise mild developmental delay, episodes of severe metabolic acidosis, hypogylcemia and hyperammonemia, seizures and cardiomyopathy. Patients should benefit from low-fat diet and carnitine supplements. Very few subjects were identified by ESI-MS/MS-based newborn screening and todate some were without any problems. We report 4 children (15 months, 3 years, 4 years and 4 years old) in whom malonic aciduria was identified by newborn screening. On conformation diagnosis all subjects had substantially increased urinary levels of malonic acid. Without a specific diet and carnitine supplement they did not present episodes of acute metabolic derangement even during catabolic stress. One child showed mild delevelopmental delay, one had a delayed speech development. The blood carnitine levels were in the low normal range and there were no signs of cardiomyopathy. In three patients pathogenic mutations were demonstrated in the *MLYCD* gene. In one patient mutational analysis was not performed so far.
Conclusion: The cases demonstrate that there are asymptomatic infants with malonyl-CoA decarboxylase deficiency and that episodes of metabolic derangement during catabolic stress are not necessarily expected.

129-P

THE ROLE OF THE BLOOD–BRAIN BARRIER IN NEUROPATHOGENESIS OF DICARBOXYLIC ORGANIC ACIDURIAS

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Introduction: Biochemically, some organic acidurias are characterised by the accumulation of dicarboxylic acids (DCAs) in tissue and body fluids, e.g. methylmalonic aciduria and glutaric aciduria type I. Clinically, affected patients often present with severe neurological impairment due to neurodegeneration. In a recent study, we have postulated that the blood–brain barrier (BBB), which is supposed to have a low permeability for DCAs, plays a crucial role in the neuropathogenesis of these diseases facilitating intracerebral accumulation of putatively toxic metabolites ('trapping hypothesis').
Methods: We investigated the transport of d3-methylmalonic acid (d3-MMA), d4-glutaric acid (d4-GA) and d5-3-hydroxyglutaric acid (d5-3-OH-GA) across the BBB in cultivated human brain endothelial cells (hCMEC/D3) co-cultured with astrocytes (SV-NRA) and in primary porcine brain capillary endothelial cells (PBCEC). Metabolites were detected by GC/MS analysis. Their influence on viability of hCMEC/D3 was determined by the release of lactate dehydrogenase.
Results: The transport experiments with deuterated DCAs did not display any saturable component. Further we did not found any cis-inhibitory and only a weak trans-stimulatory effect of p-aminohippuric acid. Moreover, DCAs showed no influence on cell viability following 24 h of incubation (100 μM–5 mM MMA, GA, 3-OH-GA).
Conclusion: Our results suggest that MMA, GA and 3-OH-GA do not influence BBB integrity and that the specific transport capacity of the BBB for these DCAs is very low. In conclusion, our results confirm some biochemical aspects of the 'trapping hypothesis' for methylmalonic aciduria or glutaric aciduria type I.

130-P

OXIDATIVE STRESS INDUCED APOPTOSIS IN PATIENTS WITH METHYLMALONIC ACIDEMIA

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Methylmalonic acidemia (MMA) is a heterogenous group of rare genetic metabolic disorders caused by defects related to intracellular cobalamin metabolism. Increasing evidence has emerged suggesting that free radical generation is involved in the pathophysiology of neurodegenerative diseases, including some inborn errors of metabolism. We have previously identified in MMA patients several differentially expressed proteins involved in oxidative stress (mitochondrial superoxide dismutase (MnSOD) and mitochondrial glycerophosphate dehydrogenase (mGPDH)) and apoptosis by a proteomic approach. We have now extensively evaluated various parameters related to oxidative stress and apoptosis in cultured fibroblasts from a spectrum of 18 patients with MMA disease (8 cbl A, 5 cbl B, 2 cbl H / cbl D-2, 2 cbl C and 2 mut). Fibroblasts from MMA patients (particularly cbl B patients) showed a significant increase in intracellular ROS level and percentage of apoptotic cells with respect to controls. Methylmalonic acid increased the ROS level and the percentage of apoptotic cells, and decreased the mitochondrial membrane potential (MMP). The decrease in MMP and the activation of caspase 9 suggest that apoptosis process primarily involves the mitochondrial/caspase-dependent pathway. In addition, significantly higher levels of MnSOD protein expression and mRNA were detected in MMA patients compared to normal individuals, suggesting a cellular response to intrinsic ROS stress. Moreover, we have demonstrated that mGPDH is an important ROS generator in MMA patients by siRNA. These findings support the possible role of oxidative stress in the pathophysiology mechanism underlying MMA neurodegeneration.

131-O

MULTIPLE OXPHOS DEFICIENCY AND MITOCHONDRIAL DNA DEPLETION IN THE LIVER OF A PATIENT WITH cbl A METHYLMALONIC ACIDURIA SENSITIVE TO VITAMIN B12

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Background: Defects of adenosylcobalamin A, which is the coenzyme of the methylmalonyl-CoA mutase (MUT) is responsible for methylmalonic aciduria (MMA) responsive to vitamin B12. A few reports have supported the hypothesis that secondary respiratory chain deficiency could be the cause of complications observed in MMA patients. **Case report:** A patient with cbl A MMA responsive to vitamin B12 (homozygous c.387, nonsense mutation in exon 2 of the *MMAA* gene) and considered to have a well-controlled metabolic disease with a very low urinary excretion of methylmalonic acid, presented with an extremely sudden and severe visual impairment due to optic atrophy without retinal degeneration. Six months later, he presented with a severe metabolic distress, with lactic acidosis and multiorgan failure leading to death. **Results**: A multiple OXPHOS deficiency was found in the patient's liver with reduced absolute activity values of mtDNA-encoded complexes (I, III, IV and V) and abnormal activity ratios. A profound mtDNA depletion was also identified in the liver, with a residual mtDNA content of 16%. **Conclusion**: We describe for the first time multiple OXPHOS deficiency and mitochondrial DNA depletion in the liver of an MMA-CblA, B12 sensitive patient. Deficient methylmalonyl-CoA mutase results in an accumulation of methylmalonyl-CoA, but also in a reduction of succinyl-CoA, which affects the activity of the succinyl-CoA synthase (SCS), known to be responsible for mtDNA depletion, and influences the overall flux of the tricarboxylic acid (TCA) cycle. This hypothesis confers a major role to the TCA cycle in the physiopathology of long-term complications in MMA.

132-P

TISSUE ACYLCARNITINE ANALYSIS IN PATIENTS WITH METHYLMALONIC ACIDURIA

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Background: Methylmalonic aciduria (MMA) is one of the inborn errors of metabolism, caused by the defect of methylmalonyl-CoA mutase or vitamin B12 insufficiency. In this disease, methylmalonyl-CoA and propionyl-CoA accumulate and conjugate with internal carnitine by carnitine acetyltransferase, and then methylmalonylcarnitine (MMC) and propionylcarnitine (PC) are excreted into urine. Thus, the administration of L-carnitine is essential for the patients of this disease, and monitoring of carnitine and acylcarnitine (AC) levels is useful for the diagnosis and assessment of the metabolic state. We have developed the method of detailed determination of ACs including MMC and PC using HPLC-ESI/MS-MS (Maeda, Ito et al., Rapid Commun Mass Spectrom, 2007;21:799–806). Here we applied this method to tissue AC analysis and measured tissue ACs in two patients with MMA.
Cases: Two girls were chemically diagnosed by GC/MS and had genetic mutations of methylmalonyl-CoA mutase gene. Both were dead because of acute metabolic decompensation and tissue samples were collected by autopsy after informed consent.
Method: ACs that were extracted from the lyophilized tissues were purified by solid phase extraction and applied to the HPLC-ESI/MS-MS system with multiple reaction monitoring.
Result: In the samples of urine and serum, PC concentrations were much higher than the MMC. The same result was seen in the kidney samples, whereas, high concentrations of MMC were determined in the liver and skeletal muscle.
Conclusion: We applied a new nethod for tissue AC analysis. The discrepancy of the AC levels in the tissues should be elucidated.

133-P

DEVELOPMENT OF PHARMACOLOGICAL THERAPIES TO TREAT METHYLMALONIC ACIDURIA (MMA) RESULTING FROM MUTASE DEFICIENCY

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The majority of mutations resulting in MMA are either missense or nonsense. Increasing the expression of the MUT gene in patients with missense mutations and low-levels of residual MCM activity could potentially be therapeutic. A bacterial artificial chromosome containing the entire human MUT functional genomic sequence was identified and an in-frame fusion between the enhanced green fluorescent protein (EGFP) reporter gene and the human MUT locus was constructed and used to create stable clones in HeLa cells. Cells were cultured in the presence of a number of chemicals and MUT-EGFP expression assessed. Cisplatin, butyric acid, propionic acid, lactate and wheat grass increased MUT-EGFP expression up to 1.3 fold of untreated.

An alternative for patients with nonsense mutations involved assessing the use of aminoglycosides to cause stop codon readthrough. HeLa cell clones stably expressing the entire human MUT locus, modified to include a nonsense mutation, together with an in-frame EGFP reporter gene were created. Cells were cultured in the presence of G418 and/or gentamicin. An increase in human MUT mRNA expression was detected by Real Time RT-PCR. Flow cytometric analysis revealed a resultant increase in green fluorescence following treatment with G418 and gentamicin alone, with an additive effect when the two were combined, giving a 1.7 fold increase in MPF after 72 h.

These in vitro data highlight the potential of pharmacological agents to alleviate the effects of the genetic defect causing MMA in some patients. Future plans involve further evaluation of these drugs in various existing mouse models of MMA.

135-P

PROPIONIC AND METHYLMALONIC ACIDEMIA: ANTISENSE THERAPEUTICS FOR INTRONIC VARIATIONS CAUSING ABERRANTLY SPLICED mRNA

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In this work we report molecular studies and antisense oligonucleotide therapy of intronic molecular defects identified in methylmalonic (MMA) and propionic acidemia (PA). The patients exhibited aberrantly spliced mRNA with an inserted intronic fragment due to a deep intronic point mutation. We have identified a 72 bp insertion between exons 6 and 7 in the *PCCB* gene (r.654ins72), in homozygous fashion and two insertions in heterozygous fashion, an 84 bp insertion between exon 13 and exon 14 in *PCCA* gene (r.1209ins84) and a 76 bp insertion between exons 11 and 12 in the *MUT* gene (r.1957ins76). The changes identified increased the Shapiro and Shenapathy score or generate consensus binding motifs for splicing factors such as SC35, SRp55 which most likely favor the intronic inclusion. Modified antisense morpholino oligonucleotides (MO) complementary to the 54 or 34 cryptic splice sites have been used to block access of the splicing machinery to the pre-mRNA. Using this antisense therapeutics we have obtained full-length mRNA after 24 h of transfection and PCC or MUT activity were rescued in patient's fibroblasts. In MUT affected patients 40% of MCM activity measured by incorporation of 14C-propionate was obtained at 48 h and 100% of PCC activity was measured in both PA patients at 72 h. Our results demonstrate that the inclusion of these intronic sequences are disease causing mutation in these patients. These findings could provide a new therapeutic strategy to correct splice mutations caused by aberrant intronic inclusions.

134-P

CURRENT DIAGNOSTIC AND THERAPEUTIC STRATEGIES IN ISOLATED METHYLMALONIC ACIDURIAS – RESULTS FROM 16 EUROPEAN METABOLIC CENTRES

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Background: Many children affected by methylmalonic acidurias (MMA) survive the acute metabolic crisis following implementation of an emergency treatment protocol, but to prevent serious complications long-term management must be optimized. The major aim of this study was to evaluate the current practice in metabolic centres. **Methods**: Standardised questionnaires were sent to 20 metabolic centres. The questionnaire included questions on confirmation of diagnosis, standard procedures to test cobalamin responsiveness, dietary protocol, continuous pharmacotherapy, and biochemical and clinical monitoring. **Results**: 16 of 20 metabolic centres (80%) returned questionnaires on 183 patients: 89 patients were classified as mut0, 36 mut-, 13 cbl A, 7 cbl B, and 38 cbl A/B. (1) Confirmation of diagnosis: All centres aimed for an assay of propionate fixation in fibroblasts, while six centres also perform mutation analysis. (2) Cobalamin response: 10 centres follow standardised protocols but there were large variations. All recognised that non-specific effects were the main pitfall when accessing responsiveness. (3) Maintenance treatment: In cobalamin-responsive patients, most centres use hydroxycobalamin (1–14 mg/week i.m. or 5–20 mg/week orally) while two centres use cyanocobalamin. In cobalamin non-responsive patients all centres supplement with L-carnitine (100 mg/kg/day). 14 centres use intestinal decontamination, 6 follow the dietary recommendations of DACH 2000, 4 use the revised safe values of Dewey, and 4 follow other guidelines. 2 centres do not use amino acid mixture. Standardized monitoring protocols are available in 7 centres, again showing a high variability. **Conclusions**: Management in MMA differs substantially between centres and prospective studies for evaluation of different strategies are required.

136-P

CARDIOMYOPATHY AS THE PRESENTING FEATURE IN A 15-YEAR-OLD BOY WITH PROPIONIC ACIDEMIA

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A 15-year-old boy was well and normally active until diagnosed with a cardiomyopathy. He was a competitive high school tennis player until shortly before diagnosis and had no symptoms of cardiac insufficiency. The family history was remarkable for a six year-old sister who died of an idiopathic cardiomyopathy 20 years earlier. Mitochondrial vitamin and cofactor cocktail therapy did not improve his initial cardiac function, nor did high dose carnitine supplementation. He was not carnitine deficient. He required donor cardiac transplant. During his pretransplant workup, an elevated propionyl-carnitine level was detected. Acyl-carnitine analysis in skin fibroblasts revealed massively elevated propionyl-carnitine, similar to that seen in severe, neonatal propionic acidemia. Direct assay of propionyl-CoA carboxylase (PCC) revealed 4% residual activity. Mutation analysis demonstrated that he had G188R and N536D substitutions in *PCCB*, resulting from c.562 G>A and c.1606 A>G mutations, respectively. These have been observed in other propionic acidemia patients and represent both severe and mild mutations. Since the transplant, he has been maintained on a normal diet with carnitine and biotin supplementation, as well as immunosuppressive medications.

137-P

DIFFERENT gDNA LESIONS CAUSING SKIPPING OF EXONS 3 AND 4 OF THE *PCCA* GENE RESULTING IN PROPIONIC ACIDEMIA

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Propionic acidemia is caused by a deficiency of propionylCoA carboxylase (PCC) and can result from mutations in either the *PCCA* or *PCCB* genes, encoding both PCC subunits. During the course of genetic analysis of *PCCA* deficient patients by RT-PCR analysis we have identified a common defect in cDNA consisting of an in frame skipping of exons 3 and 4 of the *PCCA* gene (r.184.300del). Four patients of different origins were homozygous for this defect and three heterozygous. The mutant cDNA predictably results in a protein with an internal deletion of 39 aminoacids (T62.S100del39). Expression analysis showed undetectable activity of the mutant construct which is in agreement with the severe phenotype of homozygous patients. Genomic DNA analysis revealed at least three different changes. In one heterozygous patient, a splicing mutation affecting the conserved 5′ donor site of exon 3 (IVS3+1G>C) was identified. Both the fact that the 3′ splice acceptor site in exon 4 is very poor and that intron 3 is short (104 nt) may explain why both exons 3 and 4 are skipped together. In some homozygous patients, neither exon 3 nor 4 could be amplified, suggestive of a genomic deletion. Long-range PCR using appropriate primers hybridising to sequences in introns 2 and 4 resulted in the identification of two different deletions of 8,8 and ~5 Kb. The results highlight the genetic heterogeneity of *PCCA* gene defects.

138-P

CARBAGLU EFFECTIVE IN LOWERING HYPERAMMONEMIA IN A 3-YEAR-OLD PROPIONIC ACADEMIA PATIENT

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N.K. was born in 2003 following an affected sibling diagnosed with propionic acidemia (PA). She seemed normal at birth but became lethargic after 48 h and admitted. Ketone bodies were detected 3 + in urine. PA was confirmed at 1 week of age by the typical pattern of urine organic acid chromatography using GC-MC and that of ESI-MS/MS assay in dried blood spots, which was consistent with the diagnosis of PA. Her sister born in 1998 was diagnosed at 3 months of age by GC-MS urinary organic acid chromatography showing the typical PA pattern and confirmed by enzyme assay of propionyl-CoA carboxylase in lymphocytes (Dr T Suormala and ER Baumgartner in Basel) but died at 3 years of age during an intercurrent respiratory infection despite adequate treatment and a special diet. The parents refused prenatal diagnosis. The parents originate from the same southern village and 3 other older siblings are in good health.

N.K. became ill, somnolent and apathic during a winter season viral infection, blood ammonia raised to 174 micromol/L. Carglumic acid (Carbaglu) was given the same day: 200 mg tablets = tab a day for four consecutive days and she was also treated with a more restrictive low-protein diet partly given through nasogastric tube and oral metronidazole along with her previous daily supplement of L-carnitine and sodium citrate. Plasma ammonia dropped to 55 micromol/L. Mild microcytic anemia was revealed. The child responded well to treatment.

139-P

REVERSAL OF SEVERE CARDIOMYOPATHY AFTER LIVER TRANSPLANTATION IN LATE ONSET PROPIONIC ACIDEMIA

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Background: In propionic academia (PA) cardiomyopathy may be responsible for rapid deterioration or death. As the liver is responsible for the metabolism of many of the precursors accumulating in PA, orthotopic liver transplantation (OLT) has been described as treatment option. But the role of OLT in PA and the effect on the cardiomyopathy is still unclear.
Objective: We report the reversal of severe cardiomyopathy three years after OLT in a patient with late onset PA. Case: The diagnosis was made at 18 months because of lethargy, severe metabolic acidosis and a history of failure to thrive and psychomotor retardation. On dietary treatment, carnitine and metronidazole he had only two metabolic decompensations in the first two decades. An asymptomatic cardiomyopathy was first described at the age of nineteen. Despite intensified medical treatment he developed a severe heart failure within two years (minimal ejection fraction (EF) of 20%, pulmonary hypertension, brain natriuretic peptide (BNP) of 150 pg/ml, NYHA II). After OLT at 22 years, diet and metronidazole was discontinued. Carnitine supplementation stopped 4 months later. Within few months organic acids and serum propionate normalised. Three years after transplantation the cardiac function is nearly normalized (EF 50%, BNP 7 pg/ml, NYHA I).
Conclusion: Liver Transplantation may reverse cardiomyopathy in PA. One may hypothesise that this is due to the decrease of odd chain fatty acids stores.

140-A

REDUCTION OF NA^+, K^+-ATPASE ACTIVITY FROM SYNAPTIC MEMBRANES OF CEREBRAL CORTEX OF YOUNG RATS BY ISOVALERIC ACID

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Patients affected by isovaleric acidemia (IVAcidemia) suffer from acute episodes of encephalopathy, however the mechanisms underlying the neuropathology of this disease are poorly known. The objective of the present study was to investigate the in vitro effects of the metabolites that predominantly accumulate in IVAcidemia, namely isovaleric acid (IVA), 3-hydroxyisovaleric acid (3-OHIVA) and isovalerylglycine (IVG), on important parameters of energy metabolism, such as 14CO2 production from acetate and the activities of the respiratory chain complexes I-IV, creatine kinase and Na^+,K^+-ATPase in cerebral cortex of 30-day-old rats. We observed that IVA exposition to cortical homogenates provoked a selective inhibition of Na^+,K^+-ATPase activity, without modifying the other parameters tested. Furthermore, pre-treatment of cortical homogenates with α-tocopherol and creatine totally prevented IVA-induced inhibition on Na^+,K^+-ATPase activity from synaptic plasma membranes, indicating that peroxide radicals were probably involved in this inhibitory effect. Since Na^+,K^+-ATPase is a critical enzyme for normal brain development and functioning and necessary to maintain neuronal excitability, it is presumed that the inhibitory effect of IVA on this activity may be involved in the pathophysiology of the severe neurological dysfunction of isovaleric acidemic patients.

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141-A

3-HYDROXYISOBUTYRIC ACID IMPAIRS ENERGY METABOLISM IN CEREBRAL CORTEX OF YOUNG RATS

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3-Hydroxyisobutyric aciduria (3HiBuria) is an inherited metabolic disease of valine metabolism. Tissue accumulation and high urinary excretion of 3-HiB is the biochemical hallmark of this disorder. Symptoms include repeated episodes of ketoacidosis and lactic acidemia, failure to thrive, brain dysgenesis, malformations and hypotonia. Considering that the pathophysiology of the brain alterations in 3HIBuria are poorly known, the aim of the present work was to investigate the *in vitro* effect of 3-HiB (0.1, 0.5 and 1 mM) on various parameters of energy metabolism, namely 14CO2 production from glucose and acetate, and the activities of the respiratory chain complexes I-IV, succinate dehydrogenase and creatine kinase (CK) in cerebral cortex of 30-day-old rats. We observed that 3-HiB significantly reduced complex I-III and CK activities (up to 20% and 30% respectively). Our results suggest that 3-HiB alters cellular energy homeostasis, which could explain, at least in part, the neurological alterations found in patients with 3HiBuria.

Financial support: FAPERGS, CNPq, PROPESq, PRONEX.

142-A

EVIDENCE THAT THE METABOLITES ACCUMULATING IN 3-METHYLGLUTACONIC ACIDURIA INDUCE OXIDATIVE STRESS IN RAT BRAIN

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Deficiency of 3-methylglutaconyl-CoA hydratase (3MGH) leads to the accumulation of methylglutaconic acid (MGT), 3-methylglutaric acid (MGA) and 3-hydroxyisovaleric acid (3OHIVA). The disorder is mainly characterized by mental retardation with impaired speech development and seizures. Since the pathophysiology of the disease is practically unknown, the objective of the present study was to investigate the in vitro effect of MGT, MGA and 3OHIVA on various parameters of oxidative stress in cerebral cortex homogenates of 30-day-old rats. All metabolites significantly increased thiobarbituric acid-reactive substances (TBA-RS) and chemiluminescence, suggesting an induction of lipid peroxidation. Moreover, MGA diminished the antioxidant defenses, represented by total-radical trapping antioxidant potential (TRAP) and glutathione (GSH) concentrations, whereas MGA and 3OHIVA did not alter these parameters. Finally, none of the metabolites affected the thiol content of mitochondrial membrane, indicating that the acids do not cause direct oxidation of protein thiol groups. It is therefore presumed that MGT, MGA and 3OHIVA induce oxidative stress and this may be at least one of the mechanisms responsible for the brain injury found in 3MGH deficiency.

Financial Support: CNPq, PROPESQ-UFRGS.

143-A

ACUTE ADMINISTRATION OF GLUTARIC ACID DISTURBS ENERGY METABOLISM IN MIDBRAIN OF YOUNG RATS

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A genetic mice model of glutaric acidemia type I (GAI) has recently been developed, however the mutant animals do not develop the striatal damage characteristic of patients with this disorder. Therefore, in the present work we induced high brain glutaric acid (GA) concentrations similar to those found in GAI patients through subcutaneous injections (5 μmol/g body weight) of GA in 7- to 22-day-old rats and investigated the effect of this model on energy metabolism parameters in midbrain, in which the striatum is localized. Control rats received saline in the same volumes. GA brain levels (0.72–1.08 mM) were about 8-fold lower than in plasma and 4-fold lower than in skeletal muscle, indicating the low permeability of the blood–brain barrier to GA. We verified that CO2 production from glucose and creatine kinase enzyme activity were not changed, while complex I-III activity of the respiratory chain was inhibited in midbrain (25%). These data indicate that GA acute administration mildly impairs cellular energy metabolism in midbrain of young rats.

Financial support: CNPq, FAPERGS, PROPESq/UFRGS.

144-P

EVIDENCE THAT GLUTARIC AND 3-HYDROXYGLUTARIC ACIDS ACT SINERGISTICALLY DISTURBING ENERGY METABOLISM IN RAT BRAIN CORTEX

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In the present work we initially investigated the role of glutaric acid (GA) and 3-hydroxyglutaric acids (3HGA) alone or combined on various parameters of energy metabolism, namely glucose uptake, lactate formation and 14CO2 production from labeled glucose and acetate, as well as the activities of pyruvate dehydrogenase (PDH) and creatine kinase (CK) in cerebral cortex from young rats. We first observed that 5 mM GA, 1 mM 3HGA per se did not alter these parameters. In contrast, coincubation of GA plus 3HG at the same doses significantly increased glucose uptake, decreased 14CO2 generation from glucose, inhibited PDH activity as well as total and mitochondrial CK activities. Furthermore, GA plus 3HGA-induced inhibitory effects on CK were prevented by the antioxidants glutathione and catalase plus superoxide dismutase, indicating the participation of reactive oxygen species. We also evaluated whether quinolinic acid alone or combined with GA or 3HGA could alter brain energy metabolism and found no alteration of the examined parameters. Our data indicate a synergic action of GA and 3HGA disturbing energy metabolism in cerebral cortex of young rats.

Financial support: CNPq, PROPESQ-UFRGS, FAPERGS.

145-P

3-HYDROXYGLUTARIC ACID IMPAIRS THE SUPPLY OF SUCCINATE FROM ASTROCYTES TO NEURONS

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Background: Glutaric aciduria type 1 (GA1) is caused by deficiency of the mitochondrial glutaryl-CoA dehydrogenase (GCDH) which is involved in the catabolism of lysine and tryptophan. GCDH deficiency leads to accumulation of 3-hydroxyglutaric acid (3OH-GA), and during catabolic crises to striatal neurodegeneration. Recently, we have shown that 3OH-GA is transported by the sodium-dependent dicarboxylate co-transporter 3 (NaDC3). It has been reported that NaDC3 is located in kidney proximal tubule cells as well as astrocytes. In astrocytes, an important function of NaDC3 is the supply of tricarboxylic acid (TCA) cycle intermediates to neurons. We hypothesized that 3OH-GA may interfere with this function.
Methods: To study the effects of 3OH-GA on NaDC3-mediated transport of TCA cycle intermediates in the brain, uptake of ^{14}C-succinate was examined in cultured neurons and astrocytes from wildtype and Gcdh$^{-/-}$ mice in the presence and absence of 3OH-GA. Additionally, the direct transport of 3H-3OH-GA in neurons and astrocytes was measured.
Results: The uptake of ^{14}C-succinate both into neurons and astrocytes was impaired significantly by 3OH-GA in a concentration-dependent manner, suggesting also inhibitory effects on the efflux of intermediates. Surprisingly, ^{3}H-3OH-GA was transferred both into neurons and astrocytes.
Conclusion: These data demonstrate that intracerebral accumulation of 3OH-GA may interfere with supply of astrocytic TCA cycle intermediates to neurons. Subsequently the shortage of TCA cycle intermediates may lead to reduced energy metabolism and/or mitochondrial failure in neurons. Additionally, 3OH-GA inhibits TCA cycle intermediate transport into neurons directly by yet unknown mechanisms.

146-P

ASTROCYTES TRIGGER AND AMPLIFY NEURODEGENERATIVE CASCADES IN GLUTARIC ACIDEMIA I

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Glutaric acidemia 1 (GA1) is a neurometabolic disorder caused by glutaryl CoA dehydrogenase deficiency resulting in significant accumulation of glutaric (GA) and 3-hydroxiglutaric (3OHGA) acid. The mechanisms by which a concurrent childhood infection in GA1 triggers extensive neurogedeneration in the striatum and cortex remains unknown. Since astrocytes have been shown to become reactive and contribute to neuronal death during injury, we hypothesised that astrocyte response to GA and/or 3OHGA compromises neuronal survival and myelinisation. Thus, we have studied viability, metabolic status and proliferation of cultured astrocytes challenged with pathophysiological concentrations of GA and 3OHGA, and analysed whether treated astrocytes have neurotoxic effects on cultured neurones. GA and 3OHGA strongly increased astrocyte proliferation without affecting cell survival. Co-application of both metabolites did not show additive effects. Enhanced proliferation induced by GA or 3OHGA was associated with mitochondrial depolarisation as shown by MTT and JC1 assays performed on living astrocytes. Remarkably, cell free conditioned media from 3OHGA pre-treated astrocytes added to 3–5 DIV hippocampal cultured neurons doubled the neuronal death evoked by direct addition of GA or 3OHGA to the same batch of cultured neurones. It is concluded that astrocyte proliferation and activation contribute to GAI pathogenesis triggering and/or propagating the cascades leading to neurodegeneration.

147-A

GLUTARIC ACIDEMIA TYPE 1: CLINICAL AND BIOCHEMICAL PROFILE IN TUNISIAN INFANTS

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Background: Inherited metabolic diseases are rather common in Tunisia where a high rate of consanguinity exists due to frequent marriages between close relatives.

We report clinical and biochemical distinctive profile of Tunisian infants with glutaric acidemia type I (GA-1) following a clinical orientation diagnosis.
Material and methods: Seven infants were diagnosed with GA-1 during the latest 15 years. All patients underwent plasma amino acids analysis by ion exchange chromatography and urinary organic acids profile by gaz chromatography-mass spectrometry.
Results: The age of diagnosis varied from 5 to 18 months. The sex ratio was 0.75. The consanguinity was observed in 6 cases (85%) and familial similar disorder was identified in all patients. Main clinical features include hypotonia (87%), motor delay (71%) and neurologic regression (57.1%). The dystonic-dyskinetic disorder and macrocranie were not observed. Urinary organic acids profile showed clear elevation of glutaric acid (4610 to 24 501 μmol/mmol of creatinine) and 3-hydroxyglutaric acid (46 to 225 μmol/mmol of creatinine). Glutaconic acid was detected only in 2 cases.
Conclusion: The frequency of this rare disorder (about 100 cases worldly reported) seems be high in Tunisia. However, some cases were not identified because diagnosis was based only on increased urinary glutaric acid. Ultimately, enzymatic analyses of glutaryl-CoA dehydrogenase or mutation analysis are the only methods to establish or disprove the diagnosis of GA-1 with certainty. The early diagnosis is required to institute efficient therapy and prenatal diagnosis in families with affected infants.

148-P

GLUTARIC ACIDURIA TYPE I: A REPORT OF 8 CASES

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Background: Glutaric aciduria (GA) type I is an autosomal recessive disorder due to a deficiency in glutaryl CoA dehydrogenase.
Objectives: To determine clinical symptoms, biochemical profile, mutational studies and treatment in eight patients with GA type I.
Methods: Retrospective analysis of clinical, biochemical and molecular data.
Results: The authors present eight cases of GA type I, five males and three females, whose present ages range from nine to 30 years. All patients have macrocephaly. One patient presented acute encephalitis-like symptoms; five patients presented a chronic form with motor delay, hypotonia and dystonia and two patients were symptom-free. One patient was diagnosed through the newborn screening of her son. All of the patients performed brain-imaging studies. The typical metabolic profile that characterises GA type I was detected in all of the patients. Mutational studies in six of the eight patients, revealed that all were heterozygous except for two patients who were homozygous for the mutation R402W. A new mutation was found, G390V. The R227P mutation, associated with a low level of metabolite excretion, was found in one patient (R227P/R402W).
Conclusion: Medical and dietary treatment were initiated in seven patients and clinical improvement was observed. Therefore early recognition and treatment of this disorder maybe considered important for a favourable clinical outcome.

149-P

CLINICAL OUTCOME OF GLUTARIC ACIDURIA TYPE I IN RUSSIA

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Glutaric aciduria type I (GA1) is an inherited disorder of organic acid metabolism caused by glutaryl Co-A dehydrogenase deficiency. A restriction in protein or a lysine limited diet, with riboflavin and carnitine supplementation are the recommended therapies in this disease.

We present the outcome of 7 patients diagnosed with GA I in Russia, all following clinical presentation. Age of first clinical symptoms onset range from 1–9 months. Six patients have typical GAI clinical picture: acute metabolic crisis, dystonic hyperkinesis, macrocephaly, seizures, tetraparesis. First crisis developed after infection or minor head trauma. One patient had dyskinetic cerebral palsy without encephalopathic crisis. Diagnosis was confirmed by measuring GA and 3-OH-GA in urine by GC/MS and mutation analysis in the GCDH gene. All patients were taking the diet and L-carnitine supplementation for 1–2 years. Improvement of neurological symptoms was first mentioned after 3 months of treatment and presented with decreased muscular tone and improvement motor function. During the treatment period all patients had no encephalopathic crisis and seizures; motor deficits such as dystonia and dyskinesia reduced. MRI showed improved myelinization but no changes in fronto-temporal atrophy. There was no relation between glutaric acid level in urine and neurological outcome.

150-O

DECLINE OF ENCEPHALOPATHIC CRISES IN CHILDREN WITH GLUTARIC ACIDURIA TYPE I IDENTIFIED BY NEWBORN SCREENING IN GERMANY

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Background: Glutaric aciduria type I (GA-I) is a rare neurometabolic disorder that is considered treatable if patients are identified before the onset of acute encephalopathic crises. To allow early identification of affected individuals, tandem mass spectrometry-based newborn screening for GA-I has been started in some parts of Germany in 1999 and has been included into the nationwide newborn screening in 2005. **Methods**: We prospectively followed neonatally screened patients (n = 38) with GA-I and compared the neurological outcome of this cohort with patients from a historical cohort (n = 62). Management and analysis of the neurological outcome of GA-I patients was performed using standardized protocols. **Results**: In the majority of neonatally screened children with GA-I, the onset of encephalopathic crises has been prevented (89%), whereas acute encephalopathic crises or progressive neurological impairment was common in the historical cohort. Neonatal screening in combination with intensive management is effective – even assuming ascertainment bias in the historical cohort. The number needed to prevent one encephalopathic crises was 1.50. Similar proportions of commonest mutations and biochemical phenotypes (high and low excretors) were found in neonatally screened and historical patients. However, potential predictor variables for mild clinical phenotypes are not yet known and thus a selection of these patients by newborn screening is not excluded. No patient was known to be missed by newborn screening from 1999 to 2005. **Conclusions**: In conclusion, this study confirms that newborn screening for GA-I in combination with intensive management improves the outcome of affected children.

151-O

PREDICTION AND PREVENTION OF ENCEPHALOPATHY IN A MOUSE MODEL OF GLUTARIC ACIDEMIA

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Background: Glutaric acidemia type I (GA-1) is a genetic disorder that interrupts a common branch of lysine and tryptophan catabolism through glutaryl-coenzyme-A dehydrogenase (GCDH). About one-third of affected children still suffer irreversible striatal injury despite the best clinical management available. The mechanism of injury has been unclear, and lack of an early diagnostic marker heralding brain injury has impeded intervention efforts.
Methods: Dietary supplementation of $Gcdh^{-/-}$ mice with lysine provides a model of acute striatal injury. We used proton magnetic resonance spectroscopy (MRS), to determine if changes in brain metabolite ratios can predict striatal injury in this model. The ability of lysine to trigger acute neuropathology in this model inspired a potential treatment strategy aimed at blocking brain lysine. Here we used homoarginine, which occupies the same blood–brain barrier transporter as lysine.
Results: $Gcdh^{-/-}$ mice on a 4.7% dietary lysine had $\sim$40% decrease in the glutamate/glutamine to creatine ratio (Glx/Cr) and a $\sim$20% decrease in N-acetylaspartate to creatine ratio (NAA/Cr) at 48 h following diet introduction. Striatal injury was not evident at 48 h on T2-weighted MRI, but became evident between 96–124 h. There were no significant metabolic changes among animals on a normal diet or among animals on 4.7% lysine diet also supplemented with homoarginine, or supplemented with both homoarginine and glucose.
Conclusions: These findings suggest that MRS may be used to detect impending brain injury in human GA-1. Our MRI and MRS data provide supporting evidence that combined homoarginine/glucose therapy may be protective in GA-1.

152-O

3-HYDROXYGLUTARIC ACID IS TRANSPORTED VIA THE SODIUM-DEPENDENT DICARBOXYLATE TRANSPORTER NaDC3

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Background: Patients with glutaryl-CoA dehydrogenase (GCDH) deficiency accumulate glutaric acid (GA) and 3-hydroxyglutaric acid (3OH-GA) in tissues, blood and urine. Recent investigations focussed on direct cytotoxic effects of the metabolites, whereas mechanisms of their intra- and intercellular transport remain unclear. **Methods**: To identify transporters mediating the translocation of GA and 3OH-GA through membranes, kidney tissue of $Gcdh^{-/-}$ mice has been investigated due to its central role in urinary excretion of these metabolites. Comparative microarray analyses and quantitative RT-PCR were performed in kidneys of 42-day-old mice. Dicarboxylate transport experiments were carried out in either Xenopus laevis oocytes or CHO cells. **Results**: Among others, several slc-transporter genes were found to be upregulated in $Gcdh^{-/-}$ kidney tissue. Upregulation of the sodium-dependent dicarboxylate cotransporter 3 (NaDC3) and the organic cation transporter 2 (OCT2) was confirmed by quantitative RT-PCR. Two-electrode-voltage-clamp analysis of NaDC3 in Xenopus laevis oocytes demonstrated that NaDC3 directly translocates 3OH-GA in a sodium- and concentration-dependent manner. Furthermore, tracer flux measurements in OCT2-overexpressing CHO cells revealed that 3OH-GA significantly inhibited the uptake of the OCT2 substrate methyl-4-phenyl-pyridinium, whereas 3OH-GA itself is not transported by OCT2. **Conclusions**: The data demonstrate for the first time the membrane translocation of 3OH-GA mediated by NaDC3 and the cis-inhibitory effect on OCT2-mediated transport of substrates which might allow the development of novel strategies to treat patients.

153-P

FATAL OUTCOME IN A TEENAGER WITH 2-OXOADIPIC ACIDURIA

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The deficiency of mitochondrial 2-oxoadipic acid dehydrogenase leads to accumulation of 2-oxoadipic acid, 2-hydroxyadipic acid and 2-aminoadipic acid. More than 20 patients have been reported so far. Symptoms have included mental retardation, hypotonia, hypertonia, epilepsy, ataxia, failure to thrive. Asymptomatic children have been detected as well.

We report a 17-year-old female without neurological symptoms. She was admitted to the hospital because of acute respiratory infection of 10 days duration and worsening of the state despite antibiotic treatment. Laboratory examination disclosed leucopenia and anaemia. Agranulocytosis developed in the next days. Metabolic investigation revealed positive 2,4-dinitrophenylhydrazine test, markedly increased excretion of 2-aminoadipic acid (318 mmol/mol creatinine, normal <20), 2-oxoadipic acid (762 mmol/mol creatinine, normal not detectable), 2-hydroxyadipic acid (483 mmol/mol creatinine, normal <0.4) and moderately higher excretion of 3-hydroxybutyric acid, glutaric acid, 2-hydroxyglutaric acid in urine. The intensive treatment was not successful. The patient died due to sepsis 31 days after admission.

It has been thought that 2-oxoadipic acid and 2-aminoadipic acid might be metabolic markers without clinical consequences. Unexpected clinical course in the young female shows several similarities to the recently reported girl with 2-oxoadipic aciduria and proved defective oxidative phosphorylation (Kearns-Sayre syndrome). It is proposed that key metabolites may reflect defect in mitochondrial oxidative metabolism and catabolic stress could lead to severe decompensation.

154-A

L-2- HYDROXYGLUTARIC ACIDURIA IN A BRAZILAN PATIENT. CLINICAL NEURORADIOLOGICAL AND GENETIC ASPECTS

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Introduction: L-2-hydroxiglutaric aciduria is rare a inborn error of metabolism with an autosomal recessive inheritance. The disease is characterized by a mild psycomotor delay, progressive ataxia and mental deterioration. The brain MRI shows T2-W hyperintense lesions in peripheral cerebral white matter, basal ganglia, and dentate nuclei together with cerebellar atrophy. There are rare descriptions of the MR spectroscopy findings in patients with L-2- hydroxyglutaric aciduria. We present a full clinical description and neuroimaging, including the multi-voxel-voxel proton MR spectroscopy findings, from a Brazilian boy with homozygous 529delC in the duranin gene.
Case report: A 12-year-old boy is the first and only child of a Brazilian consanguineous parents. On the neurological evaluation there was truncal ataxia, dyssynergy, dysmetria, intention tremor, myoclonus and myoquimia. The brain MRI showed a diffuse subcortical white matter loss. There was symmetrical high signal in the globus pallidus and dentate nuclei. With TE of 30, the MR spectral patterns revealed two broad peaks located at 0.9–1.6 ppm peaks, and with TE of 288 there was no lactate peak. The urinary organic acid analysis showed very large peak of 2- hydroxyglutaric acid. The cDNA sequencing was performing using five pairs of primers. We found a homozygous 529delC in the patient. The parents were heterozygous for the mutation. This mutation was first described by Vilarinho, in 2005, in a Portuguese patient.
Conclusion: We emphasize the contribution of spectroscopy in the diferential diagnosis of the L-2-hydroxyglutaric aciduria and the possibility of founder Portuguese mutation in Brazilian population.

155-P

CIS AND TRANS 3-METHYLGLUTACONIC ACID IN URINE OF PATIENTS WITH 3-METHYLGLUTACONIC ACIDURIA

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Background: 3-Methylglutaconic aciduria (OMIM: 250950 (type I); 302060 (type II or Barth syndrome); 258501 (type III or Costeff syndrome); 250951 (type IV or 'unspecified' 3MGA-aciduria)) comprises a group of metabolic disorders characterized by increased excretion of urinary 3-methylglutaconic acid (3MGA) and 3-methylglutaric acid (3MG). 3MGA occurs as cis and trans isomers that can be quantified by 1H-NMR spectroscopy. Previously we reported urinary cis/trans 3MGA ratios of 2:1 in one patient with type I and 1:1 in four patients with type IV 3MGA-aciduria. In this study, we analyzed urine from 20 patients with different types of 3MGA-aciduria to investigate whether the cis/trans 3MGA ratio is useful in differentiating the four types of 3MGA-aciduria. **Methods**: We performed urine NMR spectroscopy in patients with 3MGA-aciduria type I ($n = 3$; two late-onset form*), type II ($n = 3$), type III ($n = 4$) and type IV ($n = 10$). **Results**: Urine from the three patients with confirmed 3MGA-aciduria type I showed increased excretion of 3MGA (cis/trans values [micromol/mmol creatinine]: 62/32*; 54/24*; 89/53), 3MG and 3-hydroxyisovaleric acid (3HIVA). All other patients showed mildly increased 3MGA (range: 10–120 micromol/mmol creatinine, reference <6.5) with equal amounts of cis and trans isomers. 3MG was not detectable by NMR and the concentration of 3HIVA was normal in these patients. **Conclusion**: The 3MGA cis/trans ratio shows promise in differentiating 3MGA-aciduria type I from the other types (ratio 2:1 compared with 1:1). Neither the origin of the different isomers nor the metabolic basis of their relative amounts is known.

156-P

ASSOCIATION OF 3-METHYLGLUTACONIC ACIDURIA TYPE IV IN PATIENTS WITH GLYCOGEN STORAGE DISEASE I

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Background: Glycogen storage disease type I (GSD I) is caused by a defect of one of the enzymes/transporters involving in glucose-6-phosphate metabolism. Clinical and biochemical features of GSD I include hepatomegaly, nephromegaly, abnormal bleeding, gastrointestinal symptoms and failure to thrive, hypoglycemia, hyperlactic acidemia, hyperlipidemia, hyperuricemia, and neutropenia. Recently, 3-methylglutaconic aciduria type IV (3MGCA) has been reported in a GSD Ib patient. We report follow-up of the association of 3MGCA in fifteen GSD I patients. **Methods**: We measured the urine organic acid levels in thirteen GSD Ia and two GSD Ib patients aged from 1 to 32 years by isotope dilution gas chromatography mass spectrometry method. **Results**: Eight GSD Ia and one GSD Ib patients showed mild to grossly elevated levels of urine 3-methylglutaconate (11–133 mmol/mol creatinine, ref. range <10), 3-methylglutarate (3–69 mmol/mol creatinine, ref. range <2) and TCA cycle intermediates, but with normal levels of 3-hydroxyisovaleric acid. These patients can be grouped under 3MGCA type IV. Furthermore, preliminary data suggested that the urinary levels of 3MGC, 3MGR and other TCA intermediates changed together with blood glucose, lactate and lipids levels. **Conclusion**: This is the first report to show the common occurrence of 3MGCA type IV in GSD I patients. It may be interesting to elucidate the pathophysiology and correlations of these biochemical parameters with treatment control in a larger cohort of GSD I patients.

157-A

3-METHYLCROTONYLGLYCINURIA IN A FAMILY: LATE AND DIFFERENT CLINICAL PRESENTATION

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3-Methylcrotonylglycinuria is an inborn error of leucine catabolism with an autosomal recessive pattern of inheritance that results from a deficiency of 3-methylcrotonyl-CoA carboxylase (MCC). The phenotype of the biotin-resistant form of MCC deficiency is highly variable ranging from severe neonatal onset to asymptomatic individuals.

We report two siblings and their uncle with different clinical presentations. The older one who was 3 years old, has macrocephaly and developmental language delay. His cranial MR imaging was normal. His 1,5 year old younger brother developed seizure one month ago. His EEG shown mild partial epileptiform disturbance and his cranial MR was normal as well. The siblings had healthy nonconsanguineous Turkish parents, and were born term after an uneventful pregnancy and delivery. Their uncle has been followed with epilepsy for last 5 years. Metabolic investigations were performed in siblings and their uncle because of the similar symptoms. Urinary organic acid (by GC-MS) analysis displayed elevated 3-hydroxyisovaleric acid (3-HIVA) and 3-methylcrotonylglycine (3-MCG). The analysis of acylcarnitines in dried blood samples by tandem mass shown elevated 3-hydroxyisovalerylcarnitine. Free carnitine level was decreased. Plasma biotinidase activty was normal. MCC deficiency was suspected in cases. Enzymatic and genetic studies have been continuing.

The course of 3- MCC deficiency differs in age and clinic features. Macrocephalia is an unexpected finding for this disease. Also, the late presentation of seizure in uncle is interesting. This conditions can be a new clinical form of disease.

158-P

3-METHYLCROTONYL-COENZYME A CARBOXYLASE (3-MCC) DEFICIENCY ASSOCIATED WITH ABSENCE OF URINARY 3-METHYLCROTONYLGLYCINE (3-MCG)

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We report two patients with isolated 3-MCC deficiency whose urine was devoid of 3-MCG, the pathognomonic marker for this disorder. The first patient, a girl with trisomy 21, was detected through newborn screening with elevated C5-OH carnitine, while the second patient came to clinical attention at the age of 5 months because of failure to thrive and developmental delay. Urine organic acid analysis revealed elevated 3-hydroxyisovaleric acid (3-HIVA) but no 3-MCG in both patients. Enzyme studies in cultured fibroblasts confirmed isolated 3-MCC deficiency with residual activities of 5–7% and 12% of control means, respectively. Incorporation of 14C-isovaleric acid into intact fibroblasts was virtually normal, potentially explaining the absence of 3-MCG. Mutation analysis of the *MCCA* and *MCCB* genes revealed that both patients were compound heterozygous for a missense mutation, *MCCB*-c.1015G>A (p.V339M), and a second mutation resulting in undetectable *MCCB* mRNA. The absence of 3-MCG in urine raises the potential for misdiagnosis based solely upon routine urine organic acid analysis without plasma acylcarnitine profiling.

(First/last authors contributed equally to this work)

159-O

CRYPTIC EXON ACTIVATION BY DISRUPTION OF AN EXON SPLICE ENHANCER: A NOVEL MECHANISM CAUSING 3-METHYLCROTONYL-CoA CARBOXYLASE DEFICIENCY

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3-Methylcrotonyl-CoA carboxylase (MCC) deficiency is an autosomal recessive disorder of leucine catabolism with a variable phenotype ranging from severe neonatal to asymptomatic adult forms. The heteromeric mitochondrial MCC enzyme is composed of two non-identical subunits, biotin binding alpha subunits and smaller beta subunits, encoded by *MCCA* and *MCCB* respectively.

We report on a 3-MCC deficient proband homozygous for the mutation c.1054G>A in exon 11 of *MCCB*. Sequence analysis of *MCCB* RT-PCR cDNA revealed two overlapping transcripts. One contained the normal 73 bp of exon 11 including the missense mutation c.1054G>A (p.G352R). The other lacked exon 11, but instead contained a 64 bp sequence from intron 10 that maintains the reading frame and is flanked by acceptable splice consensus sites. We show that the transcript with the missense mutation has some residual activity while the one with the cryptic exon has no detectable activity. Analysis of the region harboring the mutation revealed that the mutation is located in an exon splice enhancer sequence. Using *MCCB*-minigene constructs and transient transfection of *MCCB*-deficient fibroblasts we demonstrate that the reduction in utilization of exon 11 associated with the c.1054G>A mutation is due to alteration of this exon splice enhancer. Further, we show that optimization of the weak splice donor site of exon 11 corrects the splicing defect.

This is the first demonstration of a point mutation disrupting an exon splice enhancer that activates utilization of a cryptic exon and causes disease.

160-P

BIOTINIDASE DEFICIENCY: CLINICAL AND MRI FINDINGS CONSISTENT WITH RHOMBENCEPHALOMYELITIS

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Introduction: The clinical features of biotinidase deficiency include seizures, hypotonia, ataxia, respiratory problems, skin rash, alopecia, hearing loss, and developmental delay. Children with delayed onset have features of spastic paraparesis, visual disturbance and limb weakness. Case report: A 4-year-old girl was admitted because of intermittent ataxia and hearing impairment five weeks after an infectious episode. At this time, clinical examination was normal. Evaluation found normal brain CT scan, EEG, CSF cell count, protein and glucose. One month later, she was hospitalized, examination revealed an erythematous squamous rash, alopecia, ataxia and tetraparesis. MRI revealed Rhombencephalomyelitis. Urinary organic acid profile showed increased level of 3-hydroxyisovaleric acid. Serum biotinidase activity was undetectable. Treatment was started with 10 mg/day biotin. Follow up examination after 4 weeks revealed no skin lesions and improved hair growth. The neurological symptoms disappeared progressively but loss of hearing persist bilaterally (–30 DB). Our patient findings suggest that she manifested an atypical form. The onset in the fourth year of life exhibited features seen in children with early onset biotinidase deficiency. Furthermore, she manifested tetraparesis, confrmed by spinal MRI, a finding observed in children with delayed- onset.
Conclusion: These findings suggest that: (1) In a patient presenting myelitis the diagnosis of biotinidase deficiency should be always considered even after an infectious episode and regardless of age of presentation. (2) As well as myelitis, the brain stem injury should be equally considered as a feature of biotinidase deficiency.

161-A

BIOTIDINASE DEFICIENCY PRESENTING AS STATUS EPILEPTICUS RESISTANT TO CONVENTIONAL ANTI-EPILEPTICS

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Introduction: Biotinidase deficiency is a treatable inborn error of biotin metabolism. Main symptoms and signs are feeding difficulties, neurological abnormalities (hypotonia, impaired consciousness, seizures, ataxia) and cutaneous changes (rash, alopecia). However, the clinical presentation and age of onset are extremely variable, and organic aciduria may initially be absent. Therefore, enzyme estimation remains the gold standard.
Case Report: We report ZJ, a 9-month-old female child ex premature product of 1st degree cousins. She presented with status epilepticus, admitted to PICU and given conventional treatment for status epilepticus with poor response. She has a brother who died at the age of 3 months from metabolic acidosis and acute encephalopathy and confirmed to have biotidinase deficiency. LJ lab results showed metabolic acidosis with increased anion gap. Ammonia, liver functions, serum amino acid, and urine for organic acid were normal. Her biotidinase level was undetectable. Supplementation with biotin resulted in marked cessation of the seizure and clinical improvement with normalization of metabolic parameters.
Conclusion: As biotinidase deficiency is a treatable disorder, it should be considered in children presenting with intractable seizure especially in the presence of other characteristic neurological or cutaneous features.

162-P

PROFOUND BIOTINIDASE DEFICIENCY MANIFESTING AS NEONATAL-ONSET INTRACTABLE SEIZURES AND DIFFUSE LEUKOENCEPHALOPATHY

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Background: Biotinidase deficiency is an autosomal recessive disorder of biotin recycling. Symptoms of profound biotinidase deficiency usually develop at a few weeks to several years of age and are variable with respect to frequency and severity. **Methods**: Case study of an 8-month-old female with profound biotinidase deficiency who presented early after birth with intractable seizures and leukoencephalopathy on cerebral imaging with remarkable improvement after treatment with biotin **Results**: The patient was born at term to consanguineous parents after uneventful pregnanacy. At the age of 5 days, she developed clonic seizures of upper and lower limbs and breathing problems (frequent chocking and stridor). She was hospitalized at age of 45 days due to persistence of seizures despite trying various anticonvulsants. Physical examination showed spasticity of upper and lower limbs, axial hypotonia, stridor and tachypnea. Electroencephalogram showed abundant frank epileptic activity over both temporoparietal areas. Cerebral imaging showed diffuse leukoencephalopathy. Urine for organic acids showed moderate excretion of beta-hydroxyisovaleric acid and 3-methylcrotonylglycine. Serum biotinidase level was zero nmol/min/ml. Other normal studies included serum ammonia, lactic acid, plasma very long chain fatty acids, plasma amino acid chromatography, and enzymatic assay of Krabbe disease. There was cessation of seizures and breathing abnormalities after 5 days of initiation of biotin (20 mg per day) with significantly less frequent epileptic activity on electroencephalogram. Cerebral imaging performed at 6-month of age showed dramatic resolution of the leukoencephalopathy. **Conclusion**: Biotinidase deficiency should be considered in any patient with unexplained white matter disease

163-P

EFFECT OF BIOTIN LEVELS ON ACTIVITY AND GENE EXPRESSION OF BIOTIN DEPENDENT CARBOXYLASES IN HUMAN CELL LINES

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The role of biotin as prosthetic group of carboxylases is well known and, in addition, evidence has accumulated of its role in regulating gene expression, thus participating in cell signalling, immune response and other important cellular processes. We have studied the effect of biotin on the genetic expression of the biotin binding subunits of propionyl CoA carboxylase (*PCCA* gene) and methylcrotonyl CoA carboxylase (*MCCA* gene) in different human cell lines (fibroblasts, hepatoma, lymphoblasts), analyzing mRNA levels, biotinylated proteins and carboxylase activities. The possible effect of biotin as cofactor that may stabilize apocarboxylases was also studied analyzing total carboxylase proteins. No difference in mRNA levels for *MCCA, MCCB, PCCA* nor *HLCS* (holocarboxylase synthetase) was observed between biotin-deficient and biotin-supplemented hepatoma cells, correlating with the results reported by other authors using microarrays. In fibroblasts, no significant differences in carboxylase activities were detected. However, in supplemented hepatoma cells, significantly increased levels of biotinylated proteins and carboxylase activities were observed. This increase can be achieved by addition of biotin to biotin-deficient cells and is independent on translation, pointing to the conversion of apoenzyme to active holoenzyme as major underlying mechanism. This confirms previous reports that in liver the levels of active holocarboxylases correlate with biotin supply.

164-P

2-METHYLBUTYRYL-COENZYME A DEHYDROGENASE DEFICIENCY: FUNCTIONAL AND MOLECULAR STUDIES ON A DEFECT IN ISOLEUCINE CATABOLISM

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Background: 2-Methylbutyryl-CoA dehydrogenase (MBD; coded by the *ACADSB* gene) catalyzes the step in isoleucine metabolism that corresponds to the isovaleryl-CoA dehydrogenase reaction in the degradation of leucine. Deficiencies of both enzymes may be detected by expanded neonatal screening with tandem-mass spectrometry due to elevated pentanoylcarnitine (C5 acylcarnitine) in blood, but little information is available on the clinical relevance of MBD deficiency.
Methods: We biochemically and genetically characterize six individuals with MBD deficiency from four families of different ethnic backgrounds.
Results: None of the six individuals showed clinical symptoms attributable to MBD deficiency although the defect in isoleucine catabolism was demonstrated both *in vivo* and *in vitro*. Several mutations in the *ACADSB* gene were identified, including a novel one, which has an allele frequency of approx. 2% in the Turkish and Lebanese populations, but was not found in German controls.
Discussion: MBD deficiency may be a harmless metabolic variant although significant impairment of valproic acid metabolism cannot be excluded and further study is required to assess the long-term outcome of individuals with this condition. The relatively high prevalence of *ACADSB* gene mutations in control subjects suggests that MBD deficiency may be more common than previously thought but is not detected because of its usually benign nature.

165-P

IN VIVO AND *IN VITRO* STUDIES ON THE INTERACTION OF VALPROIC ACID AND METABOLITES WITH THE LEUCINE OXIDATIVE METABOLISM

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Background: Many biochemical systems including the branched-chain amino acid (BCAA) oxidative pathway are affected *in vivo* by valproic acid (VPA) therapy. Both BCAAs and VPA undergo b-oxidation generating structurally similar metabolites.
Aims: To study the effect of VPA on BCAA metabolism and investigate the potential inhibitory effect of valproate and some of its mitochondrial metabolites on the activity of isovalery-CoA dehydrogenase (IVD).
Methods: The *in vivo* profile of organic acids was analyzed by GC-MS, in urine samples from patients under VPA therapy. IVD activity was measured using human control fibroblasts and purified human enzyme making use of an optimized HPLC procedure. The effect of VPA and some of its metabolites on the activity of IVD was further studied.
Results: Significantly increased levels of 3-hydroxyisovaleric acid were found in urine of patients after VPA treatment ($n = 16$) when compared to controls ($n = 28$): 163.3 ± 94.6 and 45.6 ± 28.9 mmol/mol creatinine ($p < 0.05$), respectively. In fibroblasts, IVD activity was inhibited by valproyl-CoA and by valproyl-dephosphoCoA, but not by the free acids, VPA and 3-keto-VPA. The IVD activity was then characterized using purified human enzyme (Vmax = 17.0 U/ml and Km = 0.49 mM). Both valproyl-CoA and valproyl-dephosphoCoA induced a clear inhibition of IVD by a pure competitive mechanism with KI-values of 139.2 mM and 215.8 mM, respectively.
Conclusions: The observed direct effect on IVD activity accounts for the decreased rate of leucine oxidation and for the increased excretion of 3-hydroxyisovaleric acid in VPA-treated patients.

166-P

NEURODEGENERATION IN 2-METHYL-3-HYDROXYBUTYRYL-CoA-DEHYDROGENASE (MHBD) DEFICIENCY IS UNRELATED TO ENZYME FUNCTION

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17-Beta-hydroxysteroid dehydrogenase type 10 (HSD17B10) is a mitochondrial enzyme involved in the beta-oxidation of isoleucine. Mutations in the *HSD17B10* gene cause 2-methyl-3-hydroxybutyryl-CoA dehydrogenase (MHBD) deficiency, a rare metabolic disease with an unusual neurodegenerative course. In addition, HSD17B10 has been reported to bind amyloid-beta and to mediate mitochondrial dysfunction in the pathogenesis of Alzheimer disease. The pathogenetic mechanisms of HSD17B10 dysfunction have not yet been elucidated. Most patients with MHBD deficiency are hemizygous for the same recurrent mutation R130C in the HSD17B10 gene; this mutation does not involve a hypermutable CpG site and the reason for its high frequency is unknown. Observations in two novel families with MHBD deficiency now indicate that the clinical features are unrelated to the enzymatic function of HSD17B10 but are mediated through an as yet uncharacterised protein function. One boy presented in the neonatal period with evidence of severe mitochondrial dysfunction; he died with progressive hypertrophic cardiomyopathy at the age of seven months. He was hemizygous for a novel mutation D86G in the HSD17B10 gene; enzyme studies showed high residual activity of approx. 30%. Two cousins in the other family had no residual enzyme activity in fibroblasts and were hemizygous for a novel mutation Q165H in the HSD17B10 gene. One of them had severe failure to thrive but is neurologically normal at age 3 years whilst his cousin is asymptomatic at age 6 years. Work is in progress to characterise the role of HSD17B10 in neurodegeneration both in MHBD deficiency and Alzheimer disease.

167-P

3-HYDROXY-3-METHYLGLUTARIC ACIDURIA: REPORT OF 15 BRAZILIAN PATIENTS

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Background: 3-Hydroxy-3-methylglutaric aciduria is an autosomal recessive inborn error caused by deficiency of 3-hydroxy-3-methylglutaryl-CoA lyase (HL) activity that catalyzes the final step of leucine degradation and plays a key role in ketone body formation. Deficiency of the enzyme activity results in metabolic acidosis, hyperammonemia, and hypoketotic hypoglycemia in the neonatal period or in infancy. Except in Saudi Arabia and Portugal, where HL deficiency is frequent, this organic acidemia is quite rare, with approximately 60 cases described to date.
Methods: We reported here 15 Brazilian patients with an urinary organic acid profile characteristic of 3-hydroxy-3-methylglutaric aciduria.
Results: Urine from all patients contained large amounts of 3-hydroxy-3-methylglutaric, 3-methylglutaconic, 3-hydroxyisovaleric and 3-methylglutaric acids, whereas 3-methylcrotonylglycine was also observed in 13 patients. The number of cases of 3-hydroxy-3-methylglutaric aciduria diagnosed comprehended 8.5% of total organic acidurias detected in our laboratory in the last few years, suggesting a high incidence of this disorder in our population. The main symptoms of clinical presentation in our patients were hypoglycemia (12 patients), seizures (10 patients), metabolic acidosis (9 patients), vomiting (6 patients), and hepatomegaly (5 patients). Interestingly, all except two patients were of Portugueese ancestry.
Conclusions: Our findings significantly expand the number of reported cases and enhances our understanding on the clinical phenotype of this disorder.

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168-P

SHORT/BRANCHED-CHAIN ACYL-CoA DEHYDROGENASE DEFICIENCY IN A TAIWANESE INFANT IDENTIFIED BY MS/MS NEWBORN SCREENING

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Background: Short/branched-chain acyl-CoA dehydrogenase (SBCAD) deficiency (OMIN No. 600301) is an inherited metabolic disorder of L-isoleucine catabolism. Mutation in the gene encoding short/branched-chain acyl-CoA dehydrogenase (*ACADSB*) leads to accumulation of 2-methylbutylglycine in urine and 2-methylbutyl (C5) carnitine in blood, by which SBCAD deficiency is also known as 2-methylbutyryl-CoA dehydrogenase (2MBCD) deficiency (OMIM No. 610006). Newborn screening by MS/MS technology in Taiwan is officially expanded for diseases with elevated C5 carnitine in July, 2006. In this study, an asymptomatic newborn with SBCAD deficiency was detected by the newborn screening program.
Methods: Samples from infant with elevated C5 carnitine were analyzed by GC/MS for urine organic acids. Mutations in the *ACADSB* gene were identified by PCR-based direct sequencing.
Results: This patient showed persistent elevation of C5 carnitine in 4 separate blood spot samples at age of 2, 8, 10 and 15 days. Organic acids analysis did not show accumulation in the urine at age of 16 days, however, an elevated 2-methylbutyrylglycine was detected in the urine at age of 32 days without clinical presentations. Molecular genetic analysis for this patient revealed a compound heterozygote of c.275C>G (p.Ser92X) and c.655G>A (p.Val219Met) in the *ACADSB* gene. These two novel variations were absent among 50 normal individuals and confirmed the SBCAD deficiency.
Conclusions: Asymptomatic SBCAD deficiency could be identified by MS/MS screening for newborns. Urine organic acids should be followed up in different life stages for newborns with elevated C5 carnitine to confirm diagnosis of SBCAD deficiency.

169-P

A NEW CASE OF SHORT/BRANCHED-CHAIN ACYL-CoA DEHYDROGENASE DEFICIENCY CAUSED BY TWO NOVEL MUTATIONS

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Short/branched-chain acyl-CoA dehydrogenase deficiency is an autosomal recessive defect in the S-pathway of isoleucine catabolism. Although few patients have been reported so far, they show a variety of clinical presentations. Some were diagnosed after metabolic/neurological presentation; others were diagnosed by neonatal screening and treated presymptomatically; others remain asymptomatic without any treatment. We report a new symptomatic case of SBCADD of Italian origin, identified by MS/MS newborn screening. Acylcarnitine and amino acid MS/MS analysis of patient's neonatal blood spot, showed an isolated increase of C5-acylcarnitine (0.96 μM; cut-off: 0.48 μM) and C5/C2, C5/C3 ratios. An elevation of 2-methylbutyrylglycine associated to an increase of 2-ethylhydracrylic acid in urine obtained by GC/MS organic acid analysis, suggested the diagnosis of SBCAD deficiency. *ACADSB* gene sequencing analysis showed a missense mutation in exon 6 P262L (c.785 C>T) and an insertion at the donor splice site in intron 9: (g.42159_42160insT). Both mutations were not found in 116 normal chromosome. RT-PCR of full length cDNA showed the presence of two transcripts. Their analysis by sequencing showed the skipping of exon 9 in the shorter fragment. The present patient was born from a triple pregnancy. A prenatal ultrasonography at 30 weeks of gestation revealed shunting of blood between two fetuses. The intrauterine death of the donor fetus occurred at 31 weeks of gestation and a caesarean section was performed for fetal distress. The clinical presentation in this patient was therefore complicated by cerebral prenatal damage and we think that this serious illness could overlap the adverse effect of SBCADD.

170-P

OXIDATIVE STRESS IS INDUCED IN SUCCINATE SEMIALDEHYDE DEHYDROGENASE NULL MICE

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The mechanisms involved in the pathophysiology of succinate semialdehyde dehydrogenase (SSADH) deficiency remain largely unresolved. Therefore, in the present study we evaluated antioxidant defenses and lipid peroxidation in various cerebral structures (cortex, cerebellum, thalamus and hippocampus) and in the liver of SSADH deficient mice. We first observed that the tissue non-enzymatic antioxidant defenses were significantly reduced in the SSADH deficient animals, particularly in the liver (decreased total radical-trapping antioxidant potential and GSH) and in the cerebral cortex (decreased GSH), as compared to the wild type mice. Furthermore, superoxide dismutase activity was increased in the liver and cerebellum, whereas the activity of catalase was higher in the thalamus. In contrast, glutathione peroxidase activity was diminished in the hippocampus. Finally, lipid peroxidation (thiobarbituric acid-reactive substances) was markedly increased in the liver and cerebral cortex, reflecting a high lipid oxidative damage in these tissues. Our data showing an imbalance between tissue antioxidant defenses and oxidative attack strongly indicate that oxidative stress is involved in the pathophysiology of SSADH deficiency in mice, and likely the corresponding human disorder.

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171-P

^{11}C-FLUMAZENIL PET IMAGING IN PATIENTS WITH SSADH DEFICIENCY

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Background: Succinic semialdehyde dehydrogenase (SSADH) deficiency is an autosomal recessive disorder of gamma-aminobutyric acid (GABA) metabolism characterized by elevated levels of GABA and gamma-hydroxybutyric acid (GHB). Clinical findings include mental retardation with disproportionate expressive language dysfunction, hypotonia, hyporeflexia, hallucinations, autistic behaviors and seizures. Autoradiographic labeling and slice electrophysiology studies in the murine model provide evidence for use-dependent down-regulation of the GABA(a) receptor. We investigated benzodiazepine receptor (BZPR) binding in patients with SSADH deficiency using [^{11}C] flumazenil (FMZ) and positron emission tomography (PET).
Methods: FMZ binding was measured in 6 patients with SSADH deficiency,and 10 unaffected parents (obligate heterozygotes). We performed PET on a GE Advance Scanner using a reference region compartmental model, with time-activity curve from pons as the input function. Relative parametric binding potential (BP) was derived, with MRI-based pixel by pixel partial volume correction, in regions of interest drawn on co-registered MRI.
Results: In hippocampus, amygdala, thalamus, caudate, frontal cortex, occipital cortex, and cerebellar vermis, patients with SSADH deficiency had 25–45% significant reductions in FMZ BP compared to parents. There was no effect of gender.
Conclusions: SSADH deficient patients show widespread reduction in BZPR binding on ^{11}C-FMZ PET. Since previous studies of FMZ PET have shown that binding is higher in children, our results suggest that high endogenous brain GABA levels in SSADH deficiency down-regulate GABA(a)-BZPR binding site availability.

172-P

A UNIQUE CASE OF SUCCINIC SEMIALDEHYDE DEHYDROGENASE (SSADH) DEFICIENCY (GAMMA-HYDROXYBUTYRIC ACIDURIA) AND WILLIAMS BEUREN SYNDROME

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Objective: We present a unique case of gamma-hydroxybutyric aciduria (succinic semialdehyde dehydrogenase, SSADH) deficiency in association with Williams Beuren syndrome (WS). **Case report:** Metabolic and cytogenetic work-up was pursued in a 5-month-old female infant with hypersomnolence and irritability, failure to thrive, muscular hypotonia, dysmorphic facies, global developmental delay and supravalvular aortic stenosis. WS was confirmed by cytogenetic analysis (hemizygous deletion 7q11.23). A neurometabolic disorder was suspected due to global developmental delay along with hypersomnolence and failure to thrive. Organic acid analysis revealed markedly elevated gamma-hydroxybutyrate, 3,4-dihydroxybutyrate and medium chain dicarboxylic aciduria on 2 separate urine specimens. SSADH deficiency was confirmed enzymatically (patient 26 pmol/min/mg; controls 342–1509) and at the molecular level (two nucleotide duplication in exon 6, c.967.968dupCA, resulting in substitution of histidine for glutamine at position 323 and premature truncation, p.Gln323HisfsX4). Treatment included careful neuro-pediatric follow-up, nocturnal nasogastric tube feeding and monitoring of serum calcium levels. At 14 months of age, her gross motor, language, and visual-motor/problem-solving skills were at a 5 month level, but her irritability had decreased dramatically and sleep quality improved. **Conclusions:** This extremely rare co-occurrence of two unrelated genetic conditions highlights the importance of instituting comprehensive metabolic studies despite the presence of syndromic findings even in the absence of other metabolic abnormalities that may be indicative of metabolic disease such as hyperammonemia or metabolic acidosis.

173-P

R268H MUTATION IN SUCCINYL-CoA:3-KETOACID CoA TRANSFERASE (SCOT) GENE IS A TEMPERATURE-SENSITIVE 'MILD' MUTATION

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Objective: Succinyl-CoA:3-ketoacid CoA transferase (SCOT; locus symbol *OXCT*; E.C. 2.8.3.5) deficiency causes episodic ketoacidosis. We investigated molecular basis of SCOT deficiency in sibling cases (GS10 and 11) in South Africa.
Methods: RT-PCR, genomic sequencing, transient expression analysis of mutant cDNAs were performed as reported previously.
Results: They were revealed to be homozygotes of R268H mutation. Transient expression analysis revealed that R268H mutant SCOT protein was clearly detected as much as 50% wild-type, together with 40% residual SCOT activities at 37°C. R268H retained so high residual SCOT activity that R268H was first regarded as being not disease-causing mutation. Further transient expression analysis of wild-type and mutant cDNAs at 30°C, 37°C, and 40°C showed that accumulation of the R268H mutant protein was strongly temperature-dependent; residual SCOT activities were calculated to be 59.7%, 34%, and 4%, respectively, in expression at 30°C, 37°C, an 40°C in SV40-transformed GS01 fibroblasts. The difference of residual SCOT activities at these temperatures in expression analyses was due to the differences in the level of the mutant protein. These results indicated that R268H mutant protein was clearly unstable than wild-type with temperature-sensitive manner.
Conclusion: We finally concluded that R268H mutation is disease-causing one. Stability of mutant protein in transient expression analysis did not always reflect on those in patients' fibroblasts.

174-P

IDENTIFICATION OF AN ALU-MEDIATED TANDEM DUPLICATION OF EXONS 8 AND 9 IN A PATIENT WITH MITOCHONDRIAL ACETOACETYL-CoA THIOLASE (T2) DEFICIENCY

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Objective: Mitochondrial acetoacetyl-CoA thiolase (T2) deficiency (OMIM#203750) is an autosomal recessive disorder characterized by intermittent ketoacidotic crises and asymptomatic between crises. We analyzed molecular basis of a typical T2 deficient patient of which mutations had not been identified by routine mutation analysis using PCR at the genomic level.
Methods: RT-PCR analysis was then performed to obtain a clue for the molecular basis. To amplify genomic region including the recombination site was done using long-range PCR method.
Results: A single cDNA fragment larger than the normal one by 210 bp was amplified. It was revealed to have a tandem repeat of exons 8 and 9. This is why routine mutation analysis using PCR at the genomic level could not detect mutations. Genomic PCR analysis designed for specific amplification of the patient's exon 9 exon 8–exon 9 genomic sequence successfully amplified a 2 kb fragment including the recombination site.

Alu element-mediated unequal homologous recombination between an Alu-Jo in intron 7 and another Alu-Jo in intron 9 appears to be responsible for this duplication.
Conclusions: Alu-mediated unequal homologous recombination causes duplication or deletion resulting in molecular basis of T2 deficiency.

175-A

N-ACETYLASPARTIC ACID PROMOTES OXIDATIVE STRESS *IN VITRO* IN CEREBRAL CORTEX OF RATS

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N-acetylaspartic acid accumulates in Canavan disease, a severe leukodystrophy characterized by swelling and spongy degeneration of the white matter of the brain. This inherited metabolic disease, caused by deficiency of the enzyme aspartoacylase, is clinically characterized by severe mental retardation, hypotonia and macrocephaly, and also generalized tonic and clonic type seizures in about half of the patients. Considering that the mechanisms of brain damage in this disease remain not fully understood, in the present study we investigated whether oxidative stress is elicited by N-acetylaspartic acid. The *in vitro* effect of N-acetylaspartic acid (10–80 mM) was studied on oxidative stress parameters: total radical-trapping antioxidant potential (TRAP), total antioxidant reactivity (TAR), chemiluminescence, thiobarbituric acid-reactive substances (TBA-RS), glutathione content, sufhydryl content and carbonyl content in the cerebral cortex of 14 day-old rats. TRAP, TAR, reduced glutathione content and sulfhydryl content were significantly reduced, while chemiluminescence, TBA-RS and carbonyl content were significantly enhanced by N-acetylaspartic acid *in vitro*. The enhancement in TBA-RS promoted by N-acetylaspartic acid was completely prevented by ascorbic acid plus Trolox, and partially prevented by glutathione and dithiotreitol. Our results indicate that N-acetylaspartic acid promotes oxidative stress by stimulating lipid peroxidation, protein oxidation and by decreasing non-enzymatic antioxidant defenses in rat brain. This could be another pathophysiological mechanism involved in Canavan disease.

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176-P

BODY FLUID NMR SPECTROSCOPY OF XANTHURENIC ACIDURIA DUE TO KYNURENINASE DEFICIENCY

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Background: Xanthurenic aciduria (OMIM: 236800) is an IEM characterized by increased excretion of urinary xanthurenic acid (XA), 3-hydroxykynurenine (3OHKYN) and kynurenine (KYN). A diagnosis of xanthurenic aciduria was made in a young Somali boy with jaundice and vomiting a week after birth (J Inherit Metab Dis. 2007;30:248–255). The diagnosis was confirmed at the metabolite level (HPLC in body fluids), and mutations were found in the gene KYNU encoding kynureninase. In this study, we analyzed urine from the patient to investigate the potential use of NMR spectroscopy to diagnose xanthurenic aciduria.
Methods: We performed urine NMR spectroscopy before and after an oral administration of tryptophan. Assignments were based on literature data and on reference spectra of relevant authentic standards (XA, 3OHKYN, KYN, kynurenic acid (KYNA), tryptophan (TRP), N-acetyltryptophan (ATRP) and quinolinic acid (QA)).
Results: Urine from the patient showed increased excretion of XA and 3OHKYN (90 and 210 μmol/mmol creatinine, respectively). KYN was not detectable by NMR. After tryptophan administration, the urinary concentrations of XA and 3OHKYN were 570 and 3000 μmol/mmol creatinine, respectively, and four more TRP metabolites were observed (KYN, KYNA, TRP and ATRP). Furthermore, we observed two unknown peaks in the N-acetyl region of the NMR spectrum. Most likely these peaks derive from N-acetylkynurenine and N-acetyl-3-hydroxykynurenine.
Conclusion: NMR spectroscopy of urine can establish the diagnosis of xanthurenic aciduria. Our data define xanthurenic aciduria at the metabolite level providing a specific urinary profile of two accumulating kynurenine derivates (XA and 3OHKYN).

177-A

REPORT OF A NEW CASE OF MEVALONIC ACIDURIA IN WESTERN SICILY

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Objective: Mevalonic aciduria (MVA) is a rare inherited metabolic disease due to mevalonate kinase (MK) deficiency. At least 30 patients with MVA have been reported worldwide. Here we describe a new case of MVA. Patient: The patient was born from healthy unrelated parents in originating from a small village of western Sicily. This is the third case of MVA in the same village diagnosed in the last four years. The relevant clinical features were facial dysmorphisms, hypotonia, hepatosplenomegaly, anemia, marked leukocytosis and recurrent crisis of fever accompanied with lymphadenopaty, artralgia and intestinal subobstruction. Brain MRI showed cortical athropy and hypoplastic corpus callosum with no cerebellar atrophy. He was first diagnosed having a myelodysplastic syndrome. MVA was then recognized following urinary organic acid analysis that showed increased mevalonic acid excretion. Fibroblasts mevalonate kinase activity was markedly reduced. Molecular analysis performed in DNA extracted from blood lymphocytes revealed that the patient was homozygous for the mutation c.709A>T in the mevalonate kinase gene. Since diagnosis the patient received oral ubiquinone therapy (100 mg/day), vitamin E and A. prednisone (2 mg/kg) and montelukast were successfully administered during crises.
Conclusion: The occurrence of three cases of MVA in the same village of western Sicily, could be suspicious for possibility of founder gene effect, so population genetics analyses should be performed.

178-A

A GIRL WITH PERIODIC FEVER

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Clinical presentation: Belgian girl, non-consanguineous parents. Developed fever (accompanied by convulsions) at the age of 5 months after the first vaccination. Second episode of fever lasting 14 days after the second vaccination. Periodic unexplained fever every 2 months lasting for 3–4 days/period.
Clinical presentation: During episodes of fever: hepatosplenomegaly and lymphadenopathy; skin manifestations: gianotti crosti; erythema nodosum; salmon rash; arthritis (∼morbus still)/arthralgia from the age of 2–3 years. Often the patient complained of a sore throat.
Laboratory investigations: During episodes of fever: Leucocytosis: >20 x 109/L, CRP: >10 mg/dl. Persistent anaemia from the age of 4 months; No evidence of bacterial or viral infections; Rheumatic parameters: negative; serum immunoglobulines (incl. IgD): normal; strongly elevated TNF and IL-2.
Diagnosis: Organic acid analysis on urine (GC-MS) (age 7 years), collected during an episode of fever: mevalonic aciduria was found. Up to three former urine samples analysed gave 'negative' results. Confirmed diagnosis: Mevalonate kinase deficiency. Molecular genetic analysis: Compound heterozygote: 1129G>A (mutation commonly found), 439G>A mevalonate kinase (lymphocytes): 8 pmol/min*mg (controls: 103±33).
Conclusion: Mevalonate kinase deficiency, a metabolic disorder of the biosynthesis of cholesterol and isoprenoids, can give rise to an episodic fever syndrome from early childhood on, and should be considered in the differential diagnosis of unexplained fever and/or a rheumatoid disorder and should be searched for by performing organic acid analysis on urine collected during an episode of fever.

179-O

CHRONIC INFLAMMATION AND HYPER IgD/IgE IN MICE WITH TARGETED DELETION OF THE MEVALONATE KINASE (*MVK*) GENE

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MvK catalyzes the first committed step in isoprenoid/cholesterol synthesis. Human MvK deficiency manifests as severe mevalonic aciduria (MA; OMIM 610377) and hyper-IgD syndrome (HIDS; OMIM 260920), both featuring decreased MvK activity, MvK gene mutations, and chronic autoinflammatory disease. Deletion of both murine MvK alleles is lethal; conversely, MvK± mice survive and are fertile. These animals accumulate mevalonate in tissues, and manifest decreased liver MvK activity associated with immune dysfunction. The latter includes lymphocyte hyperproliferation, heightened cytokine secretion (particularly TNF-alpha, IL-6, and IFN-gamma), and elevated serum immunoglobulin levels (particularly IgA, IgG3, and IgE). Serum IgD titer was significantly elevated in age-matched young (<15 weeks; MvK+/+, 4.4±2.5 (n = 25, SEM); MvK±, 51.9±18.3 (n = 26; $p<0.01$) and older animals (>15 weeks; MvK+/+, 22±19.8 (n = 10); MvK±, 194.1±45.9 (n = 41, $p<0.04$). Serum IgD increased significantly with age for MvK± mice (p = 0.01, <15 week vs. >15 weeks old). IgE did not differ with genotype in young mice, but was dramatically elevated in aged MvK± animals (>15 weeks; MvK+/+, 7.8±2.5 mg/L (n = 10); MvK±, 155.2±64.7 (n = 40, $p<0.0001$). MvK ± bone marrow showed hypocellularity with erythroid dominance and dysplastic megakaryocytes; peripheral blood was neutropenic (with atypical lymphocytes and monocytes as the predominant white blood cell types), and elevated reticulocyte number suggested anemia, indicative of altered hematopoiesis. MvK± mice reproduce features of MA and HIDS patients (especially inflammation), and may reveal novel roles for MvK in immune function and the allergic response.

180-P

SUCCESFUL BONE MARROW TRANSPLANTATION IN A CHILD WITH MEVALONIC ACIDURIA

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Background: Mevalonic aciduria (MA) is a rare inborn error of isoprene biosynthesis caused by a deficient activity of mevalonate kinase (MVK) resulting from mutations in the *MVK* gene. The neonatal phenotype is characterised by severe periodic fever attacks, ataxia, failure to thrive and cataracts. The prognosis is poor and children die in early infancy.
Methods: We report a 2.5-year-old boy with MA diagnosed at the age of 3 months upon elevated mevalonic acid in urine, confirmed by severely impaired activity of mevalonate kinase in lymphocytes and a homozygous mutation in the *MVK* gene (G326R, exon 9). He presented with low birth weight, severe anemia, hepatosplenomegaly and liver disease. Recurrent episodes of fever were noted every two weeks, with transient rash and abdominal pain. He had failure to thrive and mild cerebellar ataxia. The effect of several anti-inflammatory drugs on febrile attacks was very transient. Because of the very severe condition of the child, we proposed a BMT from a geno-identical heterozygous sister.
Results: Hematological recovery was achieved on day 21 post-BMT, with a 96% donor chimerism. We are now 18 months since the BMT and the child did not present any fever attack and no major complication. The volumes of liver and spleen have significantly decreased. Body growth is good and psychomotor development improved. Mevalonic aciduria decreased significantly. Mevalonate kinase activity has been restored to heterozygous levels.
Conclusion: BMT is a feasible procedure in MA patients and has been proven so far efficient in preventing recurrent inflammatory attacks.

181-A

ALKAPTONURIA: A RARE METABOLIC DISORDER A REPORT OF THREE PATIENTS (TWO SIBLINGS AND OTHER PATIENT)

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Alkaptonuria is a rare hereditary disease characterized by an increased urinary excretion of homogentisic acid and blackish coloration of the urine deficiency of the homogentisic acid oxidase activity leads to accumulation of homogentisic acid (HGA) and its oxidation product benzoquinone acetic acid. Toxic metabolite causes ochronosis, inflammatory arthritis or urinary calculi. In this study has been described of alkaptonuria diagnosed in a three patients (two siblings and another patient).

Their past medical histories were from early age a gradual dark discoloration of their urine had been noticed in the diapers but routine urine analysis had not revealed abnormalities. Patient's systemic and neurological examinations, laboratory findings and x-ray examination were completely normal. Sodium hydroxide was added to freshly three patient's urine. The urine color turned dark within a few minutes. There were initially diagnosed as having alkaptonuria by the presence of positive for urinary sodium hydroxide test.

The diagnosis was confirmed by urinary HGA amount. Normal levels of HGA in urine 2.4–12 ng/ml. In these three cases urine levels showed the level of HGA to be increase 14-, 14- and 24-fold respectively.

Our patients living in Sivas, an eastern province where socioeconomic conditions are unsatisfactory and consanguineous marriages is very high. Nutrition of patients was largely derived from leavened bread, cereals, vegetable foods and negligible amount of animal protein. This finding demonstrated that our patients were showed normal growth and development because of consuming a diet with low protein.

182-P

DETECTION OF FATTY ACID OXIDATION DEFECTS USING SIMULTANEOUS MEASUREMENTS OF TOTAL FATTY ACID β-OXIDATION FLUX AND ACYLCARNITINE PROFILING IN FIBROBLASTS CULTURED WITH ^{2}H31-PALMITATE BY ISOTOPE RATIO MASS SPECTROMETRY AND ELECTROSPRAY TANDEM MASS SPECTROMETRY

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Background: Total mitochondrial fatty acid β-oxidation (FAO) flux rate and acylcarnitine profiling are two commonly used methods for making a definitive diagnosis of FAO defects (FAOD) in cultured cells. We developed a novel functional assay which combined the total FAO flux rate assay with the conventional acylcarnitine profiling method in a single tracer incubation experiment. **Methods**: Skin fibroblasts from control subjects and patients with FAOD were incubated in medium containing universal deuterium-labelled palmitate (^{2}H31-palmitate) and L-carnitine without glucose supplemetation for 96 h. The culture medium was assayed for deuterated water enrichment using isotope ratio mass spectrometry (IRMS) and acylcarnitine profiling by electrospray-ionization tandem mass spectrometry (ESI/MS/MS). **Results**: The medians of ^{2}H2O enrichment after 96 h of incubation of ^{2}H31-palmitate of the control and FAOD cell lines were 109.9 and 23.1 ppm/mg protein/96 h, respectively. All fibroblasts with FAOD except carnitine uptake defective, multiple acyl-CoA dehydrogenase and short-chain 3-hydroxyacyl-CoA dehydrogenase deficient cells were well separated from the control and could be identified by IRMS assay. Moreover, accumulations of disease-specific acylcarnitines due to blockage in the carnitine cycle and FAO spiral were also demonstrated by acylcarnitine profiling. **Conclusions**: This novel functional assay does not require radio-active substrates for FAO flux rate measurement. It is less time-consuming and relatively simple compared to other published methods, and can be used to investigate patients suspected to have FAO defects.

183-P

QUANTIFICATION OF ACYLCARNITINES AND FREE CARNITINE BY ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY/ TANDEM MASS SPECTROMETRY (UPLC-MS/MS)

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Over the last decade tandem mass spectrometry (MS/MS) has become the preferred approach for analysis of acylcarnitines in biological fluids. However, even though MS/MS has proven very successful, current methods often lack the ability to discriminate between isomeric compounds. We report a fast UPLC-MS/MS method for separation and quantification of free carnitine and acylcarnitines (including isomers) in plasma or urine without derivatisation. Free carnitine and 25 saturated and unsaturated acylcarnitines (ranging from C2-C18OH) are separated and quantified within 10 min. The method allows separation of the C5-isomers; pivaloyl-, valeryl-, 2-methylbutyryl- and isovalerylcarnitine, enabling differentiation between patients suffering from e.g. isovaleric acidemia and patients treated with pivalate containing antibiotics. Also the C4-isomers; isobutyryl- and butyrylcarnitine, the C4DC-isomers; methylmalonyl- and succinylcarnitine and the C8-isomers; octanoyl- and valproylcarntine are separated. For acylcarnitines in plasma intra-day% CV (n = 6) and inter-day% CV (n =12) were less than 6 and 11, respectively. The chromatographic method has been very useful for diagnostic purposes and follow-up in patients with e.g. MCAD, LCHAD, MAD, GCDH deficiencies. As shown elsewhere, we find that excretion of glutarylcarnitine (C5DC) in urine is a more sensitive marker than measurement of C5DC in plasma for the diagnosis of glutaric acidemia type 1 in patients with residual GCDH activity.

184-P

DETERMINATION OF UNDERIVATIZED ACYLCARNITINES WITH A TANDEM MASS SPECTROMETRY METHOD CAPABLE OF DISTINGUISHING BETWEEN ISOMERS

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Background: Rapid determination of acylcarnitines with tandem mass spectrometry has become a valuable screening method for disorders of fatty acid metabolism. Usually rapid methods lack the ability to distinguish between isomers, which may impair the possibility to make an accurate diagnosis.
Methods that separate isomers often use ion pairing agents which may cause ion suppression of other analytes analysed on the same instrument. We wanted a high performance liquid chromatography tandem mass spectrometry method capable of distinguishing between isomers without the use of ion pairing agents.
Methods: Serum proteins are precipitated with acetonitrile/methanol and the supernatant is injected on a mixed mode column, on which retention and separation are accomplished by both reversed phase and cation exchange mechanisms. The analytes are ionised and detected by positive electrospray tandem mass spectrometry.
Results: All four isomers of the five carbon acylcarnitines are sufficiently separated to be distinguished. Within a cycle time of 30 min stearoylcarnitine elutes before 22 min. All acylcarnitines quantitated (acetyl-, propionyl-, butyryl-, isovaleryl-, hexanoyl-, octanoyl-, decanoyl-, lauroyl-, myristoyl- and palmitoylcarnitine) have a limit of determination better than 0.1 μmol/L. The total coefficients of variation are below 5%, except for isovaleryl-, hexanoyl- and myristoylcarnitine ($<10\%$).
Conclusions: We have developed a high performance liquid chromatography tandem mass spectrometry method for underivatized acylcarnitines. Quick sample preparation and capability to distinguish between isomers without ion pairing agents is achieved.

185-P

TOTAL ACYLCARNITINE LEVEL DETERMINED BY *IN-VITRO* PROBE ASSAY USING ^{2}H31-PALMITATE CAN DIFFERENTIATE CARNITINE UPTAKE DEFECT FROM OTHER FATTY ACID OXIDATION DISORDERS

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Background: Acylcarnitine profiling is commonly used for making a definitive diagnosis of FAO defects (FAOD) in cultured cells. Unlike other FAOD, carnitine uptake defect or plasma membrane carnitine transporter defect (CUD) does not yield any specific diagnostic change in acylcarnitine profile and thus, it cannot be detected by this simple method. However, our preliminary data suggested that the cellular production of acylcarnitines might be lower in CUD. This study was aimed to determine the potential use of summation of even-number acylcarnitines (C4:0- to C16:0-acylcarnitines) for the differentiation of CUD from other FAOD. **Methods**: Skin fibroblasts from control subjects and patients with FAOD were incubated in medium containing universal deuterium-labelled palmitate (^{2}H31-palmitate) and L-carnitine without glucose supplementation for 96 h. The acylcarnitines were quantitated by electrospray-ionization tandem mass spectrometry (ESI/MS/MS). **Results**: The means $\pm$ SD of total acylcarnitines of the normal control (n = 16) and CUD cell lines (n = 8) were 13.1 ± 2.3 and 7.7 ± 2.1 nmol/mg protein/96 h, respectively and the difference was statistically significant ($p<0.001$). All fibroblasts with FAOD could be differentiated from CUD based either on the accumulation of disease-specific acylcarnitines or very low levels of total acylcarnitines in the case of CPTI. **Conclusions**: Total acylcarnitine level accumulated in culture medium as determined by the summation of C4:0 to C16:0 even-chain acylcarnitines can be used to differentiate CUD from other FAOD. The method is relatively simple by comparison to the radio-active carnitine uptake assay and can be used to investigate patients suspected to have carnitine uptake defect.

186-P

RAPID BIOCHEMICAL CONFIRMATION OF FATTY ACID OXIDATION DEFECTS USING WHOLE BLOOD, STABLE LABELLED PALMITATE AND TANDEM MASS SPECTROMETRY

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Objective: Development and application of a rapid and simple method for biochemical diagnosis of fatty acid beta-oxidation defects, based on incubation of whole blood with stable-labelled palmitate.
Method: Incubation with (2H)5-palmitate, ethanolic extraction, butanolic derivation and specific detection by tandem mass spectrometry (ESI-MS/MS) of induced acylcarnitines. **Results** are expressed as percentage of accumulated intermediate (2H)5-acylcarnitines after comparing patient to mean of controls. Histograms are plotted and ratios determined to facilitate interpretation. Characteristic profiles were obtained for all patients explored. Percentage values were respectively at 210–250% of (2H)5-C14 in the case of VLCAD, at 620–760% of (2H)5-C8 for MCAD and at 130–260% of (2H)5-C4 for SCAD, in comparison to controls. This method was also successfully applied to patients suffering from L-carnitine cycle disorders (CPT I and CPT II).
Conclusion: This method allows biochemical confirmation of fatty acid oxidation defects and could constitute a sensitive, specific and efficient tool to diagnose these disorders. It only requires a small blood sample, which may be extremely useful for young children and neonates. Analytical process is quite simple as neither cell culture nor lymphocyte isolation is required, and results can be obtained very rapidly.

187-P

WHOLE BLOOD PALMITATE OXIDATION AS A SCREENING TEST FOR FATTY ACID OXIDATION DISORDERS: A FIVE-YEAR EXPERIENCE

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Inborn errors of fatty acid oxidation (FAOD) are a group of recessively inherited disorders presenting with a wide spectrum of manifestations. Given the complexities involved in establishing the diagnosis of a FAOD, developing simple screening assays has been a focus of our work. A simple and rapid screening assay for FAOD using a small volume of whole blood from the patient and tritiated palmitic acid as a substrate (whole blood palmitate oxidation assay-WBPO) was developed in our center (Seargeant et al., 1999). This assay also detects some mitochondrial respiratory chain disorders.

We report here the predictive value, sensitivity and specificity of the WBPO assay at various cutoff values. We reviewed our laboratory records to identify all the patients who had undergone WBPO testing between the years 1999 and 2005. The medical records of these patients were then reviewed and data with respect to other investigations and diagnoses were collected. The best cutoff value for WBPO assay was established using the receiver operating characteristic (ROC) curve.

The sensitivity of this screening test was determined to be 87% at a WBPO assay cutoff of 70% and the specificity at this cutoff was 60%. The sensitivity of the test climbed up to 96% when a cutoff value of WBPO assay of 80% was used while the specificity dropped to 48%. Using the ROC curve the best cutoff value for WBPO assay was established to be 70%. WBPO assay is a simple, non-invasive and sensitive screening test for FAOD.

188-O

MOUSE MODELS FOR HUMAN DISORDERS OF MITOCHONDRIAL FATTY ACID OXIDATION: STUDIES ON GLUCOSE METABOLISM

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Background: Mitochondrial fatty acid oxidation (mFAO) is considered to be essential for driving gluconeogenesis (GNG) during fasting. Human disorders of mFAO are associated with hypoketotic hypoglycaemia.
Methods: We quantitatively studied glucose metabolism in vivo in 3 different mouse models for mFAO, by infusing [U-13C]glucose, [2-13C] glycerol, [1-2H]galactose, and paracetamol for 6 h, followed by mass isotopomer distribution analysis (MIDA) in blood glucose and urinary paracetamol-glucuronide.
Results: After acute pharmacological inhibition of CPT-I by TDGA, endogenous glucose production was unaffected, but metabolic clearance rate (MCR) of glucose increased over two-fold. Although the rate of de novo synthesis of G6P was slightly decreased, net hepatic glucose production remained unaffected. After acute pharmacological inhibition of MCAD by SPA, MCR of glucose was unaffected. The rate of de novo synthesis of glucose-6-phosphate (G6P) was slightly decreased, associated with a corresponding decrease of net hepatic glucose output. In MCAD-deficient mice, the rate of de novo synthesis of G6P was unaffected and endogenous glucose production tended to be decreased. MCR of glucose did not differ between the groups.
Conclusions: Depending on the position of the blockade of mFAO, hypoglycaemia was brought about by different mechanisms. Endogenous glucose production can be maintained despite defects of mFAO.

189-P

ASSESSMENT OF KETOGENESIS IN HYPOGLYCAEMIC CHILDREN BY PLASMA ACYLCARNITINE RATIOS: COMPARISON WITH LONG CHAIN FATTY ACID OXIDATION DISORDERS

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Urinary organic acids and plasma 3-hydroxybuyrate and free fatty acids provide key parameters for the diagnosis of metabolic disorders presenting with hypoglycaemia. Plasma acylcarnitine analysis is also used routinely to investigate hypoglycaemia and quantitation of hydroxybutyrylcarnitine (C4OH) and acetylcarnitine (C2) can be used to assess ketogenesis. These two metabolites are often increased during metabolic stress indicating a normal ketogenic response. However medium and long chain acylcarnitines are also often increased and may approach the diagnostic ranges for long chain fatty acid oxidation disorders such as VLCAD and CPT2 deficiencies. Using MS/MS of butyl esters we have established ranges of C2, tetradecenoylcarnitine (C14:1) and dodecanoylcarnitine (C12) in 40 cases of hypoglycaemia with increased C4OH. Ratios of C14:1 and C12 to C4OH differentiate an appropriate ketogenic response from enzymatically confirmed cases of VLCADD and CPT2 deficiency.
Results
Concentrations (micromol/L) in ketogenic patients (n = 40)
C14:1 0.03–0.60 Mean 0.32 Normal <0.30
C12 0.04–0.63 Mean 0.21 Normal <0.20
C2 11–66 Mean 29 Normal 6–27
C4OH 0.14–1.76 Mean 0.42 Normal <0.10

Ratios ketogenic patients [VLCADD (n = 3)] [CPT2def (n = 2)]
C14:1/C4OH 0.13–2.2 [8.9, 44.6, 65.4] [2.16, 10.9]
C12/C4OH 0.15–2.9 [1.9, 4.2, 3.7] [4.2, 16.6]
Conclusion: Plasma acylcarnitine ratios are useful in assessing appropriate ketogenesis in hypoglycaemic patients and excluding VLCADD (by C14:1/C4OH) and CPT2 deficiency (by C12/C4OH).

190-P

CHARACTERIZATION OF L-AMINOCARNITINE, AN *IN VITRO* MODEL FOR FATTY-ACID OXIDATION DEFECTS

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Background/Objectives: Heart failure is a common cause of death in children with mitochondrial long-chain fatty acid β-oxidation (FAO) defects. The pathogenesis of this heart failure is still unknown, but we speculate that elevated free fatty acid levels are important. *In vitro* studies using primary cardiomyocytes or myoblasts are hampered by the lack of natural mutants to asses the effect of FAO inhibition. In addition, most inhibitors of FAO are fatty acids analogs, which activate peroxisome proliferator activated receptor (PPARs). L-aminocarnitine is not a fatty acid and does not have this drawback. L-aminocarnitine inhibits carnitine palmitoyltransferase (CPT) with different sensitivities towards CPT1 and CPT2. The aim of this study is to further characterize L-aminocarnitine.
Methods: We used different human control and patient fibroblast cell lines. Acyl-carnitine profile was determined in culture medium by tandem-mass spectrometry after loading with [U-13C] palmitate for 4 days in presence of L-aminocarnitine.
Results/Conclusion: In control fibroblasts L-aminocarnitine inhibits FAO as shown by the accumulation of C16-acyl-carnitine. In carnitine acyl-carnitine translocase-(CACT) and CPT2-deficient cell lines, L-aminocarnitine did not change the already elevated C16-acyl-carnitine level, showing that CPT1 is not inhibited. In very long chain acyl-CoA dehydrogenase (VLCAD)-deficient fibroblasts C14-acyl-CoA accumulates, which is converted to C14-acyl-carnitine by CPT2. In these cell lines addition of L-aminocarnitine resulted in a decrease of C14-acyl-carnitine and an increase of C16-acyl-carnitine.

Therefore, we conclude L-aminocarnitine inhibits CPT2, making it a useful inhibitor of FAO in order to simulate a FAO defect in cells from different origin such as primary cardiomyocytes.

191-P

MITOCHONDRIAL FACTORS PREDISPOSING FOR SCAD INSUFFICIENCY: STUDIES USING RNAI AND MASS SPECTROMETRY

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Background: Several mutations in the short-chain acyl-CoA dehydrogenase (SCAD) gene may lead to SCAD insufficiency, a severe disease altering mitochondrial short-chain β-oxidation. However, only few patients have presented symptoms related to energy deficiency. Instead, the predominant clinical symptom is neuromuscular dysfunction, implying that the disease-causing nature of SCAD variant proteins is not in itself a loss-of-function, but may involve other factors. Biochemically, SCAD insufficiency is characterized by accumulation of butyrylcarnitine and ethylmalonic acid as a result of unmetabolized SCAD substrate. In order to identify susceptibility/modifier genes we have developed a cellular model for SCAD insufficiency using RNA interference (RNAi) and metabolite profiling. Since we have previously demonstrated that SCAD insufficiency is a protein misfolding disease such modifier genes could be involved in folding and maintenance of the SCAD enzyme. **Methods**: RNAi-mediated knockdown of SCAD was induced by transfecting HEK-293 cells with SCAD-specific siRNAs. Following incubation in [U-13C]-labeled palmitate and L-carnitine containing medium, the acyl-carnitine profile of the culture medium was analyzed using tandem mass spectrometry. **Results and Conclusion**: SCAD knock-down resulted in significant elevation of [U-13C]C4-carnitine in cells treated with SCAD-specific siRNAs. Even 2 days after addition of labeled palmitate increased amounts of [U-13C]C4-carnitine (2.5-fold) was observed and the effect was most pronounced after 3 days (4-fold). The results show that the RNAi treatment leads to the biochemical phenotype. The established model will be used to investigate the etiology of SCAD insufficiency by transfecting HEK-293 cells with siRNAs targeting mitochondrial factors like Lon, Hsp60, ETHE1, ECHS1, PGC1.

192-O

SYNERGISTIC HETEROZYGOSITY FOR SCAD AND ETFB VARIATIONS?

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Background: Controversy persists as to whether mutations and/or polymorphic variants in the *ACADS* gene encoding short-chain acyl-CoA dehydrogenase (SCAD) cause significant clinical disease, or merely produce the clinically asymptomatic biochemical phenotype of ethylmalonic aciduria (EMA) ± elevated butyrylcarnitine. **Methods**: Metabolic studies and sequencing of genes responsible for SCAD and multiple acyl-CoA dehydrogenase (MAD) deficiencies were performed in a 6-month-old girl presenting with acute gastroenteritis complicated by collapse, encephalopathy, acidosis, hepatopathy and renal failure. **Results**: Initial urine organic acid analysis revealed massive glutaric aciduria, marked dicarboxylic aciduria with relative hypoketonuria, and EMA, raising the possibility of MAD deficiency. Free/total carnitine levels were 6/23 μmol/L. Bloodspot acylcarnitine analysis was non-contributory. She recovered completely following intensive care and peritoneal dialysis. At age 4 years she remains perfectly normal apart from persisting EMA. The patient and mother were heterozygous for a novel *ETFB* c.577G>A (p.Ala193Thr) variation affecting a conserved residue. The patient and father were compound heterozygous for the *ACADS* c.319C>T variation previously identified in the Ashkenazi Jewish population, and the prevalent *ACADS* c.625G>A variant; the mother was homozygous for c.625G>A. No variations were found in the *ETFA* or *ETFDH* genes. **Conclusions**: These findings are consistent with the hypothesis that *ACADS* variations may remain asymptomatic in isolation, but might act synergistically with variations in genes from the same or related pathways to produce clinical disease during acute stress from intercurrent illness and/or fasting. In patients with apparently symptomatic SCAD deficiency, mutation analysis of related genes can contribute to understanding the pathogenesis of the disorder.

193-P

A DISEASE-CAUSING VARIANT OF SHORT-CHAIN ACYL-CoA DEHYDROGENASE PROMOTES OXIDATIVE STRESS

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Background: Short-chain acyl-CoA dehydrogenase deficiency (SCADD) is a rare recessively inherited metabolic disorder, affecting the mitochondrial β-oxidation. Patients are usually presenting neuromuscular features such as developmental delay, hypotonia, seizures, as well as a general failure to thrive.
Methods: To study the pathogenesis of the disease, transduced astrocytic cells stably expressing five different disease-associated SCAD protein variants were established. In the cloning process, the viability of cells expressing each one of two severe SCAD variant proteins was severely reduced, compared with cells expressing the SCAD wild-type (wt) protein. One of these was the rare variant protein Arg83Cys (319C>T), which is unable to assemble into catalytically active SCAD tetramers, as well as having aggregational tendencies in vitro. Six out of six SCAD wt cell colonies survived, whereas only four out of seven SCAD Arg83Cys colonies survived colony transfer. To investigate whether this variation inflicts with the ability of the cell to overcome a stress-full situation, cells expressing the wt or the 319C>T variation was subjected to heat stress of 400°C, and the stress-response was followed over a time period of 24 h, monitored by selected stress response genes (Hsp70, Hsp60, MnSOD (manganese superoxide dismutase) and HO-1 (hemeoxygenase-1)).
Results and Conclusion: The cell line expressing the *SCAD* Arg83Cys variant protein revealed an elevated production of MnSOD and HO-1 compared with the *SCAD* wt cells, indicating oxidative stress, elicited by the misfolded mitochondrial SCAD variant protein.

194-P

CLINICALLY PRESENTING FATTY ACID OXIDATION DEFECT WITH A MILD MEDIUM CHAIN ACYL-CoA DEHYDROGENASE DEFICIENCY (MCADD) BIOCHEMICAL PHENOTYPE AND NORMAL DRIED BLOOD SPOT OCTANOYL CARNITINE (C8), NOT DETECTED BY NEWBORN SCREENING

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A 10-month-old male infant presented with lethargy and dehydration following 3 days of gastroenteritis. Blood sugar was 2.6 mmol/L with appropriate lipolytic (free fatty acids 3.0 mmol/L) and ketogenic (3-hydroxybutyrate 4.6 mmol/L) responses. Urine organic acids showed a mild dicarboxylic aciduria with a small peak of hexanoylglycine. The plasma acylcarnitine profile showed a typically ketotic pattern, with a relatively high elevation in C8 (C8:C10 = 0.97, MCADD ratio >1.0). Hexanoylglycine when well was elevated (1.6 mmol/mmol creatinine, normal 0.1–1.1) but below the MCADD range (>1.9). Plasma C8 was elevated (0.29 mmol/L, ref <0.22), but blood spot C8 was normal (0.15 mmol/L, ref <0.3), as had been on the newborn screening blood spot (0.27 mmol/L, ref <0.5). Sequencing of all 12 exons and exon/intron boundaries of the *ACADM* gene identified only heterozygosity for the c.985A>G p.K329E mutation. Fatty acid oxidation in skin fibroblasts showed decreased flux with myristate and octanoate (46% and 32% of controls respectively), indicating reduced medium chain fat oxidation, inconsistent with simple MCADD heterozygosity. With MCADD treatment there has been no further hypoglycaemia. This child with hypoglycaemia has a significant deficiency of medium chain fat oxidation with mild biochemical features and normal blood spot C8. The genetic basis for these changes may be related to non-exon changes in the *ACADM* gene or to modifier genes.

Children with subtle but persistent biochemistry associated with MCADD should be fully investigated for fat oxidation defects even if blood spot C8 diagnostic and newborn screening tests are normal. Hexanoylglycine may be a more sensitive marker.

195-A

MEDIUM CHAIN ACYL-CoA DEHYDROGENASE DEFICIENCY: 19 CASES

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Background: Medium Chain Acyl-CoA Dehydrogenase (MCAD) deficiency is the most common defect in the mitochondrial β-oxidation pathway. It is an autosomal recessive disorder that can present itself at any age.
Objectives: To determine clinical symptoms, biochemical profile and mutational studies in 19 patients with the diagnosis of MCAD deficiency.
Methods: Retrospective analysis of clinical, biochemical and molecular data.
Results: The authors present 19 cases of MCAD deficiency, one female adult, 14 girls and four boys. 17 patients are of gypsy origin and 11 have consanguineous parentage.

The diagnosis was made in eight children due to the presence of characteristic symptoms. 11 patients were asymptomatic, seven diagnosed through the newborn screening program and four based on family history. The main clinical presentation, after an episode of fasting was vomiting, coma and hepatomegaly, as well as hypoketotic hypoglycaemia and metabolic acidosis.All patients had the typical acylcarnitine, dicarboxylic acid and acylglycine profile. Mutational studies revealed that all of the patients of gypsy origin were homozygous for the mutation A985G. One Portuguese patient was a compound heterozygote C1045T/C250T and the other patient, an Argentinean a compound heterozygote A985G/A503T.
Conclusion: Newborn screening has been extremely important in the early detection of index cases and in asymptomatic family members.

196-A

AVAILABILITY OF CARNITINE LOADING TEST IN MCADD INFANTS WITH CARNITINE DEFICIT

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We report herein a Japanese medium-chain acyl-CoA dehydrogenase deficiency (MCADD) patient, who were diagnosed by acylcarnitine profiles, enzyme activity and genetic analysis after clinical presentation. At the present time, the newborn screening by tandem mass spectrometry (MS/MS) has not been performed in all area of Japan. MS/MS is enabling to screening of some organic acidemias and fatty acid oxidation disorders including MCADD. However the current newborn screening by MS/MS cannot separate acylcarnitine isomers, and is unsuitable to more detailed diagnosis and evaluation of the treatment for these patients. Our previous reported method of high-performance liquid chromatography electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS) can separate most acylcarnitines isomers and quantify them. Using our HPLC-ESI-MS/MS method, we quantified the patient's acylcarnitines in serum and urine during fasting test and carnitine loading. Acylcarnitines were difficult to be detected under low serum carnitine level. After carnitine loading, acylcarnitine profile was characterized by increase of octanoylcarnitine (C8) and the urinary excretion of C8 was obviously excreted to urine.

In fasting test, the serum glucose level remained normal with or without carnitine supplementation, while the production of acylcarnitines with carnitine supplementation was much greater than that of without carnitine supplementation. Carnitine supplementation may be useful to conjugate and excrete toxic acyl-CoA to urine even in asymptomatic fasting. MCADD could be missed under low carnitine level, carnitine loading should be considered.

197-O

A SILENT SUBSTITUTION IN THE MCAD GENE CAUSES EXON 2 SKIPPING BY DISRUPTION OF A CRUCIAL SRP40 BINDING EXONIC SPLICING ENHANCER WHICH IS FUNDAMENTAL FOR MCAD GENE EXPRESSION

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Correct splicing of exons is determined by a finely balanced interplay between cis-acting regulatory sequences like exonic splicing enhancers (ESE) and exonic splicing silencers (ESS). Mutations that create or disrupt ESS/ESEs may disturb this balance and cause missplicing and disease.

We identified two unrelated newborns who were compound heterozygous with the prevalent c.985A>G mutation and a c.87A>G synonymous substitution (R4R). Analysis of cells from one of the newborns and her father showed nearly complete skipping of exon 2.

To investigate if exon 2 skipping is caused directly by c.87A>G or by an undetected intronic mutation we used a minigene harboring exon 2 and part of the flanking introns. This confirmed that c.87A>G causes exon skipping, and that a neighboring c.85C>T mutation (identified in a diseased patient) does not affect splicing. Computer analysis indicated that c.87A>G, but not c.85C>T, disrupts a binding motif for the splicing regulator SRp40. We used a heterologous splicing reporter minigene to confirm that c.87A>G also causes missplicing in another genetic context and tested other substitutions to further delineate the consensus sequence for this ESE. Using nuclear extracts and RNA affinity purification with wild type and c.87A>G mutant oligonucleotides we confirmed that c.87A>G disrupts binding of SRp40. This suggests that an ESE encompassing position c.87 harbors a SRp40 binding ESE, which is fundamental for correct splicing of MCAD exon 2.

The present study provides a further example on how synonymous substitutions can be deleterious by disrupting the finely tuned balance between splicing regulatory elements in constitutive exons.

198-P

INTRAUTERINE GROWTH RETARDATION IN PATIENTS WITH LCHAD DEFICIENCY

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Deficiency of long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) is the most frequent clinically expressed defect among FAO disorders in the Polish population. Out of 42 patients diagnosed, 28 stay alive. Fifteen of them were born at term with mean weight 3042 g. Above 44% were born preterm from 27 to 37 hbd (mean gestational age 33 hbd) with low birth weight from 580 g to 2300 g (mean: 1643 g). In seven premature babies significant intrauterine growth retardation (IUGR) was noticed with birth weight below the 10th percentile. One boy was born with extremely low weight 580 g in 28 hbd in deep asphyxia (1 point in Apgar scale) with intracranial haemorrhage and on artificial ventilation for 20 days. At the age of 4 months operated because of obstructed bilateral inguinal hernias, at the age of 12 months he suffered from bacterial meningitis, complicated by DIC, pneumonia and respiratory-circulatory insufficiency. This patient was found heterozygous for common c.1528G>C *HADHA* mutation. Now at the age of 4 years the patient is in good clinical condition without episodes of decompensation, with growth and weight –2.0 SD. Due to hypertrophic cardiomyopathy (147% of LV) and arrhythmias he requires cardiological treatment and monitoring.

Up to now IUGR and prematurity have been reported in some single cases of LCHAD deficiency. In one of them placental floor infarction has been documented [Matern et al]. Our study confirms high frequency of IUGR and prematurity in LCHAD deficient patients, though the reason remains to be elucidated.

199-P

CLINICAL FOLLOW-UP OF 10 CHILDREN WITH LONG-CHAIN 3OH-ACYL-CoA DEHYDROGENASE (LCHAD) DEFICIENCY

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Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD) is a recessively inherited disorder caused by a defect in the mitochondrial β-oxidation of long chain fatty acids. Patients often present with hypoketotic hypoglycaemia, hypotonia, liver enlargement, muscular pain and weakness, and sometimes cardiomyopathy. Ocular complications are common.

The available treatment consists of low fat diet with supplementation of medium chain triglycerides (MCT fat) and essential fatty acids. Avoidance of fasting and dietary carbohydrate enrichment or i.v. glucose infusion in catabolic situations is important.

Despite a complicated dietary treatment these children risk life-threatening decompensations in catabolic situations such as common febrile illnesses.

We report on the long-term (up to 17 years) clinical follow-up of 10 children, aged 4 to 17 years, with LCHAD deficiency. Age at diagnosis was 5.9 months mean (1 week–13 months). The dietary treatment was invented de novo for the first patient by our group and consisted of frequent feeds, including night feeds, low fat diet with supplementation of MCT fat as well as essential fatty acids, very similar to the currently used treatment. The younger patients have had gastrostomy and continuous night feeds early on.

The clinical outcome is variable, some patients have a developmental delay but the majority have been able to attend normal schools. All patients have developed ocular changes. Our results indicate that patients tend to develop ocular changes despite of early diagnosis and treatment.

200-O

APPLICATION OF BEZAFIBRATE FOR CORRECTION OF VLCAD DEFICIENCY AND PREDICTION OF MUTATION SEVERITY

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We recently showed that fibrates could restore FAO in patient cells harboring inborn defects in very-long-chain-acylCoA-dehydrogenase (VLCAD; mitochondrial β-oxidation), by stimulating residual enzyme activity. Given the variety of reported VLCAD gene point mutations, we investigated the response to drug as a function of genotype. 34 VLCAD-deficient fibroblast with distinct genotypes representing 50 different mutations were treated with 400 μM bezafibrate for 72 h and FAO was measured using tritiated palmitate. Untreated cells exhibited FAO rates much lower (–30 to –90%) than control. Bezafibrate induced a marked increase in FAO in 60% of the genotypes tested, and a complete correction in 15 cell lines. These data allowed to identify three groups: severely deficient cells with nonsense mutations, or missense mutations affecting residues essential for catalysis (G222, G441, R469), that were drug-resistant; a 2nd group with missense mutations compatible with a moderate response to bezafibrate; a 3rd group which harbored genotypes compatible with a full restoration of FAO by bezafibrate, pointing to mild mutations (V283A, G441D, R615Q). We also characterized changes in VLCAD mRNA and residual enzyme activity levels induced by bezafibrate, as a function of genotype. The mutations were reported in a predictive VLCAD 3-D model allowing to confirm the mild or severe mutations that were characterized in the 'bezafibrate test'. The response to bezafibrate can therefore predict the severity of VLCAD point mutations that was not documented yet and might help to identify patients for a future clinical trial.

201-P

IMPAIRED CARDIAC FUNCTION IN VERY LONG-CHAIN ACYL-CoA DEHYDROGENASE-DEFICIENT MICE AS STUDIED BY *IN-VIVO* NMR

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Background: The underlying mechanisms of cardiomyopathy in very long-chain acyl-CoA dehydrogenase deficiency (VLCADD) are unknown. Lack of energy as result of insufficient energy production from fat is one of the possible mechanisms.
Methods: Magnetic resonance imaging experiments were performed in 6 VLCAD-deficient mice and 6 wild-type (WT) littermates at the age of 15 weeks. High-resolution heart images were acquired using an ECG- and respiratory-triggered fast gradient-echo cine sequence (flip-angle: 150, echo-time: 1.8 ms, repetition-time: 4 ms, pixel-size: 117×117 microm^2, field of view: 30×30 mm^2, matrix: 128×128, acquisition-time per slice for one cine sequence: 1–2 min). Six to eight contiguous ventricular short-axis slices (slice-thickness = 1 mm) were acquired to cover the entire heart.
Results: VLCAD-deficient mice displayed impaired cardiac output as reflected by a decrease in both end-diastolic (EDV) and end-systolic volumes (ESV) (EDV and ESV were 40.6 ± 4.2 and 10.0 ± 1.8 microL, respectively; as compared to WT: 51.6 ± 3.0 and 14.2 ± 1.9 microL, respectively; mean $\pm$ SEM). Stroke volume was significantly lower in VLCAD-deficient mice (30.6 ± 2.5 and 37.3 ± 1.9 microL for WT, respectively; $p > 0.05$; mean $\pm$ SEM). We observed no alterations in left ventricular mass. Endsystolic wall diameter was increased in VLCAD-deficient mice by 10%.
Conclusions: Our findings reveal decreased cardiac output without signs of increased cardiac mass in VLCAD-deficient mice under resting conditions. Further studies after recurrent physical exercise are underway and will show, whether increased energy demand will induce cardiomyopathy development.

202-O

CORRELATION OF ACYL-CoA AND ACYLCARNITINES IN HEART AND LIVER FROM VERY LONG-CHAIN ACYL-CoA DEHYDROGENASE-DEFICIENT MOUSE

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Background: VLCAD catalyses the initial step of mitochondrial b-oxidation of long-chain fatty acids. In VLCAD-deficient mice, C14-C18 acylcarnitines accumulate, with C16:0-carnitine and C18:1-carnitine as disease-specific marker. However, data on intramitochondrial acyl-CoA concentrations and their cytotoxic effects are missing.
Methods: Approx. 30 mg of heart or liver tissue was lyophilized, homogenized in 80% v/v acetonitrile (ACN)/H2O, centrifuged and the supernatant was dried. For acylcarnitine analysis, n-butanol-acetylchlorine was added. Samples were taken up in 80% v/v ACN/H2O. C17-CoA and 2H-C16-carnitine served as internal standards. A QuattroMicro MS/MS detected acyl-CoA in multiple reactant monitoring mode and acylcarnitines in parent-scan mode (butylated acylcarnitines resulting in losses of 85 m/z). Cytotoxic effects of acylcarnitines and acyl-CoA were studied by MTT in H4IIE cells at concentrations between 100 μM and 1000 μM.
Results: VLCAD-deficient mice displayed increased C16-carnitine as compared to wildtype (WT) mice (11.04 ± 3.14 and 4.92 ± 1.39 nmol/mg wet weight, respectively). This increase was directly reflected by an increase in C16-CoA in heart from VLCAD-deficient mice (16.45 ± 5.50 and 7.32 ± 1.26 nmol/mg wet weight, respectively). In livers from VLCAD-deficient mice, C16-carnitine and C16-CoA also directly correlated, but were not significantly different from WT mice. In H4IIE cells, incubation with C16-carnitine resulted in impaired cell proliferation at IC50 = 150 μM, incubation with C16-CoA in impaired cell proliferation at IC50 = 470 μM.
Conclusions: To our knowledge, this is the first study showing that acylcarnitines and the corresponding acyl-CoA directly correlate. In the cell model, acylcarnitines are toxic at lower concentrations as compared to acyl-CoA.

203-P

TISSUE CARNITINE CONCENTRATIONS IN VLCAD-DEFICIENT MICE AFTER SUPPLEMENTATION OF MEDIUM-CHAIN FAT

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Background: Carnitine plays an important role in the transport of activated long-chain fatty acids through mitochondrial membranes, whereas medium-chain fatty acids directly pass mitochondrial membranes. In patients with very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency, more carnitine is needed to export accumulating long-chain acyl-CoA from mitochondria. Aim of this study was to analyse the effects of a medium-chain fat-diet on carnitine/acylcarnitine concentrations in tissues from VLCAD-deficient mice.
Methods: VLCAD-deficient mice were fed for five weeks with a diet containing medium-chain triglycerides (MCT) instead of long-chain triglycerides (LCT). Heart, liver and skeletal muscle tissue was lyophilized, homogenized in 80% acetonitrile (ACN)/H_2O. For analysis n-butanol-acetylchlorine was added. Samples were analyzed by MS/MS.
Results: The concentrations of free carnitine in liver and skeletal muscle are significantly reduced in VLCAD-deficient mice with an MCT-diet compared to an LCT-diet (183 ± 21 and 307 ± 53 nmol/g wet weight liver tissue and 113 ± 8 and 169 ± 12 nmol/g wet weight muscle tissue, respectively). In heart from VLCAD-deficient mice with an LCT-diet, carnitine-concentrations are already significantly lower as compared to wildtype mice (412 ± 34 and 619 ± 27 nmol/g wet weight, respectively). Cardiac free carnitine in these mice with an MCT-diet is as low as with an LCT-diet. In fact, both wildtype and VLCAD-deficient mice presented with comparably decreased cardiac carnitine-concentrations with an MCT-diet.
Conclusions: We speculate that an MCT-diet results in a reduction of endogenous carnitine production in tissues as medium-chain fatty acids pass the mitochondrial membrane not using carnitine for transport.

204-P

VERY LONG CHAIN ACYL CoA DEHYDROGENASE DEFICIENCY: RESISTANCE TO THERAPY

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Very long-chain acyl-CoA dehydrogenase deficiency (VLCAD-D) is an autosomal recessive disorder of fatty-acids oxidation (FAO). In one kindred we have diagnosed five VLCAD-D patients. We report here multiple treatment approaches in one severe myopathic patient. Case Report: A three-year-old boy was hospitalized with vomiting, diarrhea and seizures. The child remained encephalopathic in the absence of hypoglycemia. Metabolic studies revealed non-ketotic dicarboxylic aciduria, carnitine deficiency, VLCAD deficiency in fibroblasts, and homozygous VLCAD Gly222Arg mutation. He suffers from a severe myopathic form with rabdomyolysis, no cardiomyopathy nor retinopathy.Therapeutic modalities: Treatment began with a LCFA restricted diet, supplemented with MCT and L-carnitine. Within a year, he had multiple admissions for weakness, muscle pain, and rhabdomyolysis (CPK 30 000–60 000). Uncooked cornstarch, four times/day, had no effect. Over the years we introduced various therapeutic modalities in the following order: (1) Creatine monohydrate, known to improve exercise performance and increase fat-free mass. This produced no change. (2) Triheptanoin, a C7-odd-carbon fatty-acid, provides fuel to the Krebs' cycle, and is gluconeogenic. Triheptanoin caused increased abdominal pain, vomiting and diarrhea, even when it was administered in small quantities via gastrostomy mixed with L-Elemental. (3) Bezafibrate, a peroxisome proliferator-activated receptor, acting via stimulation of gene expression, was found to stimulate residual activity of FAO enzymes in fibroblasts. *In vitro* studies in the patient's fibroblasts are pending. Bezafibrate, 400 mg/day, led to increased frequency of attacks. At present, the patient, 10.5 years old, receives a low-fat, high-carbohydrate diet, MCT and L-carnitine. He continues to have frequent attacks, hoping for a new treatment.

205-O

VALPROYL-CoA INHIBITS CARNITINE PAMITOYL TRANSFEREASE 1 AND THUS INTERFERES WITH MITOCHONDRIAL FATTY ACID OXIDATION

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Background: Carnitine palmitoyl transferase 1 (CPT1) is a mitochondrial outer membrane protein responsible for the conversion of LC-acyl-CoA esters into acylcarnitines (AC) and the rate limiting enzyme of the mitochondrial fatty acid β-oxidation (FAO). CPT1 activity, and therefore FAO, is regulated by malonyl-CoA, controlling the energetic state of the cell. Valproic acid (VPA) is a C8-branched chain fatty acid that can be activated in both the intra- and extra-mitochondrial compartment.
Aim: To clarify whether valproyl-CoA interferes with the mitochondrial carnitine shuttle at the level of CPT1.
Methods: The CPT1 activity was measured as the C16-AC formation in control human fibroblasts, after in vitro incubation with 255M [U-13C]-C16-CoA, 0.5 mM L-Carnitine, 5 mM KCN, 405 g/ml digitonin and increasing malonyl-CoA (0–1 mM) in the absence and presence of valproyl-CoA (0–100 μM), for 10 min, 37°C. The AC were quantified by ESI-MS-MS.
Results: Malonyl-CoA inhibited the conversion of C16-CoA into C16-AC by CPT1, as expected, and its K_i was estimated as 0.05 mM. Valproyl-CoA was found to clearly inhibit the CPT1 activity. The apparent K_i of malonyl-CoA for CPT1 increased as the concentration of valproyl-CoA was increased in the reaction medium, thus indicating that valproyl-CoA inhibits CPT1.
Conclusions: These results clearly indicate that valproyl-CoA interacts with CPT1, interfering with its key regulator malonyl-CoA. The induced effect of valproyl-CoA on CPT1 activity can account for the decreased rate of LC-FAO associated with the drug. The microvesicular steatosis and the weight gain related with VPA therapy are clinical signs potentially reflecting the imbalance in the energetic status of the cell.

206-P

A COMMON MUTATION IN CARNITINE PALMITOYLTRANSFERASE I (CPT1) DEFICIENCY IN FINLAND: A REPORT OF 5 CASES

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Background: Carnitine palmitoyltransferase 1 (CPT1) deficiency is an autosomal recessive disorder of impaired long-chain fatty acid transport into the mitochondria. The disease manifests during the first years of life as hypoketotic hypoglycemia with hepatopathy often provoked by fasting or febrile illness, characteristic to mitochondrial fatty acid β-oxidation disorders. A variety of mutations have been detected, with only two mutations the Inuit (P497L) and Hutterite (G710E) showing the founder effect. Patients: we report the clinical, biochemical and genetic findings of 5 Finnish patients from 4 unrelated non-consanguineous families.
Results: All the patients presented the first symptoms during the first two years of their life related to viral illness and/or fasting. The clinical deteriorations varied from mild transient loss of consciousness to severe metabolic decompensation during pregnancy. In two brothers only mild disturbance of consciousness have occurred. In one patient lipid signals in brain MRS were detected during prolonged hyperammonemic and hyperlipidemic coma. One patient suffered from severe metabolic acidosis and fatty liver. One previously undiagnosed adult patient developed HELLP syndrome during her first pregnancy.

Homozygous substitution 1364A>C (K455T) in exon 12 was detected in 4 patients and the same mutation combined with 1493A>C (Y498S) substitution in exon 13 in one of the patients. Both mutations are located in the catalytic core of the enzyme.
Conclusion: We report a common mutation 1364A>C (K455T) of CPT1A deficiency in Finland. Five patients all sharing the same mutation in homozygous or heterozygous form showed great variation in the severity of the clinical course.

207-P

DIAGNOSTIC PROBLEMS IN TWO PATIENTS WITH LATE-ONSET CARNITINE PALMITOYLTRANSFERASE TYPE II DEFICIENCY

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Objective: Carnitine palmitoyltransferase type II deficiency (CPT II deficiency; MIM 255110) is an autosomal recessive disorder of carnitine dependent transport of long-chain fatty acids into mitochondrial matrix. We present two patients with late-onset CPT II deficiency, in whom tandem mass spectrometry (MS/MS) and oxidation of (2.3–3H)labeled fatty acids in lymphocytes were not able to distinguish different fatty acid oxidation (FAO) disorders. Clinical picture: Both patients have recurrent attacks of rhabdomyolysis with muscle pain and weakness since childhood. **Methods/Results**: Acylcarnitine analyses using MS/MS were suspected of FAO disorders, probably on the level of long fatty acids and/or transport into mitochondrial matrix. Analyses showed suspicion of CPT II deficiency, very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency, carnitine/acylcarnitine translocase (CACT) deficiency or glutaric aciduria type II (GA II). Oxidation of (2.3–3H)labeled fatty acids in lymphocytes showed suspicion of deficiencies of CPT I, CPT II, CACT or VLCAD, LCHAD/MTP in patient 1 and suspicion of MCAD deficiency in patient 2. Palmitate loading test in cultured skin fibroblasts reflected CPT II deficiency in both patients. Sequencing of genomic DNA has shown compound heterozygosity for the most common mutation (c.338C>T) and for a novel mutation in *CPT II* gene (c.1493G>A in patient 1 and c.1096.1098delACT in patient 2).
Conclusions: (1) Acylcarnitine analyses in blood spots and oxidation of (2,3-3H)labeled fatty acids in lymphocytes were not diagnostic for CPT II deficiency. (2) Palmitate loading test in cultivated human skin fibroblasts and DNA analyses were diagnostic. Supported by the project VZ 64165/2 of Ministry of Health of Czech Republic.

208-P

THE HUMAN CARNITINE ACYLCARNITINE TRANSLOCASE (hCACT): STRATEGIES FOR ITS HETEROLOGOUS EXPRESSION, PURIFICATION AND CRYSTALLIZATION

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CACT plays an essential role in the transport of long-chain fatty acids into the mitochondria for further β-oxidation. CACT deficiency is a rare and life-threatening autossomic recessive disease (OMIM #212138). A detailed structural and functional characterization of the human CACT is still lacking. We this aim, an expression and purification strategy to obtain a native recombinant hCACT was developed.

A prokaryotic expression system was used to produce a recombinant hCACT as a double-tagged (N-terminal 6xHis-tag and C.terminal Strep-tag.) protein with recognition sites for enterokinase and for thrombin at its N- and C-terminus, respectively. The recombinant protein (6xHis.EK.hCACT.Tr.Strep) was solubilized with 2% sarkosyl and purified by affinity chromatography in the presence of 0.2% (v/v) Brij-35. Far-UV Circular Dichroism (CD) was applied to determine the secondary structure and the conformational state of the recombinant protein. Preliminary crystallization studies were performed (4:C and 20:C) by vapour diffusion using the hanging drop method and 128 different conditions.

The developed strategy allowed the production of soluble recombinant protein with the expected Mr (38 kDa), high yield (2–3 mg/L culture) and high purity (>98%). Far-UV CD studies indicated that the obtained hCACT corresponds to a folded, mainly alpha-helical protein. After 21 days/20:C, it was possible to obtain micro-crystals with the precipitants PEG400 and Na/K tartrate. Crystal optimisation experiments are in progress.

The incorporation of the produced protein into liposomes (already ongoing) will bring new perspectives towards the full functional and structural characterization of the hCACT and the understanding of the molecular basis of CACT deficiency.

209-A

PRIMARY SYSTEMIC CARNITINE DEFICIENCY (SCD) IN THAILAND – A REPORT OF 2 CASES

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Background: Primary systemic deficiency (SCD) (OMIM # 212140) is an autosomal recessive disorder of mitochondrial β-oxidation resulting from defective carnitine transport. It is caused by heterogeneous mutations in the organic cation/carnitine transporter (OCTN2, McKusick 603377) gene *SLC22A5* located on human chromosome 5q31 (Nezu et al., 1999). Carnitine plays a major role in fatty acid metabolism, because intracellular esterification of long-chain fatty acids to the beta-hydroxyl carbon of carnitine is necessary for their entry into mitochondria. Phenotypic manifestations include hypoketotic hypoglycemia, Reye syndrome and sudden infant death, late-onset skeletal myopathy, cardiomyopathy, hepatomegaly and acute encephalopathy.
Methods: We herein report Case 1: 23-month-old Nepalese boy who presented with hepatomegaly and vomiting since age 5 months. Initially he was worked up in India with presumptive diagnosis of glycogen storage disease type IX (Fanconi-Bickel syndrome) and placed on lactose and sucrose free diet, cornstarch and frequent feedings. However, massive hepatomegaly developed within 18 months and parents brought him to Siriraj Hospital in Bangkok, Thailand for a second opinion. Liver biopsy revealed severe fatty liver. Case 2: 7-month-old Thai boy was referred to us due to hepatomegaly, intermittent hypoglycemia since age 5 months. Cardiomegaly (left ventricular hypertrophy), hepatosplenomegaly and seizure-like activity secondary to hypoglycemia were demonstrated.
Results: The acylcarnitine profile by tandem mass spectrometry demonstrated systemic carnitine deficiency; subsequently both were treated with L-carnitine with dramatic response.
Conclusions: Systemic carnitine deficiency,a rare disorder, was diagnosed in 2 infants (Nepalese and Thai) treated with L-carnitine with good response.

210-P

THE BIOCHEMICAL EFFICACY OF UNCOOKED 'SUPERSTARCH' IN LONG CHAIN FATTY ACID OXIDATION DISORDERS

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There is no published research on the efficacy of uncooked cornstarch (UCCS) in the management of long chain fatty acid oxidation disorders (LCFAOD) in preventing lipolysis and accumulation of toxic metabolites. A new 'slow release' waxy maize starch (Superstarch: Vitaflo) is available. The amylopectin matrix is modified by applying heat and moisture to control access of amylase.
Aim: To study the biochemical efficacy of 'Superstarch' by using loading (LT) and fasting tolerance tests (FTT)in children with LCFAOD.
Methods: 8 children (aged 3–9 y: median 5 years) with LCFAOD (LCHADD, n = 2; VLCADD, n = 2; CACTD, n = 2; and CPT11 deficiency, n = 1) were randomised to a Superstarch LT and FTT 3 months apart. For the LT, subjects were given 2 g/kg of Superstarch and 20 g glucose polymer at the study start. Blood samples were taken for free fatty acids (FFA), 3 hydroxybutyrate, lactate, glucose and creatine kinase hourly for 6 h. For the FTT, subjects were given 2 g/kg of glucose polymer at the start of the fast; had the same analytes measured but were studied for 5 h. Data was compared with retrospective UCCS (2g/kg) LT data for each child.
Results: FFA increased >400 micromol/L with: (1) Superstarch between 1–6 h (median 4 h); (2) 2 g/kg glucose between 4–5 h (median 4 h) and (3) UCCS between 4–6 h (median 5 h). Blood glucose remained >4 mmol/L for all subjects. All subjects (n = 6) whose FFA increased >800 micromol/L developed symptoms (lethargy, muscle ache, and headaches).
Conclusions: Superstarch did not increase fasting tolerance in children with LCFAOD.

211-P

CHANARIN-DORFMAN SYNDROME: A DISORDER WITH IMPAIRED FATTY ACID MOBILIZATION

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Background: Chanarin-Dorfman (CDS) syndrome is a rare disorder caused by mutations in the *ABHD5* gene that codes for the hydrolase AB-hydrolase domain containing protein 5, which facilitates lipolysis in cooperation with perilipin and other factors, including lipases. CDS usually presents as a nonbullous congenital ichthyosiform erythroderma, associated with variable liver enlargement, myopathy, developmental delay and decreased fasting tolerance. Here we present genetic and metabolic data of two patients with CDS.
Case reports, methods and results: Both patients had ichthyosis, liver enlargement and normal development. In the first patient we found a novel *ABHD5* mutation that affected mRNA splicing as shown by RT-PCR analysis. The second patient showed a 594insC mutation. To investigate the consequences of the absence of functional ABHD5 on fatty acid metabolism, a standardized 24-h fasting test was performed. In addition, mitochondrial fatty acid oxidation in fibroblasts was measured.

Patient 1 (10 years) had prolonged drowsiness at T = 24, despite normal glucose levels. Patient 2 (1 year) became hypoglycemic after15 h of fasting. Both patients had decreased FFA after fasting with low FFA/3-OH-B ratios reflecting abnormal fatty acid mobilization. Patient 1 had a SCAD polymorfism. Patient 2 had normal fatty acid oxidation in fibroblasts.
Conclusion: The results of the fasting test were consistent with the recent in vitro finding that ABHD5 is an activator of the adipose triacylgycerol lipase. Our data demonstrate for the first time that the *in vivo* consequences of *ABHD5* mutations are consistent with this function.

212-P

THE DEVELOPMENT OF A SCREENING PROTOCOL FOR CONFIRMATION OF CARBOHYDRATE METABOLIC DEFECTS IN SOUTH AFRICA

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Background: Carbohydrate metabolic defects are a large group of diseases which, amongst others, consist of monosaccharidosis, oligosaccharidosis and lysosomal storage diseases. The increasing evidence of high prevalence of these diseases in South Africa, for example galactocemia and glycogen storage disease has led to the implementation of a specific analytical protocol in our laboratory for screening of carbohydrate related defects.
Methods: Previously, the screening procedure for carbohydrate defects was done by thin layer chromatography and two dimensional electrophoresis. These methods are fast and qualitative, but absolute identification is problematic. We have developed a screening protocol which consist of the following additional analytical techniques: Pre-column Phenyl-methyl-pirazolone-derivatization and chromatography give an indication of a possible monosaccharidosis, oligosaccharidosis or mucopolisaccharidosis through identification with tandem mass spectrometry. An additional spectrophotometric method is implemented for the identification of fructose and related compounds. Furthermore a GC-MS analysis of urinary polyols (TMSI-derivatives) gives a clear indication of the secondary catabolism of elevated carbohydrates.
Results: To demonstrate the application of the suggested protocol, urine samples from a number of patients with carbohydrate metabolism defects were analysed accordingly and conclusive identifications were made. Amongst these were cases of galactosemia, glycogen storage disease and GM1 gangliosidoses.
Conclusion: This approach complements already routine screening procedures and may provide a more assured identification of carbohydrate metabolic diseases.

213-P

INSIGHTS INTO A LEUCINE RESTRICTED DIET IN THE MANAGEMENT OF THE HYPERINSULINISM/ HYPERAMMONAEMIA SYNDROME

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Objectives: *GLUD1* mutations impair glutamate dehydrogenase (GDH) sensitivity to its allosteric inhibitor GTP resulting in increased sensitivity to leucine its allosteric stimulator. GDH catalyzes oxidative deamination of glutamate to alpha-ketoglutarate plus ammonia and leads to β cell membrane depolarization and insulin release. Mutations cause hyperinsulinism and hyperammonaemia the HI/HA syndrome. Affected children present with seizures with or without hypoglycaemia. Most patients respond to diazoxide and a low protein or low leucine diet; however some cases continue to have unexplained non hypoglycaemic seizures and delayed developmental milestones. We compared the benefits of a low leucine diet to a low protein diet.
Methods: Case study of 7-month-old female with seizures and hypoglycaemia. Mutation analysis of *GLUD1* found de novo novel missense sequence D451V variant c.1523A>T (p.Asp451Val), a non-conservative amino acid substitution in a critical region. Despite treatment with diazoxide and a low protein diet, continued to have non-hypoglycaemic seizures. Brain imaging and electroencephalogram were normal. Plasma leucine, glucose, free fatty acids, ketones, insulin and ammonia were measured at 0, 1 and 2 h on a protein restricted diet (1.5 g/kg/day) then after 2 weeks on a leucine restricted diet (0.5 g/kg/day natural protein plus 1.0 g/kg/day of leucine-free amino acid mixture).
Results: No significant difference in ammonia levels. No hypoglycaemia detected but levels of free fatty acids and hydroxybutyrate increased at 2 h on leucine restricted diet.
Conclusion: A leucine restricted diet does not have additional benefits compared to a low protein diet but may in fact cause an accelerated fasting response.

214-P

CONTINUOUS GLUCOSE MONITORING IN HYPERINSULINISM–HYPERAMMONAEMIA SYNDROME

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We present a 5-month-old girl who was referred for investigation of intermittent seizures and hypoglycaemia. She was born at term (weight on 50th centile) and had presented to her local hospital at 5 weeks, 3 months and 4 months with seizures, on one of these occasions hypoglycaemia was documented. At 5 months she was a well-looking, active and alert baby (weight on 2nd centile). Clinical examination was unremarkable apart from a 1 cm liver edge. On admission her plasma glucose was 2.7 mmol/L and she was started on a dextrose infusion. Despite dextrose at 12.8 mg/kg/min and four hourly feeds, her plasma glucose dropped to 2.3 mmol/L. At this time she had a mild, but inappropriately, raised insulin (13 pmol/L) and persistent hyperammonaemia (162 micromol/L), although the plasma glutamine was within the normal range. In addition, subcutaneous continuous glucose monitoring, which measures glucose levels in interstitial fluid, failed to show variation in glucose concentration with feeds. In view of these findings glutamate dehydrogenase sensitivity was suspected and she was treated with diazoxide and hydrochlorothiazide, which not only restored normoglycaemia, but also the variability in glucose concentration. A mutation in the glutamate dehydrogenase gene has subsequently been identified, which has been previously described in hyperinsulinism-hyperammonaemia syndrome (OMIM: #606762).

This case highlights the use of the continuous glucose monitoring as an adjunct to random glucose measurements.

215-P

CONTINUOUS GLUCOSE MONITORING IN HYPOKETOTIC HYPOFATTY ACIDAEMIC HYPOINSULINAEMIC HYPOGLYCAEMIA WITH HEMIHYPERTROPHY

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We report a 2-year-old female who presented to her local hospital at 5 months with hypoglycaemic seizures. She was the first child of non-consanguineous parents (birth weight 3.1 kg).

On referral to the paediatric metabolic unit at 9 months for further investigation she was noted to have subtle facial hemihypertrophy which has worsened with time. Glucose monitoring using a continuous glucose monitoring system (CGMS) revealed a fasting tolerance of approximately 3 h, although she was asymptomatic consistent with impaired hypoglycaemia awareness. Investigations during episodes of hypoglycaemia showed suppressed free fatty acids, 3-hydroxybutyrate and branched chain amino acids suggestive of hyperinsulinism although the insulin, proinsulin, split proinsulin and C-peptide were appropriately low. ACTH, growth hormone, IGF1, ammonia, lactate, acylcarnitines, urine organic acids and synacthen test were normal.

She has since been maintained on 3 h feeds during the day and overnight gastrostomy feeds (0.4 g carbohydrate/kg/h), with no further episodes of seizures or hypoglycaemia. At two years of age her growth is on the 50th centile with normal development.

To our knowledge this is the second reported case of this syndrome. Hussain et al extensively investigated the original case, speculating that the cause might be up-regulation of the insulin/IGF1 receptor or defects in the signalling pathway. The pathophysiology remains unclear.

The CGMS system has been helpful in assessing fasting tolerance and optimising treatment, thereby reducing the frequency of venepuncture.

Hussain K et al. Hypoketotic hypofattyacidaemic hypoinsulinaemic hypoglycaemia in a child with hemihypertrophy? A new syndrome. Horm Res. 2004;61:222–7.

216-P

EVIDENCE OF CATAPLEROSIS IN A PATIENT WITH CLASSICAL GALACTOSEMIA

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Background: Physiopathology of classical galactosemia remains controversial; if some features are clearly linked to a toxic effect of galactose metabolites accumulation, the explanations for the neurologic long-term outcome are still not clear. **Methods and results:** We report the case of a 7-week-old girl who presented with liver failure, hypoprotidemia, ascites and generalized edemas. The biologic screening shows typical pattern of type II citrullinemia neonatal form (slight hyperammoniemia, high citrulline: 275 µmol/L, high lysine: 577 µmol/L and low glutamine: 214 µmol/L and hyperaminoaciduria) with disappearance of citric cycle intermediates (absence of oxoglutarate, succinate and citrate). Citrate treatment alone induced a clear improvement in liver and biological status. There was a partial improvement in ASAT, ALAT and protidemia levels while glutamine, citrulline and lysine normalized. 4 weeks later a galactose free diet (recommended in citrin deficiency with galactosemia) was prescribed and induced a complete normalization of the clinical and biological status. Citrin deficiency was ruled out by molecular biology, and galactose was reintroduced inducing vomiting, galactose aversion and hepatic cytolysis. The diagnosis of classical galactosemia was then established (low GALT activity and *GALT* mutations Q188R and R333W). **Conclusions**: Our case clearly shows that cataplerosis could play a role in the pathophysiology of neonatal symptomatology of classical galactosemia. Anaplerotic treatment by citrate induced a clear (but incomplete) improvement in clinical and biological status of our patient. Further studies are needed to explore the responsibility of chronic cataplerosis in the neurologic long-term outcome of GALT deficient patients.

217-P

HYPERTHYROIDISM CAUSING METABOLIC DETERIORATION IN GALACTOSEMIA

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Background: Hyperthyroidism can affect carbohydrate metabolism. We describe a case of Grave's disease causing metabolic deterioration in a patient with galactosemia.
Case report: A 21-year-old woman with classical galactosemia and who was compliant with lifelong dietary galactose restriction developed hyperthyroidism due to Grave's disease. In the 4 years prior to the development of HT, her mean (SD) galactose-1-phosphate (Gal-1-P) level was 0.468 (0.21) µmol/g Hb. When she developed hyperthyroidism, her mean Gal-1-P level increased to 1.07 (0.36) µmol/g Hb (p=0.02). After treatment of hyperthyroidism with radioactive iodine therapy, her Gal-1-P levels declined towards pre-GD values. Her dietary galactose intake did not change during this interval.
Discussion: Hyperthyroidism can affect galactose metabolism in several ways: (a) increased intestinal absorption of dietary galactose. (b) reduced activity of aldose reductase leading to reduced metabolism of galactose to galactitol. (c) altered affinity of cell surface receptors for galactose uptake.
Conclusion: Thyroid dysfunction should be considered in the differential diagnosis of metabolic deterioration in galactosemia.

218-P

CLASSICAL GALACTOSAEMIA AND DECREASED BONE MASS; CLINICAL RECOMMENDATIONS

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Background: Decreased bone mass in early childhood is an increasingly recognized problem in classical galactosaemia like in many other chronic diseases. Peak bone mass (PBM) is reached in late adolescence, thus, increasing PBM in childhood can prevent osteoporosis. Regular bone mass measurements and prevention treatment should begin already in childhood.
Objective: In the absence of evidence based guidelines, we provide a follow-up proposal to identify/treat decreased PBM based on our experience and available literature.
Guidelines: Bone mass assessment: DXA can be used. Because cooperation is required, measurements can usually be performed from age of four years. Interpretation of bone mass measurements is crucial for the diagnosis of osteopenia/osteoporosis. In children and adolescents, total body and lumbar spine bone mineral content (BMC) as well as lean tissue mass (LTM) should be measured. Comparing BMC corrected for LTM of the patient with the BMC corrected for LTM of healthy controls allows correcting for the confounding effect of bone size. In adults, lumbar spine T-scores should be used. Repeat DXA every two years in case of normal BMC, as this is the time window in which abnormalities become measurable. If BMC is between 0 and -1 SD, lifestyle factors like physical activity, calcium and vitamins K and D intake and oestrogen supplementations (in girls) should be optimized. If BMC <-1 SD we recommend to treat. Calcium, vitamins K1 and D3 supplementation or biphosphonates can be considered. Repeat DXA yearly in case of BMC <0 SD in order to notice deteriorations/improvements early.

219-P

DETERMINATION OF THE LACTOSE AND GALACTOSE CONTENT OF CHEESE FOR THE GALCTOSAEMIA DIET

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Some types of mature hard cheese contain minimum lactose and galactose and may be suitable in a galactosaemia diet. Lactose is reduced by 2 cheese manufacturing processes: (1) the separation and removal of whey; and (2) fermentation of lactose by bacteria.
Aim: To determine the lactose and galactose content of mature cheeses.
Methods: Over a 7-year period, the UK Galactosaemia Support Group Medical Advisory Panel commissioned the analysis of 99 samples of 12 different cheese types by 2 laboratories. The cheeses were purchased from retail outlets, were homogenised, and sugars extracted using water or 40% alcohol for fatty samples. The protein was removed using Carrez reagents and the filtered extract was diluted with water for enzymatic analysis using the the Boehringer Mannheim Test-Combination kit for enzymatic analysis of lactose/D-galactose. In total there were 7 different analyses performed at different times of the year.
Results: Cheeses containing undetectable quantities of lactose (<2.8 mg/100 g) and galactose were: Gruyere (5 samples); Emmental (block, sliced and grated) (16 samples); Jarlsberg (6 samples); Parmigiano Reggiano and Grana Padano Italian Parmesan (block and grated) (15 samples); and mature Cheddar cheese from the UK West Country Farmhouse Cheese Makers Association (32 samples) only. Lactose containing cheeses included: other types of mature Cheddar cheese, Gouda, Edam and soft cheeses like Brie.
Conclusions: Gruyere, Emmental, Jarlsberg, Italian Parmesan (Parmigiano Reggiano and Grana Padano), and mature Cheddar cheese produced in one area of England where the manufacturing process is standardized and guaranteed are allowed in the UK galactoseamia diet.

220-P

SIGNIFICANCE OF GALACTONATE EXCRETION IN CLASSICAL GALACTOSAEMIA (GALT DEFICIENCY)

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Background: The physiological significance of galactonate formation for galactose homeostasis in classical galactosaemia is unclear. We studied galactonate excretion in patients and comparatively in healthy subjects.
Methods: Healthy subjects aged 3–71 years (n = 261). Patients aged 2–39 years (n = 61). Classical galactosaemia was verified by full genotypic, metabolic and enzymatic characterization. Galactose metabolite concentrations were assayed in postabsorptive urine specimens by SID-GC-MS.
Results: Galactonate excretion was age dependent and the data fitted well to the growth related model y=exp[a+b*min(t,T)] (the model assumed exponential decline of excretion until adulthood (T) with a,b, and T being constants and t being age). In the patients, galactonate excretion decreased from about 12 µmol/(kg*d) in siblings to 5 µmol/(kg*d) in adults. In healthy subjects, the corresponding rates were significantly lower and amounted to 3 and 0.5 µmol/(kg*d), respectively. The relation of galactose metabolite excretion was rather constant with age. In galactosaemia, the relation of urinary excretion of galactose:galactonate:galactitol was 1:8:35. In controls, the relation was 1:5:2. Thus, the by far most abundant urinary galactose metabolite in the patients was galactitol, whereas galactonate was the leading urinary galactose metabolite in healthy subjects.
Conclusion: Galactonate formation and urinary excretion appears to be of only minor significance for whole body galactose removal in classical galactosaemia.

221-P

MONITORING OF URINARY GALACTITOL IN CLASSICAL GALACTOSAEMIA (GALT-DEFICIENCY)

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Background: In galactosaemia, galactose-1-phosphate in RBC is applied for metabolic monitoring. Whether urinary galactitol might provide an alternative was assessed by establishing putative nomograms for postabsorptive treated galactosemics and testing their applicability by comparison with random sampling.
Methods: Nomograms were based on short-term sampling of multiple urine samples (n = 14) from fully characterized female/male patients (n = 21/19, 3–38 years) with excellent compliance (gal intake <1 mg/kg/d) and analyses of galactitol by SID-GC-MS. The data were fitted to a growth rate related model comprising (1) exponential decrease during infancy, (2) progressive diminuation of decline during adolescence leading to (3) stable excretion in adulthood. Random urine samples from females (n = 204) and males (n = 223) were received for metabolic monitoring (generally in parallel with EDTA-blood).
Results: When related to percentiles 1, 5, 10, 50, 90, 95 and 99 of the nomograms, the percent of random samples below these percentiles were in females/males 5/5, 14/17, 19/22, 56/58, 82/87, 88/92, and 93/96, respectively. Thus, random samples in general fitted well to the nomograms. The obvious excess below percentile 1 and above percentile 99 might be due to the unnoticed presence of variants and poor dietary compliance, respectively. Interestingly, gal-1-p in RBC levels showed a quite different age dependence and were but poorly correlated to urinary galactitol (linear regression: female R2 = 0.10, n = 179; male R2 = 0.15, n = 196).
Conclusion: Urinary galactitol appears to be rather a complementary than an alternative parameter to gal-1-p in RBC for metabolic monitoring in classical galactosaemia.

222-P

GALACTOSEMIA: WHAT HAPPENS WITH VERBAL DYSPRAXIA AFTER CHILDHOOD

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Verbal dyspraxia is a frequent symptom in children with classic galactosemia. However, there are only limited information available about the course of verbal dyspraxia in this context.
Objective: To evaluate the outcome of patients with GALT-deficiency with respect to verbal dyspraxia.
Methods: Patients with confirmed diagnosis of classic galactosemia were evaluated using the 'Hierarchische Wortlisten', a standardized instrument for diagnosis and follow-up of verbal dyspraxia in German speaking patients. The test yields results in three categories, phonetic and phonematic items and word flow, reflecting the different types of speech disturbances in verbal dyspraxia. The maximal score in each subscale of the 'Hierarchische Wortlisten' is 96.
Results: 32 patients (13 females, 19 males) were evaluated with a mean age of 21.2+7.2 years. All patients had German as their primary language, and none had a diagnosis of hearing impairment. In this cohort, the mean score for phonetic items was 94.3 (range 84–96). Similar values were achieved for word flow (mean score 93.8, range 81–96), whereas the mean score for phonematic items was significantly lower compared to the others (mean 88.7, range 74–96; $p<0.001$). Patients had higher scores for regular words compared to pseudo-words in all three categories ($p<0.001$).
Discussion: Patients with classic galactosemia in this cohort only show distinctive features of verbal dyspraxia. Interestingly 40.6% of these patients had been diagnosed as having a speech disturbance more than 14 years ago. No differences were found for all scores between these patients and those without previous diagnosis of speech disturbance.

223-P

GALACTOSEMIA: RESULTS OF 5 YEARS STUDY ON IRANIAN PATIENTS

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Background: Classical galactosemia is an inborn metabolic disorder caused by autosomal recessive mutations in galactose-1-phosphate uridyl transferase (*GALT*) gene. The incidence of the disease varies from 1:40 000–1:60 000 among Caucasians population. To date, over 170 different mutations have been reported including point mutations, microdelitions and insertions. The most frequent reported mutation is Q188R which accounts for approximately 60% of mutant alleles in Caucasian population. In the present article results of 5 years study on galactosemia in Iran including biochemical diagnosis and molecular analysis of *GALT* gene in the Iranian galactosemia is presented. **Methods**: Twenty galactosemia patients were subjected to diagnosis of galactosemia by the determination of GALT activity in RBCs using Beutler test. DNA samples were investigated for the 5 most reported mutations including Q188R, K285N, X380R, L195P and Q169K using PCR-RFLP method. PCR-SSCP method was used for whole *GALT* gene including 11 exons and flanking intronic sequences to investigate the mutations which were not detected by PCR-RFLP method. In a retrospective study, 20 galactosemic patients, were traced and their long term outcome were evaluated. **Results**: Q188R mutation was the most observed mutation with the allelic frequency of 57.1%. The allelic frequencies for S135L, Y209S, A320T, and K285N were found to be 7.1%, 7.1%, 7.1%, and 3.57% respectively. **Conclusions**: Our results show that galactosemia is a heterogeneous disorder at the molecular level among the Iranian population. Study on long term outcome of the disease emphasis on the need of a new look and new challenges for galactosemia in Iran.

224-P

DELAYED DIAGNOSIS OF ATYPICAL MILD GLUT 1 DEFICIENCY

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Background: Dominant *SLC2A1* mutations of the glucose transporter type 1 (GLUT1) cause epileptic encephalopathy, developmental delay, acquired microcephaly, and complex movement disorder. Main diagnostic criterion is the reduced CSF/plasma glucose ratio. Treatment with ketogenic diet is effective by providing an alternative source for brain energy metabolism. **Case study:** A 10-year-old girl suffered from atypical absences and myoclonic jerks since early infancy, particularly at awakening and before meals. Longer periods of +disconnection; or sudden need to sleep were also reported. Head circumference, neurological examination, and development were normal but increasing learning difficulties were documented. She improved under combined ethosuximide and clobazam therapy but did not respond to valproate. **Results**: EEG: generalized epileptic activity, predominantly bifrontal, with slow background activity; 24 h-EEG: striking decrease of epileptic discharges and normalization of background activity after meals. CSF/plasma glucose ratio: 0.42 (normal >0.46). A known *SLC2A1* mutation confirmed the diagnosis. Under ketogenic diet with 3:1 ratio, the child became seizure-free and showed clear improvements in alertness, attention and concentration skills. After 4 months the EEG normalized and significant cognitive improvements in executive functions, verbal and graphic performances, fine motor skills, and memory were documented. **Conclusions**: This case demonstrates that mild phenotypes of GLUT1 deficiency exist but are difficult to diagnose; microcephaly and developmental delay are not obligatory features. Myoclonic and atypical absence epilepsy are a possible presentation of GLUT 1 deficiency. CSF/plasma glucose ratio should be evaluated in such patients and even slight reductions may already be suggestive of GLUT1 deficiency.

225-P

MILD PSYCHOMOTOR RETARDATION WITH DISCRETE ATAXIA AND ABNORMAL RETINAL FUNCTION IN GLUT1 DEFICIENCY SYNDROME

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Glucose transporter type 1 deficiency syndrome (Glut1-DS) is a severe disease characterized by epileptic encephalopathy starting in infancy. Glucose transport across the blood-brain barrier (BBB) is impaired as reflected by hypoglycorrachia in patients. Ketogenic diet is effective, as ketone bodies, transported across the BBB through the monocarboxylate transporter type 1 (MCT1), serve as alternative fuel for the brain.

We report a 5-year-old female child presenting with a Glut1-DS mild phenotype. Motor retardation was noted at the age of 10 months, she walked unaided at the age of 20 months and experienced two short atonic episodes between 18 months and 2 years of age. Electroencephalogram and cerebral RMN were normal. At the age of 30 months, global developmental delay and fluctuating ataxia were observed. At the age of 3 years, she presented two generalized tonico-clonic seizures successfully treated by sodium valproate. Dyschromatopsia and bad night vision were detected and electroretinogramm showed abnormal function of the cones. Hypoglychorrachia (1.2 mmol/L) was diagnosed at the age of 4 years and 6 months. Molecular analysis of the corresponding gene, *SCL2A1*, revealed the presence of a de novo heterozygous nonsense mutation (S113X), potentially resulting into a truncated protein. A ketogenic diet was then introduced and allowed significant improvement of the psychomotor skills.

The observation of retinal anomalies in this patient harbouring a *SCL2A1* mutation is in agreement with the role of Glut1 at the inner blood-retina barrier. This case report shows that Glut1 deficiency can result in retinal dysfunction.

226-P

ENDOGENOUS GLUCOSE PRODUCTION AND GLUCOSE UPTAKE AFTER OVERNIGHT FASTING: AN AGE-DEPENDENT REGRESSION MODEL

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Objective: To obtain knowledge about the exact glucose requirement in children after an overnight fast, data on endogenous glucose production (EGP) in children were pooled with adult data on EGP and used to construct an age-dependent regression model for EGP and glucose uptake. This model could be used to estimate minimal glucose requirement in children. **Methods**: Data on EGP in 16 healthy children, aged 2.5 to 17 years, were combined with data in healthy adults from our research group ($n = 37$). EGP in all subjects was quantified using the [6,6-2H2]glucose dilution method after an overnight fast. Since EGP was quantified during isotopic steady-state glucose uptake equalled EGP. Pooled data were analysed using a one-phase exponential decay curve fitting model. To validate our regression model data from literature on EGP in children and adults were analysed separately ($n = 24$). **Results**: Regression analysis of the pooled data yielded the following equation:

EGP (micromol/kg/min) = 36.11 / exp(–0.1373/age(y)) + 10.05 (R2 0.92, Sy.x 2.49)

Regression analysis of the reference data yielded the following equation:

EGP (micromol/kg/min) = 29.31 / exp(–0.07440/age(y)) + 9.00 (R2 0.80, Sy.x 4.96)

The 95% confidence intervals of the variables in both regression equations showed significant overlap. **Conclusions**: The constructed regression model accurately estimates EGP and thus glucose uptake after overnight fasting and can be used to estimate minimal glucose requirement in healthy subjects. These data are of value for the treatment of patients with inborn errors of metabolism at risk for the development of hypoglycemia.

227-P

GLUCONEOGENESIS AND GLYCOGENOLYSIS IN GLYCOGEN STORAGE DISEASES DURING FASTING

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Objective: Although endogenous glucose production (EGP) during fasting has been quantified previously in patients with different glycogen storage diseases (GSDs), the differential contribution of glycogenolysis (GGL) and gluconeogenesis (GNG) to EGP has never been established. Here we describe EGP, GGL and GNG in three patients with different GSDs.
Methods: EGP was quantified using the [6,6-2H2]glucose dilution method; fractional GNG was quantified using the deuterated water method. Patient 1 (male, 17.9 years) was glucose-6-phosphatase deficient (GSD-Ia), patient 2 (female, 15.6 years) was amylo-1,6-glucosidase deficient (GSD-III) and patient 3 (male, 3.7 years) was phosphorylase kinase deficient (GSD-IX). Fasting duration was set individually based on previously estimated fasting tolerance.
Results: Patient 1: after 2.6 h of fasting, glucose: 1.1 mmol/l; EGP: 3.9 micromol/kg/min; GGL 3.2 micromol/kg/min and GNG 0.7 micromol/kg/min. Patient 2: after 15.8 h of fasting, glucose: 3.5 mmol/l; EGP: 8.2 micromol/kg/min; GGL 2.2 micromol/kg/min and GNG 6.0 micromol/kg/min. Patient 3: after 21.9 h of fasting, glucose 3.4 mmol/l; EGP: 14.0 micromol/kg/min; GGL 4.0 micromol/kg/min and GNG 10.0 micromol/kg/min. When compared to healthy individuals of the same age EGP was decreased in all: patient 1: 29.7%, patient 2: 57.4%, patient 3: 44.1% of control values.
Conclusions: These data show decreased EGP in three different GSDs during fasting. We show for the first time that GNG can contribute up to 20% to EGP in GSD-Ia. GGL contributed substantially to EGP in all three GSD patients. These studies provide important new information on the pathophysiology of GSDs.

228-O

MUSCLE GLYCOGEN STORAGE DISEASE 0 – CARDIOMYOPATHY AND EXERCISE INTOLERANCE

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Storage of glycogen is essential for glucose homeostasis and for energy supply during bursts of activity and sustained muscle work. We have identified three siblings, children of consanguineous parents, with profound heart and skeletal muscle glycogen deficiency and a homozygous nonsense mutation (Arg462→ter) in the muscle glycogen synthase gene, *GYS1*.

The oldest brother, who also had mild epilepsy, suffered sudden cardiac death at the age of 10.5 years. An 11-year-old brother showed muscle fatigability, hypertrophic cardiomyopathy and abnormal heart rate and blood pressure response on exercise. Glucose tolerance was normal. A two-year-old sister was without symptoms, but showed subtle signs of cardiomyopathy and impaired function at rest.

Histochemical analyses of heart and liver tissue obtained post mortem from the deceased child revealed absence of glycogen in the heart but not in the liver. Morphological analyses of open muscle biopsy samples from the two younger siblings showed identical results i.e. a marked predominance of oxidative muscle fibers, accumulation of partially abnormal mitochondria and absence of glycogen. Western blot analyses showed no detectable levels of glycogen synthase in the patients. RFLP-analysis of *GYS1* exon 11 identified the parents, both grandmothers and 3/21 additional relatives as carriers. Furthermore, 1/100 in an ethnically matched control group was carrier of the mutated allele.

We conclude that the first identified cases of muscle glycogen storage disease 0 (MGSD0) with muscle glycogen synthase deficiency are caused by the identified homozygous nonsense mutation in *GYS1*, and that there is no muscle glycogen synthesis without this enzyme.

229-P

ELEVATED SERUM BIOTINIDASE ACTIVITY IN HEPATIC GLYCOGEN STORAGE DISORDERS – A CONVENIENT BIOMARKER

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Background: Glycogen storage disorders (GSD) are a group of inborn errors resulting in altered glycogen metabolism. Previous reports showed that biotinidase activity in serum of patients with GSD Ia was markedly elevated. Our aim was to evaluate biotinidase activites in additional GSD types.
Methods: Biotinidase activity was determined by a colorimetric assay using biotinyl-p-aminobenzoate.
Results: Serum biotinidase activity was measured in 54 GSD patients. The mean and range of biotinidase activity from healthy individuals was 8.7 mU/ml; 7.0–10.6; $n = 26$. We found increased biotinidase activites in following GSD types: GSD Ia (17.4; 11.4–23.2; $n = 18$), GSD Ib (21.3; 14.6–26.0; $n = 4$), GSD III (13.3; 8.0–19.1; $n = 11$), GSD VI (14.1; 17.7; $n = 2$) and GSD IX (14.9; 7.5–21.6; $n = 19$). The sensitivity was 100% for patients with GSD Ia, 73% for GSD III and 79% for GSD IX. Even though the sensitivity was 100% for GSD Ib and GSD VI, more sera of these patients need to be analyzed. In addition, we found elevated biotinidase activity in patients with Fanconi-Bickel Syndrome, a disorder that is characterized by the failure to release glucose from liver due to deficient GLUT2 transporter (mean:15.3 mU/ml; range: 11.0–19.4; $n = 5$; sensitivity = 100%).
Conclusions: Based on the sensitivity and reproducibility, serum biotinidase is a useful diagnostic biomarker for hepatic glycogen storage disorders.

230-A

SPONTANEOUS PREGNANCY IN A WOMAN WITH GLYCOGEN STORAGE DISEASE TYPE IA (GSDIa) AND LONG-TERM COMPLICATIONS OF THE DISEASE

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Introduction: GSDI in adults is presented with long-term severe complications. Increased risk of subfertility is reported and pregnancy is rare. Mother's metabolic abnormalities including glucose imbalance worse fetal outcome. **Case report**: We report a pregnancy in a woman with GSDIa complicated by severe growth retardation, remarkable hyperlipidaemia and hyperuricemia, liver adenomas (22 years), and renal hyperfiltration (24 years). The girl has been treated since the age of 3 years with frequent meals, additional oral glucose intake and cornstarch. The compliance was always poor. 3-month pregnancy was uncovered at age of 25 years at our metabolic paediatric ward, on occasion of her abdominal complaint. Started 6 months earlier ACE-I therapy was stopped, and careful obstetric care assured. Cornstarch intake was intensified. Serum lactate, urate and triglycerides concentrations improved. Renal dysfunction, hypertension and liver adenomas were stable. Fetal ultrasonography was normal. At 29 weeks steroid prophylaxis was administered. At 31 weeks the patient was admitted to maternity clinic for her closer supervision and fetus monitoring. The woman gave birth to healthy male infant (2440 g, Apgar 10) by planned caesarean section at the 36th week. Constant 20% glucose was given during the delivery. The postpartum period was uneventful. The ACE-I was re-introduced after lactation. Further compliance of this GSD-affected mother is insufficient as before. Her healthy son has developed normally with slight hyperactivity. He attended primary school on time at the age of 6 years. **Conclusion**: Possibility of successful pregnancy in GSDIa in spite of bad compliance is confirmed by our observation.

231-P

OSTEOPOROSIS IN GLYCOGEN STORAGE DISEASE TYPE 1 PATIENTS

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Background: Osteoporosis is frequently observed in patients with Glycogen Storage Disease type 1 (GSD 1). Abnormal metabolic and endocrine environment have been proposed to explain the presence of osteoporosis in GSD patients.
Materials and methods: 19 patients were enrolled in the study. Bone involvement investigation included: (1) biochemical parameters: serum levels of calcium, phosphorus, alkaline phosphatase, parathormone, calcitonin, calcium excretion. (2) Bone mineral density (BMD) with dual energy X-ray absorptiometry (DEXA), and by quantitative ultrasound of proximal phalanges (QUS).
Results: Hypercalciuria was observed in 3/12 GSD1a patients. The high calcitonin levels were observed in 1/12 GSD1a and 3/7 GSD1b patients. The Z-score of DEXA was below –2.5 DS in 3/7 GSD1b patients and in 2 other GSD1b patients the Z-score of QUS was below –2.5 DS.

In the patients with abnormal DEXA and QUS, hydroxyproline excretion was increased. Hydroxyproline excretion was increased also in 3/12 GSD1a patients that showed normal results at DEXA and QUS.
Conclusions: For a diagnosis of osteoporosis it is important to perform biochemical and instrumental investigations. The Consensus Conference of 1993 established that the technique to evaluate the bone mass is DEXA, nevertheless the QUS may also represent an useful tool to assess bone mineral status. The high prevalence of osteopenia in GSD1b patients suggests that malabsorption, due to Crohn-like colitis, may be causative of reduced bone mineralization. The detection of an increasead hydroxyproline excretion in patients without abnormalities of both DEXA and QUS may suggest that this parameter represents the first sign of bone involvement.

232-P

POSITIVE EFFECT OF GROWTH HORMONE (GH) TREATMENT IN A PATIENT WITH GLYCOGEN STORAGE DISEASE TYPE Ia (GSD Ia)

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Background: Growth failure is a common feature in GSD Ia. Dietary treatment has been shown to partially correct biochemical and hormonal abnormalities and to improve growth. However, a few patients do not respond to diet for reasons not fully understood. Historically, individuals with the highest endogenous GH production showed the poorest growth and, according to few reports on GSD Ia patients, the use of GH has shown little success.
Methods: Case report on a 13-year-old female GSD Ia patient with growth failure despite good compliance to dietary treatment. SDS for height was – 6.7 while BMI followed the 50th percentile. Bone age was 2.5 years delayed. Growth velocity was 3 cm/year and was markedly reduced. Meticulous assessment excluded additional systemic diseases, and genetic or psychosocial factors that may be related to short stature. Laboratory examinations showed normal plasma concentrations of IGF1 and IGFBP3 and a GH increase during exercise (0.35 → 5.8 5 g/L) and after arginine stimulation (2.7 → 10.8 5 g/L). On GH (0.33 mg/kg/wk), we observed an increase of the patient's height of 6 cm within the first 6 months of therapy, and an average growth velocity of 8 cm/year during the next two years. No complications were observed.
Conclusion: The dramatic growth improvement demonstrated in this case suggests that GH may be an effective treatment for some GSD Ia patients with short stature. The reason for this improvement is unclear. Since GH- and IGF1-deficiency have been excluded, hormonal resistance or other unknown effects have to be considered.

233-P

EFFECTS OF SUPPLEMENTATION WITH VITAMIN E ON NEUTROPENIA IN PATIENTS WITH GLYCOGEN STORAGE DISEASE TYPE IB

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Background: Glycogen storage disease type 1 (GSD1) is an autosomal recessive inborn error of glycogen metabolism. The clinical picture is characterized by short stature, hepatomegaly, lactic acidemia, hypoglycemia, hyperuricemia and hyperlipidemia. In addition to the classical clinical manifestation of GSD1, GSD-1b patients suffer from chronic neutropenia and functional deficiencies of neutrophils and monocytes, resulting in recurrent bacterial infections. Granulocyte-Colony Stimulating Factor (G-CSF) is used to improve neutrophil count and function and to decrease the frequence of bacterial infections.

Several apoptotic features have been demonstrated in GSD-1b neutrophils; anti-oxidants seem to revert neutrophil apoptosis. Vitamin E, an anti-oxidant, modulates the function of neutrophils.

The objective of this study has been to estimate the effect of vitamin E therapy on neutropenia and neutrophil function in GSD-1b patients.
Methods: The study has been carried out over a period of 24 months. Five patients were enrolled (3 patients referred to the Unit of Metabolic Diseases, Department of Pediatrics, University 'Federico II', Naples and 2 to the Department of Pediatrics, Ospedale San Gerardo, Monza). Vitamin E has been administered for 12 months with monitoring of neutrophil count and of the type and the number of infections and hospitalizations.
Results: The administration of vitamin E has not significantly modified hematological parameters. However during treatment with vitamin E, the severity and number of infections (26 vs 11 $p<0.05$) and hospitalizations (5 vs 0 $p<0.05$) decreased.
Conclusion: These results suggest that vitamin E may represent an additional tool for the treatment of the functional deficiency in GSD-1b patients.

234-P

GLYCOGEN STORAGE DISEASE TYPE III (GSDIII): GENOTYPE PHENOTYPE CORRELATIONS IN THE IRISH POPULATION

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Background: GSDIII results from mutations of the *AGL* gene encoding the glycogen debrancher enzyme. The disease has clinical and biochemical heterogeneity reflecting the severity of the *AGL* mutations.
Objectives: To describe the genotype-phenotype correlations in our cohort of Irish patients with GSDIII.
Results: We identified seven families (14 patients, age 2-25 years). Five patients had mild disease limited to hypoglycaemia and hepatomegaly with CK levels in the normal range (GSD IIIb). Nine patients had more severe disease with liver, skeletal and cardiac muscle involvement with elevated CK (GSD IIIa). We identified seven null mutations in this cohort including four new mutations: c.276delG, c.3682C>T (p.R1228X), c.3980G>A (p.W1327X) and the novel mutations c.1557T>A (p.Y519X), c.2207delC, 4197delA and 4221delA. c.17delAG was found in two GSDIIIb families. The family homozygous for c.4197delA had the most severe phenotype (early hypoglycaemia in infancy, massive hepatomegaly, myopathy and hypertrophic cardiomyopathy before age two), not halted by aggressive carbohydrate and protein supplementation (median CK levels 2000–3000 U/L). Two other families with GSDIIIa (homozygous for null alleles) had less fulminant, yet still severe disease.
Conclusion: The phenotypic diversity in our GSDIII cohort is explained by allelic heterogeneity. We describe four novel null mutations, including two in exon 32 in two families with severe GSDIIIa resistant to current treatment modalities. Knowledge of the specific mutations segregating in this cohort may allow for the development of new therapeutic interventions.

235-P

INACCURATE DIAGNOSIS OF GLYCOGEN STORAGE DISEASE (GSD) TYPE VI (GLYCOGEN PHOSPHORYLASE DEFICIENCY) BY ENZYME STUDIES: CORRECTION TO GSD TYPE IX (PHOSPHORYLASE KINASE DEFICIENCY) BY MOLECULAR ANALYSIS

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Discrimination of GSD VI and IX is clinically difficult and the interpretation of enzyme assays can be problematic. We investigated 13 boys and 1 girl from two centres diagnosed with GSD VI by blood cell or liver enzymology and clinical symptoms (hepatopathy and hypoglycaemia). All exons of the PYGL (GSD VI) gene were amplified from genomic DNA and directly sequenced. Where appropriate, all exons of the *PHKA2* and *PHKB* (GSD IX) were also analysed. Erythrocyte phosphorylase kinase activity was normal in all patients. Leukocyte or liver activated glycogen phosphorylase was either significantly reduced or borderline in all patients whilst levels of total glycogen phosphorylase varied from undetectable to within the normal range. In three (21%) patients (one female) compound heterozygous mutations of the *PYGL* gene were identified confirming diagnosis of GSD type VI. In the remaining 11 (79%) patients analysis of the *PYGL* found no evidence for causative mutations. Analysis of the *PHKA2* gene has thus far been performed in five patients identifying hemizygous mutations in four: c.883C>T, p.R295C, c.884G>A, p.R295H, c.3479dupG, p.I1161fs and c.133C>T, p.R45W. Analysis of *PHKB* revealed compound heterozygous c.1207+1G>T splice site and c.1969C>T, p.Q657X nonsense mutations in the fifth patient. In **conclusion**, accurate diagnosis of autosomal GSD VI versus X-linked or autosomal GSD IX is necessary to allow appropriate care and genetic counselling. Molecular confirmation of clinically or enzymatically diagnosed GSD VI is essential. Furthermore, the prevalence of normal erythrocyte phosphorylase kinase activity in both X-linked and autosomal GSD IX also makes molecular analysis indispensable.

236-P
SEVERE PICTURE IN DUTCH PATIENTS WITH GLYCOGEN STORAGE DISEASE TYPE IX?

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Background: Characteristic features of glycogen storage disease type IX, a defect of phosphorylase b kinase, are a specific growth pattern and hepatopathy. Typically, symptoms tend to resolve with age. We studied clinical and biochemical aspects and dietary advises in 10 Dutch patients.
Results: We studied 10 patients (8 boys and 2 girls) with a median age at diagnosis of 2 years (0.8–5.8). Median follow-up period was 1.3 years (0.8–4.8). At the time of diagnosis, all patients had hepatomegaly and feeding problems, 4/10 had short stature, 5/10 suffered from diarrhoea. Two of them had hypoglycaemias. All patients had a X-linked inheritance pattern. Median enzyme activity was 1.3 nmol/min^2/mgHb (0–4.4) (controls 6–19). Transaminases were elevated in all patients (2–40 times). Cholesterol, lactic acid and triglyceride concentrations were elevated in 4, 8 and 8 patients respectively. Tetraglucoside excretion was increased in 8 patients. Dietary management consisted of raw cornstarch suppletion 1 g/kg in 9/10 patients. Fructose and lactose intake were restricted in 5 and 7 patients respectively, resulting in a normalisation of stools in all patients. Nine patients received a late evening feeding, two nocturnal tube feeding.
Conclusions: We detected serious clinical and biochemical symptoms in our patient group, including two females, necessitating dietary therapy and even nocturnal tube feeding in order to prevent hypoglycaemias and chronic liver dysfunction. Half of the children suffered from diarrea treated with lactose restriction. The observed phenotype in our patients appeared to be more severe than described in literature previously

237-P
THYROID AND GLYCOGENOSIS

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Background: Recent studies have demonstrated an increased prevalence of thyroid autoimmune disorders and hypothyroidism in patients affected by glycogen storage disease (GSD).
Aim of the study was to evaluate the prevalence of thyroid-related diseases in patients affected by GSD and followed in our Centre.
Methods: Fifteen patients affected by GSD type I (7 GSD-IA, 8 GSD-IB) and 5 patients affected by GSD type III (aged 6–27 years) were investigated. Patients were aged between 5.2 and 27.5 years (mean 18.3 years, median 19.9 years, 12 M and 8 F). Growth (weight and length) parameters were within norm in all patients. Thyroid function was investigated by the serial determination of T3, T4 and TSH with electro-immune-chemiluminescence methods and FT3, FT4 and TSH with RIA methods. In patients with increased TSH plasma levels the anti-thyroid autoimmune profile was also investigated (thyroid peroxidase and thyroglobulin autoantibodies with chemiluminescence methods). In case of thyroiditis, a thyroid ultrasound scan was performed.
Results. Subclinical hypothyroidism (with negative autoantibodies) was found in 2 patients (one affected by GSD-IA and one by GSD-IB, respectively). Other 2 out of 5 GSD III patients showed thyroiditis. One patient required an hormonal substitutive therapy with Levo-thyroxine. Thyroid ultrasound didn't show pathologic remarks in either subject with thyroiditis. Growth (weight and length) parameters were normal in all patients.
Conclusions. Our study confirms an increased prevalence of thyroid autoimmunity and hypothyroidism in patients affected by GSD. The hypothesis of a damage of the hypothalamo–hypophisis–thyroid axis needs further studies.

238-P
GLYCOGEN STORAGE DISORDERS: CLINICAL, BIOCHEMICAL AND DIETARY MODIFICATION THERAPY IN INDIA

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Introduction: Glycogen storage disorders (GSD) are a group of inherited disorders of glycogen metabolism characterized by the presence of abnormal glycogen, qualitative or quantitative. There are many types of GSD and correct diagnosis is essential for appropriate diagnosis, treatment and counseling of families.
Materials and methods: Patients referred for the evaluation of glycogens storage disorders at the Genetic Department of Sir Ganga Ram Hospital were evaluated by a detailed history, clinical examination, biochemical investigations including glucose and glycogen tolerance tests, RBC glycogen content enzyme estimation, histopathology on liver biopsy, and molecular tests where possible. These patients were managed by dietary therapy and followed up over the study period.
Results: Glucose tolerance test with estimation of glucose and lactate levels contributed to the diagnosis of the GSD affecting the liver. Enzyme estimation was done for GSD type II and IX. Of the patients evaluated, fifteen were confirmed to have GSD. Of these two were diagnosed to have GSD type I, five GSD type II, six GSD type III, one GSD type V and one patient GSD type IX.
Conclusions: Glycogen storage disorders are not uncommon in India. It is possible to diagnose the different types of GSD by utilizing commonly available biochemical tests thereby allowing management of the patients by dietary manipulation.

239-P
MULTISYSTEM INVOLVEMENT: A RARE AND UNUSUAL PRESENTATION OF GSD TYPE IV

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GSD Type IV, also known as Anderson disease or amylopectinosis, is a rare autosomal recessive disorder caused by deficiency of GBE activity (α-1,4-glucan 6-glycosyl-transferase). This disease is extremely heterogeneous in terms of tissue involvement, age of onset and clinical manifestations.

We present a 13-year-old boy patient with GSD Type IV with multisystem involvement. He was born to consanguineous parents at full term without any complications and his maternal perinatal history was uneventful. His parents were cousins. He had normal growth and development. His sister died from unexplained cardiomyopathy at the age of 8. Our patients initial symptom was severe heart failure. Also he had complainted muscle weakness so that EMG was performed and showed muscle involvement. The diagnosis was suggested by muscle biopsy tissue showing intracellular basophilic diastase resistant PAS positive inclusion bodies, and was confirmed by showing the completed branching enzyme deficiency. Similar vacuoles were found in liver biopsy. The patient died from an intercurrent infection. Postmortem endomyocardial biopsy revealed intracellular basophilic diastase resistant PAS positive inclusion bodies. GBE gene sequence was normal.

From the information gathered so far it is evident that GSD-IV manifests with an unusual array of presentations both clinically and biochemically. Our case pointed out that GSD Type IV should be kept in mind in young patients with heart involvement.

240-P

MOLECULAR GENETIC DIAGNOSIS OF HEREDITARY FRUCTOSE INTOLERANCE (HFI): ALDOLASE B (*ALDOB*) MUTATION ANALYSIS USING DRIED BLOOD SPOTS AND THE LUMINEX X-MAP TECHNOLOGY

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Hereditary fructose intolerance, an autosomal recessive disorder of carbohydrate metabolism with a prevalence of 1:26 000 results from congenital aldolase B deficiency. To date, there are 35 reported mutations; the three most frequent ones (p.A150P, p.A175D, p. N335K) however, are found on 84% of chromosomes of HFI patients. Since conventional diagnostic methods are invasive, molecular genetic diagnosis has increasingly been recommended and, since there is no acumulation of metabolites in the newborn period, mutation analysis has also been considered to be a method for neonatal screening.

The Luminex X-Map technology provides a convenient tool for the simultaneous analysis of multiple mutations in one run. Differently coloured beads (hues of red) are recognized by laser excitation and, when coupled with various oligonucleotides, allow the identification of multiple analytes from the same well. For our purpose, we immobilized oligonucleotides carrying the common *ALDOB* mutations and their wild-type counterparts on 6 different beads. In addition, for background analysis, we added a bead set carrying a nonsense oligonucleotide. Analysis of 7 HFI patients using either isolated DNA or PCR products directly obtained from dried blood spots without further purification steps yielded positive results for all cases. Controls were clearly negative in this assay. Analysis time was less than 30 s on the Luminex analyser with an approximate preparation time of 2 h.

In summary, we were able to show that the technique is robust and rapid. It requires only few preparatory steps making it useful for the diagnosis of patients and for neonatal screening for HFI.

241-P

METHODS FOR ENZYME ACTIVITY AND MUTATION ANALYSIS OF FRUCTOSE-1,6-BISPHOSPHATASE DEFICIENCY (FBP1 #MIM 229700)

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Background: Fructose-1,6-bisphosphatase (FBPase) is a key enzyme in the gluconeogenetic pathway. FBPase deficiency is a recessive inborn error of metabolism. Children with FBPase deficiency may react with hypoglycaemia, ketonemia and acidosis during infections or periods of low food intake.
Methods: We have modified published methods and established a radiochemical enzyme assay based on calcitriol stimulated monocytes and a mutation analysis where we investigate the expression of the *FBP1* gene. Two patients were studied using these methods.
Results: Patient 1 was diagnosed as a child with several oral load tests and FBPase activity measurement in tissue from a jejunal biopsy. Isolated leucocytes from the parents showed intermediate enzyme activity. The patient is now 36 years old, healthy and does not suffer from his metabolic defect. The diagnosis was confirmed; no detectable level of FBPase activity was found in stimulated monocytes. Mutation analysis showed that he was heterozygous for two mutations predicting amino acid exchanges at position G259R and G293E.

Patient 2 is 7 years old and he has experienced several episodes with hypoglycaemia and high blood levels of glycerol and lactate. We found no detectable FBPase activity in stimulated monocytes. His parents had a decreased enzyme activity compared to the normal controls. In the mutation analysis no transcript from the FBP1 gene was detected but at the DNA level we found a C659G mutation in exon 5 predicting a premature stop codon.

In **summary,** we have established a system for the diagnosis of FBPase deficiency based on a single blood sample.

242-P

NOVEL MUTATIONS IN FRUCTOSE-1,6-BISPHOSPHATASE DEFICIENCY: NO CORRELATION BETWEEN GENOTYPE AND BIOCHEMICAL AND CLINICAL PHENOTYPES

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Fructose-1,6-bisphosphatase catalyses the unidirectional hydrolysis of fructose-1,6-bisphosphate to fructose-6-phosphate during gluconeogenesis. Deficiency leads to crises with hypoglycaemia, lactic acidosis and glyceroluria, and hepatomegaly.

We investigated three patients with enzymatically confirmed fructose-1,6-bisphosphatase deficiency, presenting with hypoglycaemia, elevated lactate, and hepatopathy. Patient 1 presented neonatally and is fructose intolerant. Patients 2 and 3 presented in their second year. Glyceroluria was found in patients 1 and 2. Leukocyte enzyme activity was 0% in patient 1, 124% in patient 2 and 5% in patient 3 (relative to lower limit of normal). Liver enzyme activity in patient 2 was 11% of control.All exons of the *FBP1* gene were amplified from genomic DNA and directly sequenced. Residues affected by missense mutations were assessed for evolutionary conservation by alignment of amino acid sequences using ClustelW and mapped on the crystallographic structure (1FBP) using MacPymol.

Patient 1 was compound heterozygous for two novel missense mutations, c.365A>C, p.D122A and c.843G>A, p.E281K affecting two highly conserved residues predicted to interact with the substrate in the enzyme active site. Patient 2 was homozygous for a novel small deletion, c.618delA, p.G207fs. A large homozygous deletion was suspected in patient 3 as the exon 1 PCR failed repeatedly. An inverse PCR protocol with primers in intron 1 defined the deletion as c.-50.170+5192del covering part of exon 1 and into intron 1.

Fructose-1,6-bisphosphatase deficiency results from a variety of genetic mechanisms. Molecular analysis gives a clear and accurate diagnosis and allows family studies but has revealed no clear genotype/phenotype correlation.

243-P

EVALUATION OF URINE D-ARABINITOL LEVEL (AS RATIO D-/L-ARABINITOL) IN NEWBORNS AFTER BIRTH AND 2 DAYS LATER

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Background: D-arabinitol (acyclic polyol) as a product of yeast Candida spp. metabolism is present in fungal infected children's urine and serum. The elevated level of D-arabinitol, measured as ratio D-/L- arabinitol, (pathogenic value >4.2) is considered as a marker of Candida infection. In healthy children D-arabinitol is also present, normal value of D-/L-arabinitol levels for infants group (1–12 months age) is mean ± SD; 2.48 ± 0.58. However, the origins of polyols, mainly D-arabinitol and ribitol, are not known. It can be assume that the source of D-arabinitol in healthy humans could be Candida spp. colonization or endogenous origin.
Methods: To prove this hypothesis we measured D-/L-arabinitol, by gas chromatography, in first urine sample in 50 newborns and subsequently in the same populations 2 days later. Mothers of the newborns were healthy and had no clinical symptoms of either superficial or invasive candidiasis.
Results: Our data show that in first urine D-arabinitol is present, the level D-/L-arabinitol, mean ± SD = 1.58 ± 0.31. In the samples collected in the second day of life the D-/L-arabinitol level was higher (2.33 ± 0.38), as it is in (1–12 month) old group of healthy children.

One can hypothesize that the D-arbinitol in the first urine samples is from the endogenous metabolic pathway. Increased levels of D-arabinitol on the second day samples could be from exogenous source, i.e. dietary or colonization.

245-O

SEDOHEPTULOKINASE DEFICIENCY DUE TO A 57-kb DELETION IN CYSTINOSIS PATIENTS CAUSES ACCUMULATION OF SEDOHEPTULOSE: ELUCIDATION OF THE *CARKL* GENE

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Background: Recently we described two new defects in the pentose phosphate pathway (PPP): Transaldolase deficiency and Ribose-5-phosphate isomerase deficiency (Verhoeven et al., 2001; Huck et al., 2004). Here we report additional elucidation of another gene involved in the PPP. The most common mutation in the cystinosis gene, *CTNS*, is a homozygous 57-kb deletion that also includes an adjacent gene *CARKL*. The *CARKL* gene encodes a protein that is predicted to function as a carbohydrate kinase. In a cystinosis patient with the 57-kb deletion we found elevations of sedoheptulose which suggested involvement of *CARKL* in the PPP. **Methods**: Sedoheptulose was analysed in 8 cystinosis patients 3 with and 5 without the 57-kb deletion. Sedoheptulokinase activity was determined in fibroblasts, from 3 with and 2 without this deletion and 3 controls, by measuring the production of sedoheptulose monophosphate with LC-MS/MS after incubation with sedoheptulose. **Results**: Cystinosis patients with the common 57-kb deletion had strongly elevated urinary concentrations of sedoheptulose (28–451 mmol/mol creatinine; controls and other cystinosis patients <9) and only 20% sedoheptulose phosphorylating activity compared to cystinosis patients with other mutations and controls. **Conclusions**: We have identified the function of *CARKL*. It catalyses: Sedoheptulose + ATP –> Sedoheptulose monophosphate + ADP. We have shown decreased sedoheptulokinase activity in cystinosis patients with an absence of the *CARKL*-encoded protein and normal activity in cystinosis patients without the 57-kb deletion. This indicates that the *CARKL*-encoded protein is responsible for the phosphorylation of sedoheptulose. Deletion of *CARKL* causes accumulation of sedoheptulose.

246-P

TRANSALDOLASE DEFICIENCY: 7TH CASE PRESENTING WITH NEONATAL MULTIORGAN INVOLVEMENT, RICKETS AND DEAFNESS

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Background: Transaldolase (TALDO) deficiency is a rare inborn error of the pentose phosphate pathway. So far six patients (3 families) have been described all from Turkish heritage, manifesting a multiorgan involvement with liver dysfunction, splenomegaly, dysmorphic features and haemolytic anemia. Here we present a new case of TALDO deficiency in a 2-year-old boy from the United Arabic Emirates.

He is the 4th child of first cousin parents, 28-week twins died (prematurity) and a 9 year old sister is doing well. One week prior to delivery ultra sound showed oligohydramnios and splenomegaly. In the neonatal period he had hemolytic jaundice, hepatosplenomegaly, pancytopenia, mild dysmorphic features, bilateral inguinal hernias, small patent ductus arteriosus and rickets. At 1 year he had recurrent respiratory infections (RSV, CMV), easy bruising, mild developmental delay, hepatosplenomegaly. A liver biopsy at the age of 14 months showed cirrhosis, cause not evident. At 2 years there was a speech delay associated with a mixed sensorineural and conductive deafness. Extensive investigations for causes of neonatal onset liver disease were negative.

Urine analysis of sugars and polyols showed elevated concentrations of erythritol, ribitol, arabitol and sedoheptulose, indicating a transaldolase deficiency. The *TALDO* gene showed a homozygous mutation resulting in a pathogenic change of arginine 192 to cystine.

Conclusions: The seventh patient (4th family) with TALDO has been diagnosed. Most of his clinical symptoms are similar to other TALDO patients; however he is the first non-Turkish patient and the first presenting rickets and deafness.

247-P

A RARE CAUSE OF HEPATOSPLENOMEGALY – TRANSALDOLASE DEFICIENCY

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Transaldolase deficiency, a multi-system disorder of the pentose phosphate pathway, was first described in 2001. Up to now, 7 patients were reported in the literature. Typical clinical features include hepatosplenomegaly, liver cirrhosis or failure with or without cardiomyopathy. No neurological involvement is present. Our patient was the first boy of a consanguineous Pakistan couple. Intrauterine growth retardation was present in the antenatal period. His birth weight was 1.67 kg at 36 weeks of gestation. Post-natal period was complicated by recurrent hypoglycaemia, thrombocytopenia, deranged liver function and coagulation, hepatosplenomegaly and bilateral undescended testes. Subsequently, he developed failure to thrive, transient hypoglyaemia, persistently mild thrombocytopenia and mild global developmental delay. The latest physical examination at 19 months revealed no dysmorphic features but hypertrichosis and non-progressive hepatosplenomegaly (each measured 4 cm below the costal margin). Examination of other systems was unremarkable. There was catch up of his developmental milestones. Extensive investigations for inborn error of metabolism including bone marrow biopsy were unrevealing except increased Kreb cycle metabolites in urine. His liver parenchymal enzymes were mildly deranged. Computerized tomography scan of the abdomen showed suspected liver cirrhosis. Magnetic resonance imaging of the brain showed mild white matter changes at bilateral parieto-occipital regions. 3 urine samples for polyol analysis revealed persistently raised erythritol, arabitol and ribitol with a pattern consistent with this disease. Genetic testing is pending. Urine for polyols analysis may be considered in patients with unexplained hepatosplenomegaly and/or liver cirrhosis.

248-P

RETROSPECTIVE DETECTION OF TRANSALDOLASE DEFICIENCY IN AMNIOTIC FLUID

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Background: Transaldolase (TALDO) deficiency is a rare inborn error of the pentose phosphate pathway resulting in the accumulation of erythritol, ribitol, arabitol, sedoheptitol, perseitol, sedoheptulose and mannoheptulose in urine and sedoheptulose-7P in urine and blood[1]. So far six patients have been described, manifesting a multiorgan involvement with liver dysfunction, splenomegaly, dysmorphic features and hemolytic anemia as major presenting signs. We reported recently a proven TALDO deficiency in three children and one fetus from the same family[2]. The fetus presented with an early hydrops associated to oligoamnios and this pregnancy was medically terminated at 28 weeks of gestation. We report here our findings in the amniotic fluid from this terminated pregnancy.

Methods: Polyols, heptuloses and sedoheptulose-7P were measured using liquid chromatography tandem mass spectrometry in the amniotic fluid of the affected fetus and of controls.

Results: The concentrations of ribitol and especially sedoheptulose were elevated at 7 µmol/L (control range 1–4 µmol/L; $n = 11$) and 13.5 µmol/L (control range <1; $n = 12$) respectively. No differences in the concentrations of erythritol, arabitol sedoheptitol, perseitol, mannoheptulose and sedoheptulose-7P were found.

Conclusions: TALDO deficiency results in abnormal metabolite levels of sedoheptulose and ribitol in amniotic fluid, which may cause alterations in early embryogenesis. Moreover this characteristic profile of polyols in the amniotic fluid may allow prenatal diagnosis for families with a case-index or for pregnancies complicated with early hydrops associated to oligoamnios.

[1]Wamelink et al. J Inherit Metab Dis. 2007; in press.
[2]Valayannopoulos V et al. J Pediatr. 2006;149:713

249-O

MUTATION IN THE KEY ENZYME OF SIALIC ACID BIOSYNTHESIS CAUSES SEVERE GLOMERULAR PROTEINURIA AND IS RESCUED BY N-ACETYLMANNOSAMINE

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Mutations in the key enzyme of sialic acid biosynthesis, UDP-GlcNAc 2-epimerase/ManNAc kinase (GNE/MNK), result in hereditary inclusion body myopathy (HIBM), an adult-onset, progressive neuromuscular disorder. We created knock-in mice harboring the M712T Gne/Mnk mutation. Homozygous mutant (GneM712T/M712T) mice did not survive beyond postnatal day 3 (P3). At P2, significantly decreased Gne-epimerase activity in GneM712T/M712T muscle, but no myopathic features were apparent. Rather, homozygous mutant mice had glomerular hematuria, proteinuria, and podocytopathy. Renal findings included segmental splitting of the glomerular basement membrane, effacement of podocyte foot processes and reduced sialylation of the major podocyte sialoprotein, podocalyxin. N-acetylmannosamine (ManNAc) administration yielded survival beyond P3 in 43% of the GneM712T/M712T pups. Survivors exhibited improved renal histology, increased sialylation of podocalyxin, and increased Gne/Mnk protein expression and Gne-epimerase activities. These findings establish this GneM712T/M712T knock-in mouse as the first genetic model of podocyte injury and segmental glomerular basement membrane splitting due to hyposialylation. The results also support evaluation of ManNAc as a treatment not only for HIBM, but also for renal disorders involving proteinuria and hematuria due to podocytopathy and/or segmental splitting of the glomerular basement membrane.

250-P

DIAGNOSIS OF CONGENITAL DEFECTS OF GLYCOSYLATION BY ISOELECTRIC FOCUSING OF SERUM TRANSFERRING IN PATIENTS WITH GLOBAL DEVELOPMENTAL DELAY OR MULTIPLE BIRTH DEFECTS OR MULTI-SYSTEMIC DISEASES

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Congenital disorders of glycosylation (CDG) are a recently described but rapidly expanding group of inborn errors of metabolism due to deficient glycosylation of N-linked oligosaccharides. The most widely used diagnostic tests for CDG is isoelectric focusing (IEF) of serum sialotransferrins. We had performed IEF of serum sialotransferrin on 162 patients who were referred for further evaluation of global developmental delay with or without multiple birth defects or multiple systemic involvements in the past two years. Eleven patients (7%) were diagnosed having CDG. The estimated incidence is about 1 in 90 000 live births. This could be the tip of an iceberg. Six patients (65%) showed Type I pattern and 5 of them had type II pattern. Six were female and five were male. All of them (100%) had global developmental delay of variable severity, 4 had microcephaly and 1 had macrocephaly, 7 (63%) had dysmorphic features, 4 patients had multi-systemic involvements and birth defects. 5 patients (45%) demonstrated cerebellar atrophy in MRI, two had cerebral atrophy and one had white matter changes. One of the patients with type II pattern had an unbalanced chromosomal 11 rearrangement due to inversion resulting in deletion of 11q25. Four patients (36%) had isolated non-specific global developmental delay with spasticity and 2 patients had only cerebellar ataxia beside developmental delay. In conclusion, IEF of serum transferrin should be considered as one of the tests in the evaluation of patients with global developmental delay, with or without multi-systemic involvement, or multiple birth defects or cerebellar atrophy.

251-P

HPLC AS A GOOD METHOD FOR CDG SCREENING

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Congenital disorders of glycosylation (CDG) are a group of inherited defects in the synthesis and processing of the linked glycans of glycoproteins. The most useful method for diagnosis is isoelectric focusing of serum transferrin (Tf-IEF) and detection with Comassie Blue staining. The transferrin isoforms can also be separated by HPLC anion-exchange chromatography and quantified by absorbance at 470 nm. We have validated HPLC as a good method for CDG screening due to its reproducible separation and quantification of the different glycoforms of transferrin.

Due to its high selectivity, Tf-IEF is regarded as a reference method, but it is laborious, and only few sera can be processed together (in one gel). The HPLC system equipped with autosampler permits running longer series of samples, and its easier process lets us increase the number of screened patients. In addition, interpretation of the Tf-IEF patterns is often subjective, while HPLC allows us to quantitate the percentage of each separated glycoform.

The method has been validated by the analysis of controls, transferrin variants, immature babies, alcoholics, untreated patients with galactosaemia and fructosaemia, and several CDG patients (CDG-Ia, CDG-Ib, CDG-Ie, CDG-IIh/Cog8, CDG-IIx).

In a CDG-Ia patient with moderate clinical features and very slighty altered Tf-IEF pattern, almost undistinguishable from controls (little increase of disialotransferrin and no detectable increase of asialotransferrin), HPLC was able to quantify this little increase of disialotransferrin (4.2%, controls 1.16% SD 0.25).

In **conclusion**, HPLC is a good tool for CDG screening, as it permits long series of analysis and objective interpretations.

252-P

VALIDATION OF A SPECIFIC IMMUNOPRECIPITATION METHOD FOR ISOLATING INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-3 (IGFBP-3) FROM SERUM TO STUDY IGFBP-3 ISOFORMS IN GALACTOSEMIA, CDG (CONGENITAL DEFECTS OF GLYCOSYLATION) AND CONTROLS

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Background: Patients with classical galactosemia are at risk for diminished bone mass and growth without evidence of nutritional deficiencies. We hypothesize that dysglycosylation of glycoproteins of the growth hormone/IGF-1 (insulin-like growth factor type I) axis play an important role in galactosemia as in CDG. IGF-1 is over 75% bound with IGF-binding protein-3 (IGFBP-3). The aim is to investigate glycan abnormalities of IGFBP-3 through immunoprecipitation followed by high resolution mass spectrometry. **Methods:** Monoclonal and polyclonal IGFBP-3 antibodies were covalently immobilised on protein-A Sepharose beads using dimethyl-pimelimidate as cross-linker to isolate IGFBP-3 from serum. Isolated proteins were separated by one-dimensional (1-DE) and two-dimensional gel electrophoresis (2-DE) and visualised by Western blotting. The immunoprecipitation method was validated using Western blotting and enzyme-linked immunosorbent assay (ELISA). In a pilot study, 2-DE Western blots of a CDG patient and a control were compared. **Results:** The 1-DE and ELISA results illustrated that an optimal isolation was performed using phosphate buffered saline for the incubation with serum. Laemmli sample buffer, containing 2-Amino-2-(hydroxymethyl)-1,3-propanediol, hydrochloride and sodium dodecyl sulphate, or ([3-[(3-cholamidopropyl)-dimethylammonio]propanesulfonate]) was optimal for the elution. The intact IGFBP-3 and fragments were clearly visible on 1-DE and 2-DE Western blots. **Conclusions:** A specific immunoprecipitation method to isolate IGFBP-3 was optimized and validated to study isoforms of IGFBP-3. More basic and acid fragment isoforms and less acid intact isoforms were seen for the CDG patient compared to the control. In future experiments, Western blots of galactosemia, CDG patients and controls will be compared and the discriminating (glyco)proteins will be identified.

253-O

PROTEOMIC AND GLYCOMIC TOOLS APPLIED TO THE DIAGNOSIS OF CDG REVEALED AN UNEXPECTED ABNORMALITY IN GALACTOSYLATION OF CORE 1 MUCIN TYPE O-GLYCOPROTEINS

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Congenital disorders of both N- and O-glycosylation are a growing group of inherited diseases sharing heterogeneous clinical, biochemical and genetic features. We present two new cases of N- and O-glycans biosynthesis disorders allowing to address specifically the potentials of proteomics and glycomics tools for the screening and the better understanding of these diseases. First, two dimensional electrophoresis (2-DE) of mucin type O-glycosylated apolipoprotein C-III (apoC-III) and other N-glycoproteins enabled rapid detection and further characterization of typical O- and N-glycan abnormalities. One patient showed a strong overexpression of the asialylated isoform of apoC-III while the second exhibited a discrete overexpression of the monosialylated isoform; both observations being associated with abnormal transferrin patterns. Second, MALDI-TOF MS of N- and O-glycans released from serum glycoproteins allowed us not only to corroborate data from 2-DE but also to orientate towards potentially disturbed enzymatic steps. In the two cases, MS showed abnormalities affecting galactosylation of mucin type O-glycoproteins and/or of N-glycoproteins.. Moreover, when applied to fibroblasts of one patient, brefeldin-A functional assay allowed us to appreciate the potential involvement of Golgi trafficking defects in the related disease. Concerning the potential involvement of the COG complex, western blotting of each subunit showed no abnormalities. It appears that (i) 2-DE and MS are interesting analytical tools which can be successfully applied to the screening and to the characterization of CDG affecting both N- and O-glycosylation and (ii), these results strongly suggest the already proposed involvement of Golgi trafficking defects as an important part of these glycosylation diseases.

254-O

A DEFECT IN DOLICHOL PHOSPHATE BIOSYNTHESIS CAUSES A NEW INHERITED DISORDER WITH DEATH IN EARLY INFANCY

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The paper describes the discovery of a new inherited metabolic disorder, dolichol kinase (DK1) deficiency. DK1 is responsible for the final step of the de novo biosynthesis of dolichol phosphate. Dolichol phosphate is involved in several glycosylation reactions, such as N-glycosylation, glycosylphosphatidylinositol (GPI)-anchor biosynthesis, and C and O-mannosylation.

We identified four patients who were homozygous for one of two mutations (c.295TrA[99CysrSer] or c.1322ArC [441TyrrSer]) in the corresponding *hDK1* gene. The residual activity of mutant DK1 was 2%–4% when compared with control cells. The mutated alleles failed to complement the temperature-sensitive phenotype of DK1-deficient yeast cells, whereas the wild-type allele restored the normal growth phenotype.

Affected patients present with a very severe clinical phenotype, with death in early infancy. Two of the patients died from dilative cardiomyopathy.

DK1 deficiency is the first member of a newly discovered group of metabolic disorders caused by defects in dolichol phosphate biosynthesis.

255-A

CONGENITAL DISORDER OF GLYCOSYLATION TYPE I A

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Background: Congenital disorders of glycosylation (CDG) are a group of autosomal recessive genetic disorders caused by defects in the assembly and processing of glycoproteins – CDG-I and CDG-II. Of these CDG-I a is the most frequent disorder caused by deficiency of phosphomannomutase-2 enzyme (PMM2).
Methods and **Results**: We report the first case of CDG-Ia (congenital disorders of glycosylation) from India diagnosed by enzyme and molecular mutation analysis

The patient, a 7-month-old boy, had all the classical clinical features of developmental delay, failure to thrive, and hypotonia.There was lipodystrophy and inverted nipples which prompted investigation for the disorder. There was hepatomegaly and altered liver enzymes at initial presentation.

IEF and CZE of serum sialotransferrins were consistent with the diagnosis of CDG. Enzyme assay for phosphomannomutase in cultured fibroblasts was low (1.03 Mu/mg protein). This confirmed the clinical diagnosis of CDG. Mutation analysis in *PMM2* gene showed that the child was homozygous for mutation C.623G > C.

The couple was counseled about 25% risk of recurrence in the next pregnancy and its prevention by doing mutation analysis in chorionic villi at 10–11 weeks of pregnancy. Based upon this information the couple opted for prenatal diagnosis during next pregnancy. The fetus was found to be affected and the couple chose to terminate the pregnancy.
Conclusions: CDG is a rare disorder of glycosylation pathway and correct diagnosis is helpful in prenatal diagnosis in subsequent pregnancies so as to take reproductive decision.

256-P

RISK ASSESSMENT OF ACUTE VASCULAR EVENTS IN CONGENITAL DISORDER OF GLYCOSYLATION TYPE IA

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Background: The congenital disorder of glycosylation type Ia (CDG-Ia) presents a broad clinical spectrum. Some patients suffer from acute vascular events (AVE; thrombosis and bleeding) and stroke-like events. No correlations have been made between the marked haemostasis abnormalities of CDG-Ia and the occurrence of acute vascular events.
Methods: We analyzed the clinical and haemostasis data of 39 CDG-Ia patients, to determine risk factors for acute vascular events in CDG-Ia.
Results: Acute vascular events occur in patients younger than 15 years, especially when there is fever and prolonged immobilization. Haemostasis and liver cytolysis are characteristically abnormal in these patients, but improve with age. By comparing groups of patients with past history of AVE (E) or without (EF), we noticed higher factors VIII and IX activities in the E cluster ($p = 0.03$) compared to the EF cluster. Moreover, the activity/antigenicity ratio for protein C was higher in the E group ($p = 0.02$).
Conclusion: CDG-Ia patients younger than 15 years old are at risk of acute vascular events.

The paradoxical results – abnormal VIII and IX factors in EF patients and normal results in E patients, while XI, AT, PC and ASAT, ALAT are abnormal in both groups – could suggest a disequilibrium between prothrombotic and antithrombotic factors in the E group. Vascular events may also occur in patients where glycoproteins are proportionally more hypoglycosylated, particularly protein C.

257-P

PRIMARY SKELETAL DYSPLASIA AS A MAJOR MANIFESTING FEATURE IN AN INFANT WITH CONGENITAL DISORDER OF GLYCOSYLATION TYPE IA

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Background: The congenital disorders of glycosylation (CDG) display a multisystem clinical phenotype. We present a term infant with CDGIa with a severe skeletal phenotype reminiscent of a type II collagenopathy. **Case Report:** Clinical features included large ears, buttock fat pads, inverted nipples, hydrops fetalis, hypertrophic biventricular cardiomyopathy, transient hyperinsulinism, and renal cortical echogenicity. Birth parameters were weight 3859 g, length 54 cm and head circumference 39 cm (all >98%). A type 1 transferrin isoelectric focussing pattern was noted and peripheral leucocytes phosphomannomutase activity was markedly reduced (0.3 nmol/min/mg protein (RR 3.6–9.0). *PMM2* genotyping is pending. Skeletal survey revealed short long bones with 'dumbbell' appearance, generalised epiphyseal ossification delay, ovoid, anteriorly beaked vertebral bodies, hypoplastic cervical vertebrae, 13 rib pairs, hypoplastic pubic bones, and bullet shaped short tubular bones. These appearances were reminiscent of a primary skeletal dysplasia closest to Kniest dysplasia or spondyloepiphyseal dysplasia congenita (type II collagen disorders). **Discussion:** Skeletal manifestations in CDGIa are under recognised, and sparsely reported. Osteopenia, a 'dysostosis multiplex like phenotype', C1-C2 subluxation, platyspondyly, and a 'bone-in-bone' appearance have been described. Numerous extracellular matrix proteins, including collagen type II and components in the NOTCH signalling pathway undergo glycosylation. Given the radiographic phenotype of a type II collagenopathy observed in our patient, we postulate that defects in post translational glycosylation of collagen type II might underlie this phenotype. This skeletal phenotype is the most severe reported in CDGIa and raises further research questions regarding collagen type II glycosylation in these disorders.

258-P

IS CORTICAL BLINDNESS PART OF CDG-IA? FURTHER EXPANSION OF CLINICAL MANIFESTATIONS IN CDG-IA

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Congenital disorders of glycosylation (CDG) are a group of disorders of abnormal glycosylation of N-linked oligosaccharides. Clinical manifestations depend on the tissues in which the defective enzymes are expressed: symptoms and findings range from normal development to severe developmental delay with abnormal brain development. Deficiencies in thirteen different enzymes in the N-linked oligosaccharide synthetic pathway are known. CDG-Ia is the most common form and is characterized by cerebellar hypoplasia, facial dysmorphism, psychomotor retardation, and abnormal fat distribution. CDG-Ia is due to deficiency of phosphomannomutase 2 (PMM2). Serum transferrin analysis by isoelectric focusing (IEF) is widely used for diagnosis of CDG. Diagnosis of CDG-Ia can be made based on enzyme assay of PMM2 or mutation analysis of *PMM2* gene. We identified a 10-year-old Hispanic male with early infantile developmental delay, seizure, microcephaly, and cortical blindness (diagnosed at age 3 years). CDG IEF testing showed abnormal mono-oligo/di-oligo ratio 3.255 (ref: 0–0.074) and a-oligo/di-oligo ratio 1.912 (ref: 0–0.022). The PMM2 activity was reduced to 35% of normal value. Molecular analysis on cDNA of *PMM2* showed one heterozygous mutation (c.422G>A; p.R141H) and no other abnormalities were identified. Hence, the second mutation currently escapes detection. No mutations were identified by analysis of CDG-Ie gene (*DPM1*). The presented case with cortical blindness may expand clinical manifestations observed in patients with CDG-Ia.

259-P

CLITORIS HYPERTROPHY AND HIRSUTISM IN AN ADULT FEMALE PATIENT WITH A CONGENITAL DISORDER OF GLYCOSYLATION (CDG) TYPE IA

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Background: CDG syndrome type I a is a genetic multisystem disorder characterized by impaired N-glycosylation of glycoproteins. Affected individuals present with severe neurologic impairment. Furthermore, many patients with CDG-I a have presented with endocrine abnormalities due to abnormal hormone or hormone receptor function. Many of these patients show an impairment of their sexual development due to a gender- and age-dependent hypergonadotropic hypogonadism.
Case report: In this report, we describe a mentally retarded 17-year-old female patient with CDG type Ia in whom two previously unknown missense mutations of the phosphomannomutase gene were detected. The residual phosphomannomutase activity was 8.5%. She has had no sexual maturation and has not had her menarche. She showed marked hirsutism and the most striking finding was significant clitoris hypertrophy. Laboratory investigations showed hypergonadotropic hypogonadism and increased plasma concentrations of androgens. Her karyotype was 46 XX. Ultrasound examination revealed small ovaries without follicular activity. The 13-year-old sister of the patient is also affected by the CDG syndrome; she carries the same mutations. Also she has no sexual development but she does not have any signs of virilization.
Conclusion: Although female adults with CDG syndrome type I a have been repeatedly found to present with a clinical picture comparable to that of the polycystic ovary syndrome, including an augmented ratio of LH/FSH, anovulation, hirsutism, hyperprolactinemia and insulin resistance, a constellation with a profound clitoris hypertrophy has not been previously described.

260-P

CONOTRUNCAL HEART DEFECTS IN THREE PATIENTS WITH CONGENITAL DISORDER OF GLYCOSYLATION TYPE IA

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Background. Typical clinical features of CDG-Ia are psychomotor retardation, cerebellar ataxia, coagulation and liver abnormalities, convergent strabismus, inverted nipples and abnormal fat distribution. Here, we report on for the first time conotruncal malformations (CTMs) associated with CDG-Ia in three patients.

Patients: Patient 1 is a girl born at term. Her brother died at two years of Tetralogy of Fallot (TOF). He also presented ataxia, strabismus, inverted nipples and abnormal fat distribution. Patient's pregnancy was marked by antenatal diagnosis of truncus arteriosus. She was operated of her heart defect at one month. At 12 months, she presented severe psychomotor delay, hypotonia and failure to thrive. Brain imaging revealed cerebellar hypoplasia/atrophia. Laboratory investigations revealed a transferrin pattern consistent with CDG type Ia and PMM activity was undetectable in the patient's leucocytes. Mutation analysis revealed a compound heterozygous *PMM2* gene mutation (E139K/f157S). Patient 2, a girl, is the first child of non consanguineous parents. CTMs (TOF with absent pulmonary valve) was antenataly diagnosed. Heart defect was curved at two weeks of age. She presented with poor sucking, failure to thrive and severe hypotonia. Brain imaging showed cerebellar atrophia/hypoplasia leading to the diagnosis of CDG-Ia. In the patient's leucocytes, PMM activity was undetectable and mutation analysis revealed a compound heterozygous *PMM2* gene mutation (E139K/R141H).
Conclusion. CTMs are usually associated with microdeletion 22q11 or chromosomal abnormalities. Here we reported on three patients with CDG-Ia presented with CTMs as the presenting symptom suggesting to look out for CDG in patients with CTMs.

261-P

CONGENITAL DISORDER OF GLYCOSYLATION PRESENTING WITH ICHTHYOSIS, COLABOMAS AND DEVELOPMENTAL DELAY: EXPANSION OF PHENOTYPE OR A NEW CONDITION?

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Background: Congenital disorders of glycosylation (CDG) are a diverse group of conditions arising as a consequence of abnormal incorporation of oligosaccharides into glycoproteins. There are several types described, and the best so far studied group affects the process of N-glycosylation.
Methods: We are reporting a case of a patient with CDG diagnosed at age 2, who presented with prominent ichthyosis and facial dysmorphism.
Results: She also had microphthalmia, optic nerve coloboma, cataracts, nystagmus, hypotonia and global developmental delay. Transferrin isoforms measured by tandem mass-spectroscopy showed a significant increase in hypoglycosylated species, consistent with CDG. In addition, laboratory investigations disclosed mild anemia, elevated liver transaminases, slight elevation of TSH with normal free thyroxine and decreased activity of antithrombin III. The patient was the product of a consanguineous marriage (first cousins), but family history was negative.
Conclusions: Patient's phenotype was reminiscent of CDG 1f, caused by *MPDU1* mutations, but in addition to ichthyosis and developmental delay, well known to be present in these patients, she also had microphthalmia and bilateral optic nerve coloboma, which have not previously been noted in patients with this CDG type. Additional genetic evaluations are underway to try to elucidate the etiology in this interesting patient.

262-O

A MOUSE MODEL FOR CONGENITAL DISORDER OF GLYCOSYLATION IA

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Congenital disorders of glycosylation (CDG) comprise a rapidly growing group of multisystemic human diseases caused by defects in glycoprotein biosynthesis. The molecular defect of the most frequent type of the CDG, CDG-Ia, is localized in mutations in the phosphomannomutase 2 (*PMM2*) gene. The enzyme catalyzes the conversion of Mannose-6-phosphate to Mannose-1-phosphate in the cytosol. Deficiency leads to severe reduction of GDP-mannose, a key substrate in glycoprotein biosynthesis. The clinical phenotype of CDG-Ia is characterized by psychomotor and mental retardation, peripheral neuropathy, cerebellar atrophy, retinitis pigmentosa, hepatopathy and blood clotting problems.

To investigate the pathophysiology of the disease and to develop therapeutic approaches, an animal model for CDG-Ia is absolutely recommended. Therefore, we generated mice with either a complete loss of Pmm2 activity or a weak residual enzyme activity as has been observed in all CDG-Ia patients known so far. Total loss of Pmm2 activity in mice lead to early embryonic lethality around day 2.5. Moreover, mating of heterozygous Pmm2-deficient mice with WT mice revealed that maternal transmission of the Pmm2 null allele is severely impaired. The generation of a mouse model with a weak residual Pmm2 activity by introduction of point mutations which are known from CDG-Ia patients reveals a broad spectrum of phenotypes ranging from early embryonic death to normal viability.

263-P

EFFECT OF FREEZING ON LEUKOCYTE PHOSPHOMANNOMUTASE (PMM) AND PHOSPHOMANNOISOMERASE (PMI) ACTIVITIES

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Background/Objectives: Congenital disorders of glycosylation are a group of diseases with abnormal glycosylation of glycoconjugates. The majority of patients have CDG-Ia, which is due to PMM deficiency. PMM is usually measured on fibroblasts, although determination on leukocytes has reportedly given clearer results. Our laboratory is a reference center and we receive frozen leukocyte preparations from all over the country for lysosomal enzyme studies. As we have recently started performing PMM and PMI determinations, we decided to study the effect of freezing on its activities, an information not found in the literature.
Methods: Heparinized blood was drawn from 7 controls, one PMM heterozygote, and 5 patients with clinical picture of CDG and presenting CDG-I pattern on isoelectric focusing. After lysis of leukocyte pellet and protein determination, PMM and PMI activities were performed. The lysate was then frozen for a few days and a second PMM and PMI determination was carried out.
Results: After freezing there was a decrease in PMM activities, which ranged from 54% to 88.3% in controls, from 32.1 to 56% in patients and 75.9% in the heterozygote. Decreases were also seen in PMI activities. They ranged from 15.6% to 68% in controls, from 17.6% to 64.6% in patients and 71.2 in the heterozygote.
Conclusions: Freezing has a significant effect on both PMM and PMI leukocyte activities. Frozen samples should not be used, for it might give misleading results.

264-P

DIAGNOSIS AND FOLLOW-UP OF A PATIENT WITH CONGENITAL DISORDER OF GLYCOSYLATION TYPE IB (CDG-IB)

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CDG-Ib (OMIM 602579) is caused by a deficiency of mannose-6-phosphate isomerase (PMI), due to mutations in the *MPI* gene. The clinical phenotype is characterized by gastro-intestinal and hepatic symptoms. It's a potentially treatable disorder. Here we report the early diagnosis of a new CDG-Ib case that showed an initially poor response to mannose treatment until a gluten-free diet was introduced. Patient is a first child of healthy unrelated parents. First symptom-failure to thrive- appeared at age 4 months. Two mos. later he presented vomiting, hypoglycaemia (39 mg/dl), hepatomegaly and hypertransaminemia in the course of a respiratory infection. Serum% CDT was found to be increased (24%) and IEF of transferrin showed type 1 pattern. Although mannose (4×150 mg/kg) supplementation was introduced the patient presented prolonged diarrhoea. Then, a celiac disease was suspected by detecting positive gliadine antibodies and a subtotal villous atrophy. After introduction of a gluten-free diet, the patient showed catch-up growth and resolution of diarrhoea but, hypertransaminemia, altered coagulation parameters and abnormal transferrin pattern persisted. These biochemical alterations were resolved on a higher dose of mannose (5×200 mg/kg). Psychomotor development was always normal. A CDG-Ib was diagnosed by detecting a deficient PMI activity in fibroblasts (12% of control value) and by molecular analysis of *MPI* gene revealing two mutations (R219Q and R56fs) in heterozygous fashion. Genetic study of celiac disease was negative. We want to highlight the resolution of diarrhoea in a CDG-Ib patient under a gluten-free diet; however the hepatopathy didn't improve until mannose was given at 1 g/kg/day.

265-O

CONGENITAL DISORDER OF GLYCOSYLATION Ik: NOVEL MOLECULAR AND CLINICAL FINDINGS AND AN EXAMPLE FOR PHENOTYPIC COMPLEXITY IN SINGLE GENE DISORDERS

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Background: Congenital disorder of glycosylation type Ik (CDGIk) is caused by mannosyltransferase1 (MT1) deficiency due to mutations in the *hALG1* gene on chr16p.13.3. So far, in 4 unrelated males CDGIk was diagnosed and associated with an extremely severe phenotype including death in the neonatal period. **Aim**: Characterization of the clinical phenotype and identification of the molecular and biochemical defect in 5 CDGIx affected individuals (2 m, 3f; 4 y–22 y; 3 nuclearfamilies) within one highly consanguineous Turkish pedigree. **Methods**/**Results**: A genomewide SNP scan (AffymetrixGeneChip10K2.0.) revealed a LOD-score of 5.5 between SNP A-1516465 and A-1517315 on chr16p13.3. All 5 affected individuals are homozygous for a novel c.1107C>T transition of the *ALG1* gene resulting in p.S359L. All 6 parents are heterozygous for this mutation, whereas it was not identified in 130 alleles of healthy Turkish individuals. Specific defect of MT1 was confirmed by reduction of the ability to elongate GlcNAc2-PP-dolichol in the presence of unaffected Man1(14C)GlcNAc2-PP-dolichol elongation. Structural modelling showed S359 within the catalytic site of the MT1protein whereas the previously reported *hALG1* mutations are not. The p. S359L mutation in this consanguineous pedigree is associated with a clinical phenotype much less severe compared with the previously reported patients. CDG Ik in 9 individuals manifests with marked inter/intrafamilial phenotypic complexity in severity and in organ involvement. **Conclusion**: Our results double the number of diagnosed patients, add to the molecular and clinical phenotype of CDG Ik, and highlight an example for phenotypic complexity in single gene disorders. We therefore strongly recommend to (i) exclude CDG Ik in all CDG I patients with unknown biochemical defect, (ii) consider phenotypic complexity when counseling families for CDG.

266-P

CDG TYPE I AND MYOPATHY WITH AUTOPHAGIC VACUOLES – A CASE REPORT

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Background: Congenital disorders of glycosylation (CDG) are multisystemic diseases. Although muscular involvement has been described, myopathy has not been a clinical feature.

Methods and **Results**: Our patient a 20-year-old male had apparently normal development until 13 months of age when, after an episode of gastroenteritis, he had global regression. When first observed at 5 years he had mental retardation, myopathic syndrome, ophtalmoparesis and pyramidal signs. He had a first generalized seizure at 17 years.

On our last examination we observed that he had inverted nipples, peculiar fat distribution, ophtalmoparesis, facial diparesis, tetraparesis (mainly proximal with distal amyotrophies) and bilateral pyramidal signs. He was able to walk but needed assistance.

The muscle biopsy showed fibers with central and peripheral vacuoles with basophilic areas that were PAS positive, with high oxidative staining. In electron microscopy these vacuoles had cytoplasmatic debris, electron dense material, large myeloid structures. Mitochondria where normal.

A partial deficit of complex I of mitochondrial chain (37%) was found in muscle.

He had a CDT of 6.5% of total transferrin (normal <2.6%), with a type I pattern in transferrin isoelectric focusing. Phosphomanomutase and phosphomannose-isomerase were normal.

Conclusions: This patient has a N-glycosylation disorder, with an unknown enzymatic defect and Has an autophagic myopathy. The only known causative gene in these lysosomal membrane disorders myopathies is *LAMP2*, a highly glycosylated protein that requires the use of most of the N-linked glycosylation. A similar defect must explain the autophagic myopathy in our patient.

267-P

FORGET THE CLASSIC CDG PHENOTYPE; A BROAD SPECTRUM OF CONGENITAL ANOMALIES IN CDG TYPE II

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CDG type Ia, the most common form of congenital disorders of glycosylation presents with characteristic symptoms of hypotonia, strabismus, arachnodactyly, cerebellar hypoplasia, abnormal lipid distribution and gastrointestinal, endocrine and coagulation abnormalities. Patients with CDG type II, diagnosed with a glycosylation disorder due to Golgi dysfunction and abnormal transferrin isoelectric focusing in blood, have very different clinical features. Mutations in COG7, coding for one of the 8 subunits of the Conserved Oligomeric Golgi complex, lead to a new clinical syndrome of growth retardation, severe progressive microcephaly, adducted thumbs, gastrointestinal pseudo-obstruction, cardiac anomalies, wrinkled skin and episodes of extreme hyperthermia. Features in COG1 deficient patients include hypotonia, developmental delay, ventricular hypertrophy/cardiac dysfunction and a rhizomelic short stature. The single patient described so far with COG8 mutation showed a phenotype similar to that in mitochondrial disease. Other defects affecting the biosynthesis of both N- and O- linked glycosylation with hyposialylation include a new subtype of autosomal recessive cutis laxa syndrome with neonatal cutis laxa, microcephaly with large fontanel, hypotonia and failure to thrive. Despite the clinical and biochemical diagnosis in an increasing number of patients with this form of CDG type II the gene defect has not been discovered yet. Based on the recently defined clinical syndromes with N- and O-linked glycosylation defects one should consider CDG syndrome in patients with a broad spectrum of different features, including pachygyria, hypotonia with adducted thumbs, cutis laxa, cardiac and cranio-skeletal anomalies.

268-O

SCREENING FOR COG DEFECTS IN CDG-IIX PATIENTS

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Congenital disorders of glycosylation type II (CDG-II) is a group of genetic, multisystem disorders caused by defects in the processing of an oligosaccharide precursor into a mature, complex N-glycan by glycosidases and glycosyltransferases. There is a large number of putative CDG-II patients where no underlying defect has been found yet. We classify these patients as CDG-IIx. The recognition of a defect in the Conserved Oligomeric Golgi (COG) complex as the first vesicular trafficking defect causing a glycosylation disorder opened a new era in CDG research. A defect in one of the subunits of the COG complex, which is involved in retrograde trafficking from Golgi apparatus to endoplasmic reticulum (ER), causes mislocalization of the glycosylation machinery.

We checked 35 patients with CDG-IIx for defects in the subunits of the COG complex by western blot of at least 6 subunits. We found 5 patients with a defect in one of the COG subunits (Cog1, Cog4, Cog7 and Cog8). Subsequently, mutations in the respective genes were found. In these cases the diagnosis was thus confirmed at the molecular level. Direct sequencing of the COG subunits of the remaining 30 patients failed to discover pathogenic mutations. Five patients showed one heterozygous variation with amino acid change which was not known in the SNP database. These variations are unlikely to be pathogenic. Two patients were identified with a homozygous intronic variation that needs further exploration.

Conclusion: We found COG defects in about 1 out of 7 patients with CDG-IIx.

269-P

THREE FRENCH PATIENTS WITH CONGENITAL MUSCULAR DYSTROPHY DISEASE AND NEW MUTATIONS IN *FCMD*

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Fukuyama congenital muscular dystrophy (FCMD) is one of the most common autosomal recessive disorders in the Japanese population mainly related to a founder mutation, a 3 kb retrotransposal insertion in the 3′ UTR of *FCMD*. This disorder, associated with abnormal 0-mannosylation of alpha-dystroglycan, is characterized by severe congenital muscular dystrophy and mental retardation. Until now only few FCMD patients have been reported in non Japanese populations: two Turkish patients with severe WWS-like phenotype and three other patients sharing Israeli origin with no brain involvement. We report three newly diagnosed French patients with congenital muscular dystrophy and severe hypoglycosylation of skeletal muscle alpha-dystroglycan. Two were 35- and 18-year-old sisters with congenital muscular dystrophy and mental retardation, not related to the third one, a 5 year-old boy showing calf hypertrophy, myalgia but no mental retardation. In the two unrelated families, sequencing of *FCMD* led to two already described and two others up to now undescribed mutations, one in each family. The former ones were the c.1112A>G (p.Tyr371Cys) in exon 9 and c.139C>T (p.Arg47Stop) in exon 3. The latter were missense mutations: c.509C>A (p.Ala170Glu) in exon 5 and c.736A>G (p.Arg246Gly) in exon 6. The two bases modifications were predicted to affect protein function by phenotype prediction programs. Allelic inheritance was confirmed by parental study. Screening of 100 healthy individuals for the two new missense mutations revealed no common SNP. Since *FCMD* mutations are known to be associated with steroid-responsive muscular dystrophy, therapeutics can be eventually proposed to these two families.

270-O

MOLECULAR STUDY OF 47 CASES OF LISSENCEPHALY TYPE II

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Type II lissencephaly (LIS II) is related to a group of autosomal recessive congenital muscular dystrophies associated with defects in a-dystroglycan O-glycosylation which comprises Walker-Warburg syndrome, Fukuyama cerebral and muscular dystrophy or muscle–eye–brain disease. The most severe forms of these diseases often have a fetal presentation and lead to a pregnancy termination.

We report here the first molecular study on fetal LIS II in a series of 47 fetuses from 41 unrelated families. Sequencing of the different genes known to be involved in a-DG O-glycosylation allowed the molecular diagnosis in 22 families: *POMT1* mutations were demonstrated in 32%, *POMGNT1* and *POMT2*, in 15% and in 7%, respectively. Further sequencing of *FKRP, FCMD* and *LARGE* did not reveal any mutation. Therefore we sequenced three other genes not implicated until now in LIS II, but which glycosyltranferase activity is involved in O-mannosylation two b1,4 galactosyltransferase genes: *b4GALT1* which deficiency also causes Congenital Disorder of Glycosylation IId, *b4GALT2*, a key regulator of glycosylation of the proteins involved in neuronal development, and one b1-6-NAcetylglucosaminyltransferase gene, *MGAT5B* or *GnT-IX,* which is specifically expressed in the brain and could act on brain a-dystroglycan that displays HNK-1 immunoreactive glycoepitopes.

However, sequencing of *b4GalT1*, *b4GalT2* and *MGAT5B* did not reveal any mutation.

No definitive molecular diagnosis could be made for the half of our cases despite sequencing of 9 genes. So, further candidate genes for type II LIS, involved in O-mannosyl glycan synthesis remain to be identified.

271-O

A NOVEL GENETIC SCREEN FOR MITOCHONDRIAL PHENOTYPES IN EMBRYONIC STEM CELLS

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Background: Greater than 1000 different proteins are thought to constitute the mitochondrion, however only a small fraction of these are known to cause mitochondrial disorders. In order to identify novel genes that may cause mitochondrial disorders by perturbing basic mitochondrial functions we have developed a retroviral gene trap screen for genes that lead to altered mitochondrial mass and/or membrane potential.
Methods: Mouse embryonic stem cells stably expressing a mitochondrial yellow fluorescent protein (YFP) were infected with a retroviral gene trap vector that requires integration downstream of an endogenous promoter for survival in the presence of a neomycin analogue. Transduced cells were stained with the mitochondrial membrane potential-sensitive fluorescent dye HIDC then subjected to fluorescence activated cell sorting. Cells exhibiting increased HIDC fluorescence were collected and subjected to drug selection. ES cell colonies were picked and analyzed for increased YFP fluorescence. The insertion sites of those clones with increased YFP relative to the parental cell line were identified by inverse PCR.
Results: A variety of categories of genes have been identified, including those involved in signal transduction (PI3-kinase subunit), mitochondrial translation (tRNA synthetase) and mtDNA regulation (*SUCLA2*). As a proof of principle one gene, *SUCLA2*, has been used to generate chimeric mice for whole animal studies.
Conclusions: This approach promises to identify a large number of potential disease-associated genes and corresponding model organisms.

272-P

MITOCHONDRIAL ENERGY GENERATING SYSTEM IN LIVER DURING FOETAL DEVELOPMENT

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Background: Only scare data are available concerning mitochondrial energy generating system in human liver during foetal development.
Aim of study was to analyze respiratory chain complexes (RCC) and pyruvate dehydrogenase (PDH) and the amount of mtDNA in foetal liver tissue. **Material**: Samples of liver tissue were collected from 20 foetuses aborted spontaneously or after genetic indication between 12 and 28 week of gestation. 'Control liver samples' were obtained at autopsy in 10 children at the age between 1 month and 8 years.
Methods: Activities of RCC I, II, III and IV and citrate synthase (CS) were measured spectrophotometrically; PDH activity was analysed radiochemically. Western blot was used for protein analyses. The mtDNA amount was analysed by qRT-PCR.
Results: The protein amount of RCC and PDH was lower in foetal liver in comparison with postnatal tissue. Also activities of RCC I, II, III, IV, PDH and CS were markedly lower in foetal liver in comparison with postnatal tissue regardless of low (10%), high (50%) or moderate (25%) content of haematopoietic cells in liver of foetuses with different gestational age. Amount of mtDNA increased with the increasing week of gestation.
Conclusion: It may be difficult, especially in critically ill neonates, to distinguish properly between the primary genetically encoded disorders of energy provision and the secondary mitochondrial disturbances due to prematurity or environmental factors. Specific age related reference values are necessary for precise diagnostics and genetic counselling in families with deceased child in early neonatal period.

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273-P

CORRELATION BETWEEN ANTHROPOMETRIC PARAMETERS AND MITOCHONDRIAL ATP-PRODUCTION

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Background: Failure to thrive and feeding problems are common in children with primary OXPHOS defects. Secondary deterioration of mitochondrial function has been reported in patients with extreme malnutrition. Optimizing the nutritional state can improve the mitochondrial energy generating capacity in patients with secondary and primary OXPHOS defects. This raises the question if there is a linear correlation between the growth parameters and ATP-production in patients with OXPHOS disorders. **Methods**: Eighty-one patients with a suspected OXPHOS disorder were consecutively evaluated for a possible correlation between antropometric parameters and ATP-production measured in fresh muscle biopsy. The children were divided into two subgroups: with or without associated OXPHPOS-system enzyme complex deficiencies. **Results**: ATP-production rate correlated significantly with weight and height for age in all patients with mitochondrial dysfunction, especially in children without associated complex deficiencies. No significant correlation could be found in the subgroup with decreased ATP production combined with OXPHOS complex deficiencies. **Conclusion**: The biochemical finding of decreased energy production is pathognomonic for primary OXPHOS disorders however mitochondrial dysfunction could also be present secondary to an insufficient nutritional state. We found a correlation between ATP-production and growth parameters in children with the clinical diagnosis of mitochondrial disease. The ATP-production in patients with associated multiple complex-deficiencies was lower when compared to those without complex deficiencies and didn't show a direct correlation with weight and height. Growth parameters and the nutritional state should be taken to account by the interpretation of muscle biochemistry in children with suspected OXPHOS disorders.

274-P

INVESTIGATION OF PAEDIATRIC PATIENTS FOR MITOCHONDRIAL DISORDERS

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We studied the investigation of paediatric patients with suspected mitochondrial disease over a 5 year period. Inclusion criteria: Paediatric patients (under 16 years) investigated between January 2000 and January 2006, who had undergone muscle biopsy.
Results: 15 patients (age at referral to neurology: 10 days to 14 years 11 months) were identified: 3 had a diagnosis (non-mitochondrial disease, MELAS and NARP), 4 required further investigation and in one mitochondrial disease was no longer suspected.

Of the remaining 7 patients, 3 had died without a diagnosis. In the other four, it was not clear whether all the relevant investigations were complete.

These results showed that these children were not being investigated appropriately. The multisystem nature of these disorders was not recognised and there was a lack of diagnosis.

The study highlighted: (1) difficulties in identifying whether investigations were completed, or whether follow-up investigations were in progress (2) problems with handling of the muscle biopsies resulting in misleading complex IV results (3) poor recording of routine assessments (e.g. growth charts) (4) the need for more input from the clinical geneticist.

We suggest that children with mitochondrial disorders within the UK would benefit from investigation protocols that reflect the multisystem nature of these disorders, involving all services throughout the process. In our centre this has been achieved through multidisciplinary development of a rational approach to investigation, audit and yearly review of undiagnosed cases.

275-P

INVESTIGATION OF MITOCHONDRIAL MUTATIONS AND HAPLOGROUP IN IRANIAN PATIENTS WITH PARKINSON'S AND ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is the most common form of dementia in the elderly in which interplay between genes and the environment is supposed to be involved. Parkinson disease (PD) also involves the nervous system, specifically movement and control of muscles. Several reports suggest that mitochondrial (mt) dysfunction may be involved in the expression of AD and PD. Haplogroups could have important implications to understand the association between mutability of the mitochondrial genome and the disease. To assess the relationship between mtDNA haplogroup and AD, we sequenced the mtDNA HVS-I in 30 AD patients and 100 control subjects. We found that haplogroups H and U are significantly more abundant in AD patients ($p = 0.016$ for haplogroup H and $p = 0.0003$ for haplogroup U). Thus, these haplogroups might act synergistically to increase the penetrance of AD disease.

We also studied 20 unrelated PD patients and 113 control subjects for mitochondrial mutation A4336G and mutations in the complete regions of *ND1*, *tRNALeu*, *ND2* and *16s rRNA* by sequencing method. We investigated common deletion in the blood samples of these patients. Our study suggests that the A4336G mutation were not associated with an increased risk of PD in Iranian population, but other mtDNA mutations may contribute the risk factor to idiopathic PD. Lack of association was found between mtDNA haplogroups and PD, so we did not find evidence for the involvement of specific inherited mitochondrial haplogroups in conferring both risk of and protection from the common form of PD in Iranian population.

276-P

DILATED AORTIC ROOT: A PREVIOUSLY UNRECOGNIZED COMPLICATION OF MITOCHONDRIAL DISEASES

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Mitochondrial cytopathies are a genetically, biochemically, and clinically heterogeneous group of disorders associated with abnormalities of oxidative phosphorylation. The heart is highly energy dependent and is particularly vulnerable to defects in energy production. Hyperthrophic and dilated cadiomyopathy and left ventricular noncompaction are among the main cardiac manifestations occurring in mitochondrial cytopathies. Dilated aortic root is typically found in connective tissue disorders, such as Marfan and Ehlers-Danlos syndrome, however, it has not been previously reported in mitochondrial disorders. We found aortic root dilation in five patients with mitochondrial diseases. In all patients the definite diagnosis of mitochondrial disorder was based on the modified Walker criteria. The aortic root dilation was mild to moderate with z-score ranging from +2.67 to +3.65. In at least two cases the aortic root dilation was progressive and required treatment with β-blockers. The screening and follow-up of more patients with mitochondriopathies are necessary to define the real prevalence and the natural history of this newly recognized complication of mitochondrial diseases. The pathomechanism(s) leading to aortic root dilation in mitochondrial disorders is unknown. Based on the association of decreased endothelial nitric oxide (NO) production and progression of aortic complications in the Marfan syndrome animal model, we speculate that NO dysregulation may be involved. Mitochondrial dysfunction may lead to NO dysregulation and increased generation of reactive oxygen species triggering a signaling cascade of apoptosis. Therefore, we propose the increased endothelial and/or smooth muscle cell apoptosis induced by nonfunctioning mitochondria as a potential mechanism for the observed finding.

277-P

CARDIOMYOPATHY IN MITOCHONDRIAL RESPIRATORY CHAIN DISEASE

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Background: Respiratory chain diseases (MRCD) are a potential cause of cardiomyopathy. Cardiac involvement may be the first manifestation of these multisystemic disorders and is associated to poor prognosis.
Methods: In the last decade, 200 children (25 with cardiomyopathy) were evaluated for MRCD in our Hospital. Respiratory chain enzymatic complexes I-V and mitochondrial DNA analysis was done in muscle, liver and/or myocardium. Deficiencies of more than two complexes were considered generalized if involving complex II or multiple if it was not affected. In seven cases another cause for the cardiac pathology was found. According to Thorburn criteria, a primary definite MRCD disorder was diagnosed in thirteen children, whose clinical files were reviewed.
Results: Median age at onset was 7.8 months (first day of life to 2.9 years). Cardiomyopathy, one of the presenting symptoms in four children, was classified as hypertrophic in eight and dilated in five. Extra cardiac involvement was present in twelve cases. Eight children died, six in the first year of life.

Respiratory chain enzymatic complexes were evaluated in muscle, liver and myocardium in 12, 6, and 4 children, respectively. They were normal in three cases. The following deficiencies were found: multiple (three patients), isolated complex IV (three), generalized (two), and complexes I+III or I+IV (one each). No mutations of the mitochondrial DNA were detected so far.
Conclusion: In cardiomyopathy of unknown origin, MRCD should be considered, mostly if other systems are clinically affected. Searching for aetiology is important to plan treatment, establish prognosis and prepare genetic counselling.

278-P

MITOCHONDRIAL DYSFUNCTION IN ASSOCIATION WITH CARDIOLIPIN DEFICIENCY

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Cardiolipin (CL) is an essential component of the inner mitochondrial membrane. CL has a dimeric structure and contains four acyl groups, which are predominantly linoleic acid. Diminished availability of CL could affect mitochondrial function and be a potential cause of mitochondrial dysfunction. Using liquid chromatography-mass spectrometry, we have developed reference ranges for CL in skeletal muscle biopsies. The predominant form of CL was the tetralinoleoyl form and a provisional reference range of 1.2–3.4 nmol/mg protein was generated. CL levels were determined in a muscle biopsy from a 52-year-old male. This patient presented with severe muscle pain, stiffness and weakness. In addition, he has a daughter with a similar clinical picture. A muscle biopsy displayed 2 ragged red fibres and five cytochrome oxidase negative fibres. Respiratory chain enzyme analysis did not reveal an overt deficiency. However, complex II+III activity was elevated. Expression of complex I activity as a ratio to complex II+III indicated a decreased ratio (0.52 – ref range; 0.57–2.91). Analysis of CL revealed reduced CL levels (0.49 nmol/mg protein). In conclusion, we postulate that CL deficiency is associated with loss of mitochondrial function and may be an important contributing factor to the clinical picture described here. A therapeutic trial with linoleic acid is being considered.

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279-O

A LETHAL AUTOSOMAL DOMINANT DEFECT OF MITOCHONDRIAL AND PEROXISOMAL FISSION

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Background: The eukaryotic cell must control the fusion and fission of its organelles in order to maintain an ordered and dynamic subcellular organization. Key proteins in these processes are members of the dynamin superfamily; large conserved GTPases that participate in various cellular processes, including fission of mitochondria and peroxisomes. We identified a defect in this process as underlying cause for a lethal presentation accompanied with microcephaly, abnormal brain development, optic atrophy, hypotonia, persistent lactic academia, and raised plasma very long chain fatty acids.
Methods: Fibroblasts and muscle biopsy of the deceased female infant were subjected to extensive mitochondrial and peroxisomal studies. Genetic studies involved standard DNA sequencing and cDNA over-expression studies.
Results: The patient cells showed a defect in the fission of both mitochondria and peroxisomes due to a single heterozygous, but dominant negative mutation in the *DLP1* gene, which codes for the dynamin-like protein DLP1. Over-expression of the mutant *DLP1* in control fibroblasts resulted in the aberrant mitochondrial phenotype, whereas over-expression of wild-type *DLP1* in the patient fibroblasts reversed the aberrant mitochondrial phenotype to normal. The autosomal dominant inheritance of the observed defect was confirmed by the absence of the mutation in the parental *DLP1* genes.
Conclusions: Our finding represents the first patient from a new class of diseases with a combined defect in both mitochondria and peroxisomes. The combination of clinical symptoms, persistent lactic academia, elevated very long chain fatty acids but normal enzymology may identify additional patients with a defect in mitochondrial and peroxisomal fission.

280-P

EFFECT OF CYCLOSPORINE A ON MITOCHONDRIAL FUNCTION IN ENDOTHELIAL CELLS

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Background: Although CSA is considered to be a good immunosuppressive compound in transplantation, it has been suspected to alter mitochondrial function in various tissues.

We evaluated the effect of CSA on mitochondrial fatty acid oxidation (FAO) and respiratory chain (RC) enzymes in human umbilical vein endothelial cells (HUVEC).
Methods: Overall FAO was measured radiochemically by quantification of the amount of [14C] CO_2 and of acid-soluble products. Additionally, the pattern of acylcarnitines in the medium was determined by tandem MS to assess overall FAO. To determine activities of VLCAD and SCAD, substrates and products were separated and quantified by HPLC. The activity of CPT2 was measured radiochemically. Activities of RC complexes were measured spectrophotometrically. The dose-dependent effect of CSA was tested using 0, 0.1, 1, 10 and 20 μM of CSA.
Results: CSA profoundly altered overall FAO (radiochemically and by acylcarnitine profiling) in intact cultured endothelial cells. First results of RC complex- activities indicated an impaired activity of complexes I +II, I+III and IV in a dose-dependent manner.

The effect of increasing CSA concentrations on VLCAD, SCAD and CPT2 activities was not significant.
Conclusions: It can be concluded that part of immunosuppressive treatment toxicity may arise from alterations in energy metabolism. Although only demonstrated in this study for HUVEC, it is highly conceivable that such an effect may also alter the energy metabolism of other organs like brain, kidney and liver. CSA should probably be used with caution in patients suffering from metabolic diseases with compromised energy metabolism.

281-O

POTENTIAL OF EXISTING DRUGS IN THE TREATMENT OF RESPIRATORY CHAIN (RC) DISORDERS: FIBRATES CAN STIMULATE RESIDUAL CAPACITIES IN RC-DEFICIENT CELLS

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The mitochondrial respiratory chain (RC) disorders are the largest group of inborn errors of metabolism and still remain without treatment in most cases. We tested whether bezafibrate, a drug acting as a peroxisome proliferator activated receptor (PPAR) agonist, could stimulate RC residual capacities in four patient cell lines carrying mutations in the Fp (flavoprotein, complex II, CII), or *BCS1* (complex III, CIII), or *SURF1* (complex IV, CIV), or *COX10* (CIV) genes. Exposure to bezafibrate (400 μM, 72 h) was found to increase (+38 to +50%) the CII, CIII and CIV activities in control fibroblasts, and immunoblots showed parallel increases (+82 to +150%) in Fp, Core 2, SURF1, COX2 and COX4 protein levels. A similar treatment by bezafibrate improved RC capacities in BCS1- and COX10-deficient fibroblasts, as indicated by the stimulation of CIII (+133%) and CIV (+71%) enzyme activity, and by the higher expression of representative proteins. This was related to a drug-induced augmentation in the mRNA of the mutated *BCS1* or *COX 10* gene. Fp and *SURF1* mRNA were also induced by bezafibrate, but without changes in RC residual capacities due to the mutated protein instability. Additional data were obtained in myoblasts from patients presenting a myopathic form of COX deficiency with unknown molecular basis in which bezafibrate fully corrected the COX defect (–60% compared to control). These data indicate that bezafibrate trigger the expression of deficient RC complexes in patient cells and therefore suggests a possibly new approach for the correction of moderate RC disorders due to mutations in nuclear genes.

282-P

IDEBENONE TREATMENT IN PEDIATRIC AND ADULT PATIENTS WITH FRIEDREICH ATAXIA: LONG-TERM FOLLOW-UP

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Background: Antioxidant therapy is a new therapeutical approach for patients with Friedreich ataxia. We study the effectiveness of long term idebenone treatment in Friedreich ataxia patients.
Methods: Open-labeled non randomized study. Ten pediatric patients (age range 8–18 years) and 14 adults (age range 18–46 years) with genetic diagnosis of Friedreich ataxia were treated with idebenone (10–20 mg/kg/day) for 3 to 5 years. Neurological evolution was evaluated using the International Cooperative Ataxia Rating Scale (ICARS), and cardiological outcome using echocardiography.
Results: In pediatric patients, no significant differences were observed in ICARS scores and echocardiographic measurements when comparing baseline status and after 5 years of follow-up. Concerning adult cases, significant differences were observed when comparing ICARS score at baseline and after 3 years of idebenone therapy (Wilcoxon test, $p = 0.005$), while hypertrophic cardiomyopathy was not modified.
Conclusions: The effect of idebenone was remarkable for heart parameters. Echocardiographic measurements in several patients remained within reference ranges during five years and can influence in the mortality of this disease. Neurological outcome was stable or improved in half of our pediatric patients, but not in adults, suggesting that age when idebenone treatment is started may be a critical factor in the effectiveness of the therapy. Improvement on oculomotor disorders, in pediatric patients and stabilisitation in adults reduced the morbidity of our patients. Considering the lack of adverse effects, an increment in oral idebenone to 20 mg/kg/day doses should be recomended. Improvement on morbidity and reduced mortality is expected in Friedreich patients on this antioxidant therapy.

283-P

MITOCHONDRIAL DNA MUTATIONS IN PATIENTS WITH PROBABLE MITOCHONDRIAL DISORDERS

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Mitochondrial disorders are increasingly being diagnosed among patients with multiple, seemingly unrelated neuromuscular and multi-system disorders. The clinical features presented are usually nonspecific such as seizure, developmental delay, poor feeding, lethargy, floppy, optic atrophy, acute metabolic crisis and neuro-degeneration, which often make diagnosis a considerable challenge. The most common biochemical marker is abnormal organic acid profile. It is well known the molecular defect can either be of mitochondrial DNA or nuclear DNA associated with mitochondrial functions. In developing countries, diagnostics facility such as respiratory chain enzyme analysis is not easily available. We are investigating the yield of using DNA investigation as the first line of approach. We have analyzed blood samples from 123 patients with clinical features suggestive of mitochondrial disorders, requested for whole mitochondrial DNA mutations. Mutation detection was first carried out on known mutation hotspots for Leigh syndrome and our subsequent strategy involved bi-directional sequencing of whole mitochondrial genome followed by nuclear gene analysis. We report here our findings on sequence variations in mtDNA in nine (7.3%) patients, three (33%) of which are known mutations and six (67%) are probable novel mutations. One patient is identified as having hotspot mutation for LS while another with hotspot mutation secondary to LHON. Novel mutations are detected in ATP 6 (1), ND5 (1), ATP8 (2) and ND1 (2). In conclusion, stepwise investigation with respiratory chain enzymatic and proteomic analysis should still be considered as first line of investigation prior to DNA mutation analysis. This strategy would reduce costs incurred considerably.

284-P

DIAGNOSTIC DIFFICULTIES IN PATIENTS WITH mtDNA DELETIONS IN MUSCLE BIOPSY

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Mitochondrial DNA large-scale deletions are common findings in muscle of patients with several mitochondrial disorders but they may be also present in patients with non-mitochondrial disorders or as the consequence of aging.

Material and methods: In 93 patients at the age between 9 and 73 years (49 females, 44 males) with mtDNA large-scale deletions detected in muscle biopsy using amplification of the whole mtDNA (LX-PCR), we evaluated the relationship between clinical symptoms, results of spectrophotometric and protein analyses and histochemical findings.
Results: Molecular analyses recognized the primary mitochondrial disorders in 14 patients (10 Kearns-Sayre, one MNGIE, 2 MELAS, one MERRF syndrome). Profound deficiency of respiratory chain complexes (RCC) – isolated or combined – was found in 21 patients with mitochondrial myopathy combined mostly with encephalopathy, ptosis, visual impairment, cardiomyopathy or deafness. Using histochemical methods, non-mitochondrial disorders(muscular dystrophies, polymyositis, steroid myopathy, nonspecific myopathic changes) were found in 22 patients, but in seven of them had a significant decrease of one or more RCC activities. In the last group of 36 patients with mtDNA deletions and exercise intolerance, muscle pain, ptosis or visual disturbances the activities of RCC were only mildly decreased or even normal.
Conclusion: The results of our study indicates, despite of the finding of mtDNA large-scale deletions in muscle biopsy, that the distinguishing between the primary and the secondary mitochondrial disorders remains difficult, at least in patients without molecular diagnosis.

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285-P

VISUALIZING OXIDATIVE PHOSPHORYLATION DEFECTS IN SKELETAL MUSCLE OF PATIENTS WITH MITOCHONDRIAL tRNA GENE MUTATIONS USING IMMUNOHISTOCHEMISTRY

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Background: Various clinical phenotypes originate from disturbed protein synthesis caused by mutations in the mitochondrial DNA (mtDNA) encoded tRNA genes. The myopathological abnormalities associated with tRNA gene mutations are most often non-specific. We evaluated oxidative phosphorylation (OXPHOS) immunodetection in patients with different mtDNA encoded tRNA gene mutations, to identify common and specific staining patterns. **Methods:** Skeletal muscle biopsies were obtained from controls ($n = 10$), and patients ($n = 6$) with mutations in mtDNA tRNA genes causing different muscle disorders. Sections from frozen and paraffin embedded tissues were immunostained with antibodies directed against OXPHOS subunits, and markers for mitochondrial proliferation. **Results:** Antibodies best suited for OXPHOS immunohistochemistry were directed against four nuclearly encoded (NDUFS7, SDHB, UQCRC2 and ATP5A1) and one mtDNA encoded (MTCO1) gene product. Normal skeletal muscle displayed limited variation in staining intensity due to the different mitochondrial load of myofibers type I and II. In contrast, sections from patients displayed a heterogeneous staining pattern for complex I (6/6) and IV (4/6): a mosaic of negative and positive myofibers along side, and all possible staining intensities in between. Ragged red fibers (RRFs) could be identified as complex II, III and V hyper-reactive fibers, and with antibodies against porine and double stranded DNA. In MERRF patients, all RRFs were MTCO1– NDUFS7–, while in MELAS patients the majority of RRFs were MTCO1+ NDUFS7–. **Conclusions:** We concluded that immunohistochemistry could represent a valuable additional diagnostic tool. All six patients, including two patients that stayed undetected with routine histochemical evaluation, showed deficient immunostaining for complex I.

286-P

EARLY ONSET SEVERE MYOPATHY DUE TO THE A3302G MUTATION IN THE MITOCHONDRIAL tRNA LEU(UUR)

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Background: The mitochondrial tRNA mutation A3302G leads to abnormal mitochondrial RNA processing and is associated with adult onset progressive myopathy and cardiorespiratory problems. **Objective**: To present another patient with this tRNALEU(UUR) mutation with an early onset, severe phenotype. **Case:** The patient was first investigated at the age of 4 years because of increased fatigability since two years, but otherwise normal psychomotor development and normal cardiac function. At rest there was a generalized moderate hypotonia but after a short activity strong fatigability and loss of head control occurred. The disease progressed rapidly to a severe myopathy with respiratory involvement. Investigations: lactic acidosis, elevated CK and paradoxical ketosis were present; cerebral MRI/MRS was normal. Muscle biopsy showed ragged red fibers and 10% residual complex I (CI) activity. Analysis of the mitochondrial genome from skeletal muscle revealed a homoplasmic A3302G transition in the tRNA. Fibroblasts CI activity was only slightly below the control range, which correlated with the respirometric findings. **Discussion**: This mutation is usually associated with a juvenile or adulthood onset, slowly progressive myopathy. This patient enlarges the clinical spectrum of this mutation, being the youngest patient described with a severe phenotype. Similarly to the other published cases, this mutation affects CI activity in skeletal muscle but not in fibroblasts. The severity of the phenotype may by related to the mutation load of the affected tissue. **Conclusion**: This patient widens the clinical spectrum caused by this specific mt tRNA mutation, with important consequences for genetic counselling in affected families.

287-P

INVESTIGATION ON MITOCHONDRIAL tRNALEU/LYS, NDI AND ATPASE 6/8 IN IRANIAN PATIENTS WITH NEURODEGENERATIVE DISORDERS

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As with chromosomal DNA, the mitochondrial DNA (mtDNA) can contain mutations that are highly pathogenic.In fact, many diseases of the central nervous system are known to be caused by mutations in mtDNA. Dysfunction of the mitochondrial respiratory chain (RC) has been shown in patients with neurological disease including Huntington disease (HD), multiple sclerosis (MS), Friedreich's ataxia (FA) and Ataxia telangiectasia (AT). Considering this importance, we decided to investigate several highly mutative parts of mtDNA for point mutations as MT-LTI (tRNALeucine1(UUA/G)), MT-NDI (NADH dehydrogenase subunit 1), MT-COII (cytochrome c oxidase subunit II), MT-TK (tRNALysine), MT-ATP8 (ATP synthase subunit 8) and MT-ATP6 (ATP synthase subunit 6) in 80 Iranian patients affected with MS, FA, HD and AT (20 in each group) and 80 age-matched control subjects by PCR and automated DNA sequencing to evaluate any probable point mutations. Our results revealed some new mutations in each disease. This study suggests that mitochondrial mutations may be important in pathogenesis of these neurodegenerative diseases. Understanding the role of mitochondria in the pathogenesis of neurodegenerative diseases could potentially be important for the development of therapeutic strategies.

288-O

THE VALUE OF FUNCTIONAL INVESTIGATION OF INTACT MITOCHONDRIA FOR THE DIAGNOSIS OF MITOCHONDRIAL DISORDERS

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The mitochondrial energy metabolism consists of various enzymatic reactions, several of them are transport processes and depend on functionally intact mitochondrial membranes. However, diagnostics of mitochondrial disorders is based on morphological and biochemical investigation of frozen tissue in many centers.

We investigated intact mitochondria from 273 fresh muscle biopsies by respirometry and/or radiochemical substrate oxidation analysis. In 51 patients a defect of the mitochondrial energy metabolism was detected. 34 of them had respiratory chain defects, 11 a disturbance of pyruvate oxidation and 6 had a deficiency in ATP synthesis. 5 patients with deficient ATP synthesis had a decreased amount of the F1Fo-ATP synthase protein in Blue Native polyacrylamide electrophoresis. One turned out as a novel defect of the mitochondrial phosphate carrier. Investigation of pyruvate dehydrogenase complex revealed normal activity in 3 patients who showed a deficiency in pyruvate oxidation. This points to another novel disorder, probably in the import of pyruvate.

Taken together 1/3 of our patients with defective energy metabolism had disorders of either the ATP synthesis or pyruvate oxidation. 23% of them had normal activities in single enzyme investigations from frozen material. These data underline the importance of functional investigations of intact mitochondria. Patients with clinical symptoms indicative for a mitochondrial disease and normal respiratory chain enzymes should be considered for functional investigations of intact mitochondria.

289-P

SEVERE INFANTILE ENCEPHALOPATHY AND COMPLEX I DEFICIENCY ASSOCIATED WITH NOVEL MUTATIONS IN mtND1

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Pathogenic missense mutations in the mtDNA encoded subunits of complex I of the respiratory chain have been associated with disorders such as Leber hereditary optic neuropathy, MELAS and Leigh syndrome.

We have investigated two children with complex I deficiency in muscle mitochondria. Patient one had congenital lactic acidosis and developed profound learning disability. Additional features were postnatal growth retardation and later she developed hypertrophic cardiomyopathy. She died at 26 years of age because of cardiac insufficiency. Patient two had a mild developmental delay, cerebellar ataxia and squint since early infancy. At 2 years of age during a gastroenteritis she suddenly deteriorated with unconsciousness and respiratory insufficiency. Laboratory investigations showed increased lactate levels in blood and CSF. MRI of the brain showed increased signalling on T2-weighted sequences from nucleus subthalamicus and the brain stem compatible with Leigh syndrome. The course was rapidly progressive with frequent exacerbations and death at 2 years and 10 months of age.

Sequencing analysis of the mitochondrial encoded ND genes (MTND) showed a novel de novo mutation in MTND1 in both patients. Patient one had a heteroplasmic (36% in muscle) G3481A mutation, which is predicted to change a glutamic acid to lysine (E59K). Patient two had a heterplasmic (95% in muscle) G3890A mutation, predicted to change an arginine to glutamic acid (R195Q). These mutations add to previously identified mutations in MTND1 and the results in patient one show that MTND mutations may be pathogenic even at low levels of heteroplasmy.

290-P

DIAGNOSIS OF PAEDIATRIC PATIENTS WITH COENZYME Q10 DEFICIENCY

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Objective: To diagnose CoQ deficiency in muscle biopsies of paediatric patients with mitochondrial disorders.
Material and Methods: We studied 140 muscle biopsies of paediatric patients with suspicion of a mitochondrial disease (age range: 1 month–18 years). **Results** were compared with 28 muscle biopsies of pediatric controls (age range: 2–16 years). We determined the total CoQ concentrations by HPLC with electrochemical detection and mitochondrial respiratory chain (MRC) enzyme activities were determined using described spectrophotometric methods.
Results: Five patients presented muscle CoQ deficiency (range 1.1–1.9 nmol/citrate synthase units; Reference values: 2.2–7.9 nmol/citrate synthase units). CoQ deficiency was confirmed in fibroblasts from all cases (range: 20–50% of CoQ deficiency compared with the lower limit of reference values). Clinical phenotype was very broad, including mild ataxic forms, a severe epileptic encephalopathy, rabdomiolisis and a case with a fatal hepathopathy presenting a mitochondrial DNA depletion (74%). Investigations of mutations in genes related with CoQ biosynthesis are being performed.
Conclusion: Determination of total CoQ concentration in cultured fibroblasts is a useful tool for the diagnosis of CoQ deficiency. CoQ deficiency was associated with different clinical phenotypes, including a mitochondrial DNA depletion syndrome.

291-P

BLUE NATIVE POLYACRYLAMIDE GEL ELECTROPHORESIS IS A POWERFUL TOOL FOR SCREENING OF MITOCHONDRIAL RESPIRATORY CHAIN DISORDERS

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Background: Congenital and primary lactic acidosis is one of the most frequent inborn errors of metabolism, of whom only 30% have had its precise cause identified. Our aim is to make a prompt and correct diagnosis of mitochondrial respiratory chain disorders, the most frequent cause of congenital lactic acidosis, using Blue Native polyacrylamide gel electrophoresis (BN-PAGE).
Methods: Mitochondria were isolated from skin fibroblasts of 25 candidate patients, solubilised in n-dodecyl-maltoside and subjected to 4–13% BN-PAGE and western blotting using monoclonal antibodies specific for Complex I to IV subunits. Using densitometry,%assembled Complex I relative to Complex II was calculated. In gel enzymes straining for Complex I, II and IV, V were also performed.
Results: Abnormal assembled pattern for Complex I was identified in 13 out of 25 cell lines. Among those, four groups were categorized by amount and size of assembled Complexes. Grossly deficient of fully assembled complex I of 900 kDa species ($<30\%$) was in 2 cell lines, moderately decreased of 900 kDa species (30–70%) was in 8 cell lines, smaller sub-supercomplexes was in 2 cell lines and deficient of all respiratory complexes was in 1 cell line.
Conclusion: BN-PAGE is a useful guide to prompt and correct diagnosis, and future molecular analysis for categorizing respiratory chain disorders.

292-P

DETECTION OF COMPLEX III DEFICIENT PATIENTS USING A NOVEL ACTIVITY STAINING METHOD IN THE BN-PAGE GEL

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Background: Blue Native polyacrylamide gelelectrophoresis (BN-PAGE) has proved to be a powerful tool in the study of oxidative phosphorylation (OXPHOS) deficiencies. Following BN-PAGE the enzyme activities of the OXPHOS complexes I, II, IV and V can be tested using specific staining solutions. Until presently, there was no staining method to test ubiquinol cytochrome c oxidoreductase (complex III) activity in the gel. Our objective was to develop a stable staining method for visualisation of complex III activity following BN-PAGE, in mitochondrial isolates obtained from different human tissues.
Methods: Mitochondria isolated from skeletal muscle, heart, liver and cultured skin fibroblasts were solubilized and separated using BN-PAGE. In-gel activity staining of complex I, II, IV and V was performed as previously described. A method for visualization of complex III activity was developed based on the peroxidase activity of the heme group.
Results:We examined the possibilities of this new staining method on a variety of tissues obtained from controls and from patients with deficient complex III activity. Mitochondrial isolates from patients with a mutation in the human bc1 synthesis like gene (*BCS1L*), patients with mtDNA depletion caused by selective mutations in the deoxyguanosine kinase (*DGUOK*) and mitochondrial DNA polymerase gamma (*POLG*), and one patient with a mutation in the tRNATyr gene were tested. In all these patients, reduction of complex III activity can be demonstrated by this staining method.
Conclusions: In addition to spectrophotometry, this new staining technique was shown to be a valuable instrument to detect and study complex III deficient patients.

293-P

A NOVEL HETEROPLASMIC MUTATION IN COXI LEADS TO DEVELOPMENTAL DELAY, SEIZURES AND STROKE-LIKE EPISODES

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An eleven-year-old female presented initially with a seizure followed two months later with tonic-clonic seizures, weakness and aphasia. MRI of the cerebral hemispheres showed multiple infarcts. Previous history suggested gross and fine motor control deficits with learning difficulties. A muscle biopsy performed because of persistently increased blood lactate showed a specific decrease of cytochrome oxidase (COX) with decreased COX staining in all fibres and pleomorphic mitochondria. Family history showed numerous individuals with IDDM on the paternal lineage but only mild learning problems appearing in two individuals on the maternal lineage. Fibroblasts showed an initial COX activity below normal which rapidly came up to the normal range on culture. Sequencing of mtDNA revealed an heteroplasmic 7023G>A mutation in COX I. The heteroplasmy was (96% in muscle, 70% in blood, 50% in the initial skin fibroblast culture dropping to 10% in later passages.. The mutation was present in the critical region of the COXI gene, the V373M change being close to the two histidine residues, His376 and His378 co-ordinating with the Heme a and a3, and histidine 367 which co-ordinates a magnesium ion. This case a shows that protein coding mutations in mtDNA can decrease rapidly in conditions of normal tissue culture and that a MELAS-like syndrome can occur with isolated COX deficiency.

294-P

THE PRESENCE OF COMPLEX V SUBCOMPLEXES IN PATIENTS WITH DEFECTIVE INTRAMITOCHONDRIAL PROTEIN TRANSLATION

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Background: Following Blue Native polyacrylamide gel electrophoresis (BN-PAGE), the activities of the OXPHOS complexes can be visualized. In part of the patients with OXPHOS defects, catalytically active subcomplexes of complex V are detected. Complex V is peculiarly fragile to defects in mitochondrial protein synthesis, as its two mtDNA encoded structural subunits are anchoring proteins linking the catalytic domain to the mitochondrial inner membrane. We speculated that complex V subcomplexes are selective indicators of defects in mtDNA affecting intra-mitochondrial protein synthesis.
Methods: To test this hypothesis, we evaluated in retrospect the BN-PAGE activity patterns in tissue samples from the patients with either deficient OXPHOS complex activities documented by spectrophotometric analysis and from the patients suspected on a clinical basis of having a mitochondrial defect. The BN gels were incubated with specific staining solutions to evaluate OXPHOS complex activities, and to detect catalytically active complex V subcomplexes. Patients with complex V subcomplexes were screened for abnormalities in the mtDNA.
Results: More then 460 tissue samples originating from 440 patients (227 muscle samples, 186 fibroblast strains and 51 heart and liver samples) were investigated. In 51 tissues originating from 42 patients, complex V subcomplexes were detected. In 22 of these patients (53%), a defect in mtDNA was detected: tRNA mutations (10), mtDNA deletion (1), ATP6 mutation (4), and mtDNA depletion (7).
Conclusions: BN-PAGE combined with in-gel activity staining is a good screening method for detection of OXPHOS defects resulting from mtDNA alterations. The presence of complex V subcomplexes predicts defective intra-mitochondrial protein synthesis.

295-P

MICRODELETION 9205ΔTA IN *ATP6* GENE OF MITOCHONDRIAL ATP SYNTHASE

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Missense mutations in mtDNA *ATP6* gene are frequent cause of severe dysfunction of mitochondrial ATPase manifesting as NARP or MILS syndromes. We have investigated another type of *ATP6* mutation, 2bp microdeletion 9205ΔTA that presents as severe encephalopathy. The mutation alters ATP6 stop codon and prevents processing of the *ATP6/COXIII* bicistronic transcript and thus down-regulates the biosynthesis of ATP6 (subunit a) and COXIII subunits. As a result, patient mitochondria contain incomplete complexes of ATPase and decreased amount of cytochrome c oxidase (Jesina et al., Biochem J. 2004;383:561–71). We have found that ATP6-deficient fibroblasts display marked instability of the ATPase complex upon solubilisation with increasing concentration of mild detergent laurylmaltoside that was apparent as decrease in 'full size' Fo-F1 complex but accumulation of unstable subcomplexes of ATPase. Enzyme solubilisation was also associated with pronounced loss of ATPase activity. ATP6-deficient patient cells further showed increased levels of mitochondrial membrane potential at state 3-ADP and increased content of mitochondrial Mn-SOD while the cytoplasmic Cu/Zn-SOD was unchanged. Construction of transmitochondrial cybrids with microdeletion 9205ΔTA fully confirmed mitochondrial origin of the defect and allowed for characterization of the enzyme defect dependence on the 9205ΔTA mutation load using the cybrid clones carrying 50–100% of mutated mtDNA. Our results demonstrate that mitochondrial disorder due to low content of ATP6 subunit leads to altered interaction between F1 and Fo parts of the enzyme. Upregulation of Mn-SOD indicates that inefficient discharge of mitochondrial membrane potential by nonfunctional ATP synthase results in increased ROS production, similarly as in other types of ATPase disorders.

296-P

ATP SYNTHASE DEFICIENCY PRESENTING AS SEVERE NEONATAL LACTATE ACIDOSIS, NON-COMPACTION CARDIOMYOPATHY AND 3-METHYLGLUTACONIC ACIDURIA – A NEW CLINICAL ENTITY?

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Background: The characteristic symptom triad of severe neonatal lactic acidosis, neonatal non-compaction cardiomyopathy (NCCM), and 3-methylglutaconic aciduria (3-MGA) may represent a new clinical entity caused by mitochondrial ATPase deficiency.
Results: In a gypsy pedigree, three siblings (two females, one male) presented with lactic acidosis, NCCM, and 3-MGA at birth. There were two further abortions and two healthy sibs. The affected male died at the age of 2 weeks. 3-MGA was present in the clinically non-affected mother. Diagnostic work-up for mitochondriopathies (respiratory chain complexes I-IV) and Barth syndrome was normal. Blue native PAGE and SDS-PAGE in muscle tissue from the index patient revealed an almost complete deficiency of the mitochondrial ATP synthase, both at the protein and activity level. The uniform clinical course of the disease is characterized by severe neonatal onset, with predominant insufficient myocardial function, and subsequent stabilization after surviving the critical neonatal phase. The lactic acidosis disappears but 3-MGA persists. Improvement of myocardial function in the index patient was concordant with idebenone treatment. The 7-year-old sister is mentally retarded but otherwise clinically well. Others have observed the same symptom triad in several other cases. All cases were similar in their clinical course, the ATPase deficiency, and the genetic background of gypsy ancestry. The molecular basis has so far not been elucidated.
Conclusion: Neonates presenting with lactic acidosis, NCCM, and 3-MGA should be investigated for mitochondrial ATPase deficiency. All treatment attempts should be undertaken to enable affected patients to survive the critical neonatal period.

297-P

PYRUVATE DEHYDROGENASE COMPLEX DEFICIENCY: IDENTIFICATION OF FOUR NOVEL MUTATIONS IN PORTUGUESE PATIENTS

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The catalytic activity of the pyruvate dehydrogenase complex (PDHc), an intramitochondrial multienzyme system, which catalyses the physiological oxidative decarboxylation of pyruvate to acetyl-CoA, is maintained by four subunits: E1 (alfa and beta), E2, E3 and E3BP.

The present report refers to the study of three Portuguese patients, in whom clinical and biochemical evaluations were compatible with PDHc deficiency, Molecular characterization leads to the identification of four different novel mutations located in different genes (*PDHA1, PDX1* and *DLD*).

Case 1: 6-year-old boy, with mild neurological involvement, who displayed a low PDHc activity (11% activity of normal control) with concomitant absence of immunoreactive E1alfa protein; genomic DNA and cDNA analysis of *PDHA1* gene revealed a novel mutation corresponding to an AGA→GGA transition, at nucleotide 757, that originate a mutant protein with a glycine replacing arginine at codon 224 of the mature protein (R224G).

Case 2: 6-year-old female patient with neonatal lactic acidosis and PDH deficiency (21.5% activity of the day control); molecular approach revealed a novel homozygous missense mutation CGA→TGA in the *PDX1* gene that introduces a premature codon stop (R284X), producing a truncated E3BP shorter than normal.

Case 3: 13-year-old girl with neonatal hyperlactacidaemia and concomitant low PDHc activity (19% activity); direct sequencing of cDNA *DLD* gene revealed a compound heterozygoty: a splice mutation inducing the expression of the whole intron 13 and one point mutation (AGA→GGA at nucleotide 459), substituting arginine for glycine at position 154 (R154G).

298-P

FUNCTIONAL ANALYSIS OF TWO NOVEL SYNONYMOUS MUTATIONS OF THE PYRUVATE DEHYDROGENASE E1 ALPHA GENE (*PDHA1*)

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Background: Mutational analysis of *PDHA1* gene only disclosed silent single nucleotide substitutions of exon 5, i.e. [c.483C>T; pTyr161Tyr] and [498C>T; pIle166Ile] variants, respectively, in two unrelated boys and a girl with reduced pyruvate dehydrogenase complex activity and immunoreactive E1 alpha protein in cultured fibroblasts. Silent mutations within exons may cause aberrant splicing by disrupting exonic splicing enhancers (ESE) motifs in the vicinity of non-consensus splice sites. **Methods** and **Results:** Analysis of transcripts in emetine-treated fibroblasts of patients revealed the presence of both normal and truncated cDNAs with splicing out of exon 5, that was predicted to result in a frameshift and premature termination (p.Gly140fs10X), which may cause nonsense mRNA mediated decay. In silico analysis revealed that each variant disrupted a SRp55 binding site and that intron 5 donor splice site (5′ss) contained a weak splicing signal. Transient transfection of hybrid minigene constructs, containing wild-type or mutant *PDHA1* exon 5, in COS-7 cells or HELA cells and study of RT-PCR products demonstrated that each variant resulted in incomplete inclusion of *PDHA1* exon 5 in the cDNA, corrected when a perfect consensus sequence of the 5′ ss was rescued by site-directed mutagenesis. **Conclusion:** It is proposed that the weak intron 5 donor site of *PDHA1* is normally compensated for by an ESE. The two novel synonymous mutations described herein expand the spectrum of the rare *PDHA1* gene mutations affecting non canonical splice sites. Only one silent mutation causing exon 6 skipping has been previously reported (Kupper et al., 2002).

299-P

SOMATIC MOSAICISM FOR A *PDHA1* MUTATION IN A FEMALE WITH PYRUVATE DEHYDROGENASE DEFICIENCY

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Background: Pyruvate dehydrogenase deficiency was diagnosed in a girl with cerebral malformation, hypotonia and elevated blood and CSF lactate. Activity in fibroblasts was ∼50% of normal, with a mosaic pattern of E1alpha subunit immuno-staining. Screening of PDHA1 cDNA and the exons and intron-exon boundaries gave only normal sequence, apart from heterozygosity for a 4 bp insertion in intron 10. This insertion was present in genomic DNA from the mother, who has normal enzyme activity and immunochemical staining.

Methods: Normal and deficient clones of transformed patient fibroblasts were generated for further genetic analysis.

Results: Sequencing of cDNA from a deficient clone revealed an insertion of 121 bases of intron 9 in the coding region. Genomic DNA from this clone had a G>A substitution in the acceptor splice site of intron 9, resulting in activation of a cryptic upstream splice site. This mutation was not present in a clone with normal enzyme activity. The primary fibroblasts were reinvestigated and detailed analysis showed that the patient is a somatic mosaic with three different alleles: a normal paternal allele, a maternal allele with the intron 10 insertion and a second maternal allele with both the intron 10 insertion and the splicing mutation. The patient's karyotype was normal.

Conclusions: Genetic diagnosis in this unique case of *PDHA1* somatic mosaicism was complicated by absence of the abnormal transcript in primary fibroblasts and the presence of three different alleles. The fortuitous co-existence of an intronic insertion on the maternal X chromosome was key to the mutation detection.

300-P

OUR EXPERIENCE AND UNRESOLVED QUESTIONS IN THE DIAGNOSIS OF PDH-E1 DEFICIENCY

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The most commonly reported causes of PDH deficiency are E1α defects caused by mutations in *PDHA1* which is X-linked. We screened for *PDHA1* mutations in genomic DNA from 51 patients with PDH deficiency, and found changes in the *PDHA1* sequence of twenty five. Seven patients presented with five known mutations: p.R127Q, p. R263G, p.R378C, p.R302C, and c.1145-1146ins4. The latter two changes were found in two patients each: three heterozygous girls and a mosaic male for p.R302C. We detected another eight mutations: p. Y243del, p.H113D and p.Y369Q which have only been reported in our patients (Lissens et al 2000), and p.P172L, p.A169V, p.A198T, c.1143-1144ins24 and IVS5-30G>A are new. This latter mutation is a new undescribed cause of exon 6 skipping. Only one of the 18 mothers was found to be a carrier (p.R263G). Mutation p.A198T is present in a girl who is compound heterozygous with UTR5′-98C>T; this is an undescribed change absent in 100 controls but it is also present in the patient's mother.

In two patients with clear PDH deficiency, the only alterations detected in genomic DNA were the silent changes c.498C>T (p.I166I) and c.396A>C (p.R132R). The other eight PDH deficient patients carry combinations of infrequent polymorphisms (UTR5′-88G>A, IVS1-84C>T and the minihaplotypes [IVS7+26(ggccaa)2/IVS7-15A/c795G] and [IVS7+26(ggccaa)4/IVS7-15C/c795A]). The overrepresentation of these polymorphisms among our 51 PDH deficient patients is remarkable: UTR5′-88G>A in 7.8% and the minihaplotypes in 23.5%, as compared to controls (3% and 10.5%, respectively). The importance of these changes on PDH activity is unclear.

301-P

MRI FINDINGS IN THREE PATIENTS WITH PDHC (E1α) DEFICIENCY AND KETOGENIC DIET

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Ketogenic diet is generally accepted as a standard treatment of pyruvate dehydrogenase complex (PDHC) deficiency. Two mechanisms may play a role in the therapeutic efficacy: (1) provision of alternative energy sources for CNS and other tissues by increasing ketone bodies and free fatty acids, and (2) reduction of lactate and pyruvate in blood and intracellular fluids due to removal of exogeneous carbohydrates.

However the use of ketogenic diet is based only on few uncontrolled case reports. Furthermore improvement of brain MRI lesions was described only in one patient with Leigh disease (Neuropediatrics 1992;23:147–52) and the question remains whether this was due to spontaneous regression or consequence of therapy.

We describe three boys, with PDHC deficiency and mutations in the *PDHA1* gene (Ile368.Ile384dup/R88C/R263G, respectively). Age at onset was 6 months/2 years/2 years, all patients had clinical signs and initial MRI findings compatible with Leigh syndrome. A standardized ketogenic diet was started at age 13 months/5 years/4 years, composition 5:1/3:1/4:1. All patients showed a clear clinical improvement over a follow up of 4/3/3 years and no severe metabolic crisis. MRI after one and/or more years of treatment clearly demonstrated reversibility of brain lesions to a certain extent in all patients.

In summary, the findings in our patients do not replace a systematic study, which would be necessary to clarify the value and optimal composition of ketogenic diet in PDHC deficiency. Nevertheless clinical improvement and stability of the patients together with the regression of CNS lesions prove the effectiveness of ketogenic diet.

302-O

THIAMINE-RESPONSIVE PYRUVATE DEHYDROGENASE DEFICIENCY

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A small number of patients with pyruvate dehydrogenase (PDH) deficiency, have responded significantly to high doses of thiamine. All have all had mutations in the gene for the E1 alpha subunit, but the reason for the thiamine responsiveness has not always been established.

PDH kinetic studies and mutation analysis were performed on cultured fibroblasts from a boy who presented at 2.3 years with an episode of acute ataxia. Prior to this, developmental delay had been noted. He had two subsequent ataxic episodes, and was observed to be hypotonic with poor head control and difficulty walking. Lactate was mildly raised in blood and CSF. On a high fat diet and 100 mg thiamine thrice daily, his development progressively improved and he resumed walking. There have been no further ataxic episodes.

PDH activity was within the normal range, even without thiamine pyrophosphate (TPP) in the reaction mixture, however the activity decreased to a greater extent in the absence of added TPP than in normal controls. A missense mutation in the *PDHA1* gene resulting in substitution of methionine for isoleucine 87 was identified. Missense mutations in the adjacent amino acids 88 and 89 have been found in other thiamine-responsive patients, although this region is not directly involved in TPP binding.

The relatively mild presentation and normal in vitro enzyme activity suggests that the patient's mutation only minimally impairs cofactor binding or function. Detailed kinetic analysis and comparison between adjacent mutations will help to determine why these patients respond so favourably to thiamine supplementation.

303-P

UNSUCCESSFUL TREATMENT OF SEVERE PYRUVATE CARBOXYLASE DEFICIENCY WITH TRIHEPTANOIN

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Pyruvate carboxylase has an anaplerotic role in the Krebs cycle. Triheptanoin, citrate and aspartate have been used as 'anaplerotic therapy' in pyruvate carboxylase deficiency. The only previous patient receiving triheptanoin appeared to benefit but died aged 6 months (Mochel et al., 2005).

Our patient presented aged 8 h with lactic acidaemia (20 mmol/L), ketonuria and hyperammonaemia (180–330 μmol/L). Plasma citrulline was elevated with a decreased glutamine. Pyruvate carboxylase deficiency was demonstrated in fibroblasts (0.42 nmol/h/mg protein, reference range 6–40). Cranial MRI showed a periventricular cyst, abnormal myelination and hypoplasia of the pons and cerebellum. The blood lactate concentration stabilised at 10 mmol/L but there were recurrent episodes of severe metabolic acidosis, requiring intravenous bicarbonate (up to 100 mmol/kg/day). During these, the lactate concentration changed little, the plasma 3-hydroxybutyrate concentration increased to around 2.7 mmol/L and the urine was inappropriately alkaline (pH 6.2), indicating renal tubular acidosis.

After treatment with citrate, aspartate and dichloroacetate, the plasma ammonia fell to normal but there were no other changes. Triheptanoin was commenced at 3 weeks without side effects (30–45% total energy in a modular feed). The episodes of severe acidosis continued. There was no improvement in plasma or CSF lactate or glutamine concentrations.

The patient showed no psychomotor progress. She had sensorineural deafness, cortical visual impairment and mild optic atrophy. MRI brain at 5 months showed cerebral atrophy and delayed myelination. She died aged 7 months with pneumonia.

Although our patient showed no clear response to triheptanoin, this treatment might be beneficial in less severely affected patients.

304-P

PYRUVATE CARBOXYLASE DEFICIENCY DIAGNOSED BASED ON BASIC METABOLIC EXAMINATIONS

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Background: The clinical presentation of a neonate with congenital lactic acidosis varies from an overwhelming, fatal condition with generalized illness to a mild presentation with only organ specific dysfunctions. These variations are related to both the enzyme system involved and its residual activity.
Methods: We report a case of 3-day-old baby girl from non consanguineous parents. She was referred to our hospital with the diagnosis of sepsis. She was born at term by sectio caesarian on social indication with birth weight 2600 g and birth length 49 cm. On the third day she looked lethargic and developed respiratory distress.
Results: After exclusion of secondary origins of lactic acidosis, a metabolic defect screening was done. The measurements of blood gas analysis showed severe metabolic acidosis, blood glucose showed hyperglycemia with high ammonia and lactate level. Urine examination revealed ketonuria. Based on the physical examination and laboratory datas, we diagnosed her as having pyruvate carboxylase deficiency. Because of not having the facility to do the screening of serum amino acids and urinary organic acids, we then sent the DBS screening in other country. The result of DBS screening is marked elevation of citrulline and its associated ratios. The other urea cycle amino acid, ornithine, arginine and arginosuccinic acid were within the normal range. The pyruvate carboxylase activity of fibroblast culture was grossly deficient and propionyl CoA carboxylase activity was normal. These findings were in accordance with pyruvate carboxylase deficiency.
Conclusions: We managed the life threatening acidosis by giving bicarbonate, giving the adequate and balanced caloric intake to avoid ongoing catabolism, and supplementation of biotin, thiamine, citrate, carnitine. The patient died at the age of one year.

305-P

CLINICAL STUDY: CHARACTERISATION OF PATIENTS WITH LEIGH SYNDROME

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Background: Leigh syndrome (LS) is an early-onset progressive neurodegenerative disorder with a characteristic neuropathology consisting of focal, bilateral, symmetrical lesions in one or more areas of the basal ganglia and/or the brainstem. Clinical symptoms depend on which area of the nervous system is involved. Typically patients present with developmental delay, or even regress, and signs of basal ganglia and/or brainstem dysfunction. Common symptoms are hypotonia, dystonia, ataxia, strabism, optic atrophy and respiratory abnormalities. Onset of disease is typically in the first year of life and progress to death within some years, but later onset and slower progression occurs quite frequently. Common causes of LS are defects in the mitochondrial respiratory chain, particularly defects in complex I and IV, and in the pyruvate dehydrogenase complex.
Methods and **Results**: We have used biochemical and genetic methods to identify the molecular aetiology for LS in a cohort of 25 clinically well-characterized patients. In 56% (13/23) of the patients we found a decreased ATP production rate and/or a decreased activity in one or several of the complexes in the respiratory chain. The spectrum of the biochemical findings was however broad. Pathogenic mutations in mtDNA were identified in eight patients, and in one patient mutations in the *POLG1* gene was found. In total we could identify the molecular aetiology in 36% of the patients.
Significance: The investigation will possibly give us better tools for prognosis prediction, genetic counselling and prenatal diagnosis in LS patients.

306-P

LEIGH'S SYNDROME IN 17-YEAR-OLD FEMALE: LATE ONSET WITH FATAL OUTCOME

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Background: Leigh's syndrome usually presents in early years with fatal outcome during the first decade. We present two relatives with a very late onset.
Cases: A 17-year-old female presented with progressive inability to walk due to muscle weakness and enuresis after some period of mental stupor, fatigue, muscle pain, coldness and depression considered due to psychosocial reasons. Family history especially revealed club feet in her further asymptomatic mother and mental retardation in her younger sister. Physical examination revealed abnormal breathing, bradyphrenia, disconjugate eye movements, symmetrical Babinski's sign, absent ankle reflexes and club feet. The laboratory investigations showed respiratory alkalosis with lactate: 2.8 mmol/L (blood) and 3.4 mmol/L (CSF) without signs of infection. EEG: signs of mild encephalopathy without epilepsy. Cerebral MRI/MRS: symmetrical T2-hyperintensity in putamina, mesencephalon and pons with increased lactate. After some initial recovery – on ketogenic diet and supplements – she died 4 weeks later.

Muscle biopsy showed ATP production and oxidation rate of pyruvate without carnitine at 2/3 of normal with normal complex I-IV activities (muscle complex V, fibroblasts and DNA are pending). Neurological investigations of the mother showed comparable club feet and reflexes. Cerebral MRI showed symmetrical increased signals in athrophic putamina with normal MRS. Tıssues are not yet available.
Conclusion: Although there is no definite prove yet, data seem to be consistent with a late presenting but fatal Leigh's syndrome, the mother showing an even milder course. Therefore, even Leigh's syndrome can present at any age with variable course.

307-P

THE MOLECULAR FINDINGS IN A SERIES OF LEIGH SYNDROME PATIENTS WITH *SURF1* GENE MUTATIONS IN RUSSIA

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Background: Leigh syndrome (LS) is one of the most frequent mitochondrial diseases in infancy and childhood. The manifestation of LS is often in the first year of life, with failure to thrive, ataxia, disarthria, abnormal eye movements, psychomotor regression. The disease is determined by mutations either in mtDNA or in nuclear genes. Mutations in *SURF1* gene have been shown to be an important cause of LS with cytochrome c oxidase (COX) deficiency. Most of known *SURF1* mutations predict a truncated protein product.
Methods: We made SSCP and sequence analysis in a series of 18 Russian and 2 Ukrainian patients with *SURF1* mutations.
Results: We found following mutations in our patients: 845delCT, 311.321del10insAT, 868insT, 574.575insCTGC in different combinations. We found that polymorphism 54+58T is associated with the most frequent mutation in Russian patients with Leigh syndrome (845delCT). In one Russian patient we detect a new mutation in *SURF1* gene. It is related to the group of complex rearrangements and represents a 114 bp duplication with the 2 bp deletion inside of the duplicated fragment [dupIVS8-45.749delAG.816]. The duplicated fragment starts with 45 bp upstream exon 8 and ends at nucleotide 816. This fragment except nucleotide AG at the position 749 (749delAG) is inserted after nucleotide 816. The duplication shifts the reading frame and predicts a preliminary stop-codon at the 278 a.a. position and as a consequence skipping exon 9 of the gene. The second mutant allele patient has is [312-321del10insAT]. The phenotypic features of the patient are typical for Leigh syndrome.

308-P

NEW MUTATIONS IN THE *BCS1L* GENE IN A PATIENT WITH A LETHAL SYNDROME WITH CHOLESTASIS, LIVER FAILURE, TUBULOPATHY AND MICROCEPHALY

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GRACILE syndrome, caused by mutations in the *BCS1L* gene, is a neonatal metabolic disorder characterized by: fetal growth retardation, aminoaciduria, cholestasis,iron overload, lactic acidosis, and early death. The incidence in Finland is estimated to be 1 in 56 000 newborns, with a carrier frequency of 1 in 120, with autosomal recessive inheritance. Other patients reported with mutations in *BCS1L* have different clinical phenotypes with encephalopathic symptoms associated to hepatorenal disease. Patient: He presented with failure to thrive, cholestasis, liver failure, ascites, and a severe renal Toni-Fanconi-Debré syndrome. Laboratory determinations showed high levels of transaminases, bilirubin, alkaline phosphatase, very high alfa-fetoprotein, metabolic acidosis with hyperlactacidemia and hyperaminoaciduria, hypocarnitinemia, fasting hypoglycemia with hyperglycemia after feeding or i.v. glucose, severe anaemia with normal transferrin and a bleeding disorder. Microcephaly was evident at the age of 6 months but brain MR spectroscopy was normal. At age 11 moths he died because of liver failure, increased ascites and a septicaemia. Liver biopsy showed cholestasis and fibrosis ducts proliferation without iron deposits, glycogen or lipids storage. Muscle biopsy showed increased glycogen and an isolated mitochondrial respiratory chain complex III deficiency. **Methods**: The *BCS1L* gene was investigated by direct sequencing and RFLP analysis. The patient is a heterozygous compound for the R56X mutation and for two new mutations that predict structural alterations in the 5′UTR region of the BCS1L mRNA. **Conclusions**: Mutations in the *BCS1L* gene could be underdiagnosed in non-Finnish patients with neonatal onset of hepatorenal diseases or variants of GRACILE syndrome.

309-O

SUCLA2 (ADP-FORMING SUCCINYL-CoA SYNTHETASE) DEFICIENCY: CLINICAL COURSE IN 15 FAROESE CHILDREN

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Background: Mutations in the gene encoding the beta-subunit of the ADP-forming succinyl-CoA synthetase (*SUCLA2*) can cause mitochondrial DNA depletion in association with an early infantile Leigh-like encephalomyopathy. A large family pedigree with at least 15 affected individuals was found in the Faroe Island. Further cases of *SUCLA2* deficiency with other mutations than the Faroese cases were identified in Italy, Denmark (child of Pakistanian descent) and in Israel.
Methods: All available hospital records of the Faroese cases were reviewed and supplemented by private notes of the parents. **Results**: Onset and progress of the disease were similar in all patients. No dysmorphic features were present at birth. Feeding problems were noted mostly from the neonatal period, tube feeding was necessary in most cases for failure to thrive. The patients had a severe muscle hypotonia with progressive areflexia leading to profound motor developmental delay, none of them learned to sit or stand without support. The children developed a hyperkinetic-dystonic movement disorder and external ophthalmoplegia and/or ptosis. All patients were diagnosed with profound neurosensorial deafness. The general mental development seems to be normal. Death occurred after recurrent gastrointestinal and/or respiratory infections. The mean of survival time was 11 years with a range of seven months to 21 years. **Conclusion**: *SUCLA2* deficiency leads to a distinct clinical pattern which should give rise to search for the disorder. With proofed cases from different countries it seems to be a disorder which has to be expected worldwide.

310-O

METABOLITE ANALYSIS IN SUCCINYL-CoA SYNTHETASE DEFICIENCY: ELEVATED SUCCINYLCARNITINE IS A BIOCHEMICAL HALLMARK IN SUCLA2 DEFICIENT PATIENTS

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Background: ADP-forming succinyl-CoA synthetase (SCS-A; EC. 6.2.1.5) is a heterodimeric mitochondrial protein that catalyzes the conversion of succinyl-CoA to succinate in the tricarboxylic acid cycle. Recently, we have demonstrated that mutations in the *SUCLA2* gene, which encodes a β-subunit of this enzyme, lead to SCS-A deficiency in association with Leigh-like encephalomyopathy, dystonia and deafness. **Methods**. Plasma, urine and CSF samples of patients with (1) genetically-proven *SUCLA2* deficiency, (2) cobalamin C deficiency, (3) classical methylmalonic aciduria and (4) unexplained methylmalonic acidemia were subjected to standard organic acid analysis by GC-MS, as well as to LC-MS/MS analysis of the butylated carnitine esters, including a HPLC separation of the C4-dicarboxylic carnitine (C4DC) isomers. These isomers were identified by comparing their retention times and mass spectra with methylmalonylcarnitine and succinylcarnitine (synthesized by dr. H. ten Brink), respectively, and both isomers were quantified. **Results**. *SUCLA2* patients suffer from lactic acidosis and (mild) methylmalonic acidemia. In urine of all *SUCLA2* patients, we identified increased levels of methylmalonate, 3-hydroxyisovalerate, succinate and, in most cases, other TCA cycle intermediates (fumarate, 2-ketoglutarate). Detailed LC-MS/MS analysis revealed that in *SUCLA2* patients, the concentration of C4DC is clearly elevated and that succinylcarnitine amounted to >80% of C4DC in all body fluids. In CSF, the C4DC fraction appeared to be exclusively succinylcarnitine. **Conclusions**. By application of LC-MS/MS we were able to unequivocally differentiate *SUCLA2* deficient cases from patients with other forms of methylmalonic acidemia. A predominant biochemical hallmark of *SUCLA2* deficient patients is the accumulation of succinylcarnitine in body fluids.

311-P

RAPID mRNA DEGRADATION DUE TO A NEW *SUCLA2* MUTATION IN TWO SIBS WITH MILD METHYLMALONIC ACIDURIA

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Background: Fourteen patients with mild methylmalonic aciduria have been recently associated with mutations in *SUCLA2* gene.
Objectives: To extend knowledge on the defects of *SUCLA2* gene.
Patients and Methods: Two boys, born to Norwegian unrelated parents, showed delayed development, hypotonia/dystonia, dysphagia, deafness, lactic acidemia and mild methylmalonic aciduria. Brain MRI showed symmetrical changes and atrophy in the basal ganglia in both patients. The first child died at 4 years. Determination of respiratory chain enzymes in muscle from patient #1 showed a defect of complexes I and IV.
Results: Southern blotting in muscle homogenate from patient #2 revealed reduced amount of mtDNA (46% of controls). Sequence analysis of *SUCLA2* revealed a heterozygous c.1106insA in exon 8, which predicts a premature translation termination at amino acid residue 386 (p.D386X). No other mutations were found in the remaining exons. Messenger RNA level and size were normal, though the c.1106insA mutation was homozygous in cDNA extracted from cultured skin fibroblasts, consistent with the occurrence of a rapid mRNA degradation of the second mutated allele. Western blotting of the *SUCLA2* gene product in muscle homogenate showed a reduction of about 50%.
Conclusions: Our findings enlarge the spectrum of *SUCLA2* mutations in MMA. The phenotype is consistent with previously described cases, characterized by Leigh-like abnormalities, dystonia and deafness. Interestingly, we showed for the first time data suggestive of a rapid mRNA degradation, although the precise mutation remains to be determined.

312-O

DEFICIENCY OF THE ALPHA SUBUNIT OF SUCCINATE-CoA LIGASE CAUSES FATAL INFANTILE LACTIC ACIDOSIS WITH MTDNA DEPLETION

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Fatal infantile lactic acidosis is a severe metabolic disorder, characterised by the onset of lactic acidosis within the first day of life and early death. We found a combined respiratory chain enzyme deficiency associated with mtDNA depletion in a small consanguineous family with this disorder. The affected children were born at term with a low birth weight, hypotonia, hypothermia, severe lactic acidosis in the first day of life, and death at age two to four days. To identify the disease-causing gene, we performed homozygosity mapping with DNA from two affected children. We found homozygous regions on four chromosomes. The Mitop2 database was used to search for genes in these regions that encode proteins targeted to the mitochondria. DNA sequencing revealed a homozygous 2 bp deletion in *SUCLG1*, a gene that encodes the alpha subunit of the Krebs cycle enzyme succinate-CoA ligase. Mutations have not previously been reported in this gene, but mutations have been found in *SUCLA2*, which encodes a beta subunit of the enzyme. Patients with *SUCLA2* mutations have symptoms that are similar to those found in the patients with *SUCLG1* mutations, albeit milder. The mtDNA depletion is likely explained by decreased mitochondrial nucleoside diphosphate kinase activity, resulting from the inability to complex with succinate-CoA ligase.

313-P

DEOXYGUANOSINE KINASE DEFICIENCY: TWO DISEASES OR ONE?

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Background: Deoxyguanosine kinase (DGK; MIM 601465) is a nuclear gene that along with thymidine kinase-2 (TK2; MIM 188250) salvages deoxyribonucleotides (dNTPs) for mtDNA synthesis. Deficiency of either of these genes causes a mitochondrial depeletion syndrome.
Methods: We have undertaken a retrospective analysis of one center's 7 kindreds representing 13 mutations, 10 of which are unpublished. These are compared with previously published cases to establish genotype/phenotype.
Results: DGK mutations are associated with both isolated hepatic and hepato-cerebral forms. In all patients in our series hepatocerebral disease was associated with an abnormal newborn screen, early onset of nystagmus and early death. Conversely, the absence of a neurological phenotype is predictive of long term survival independent of liver transplantation. The N46S mutation is associated with isolated hepatic disease in all ethnicities.
Conclusions: Mitochondrial depletion caused by mutations in DGK should be considered in children with hepatic dysfunction or cholestasis even without neurological findings. Full gene sequencing is warranted if DGK mutations are suspected.

314-P

SEVERE CLINICAL COURSE IN A PATIENT WITH HOMOZYGOUS mtDNA POLYMERASE GAMMA MUTATION (A467T) AND HETEROZYGOUS THYMIDINE PHOSPHORYLASE MUTATION (A465T). A POSSIBLE DIGENIC EFFECT?

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Background: Mutations in mitochondrial DNA polymerase gamma (*POLG*) produce a broad clinical spectrum. Childhood presentations do have a clear male predominance and interestingly patients with homozygous A467T mutation seem to have a milder clinical course. **Objective**: Present a 12-year-old female patient with valproate induced liver failure, liver transplantation and subsequent severe clinical deterioration. Case: A 12-year-old girl with normal development received valproate for generalized seizures. After 4 months she developed acute liver failure (ASAT 274U/L, ALAT 389U/L, NH_3 219 µmol/l, factor V 33%, INR 2.0) and underwent liver transplantation. After 1 week she had a stroke with diffuse lesions occipital, frontal and in the thalamus. She developed continuous generalized myoclonus, neuropathy, cortical blindness and intestinal pseudo-obstructions needing continuous feedings. **Results**: Investigations revealed persistent elevated lactate. Respiratory chain complex I, III, and IV activities of 8%, 50% and 20% respectively in liver. Activities in muscle and fibroblasts were normal. Mutational analysis of *POLG* showed a homozygous A467T mutation and a heterozygous (A465T) mutation in the thymidine phosphorylase gene. **Discussion:** Reported cases in childhood are mostly male Patients with homozygous A467T mutation mainly develop encephalopathy, none received valproate and did not develop liver failure. Liver failure with encephalopathy seems a main characteristic in compound heterozygous patients. We hypothesise that the severe clinical course of our patient is due to valproate administration (environmental effect) and to the additional heterozygous mutation in the TP gene (a protein known to induce also mtDNA depletion) that may increase disease expression (digenic effect).

315-P

CLINICAL SPECTRUM OF ALPERS SYNDROME ASSOCIATED WITH MUTATIONS IN MITOCHONDRIAL POLYMERASE γ GENE

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Alpers syndrome is usually characterized by a clinical triad of psychomotor retardation, intractable epilepsy and liver failure in infants and young children. But clinical picture of this inherited disease is highly variable and many cases without liver failure and with adult onset are known.

We described 7 patients (two family and 2 singleton cases) with *POLG1* mutations associated with combined infantile fatal encephalopathy and hepatopathy. Only one patient had typical Alpers' syndrome. In another family with two children the disease onset was at 2 months with progressive hepatic failure, hypoglycemia and metabolic acidosis. Four patients (including 3 sibs) developed progressive myoclonic seizures without liver damage.

MRI was performed for 3 patients and was similar in all cases, showing mild brain cortical and cerebellar atrophy. One patient had subdural hematoma in the left temporo-parieto-occipital regions due to severe damage of liver with coagulopathy.

EEG: 4 patients with seizures have multifocal spikes in different regions; one child showed low amplitude polyspikes, polyspike waves and slow waves of high amplitude alternating with a trace of burst-suppression activity.

Laboratory examination: only two patients with hepatopathy have high levels of serum transaminases and high blood levels of lactate (11.0 mmol/L). The following mutations were found in our patients: A467T (in 2 patients), W747S (in 2 patients), W848S, G268A.

Diagnosis of Alpers disease can be considered in children with unexplained early developmental delay and liver failure or partial seizures, especially epileptic status.

316-P

POLG1 ARG953CYS MUTATION: AN EXPANDED PHENOTYPE AND RECESSIVE INHERITANCE IN A BRAZILIAN FAMILY

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Background: Some mitochondrial diseases are caused by mutations in nuclear genes that affect the mitochondrial DNA (mtDNA)stability. Mutations in the polymerase gama gene (*POLG*) cause multiple deletions or depletion of mtDNA and have been associated with diverse phenotypes, including progressive external ophthalmoplegia (PEO), sensory ataxia, neuropathy, dysarthria and ophthalmoparesis (SANDO), and Alpers syndrome. In 2004, a heterozygous C2857T mutation (Arg953Cys) of the *POLG* gene was described in a family with autosomal dominant PEO. We report a Brazilian family, in which this same mutation was transmitted as an autosomal recessive trait and caused a more complex clinical phenotype.
Methods: The proband and his siblings were clinical and neurological evaluated. The CK levels, EMG and histochemical muscle analysis were performed in the symptomatic patient. The molecular analysis were done by Southern blot analysis and sequencing of the *POLG* gene.
Results: Our patient is a 33-year-old man,who had PEO, parkinsonism, sensory neuropathy, and cardiomyopathy. Muscle biopsy showed scattered ragged-red fibers (RRF), which were SDH-positive and cytochrome c oxidase (COX)-negative. Southern blot analysis of muscle DNA showed multiple mtDNA deletions. Sequencing of the *POLG* gene revealed a homozygous Arg953Cys mutation. The patient's sister did not carry the mutation and his two asymptomatic brothers presented the same mutation in heterozygosis.
Conclusion: We have shown that the Arg953Cys mutation is autosomal recessive and can be associated with a complex phenotype, including parkinsonism, sensory neuropathy, proximal myopathy, cardiomyopathy and psychiatric symptoms.

317-P

ALPERS SYNDROME WITH *POLG* MUTATIONS: CLINICAL, EEG AND RADIOLOGICAL FEATURES

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Background: Alpers syndrome is a rare autosomal recessive hepatocerebral degenerative disorder. We describe clinical, EEG and radiological findings in Alpers syndrome with *POLG* mutations.
Methods: Retrospective review of 12 patients with Alpers syndrome and 2 pathogenic *POLG* mutations. Records were reviewed for clinical and EEG findings. Neuroimaging was reviewed in 10 children.
Results: All children had developmental delay or regression and refractory epilepsy. Other symptoms included ataxia (7), visual disturbance (4), motor paresis (3) and tremor (3). Myoclonic and focal motor seizures were common often manifesting as status epilepticus. Eleven children had EEGs. All had absent/slow posterior dominant rhythms. Interictal discharges were seen in 9 children, involving the occipital lobes in 7. Discharges were seen in 9 patients. Two had preterminal burst suppression patterns. CT in 9 patients was normal (5) or showed low attenuation (3) or diffuse cerebral atrophy (1). Four children had MRIs. All showed abnormalities including T2 hyperintensities (4), corresponding restricted diffusion (2) and focal atrophy (1). Lesions involved cortex (4), subcortical white matter (2), deep white matter (1) and deep thalamic grey matter (1). Regions affected included parietal lobes (4), occipital lobes (3), frontotemporal lobes (2) and primary sensorimotor cortex (2).
Conclusions: Developmental regression and refractory focal motor or myoclonic seizures are consistent clinical features of Alpers syndrome with *POLG* mutations. Migratory T2/FLAIR signal abnormalities involving metabolically active occipital and sensorimotor cortical regions are characteristic MRI findings. Interictal and ictal EEG patterns are more variable although occipital cortical regions are commonly involved.

318-O

MITOCHONDRIAL DEPLETION SYNDROMES IN CHILDREN EVALUATED FOR LIVER FAILURE

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In recent years, mitochondrial depletion syndromes have increasingly been recognized as a cause of hepatocerebral syndromes. As a referral center for patients with endstage liver disease, we report our experience with this group of disorders.
Methods: Retrospective analysis of medical records, quantitation of mtDNA in liver tissue, mutation analysis for polymerase-gamma (*POLG1*), deoxyguanosine kinase (*DGOUK*), and *MPV17*.
Results: Mutations of the *POLG1* gene were found in 3 patients with a typical presentation of Alpers syndrome. Retrospective analysis of another 3 patients suggestive for a mitochondrial disorder and rejected from the liver transplantation programme due to severe central nervous involvement detected *POLG1* mutations in another 2 of 3 patients. In these cases, p.A467T was by far the most common *POLG1* mutation (with all 5 patients being compound heterozygous). One child with compound heterozygosity for two *DOUGK* p.M1 mutations and mild neurologic involvement underwent liver transplantation and was found to carry a hepatocellular carcinoma in the explanted organ. A further case with severe cholestasis, lactic acidemia, elevated AFP, and cytotoxic edema of the brain cortex was found to be homozygous for an *MPV17* mutation. She is the first patient with nonsense alleles of this recently described gene on both chromosomes.
Conclusion: Mitochondrial depletion syndromes are an important differential diagnosis in children with cholestasis and liver failure. They can be diagnosed by detection of mitochondrial DNA copy number in liver and molecular analysis of an increasing number of genes.

319-P

MUTATION IN *MPV17* AS A CAUSE OF MTDNA DEPLETION IN A SPANISH BOY PRESENTING WITH LEUKODYSTROPHY AND HEPATIC INSUFFICIENCY

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Approximately 15% of the patients presenting with the hepatocerebral form of mtDNA depletion syndrome (MDS) are associated with pathogenic mutations in *DGUOK*. Recently, some authors have demonstrated that the absence or the malfunction of *MPV17* protein leads to oxidative phosphorylation failure and mtDNA depletion. These findings prompted us to analyze *MPV17* gene in a group of 30 patients with hepatocerebral MDS, where screening for *DGUOK* mutations had been negative.

From those 30 patients, only one had pathogenic mutations in *MPV17*. This patient presented at 2 months of age with vomiting, jaundice, hypertransaminasemia and failure to thrive. At 13 months of age, he started to have permanent hypoglycaemia, hepatic insufficiency and leucodystrophy. He died at 22 months of age with a multisystemic disease including: neurologic, renal, digestive, endrocrinologic and ophthalmologic affectation.

Levels of mtDNA measured by real-time PCR showed, both in liver and muscle, a reduction of 80% compared to controls. The analysis of *MPV17* at the genomic level by PCR and direct sequencing revealed a homozygous IVS1+5G>A mutation. This change resulted in the abolition of the donor site signal in exon 1, leading to the absence of wild type mRNA. In addition, we have found a novel alternative mRNA splicing lacking exon 2 in control individuals.

The description of the patient reported here provides new insights into the knowledge of the hepatocerebral form of MDS.

320-P

DIET THERAPY IS EFFECTIVE TO CONTROL LIVER DISEASE OF MITOCHONDRIAL DNA DEPLETION SYNDROME DUE TO *MPV17* MUTATION

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Background: Mutations of the nuclear gene MPV17 cause a hepatocerebral mitochondrial DNA depletion syndrome (MDS). No treatment is unfortunately currently available and often liver dysfunction rapidly progresses to cirrhosis.
Objective: To describe the clinical and biochemical findings in two *MPV17* mutated brothers and to demonstrate the benefic role of diet for disease control.
Results: One patient died of liver failure at 7 months and the other was liver transplanted at 25 months after strict dietary treatment. In the first 3 months of life the first patient showed cholestatic jaundice with elevated transaminases and very reduced fasting tolerance. Liver function tests improved on every 3-h feeding until the age of 6 months 15 days when he had gastroenteritis at home. After few days he was admitted to hospital with liver failure and died one week later. The second brother was fed every 3 h from birth and followed-up weekly. ALT and AST U/L mean (range) were respectively 61 (24–108) and 105 (35–187) while 'well being' (43 blood samples); 108 (70–198) and 235 (186–288) at admissions to hospital for intercurrent disease (8 samples); 34 (28–43) and 45.2 (38–53) after continuous glucose infusion for 3 days or more (4 samples). The three different sets of data are significantly different (Kruskal–Wallis one-way analyses of variance by ranks).
Conclusion: Fasting hypoglycemia is a common finding in young children with mtDNA depletion syndrome. However in contrast to other MDS, frequent meals and/or i.v. continuous glucose are protective against liver failure in *MPV17* patients.

321-P

HEPATOPATHY: AN ADDITIONAL FEATURE OF MEGDEL ASSOCIATION?

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MEGDEL association is a recently reported disease (Morava et al, 2006) and is considered to be a defect in oxidative phosphorylation, that comprises 3-methylglutaconic aciduria (3-MGA), sensorineural deafness and Leigh encephalopathy as key features. Here, we describe a boy with hepatopathy, as an additional main problem.

Family history, pregnancy and delivery were normal. Aminotransferases were twofold elevated since birth. At age 3 months further increase of transaminases indicated hospital work-up. We noticed craniotabes, strong tendency to hypoglycemia, high alkaline phosphatase, low phosphate, unmeasurably high alpha-fetoprotein, high gamaGT, low fibrinogen. Organic acids analysis in urine revealed elevated 3-MGA (26 to 56 mmol/mol creatinine). Lactate was normal. Liver biopsy revealed strong bile ducts proliferation, mild intrahepatic cholestasis, microvesicular steatosis, portal fibrosis with bridging septa and regeneratory hepatocyte nodules. Mitochondria were polymorphic, abnormally shaped with sparse, abnormal or missing crista. During follow-up hypoglycemia, rickets signs and liver tests gradually normalized, while progressive hypotonia and severe psychomotor retardation emerged. At the age of 16 months severe sensorineural hearing loss became evident. Brain MR revealed symmetrical lesions of caudate nucleus and putamen. In some muscle fibers there were fatty vacuoles and enlarged vacuolated mitochondria. In muscle there was a mild deficiency of ATP-synthase and normal other respiratory chain enzymes. mtDNA depletion test, sequencing of *DGUOK* gene, search for MELAS and NARP mutation were normal. This case indicates that MEGDEL association could involve liver, as suggestive by previous reports of some patients with 3-MGA (Broide et al., 1997).

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322-P

ISOLEUCINE IN ETHYLMALONIC ENCEPHALOPATHY

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Background: Ethylmalonic encephalopathy is a rare autosomal recessive metabolic disorder caused by mutation in *ETHE1* gene and presenting in infancy with psychomotor retardation, chronic diarrhea, orthostatic acrocyanosis and relapsing petechia. High levels of lactic acid, ethymalonic acid and methylsuccinic acid are detected in body fluids. A decreased cytochrome c oxydase activity has been reported in skeletal muscle.
Case Report: We report a 15 months old male born from consanguineous parents of Moroccan ancestry, presenting with a typical ethylmalonic encephalopathy, associated with CD8 lymphopenia. Lactic acidosis was permanent. Cytochrome c oxydase (COX) activity was decreased in lymphocytes. A homozygote deletion of exon 4 was found in *ETHE1* gene.

Up to date, the nature of the source of ethylmalonic acid is still unclear. Relation to isoleucin and methionine metabolism has been suggested in a few reports.
Methods: In order to study these hypotheses in our patient, we performed an oral isoleucin loading test (150 mg/kg) then an oral methionine loading test (100 mg/kg) and studied ethylmalonic excretion.
Results: As ethylmalonic acid was increased only after isoleucine loading (105 µmol/mmol creatine to 122 µmol/mmol creatine after methionine loading versus 99 µmol/mmol creatine to 242 µmol/mmol creatine after isoleucine loading), we put our patient on an isoleucine restricted diet (200 mg/d). However, we did not observe any clinical improvement nor biochemical modifications in ethylmalonic acid excretion in urine and in COX activity in lymphoblasts.
Conclusion: These results suggest that in our patient the isoleucine pathway is not involved in ethylmalonate excretion.

323-A

GLUTARIC ACIDURIA TYPE II – LATE-ONSET FORM IN THAI SIBLINGS – FIRST REPORTED CASES IN THAILAND

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Background: Glutaric aciduria type II or multiple acyl-CoA dehydrogenase deficiencies (MADD) is an autosomal recessively inherited disorder of fatty acid, amino acid and choline metabolism. GA-II has 3 heterogenous clinical presentations: a neonatal-onset form with congenital anomalies (type I), a neonatal-onset form without congenital anomalies (type II) and a late-onset form (type III). Symptoms and age at presentation of late-onset GA-II are highly variable and characterized by recurrent episodes of lethargy, hypoglycemia, metabolic acidosis and hepatomegaly, often preceded by metabolic stress. Muscle involvement e.g. pain, weakness and lipid storage myopathy also occurs.
Methods: We herein report the first 2 cases of GA-II (late-onset form) from Thailand. A 9-year-old boy presented with proximal muscle weakness; initially misdiagnosed with postviral myositis. Muscle biopsy revealed lipid storage myopathy. After one month of carnitine treatment, he still continued to have muscle weakness and unable to lift his head up nor walk. He was then reevaluated and diagnosed with GA-II; afterwhich riboflavin, glycine, carnitine and low fat diet were given. Subsequently, his sibling acylcarnitine profile revealed elevated C6, C8, C10, C12 and C14: 1 while asymptomatic and he was treated just after he developed muscle weakness.
Results: Tandem mass spectrometry (TMS) demonstrated abnormalities in both siblings. Elevated muscle enzymes and dicarboxylic acids were also identified via urine organic acid (OA) analysis. They were treated and recovered well.
Conclusion: Thai siblings with late-onset GA II diagnosed by urine OA analysis and TMS are first reported cases from Thailand.

324-O

EXPRESSION PROFILING OF ASTROCYTES FROM HYPERAMMONEMIC MICE REVEALS DOWNREGULATION OF GENES IMPORTANT FOR WATER AND POTASSIUM HOMEOSTASIS

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Acute hyperammonemia causes cerebral edema and severe brain damage in children with urea cycle disorders (UCDs) and in patients with liver failure. Chronic HA is associated with developmental delay and mental retardation in children with UCDs and with neuropsychiatric disturbances in patients with chronic liver dysfunction. The pathophysiology of the encephalopathy associated with these disorders has not yet been elucidated. Often treatment cannot prevent severe brain injury and neurological sequela. One of the cellular effects described in hyperammonemic encephalopathy (HAE) is astrocyte swelling. The swelling is considered to be causally related to the brain edema of acute HA.

By crossing GFAP-EGFP transgenic mice with Otc/spf mice, we created a mouse with a urea cycle disorder, which also expresses EGFP in astrocytes, allowing for their purification by FACS. We studied the changes in metabolic and signal transduction pathways in acutely isolated astrocytes and brain tissue of mice with urea cycle disorders using microarray analysis.

Expression analysis of FACS-purified astrocytes and cortex indicated changes in the expression of genes which mediate potassium and water homeostasis and inter-astrocyte signaling. These included Connexin-43, the potassium channel subunit Kir5.1, and Aquaporin 4. These findings indicate that proteins which facilitate potassium and water homeostasis and inter-astrocyte signaling might be potential targets for developing new therapies for HAE.

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325-P

OUTCOME OF UREA CYCLE DEFECTS: AN 8-YEAR EXPERIENCE IN MALAYSIA

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Urea cycle defects (UCD) are a group of treatable inborn errors of metabolism characterized by varying degrees of hyperammonemia and outcome. Over 8 years, 64 patients were diagnosed to have various UCD in Malaysia giving an estimated incidence of about 1 in 63 000 live births. There were 20 ornithine transcarbamylase (OTC) deficiency and 12 were partial, 11 citrullinemia, 14 arginosuccinic acidemia (ASA), 4 arginase deficiency and 5 transient hyperammonemia of newborn. The rest (10) were either CPS or NAGS deficiency. Overall survival rate was 83%. A total of 26 dialysis were performed and 77% survived. 32 of the survivals (60%) had either a normal outcome or mild neurological deficits. 15% of the survivals had moderate neurological deficits and 25% had severe or profound neurological deficits. OTC deficiency had a poor outcome in general. All the male OTC patients passed away. Only one of the surviving females (25%) had normal outcome with high risk screening and expectant therapy from birth. Most of the partial OTC deficiency (83%) had a normal outcome. 82% of the citrullinemia patients had long term survival and 55% had normal outcome or mild neurological deficits. 93% long term survival was achieved in arginosuccinic aciduria and majority (85%) had normal outcome or mild neurological deficits. Two out of 4 patients with arginase deficiency passed away after defaulting treatment. All five patients with transient hyperammonemia of newborn survived with normal outcome despite dialysis required in 2 of them. These data provide useful guide to the planning of newborn screening.

326-P

A REVIEW OF BIOCHEMICAL OUTCOMES OF ADULTS WITH OTC DEFICIENCY

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Background: Patients with late-onset ornithine transcarbamylase deficiency (OTCD) have intermittent episodes of encephalopathy, migraines and protein aversion. Treatment is by the use of dietary protein restriction and ammonia scavengers. In childhood, efficacy of such treatments can be gauged by clinical parameters such as growth. Apart from avoiding and managing acute episodes, treatment targets in adult life are poorly defined. The aim of this study was to retrospectively compare routine outpatient clinical treatment with biochemistry (81 samples) in our cohort of 18 adults (age 16–65, 1 male) over a four year period.
Results: 7 patients had either or both low serum vitamin B12 levels and elevated plasma homocysteine. Plasma amino acid profiles showed the following standard deviation and 95% confidence intervals above the mean for the population: glutamine 2.31 (1.92 to 2.70), glycine 2.60 (2.10 to 3.10) and alanine 1.90 (1.55 to 2.25.) Most of the remaining amino acids were low: phenylalanine –1.65 (–1.48 to –1.82.) and total branch chain amino acids (BCAA) –1.11 (–1.00 to –1.22.) Mean BCAA profile were lower in those on sodium benzoate –1.24 ($p = 0.003$) and sodium phenylbutyrate-1.34 ($p = 0.0003$,) than those off.
Conclusion: Patients with OTC are at particular risk of vitamin B12 deficiency. The disease and its treatment lead to disruption of the normal amino acid profile. It remains to be seen whether or not supplementation of certain amino acids improves health in this group.

327-O

DESCRIPTION AND OUTCOMES OF 316 UREA CYCLE PATIENTS FROM A 21-YEAR, MULTICENTER STUDY OF ACUTE HYPERAMMONEMIC EPISODES

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Background: Inherited disorders of urea cycle metabolism are a major cause of hyperammonemia in children. It has been difficult to amass significant epidemiologic or outcome data. Using a large longitudinal interventional study of hyperammonemic patients we collected data on their presenting symptoms and survival.
Methods: Between 1982 and 2003, data were collected on patients receiving nitrogen scavenging drugs. This drug was initially limited in use to defects in the first four enzymes of the pathway; CPSI, OTC, ASS, and NAGS. Since these drugs are the commonly accepted treatment for these hyperammonemic crises and were only available through this study, it is probable that most patients in the U.S. and Canada with these disorders are included in this dataset.
Results: As part of this data collection, 316 unique patients were identified. The specific enzyme defects were: OTC males 69 (21%), OTC females 77 (24%); ASS, 71 (22%); CPSI, 38 (12%); ASL, 7 (2%); and arginase, 2 ($< 1\%$). Of these 316 patients, only 104 (33%) presented within the first 30 days of life with a mortality rate of 32%. These 316 patients had 1045 reported episodes of hyperammonemia. The most common presenting symptom was neurologic (80%) followed by gastrointestinal (33%). This cohort is the largest collection of patients reported for these diseases and the first large cohort in the United States.
Conclusions: Surprisingly, the majority of patients with heritable causes of hyperammonemia present beyond the neonatal period. Late-onset presenting patients exhibited prolonged survival compared to the neonatal presenting group.

328-P

ARGININE TREATMENT IN UREA CYCLE DEFECTS: WHAT IS THE BEST DOSAGE IN THE LONG-TERM TREATMENT?

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Background: Large doses of arginine (up to 700 mg/kg/d) are used in patients with argininosuccinate synthase (ASS) and argininosuccinate lyase (ASL) deficiencies. Kolker et al. (JIMD 2006) pointed out that high doses of arginine, as a consequence of the large doses used in ASL and ASS, may be potentially neurotoxic. The aim of the study was to reduce the dose of arginine to reach the optimum therapeutic effect with the lowest levels of arginine.
Methods: Seven patients with neonatal ASS (age: 6–14 years) and eight patients with ASL (age: 2–21 years). All patients had protein restricted diet (0.7–1.2 g/kg/day), arginine (mean for ASL and ASS: 550 and 600 mg/kg/day, respectively) and sodium benzoate (200 mg/kg/day).

We monthly measured plasma amino acid (AA), ammonium (NH4) and aminotransferases (AST, ALT) in all patients before arginine administration, for 24 h, three times a day, for a period of one year.
Results: ASS patients: the mean of the lowest doses of administered arginine was 350 mg/kg/day and plasma arginine dropped from 133 to 94 μmol/L. ASL patients: the mean of the lowest doses of administered arginine was 320 mg/kg/day and plasma arginine dropped from 120 to 84 μmol/L. NH4 did not change despite the changes of arginine dose in both defects. AST and ALT were reduced in ASL patients. No metabolic decompensation were observed during the follow-up.
Conclusions: We demonstrated that in long-term treatment high dose of arginine are not necessary and should be tailored according to the age and specific defect.

329-P

INTRAUTERINE BENZOATE-LOADING AND POSTPARTAL HEPATOCYTE TRANSFUSION: NEW THERAPEUTIC MODALITIES IN UREA CYCLE DEFECTS (UCD): FIRST EXPERIENCE

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Metabolic decompensation with hyperammonaemia typically occurs during the first days of life in patients with UCD resulting in severe neurological damage or death. We describe one male patient with the prenatal diagnosis of OTC-deficiency who was loaded with benzoate before birth and was additionally transfused with human hepatocytes post partum.

The mother, who is carrier of an OTC-mutation, received 7 g of benzoate 2 h before birth. This was done on the assumption that catabolism already starts pre-/perinatally. Therapeutic levels of benzoate were found in umbilical cord blood as well as in the boy's blood post partum.

In the boy with OTC-deficiency metabolic decompensation seemed likely despite a conservative therapeutic regimen. Therefore, we decided to provide him with human hepatocytes immediately after birth in addition to prenatal benzoate-loading. A total of 640 million viable hepatocytes were transfused via an umbilical vein catheter into his portal vein in 3 fractionated doses at the age of 6, 24 and 30 h, respectively. Immunosuppression was initiated. Ammonia levels reached a maximum of 240 μM on the 2nd day of life and remained normal thereafter. The post interventional course was complicated by brief self-limited hypophosphataemic seizures at day 10. The boy shows normal psychomotor development under a diet with 0.9 g/kg natural protein per day supplemented with essential amino acids, arginine and 350 mg/kg per day of butyrate at the age of 8 weeks.

330-O

LIVER CELL TRANSPLANTATION (LCT) FOR CARBAMOYLPHOSPHATE SYNTHASE 1 (CPS1) DEFICIENCY AND CITRULLINAEMIA

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Background: LCT has so far been used in five children with urea cycle disorders (UCD). We here report on the first two UCD patients treated in Germany and the first use of stable isotope techniques for the detection of transplanted cells.
Methods: Patient 1 suffered from neonatal CPS1 deficiency with frequent hyperammonaemic crises despite intensive pharmacological and dietary treatment. He was not suitable for liver transplantation because of severe cardiomyopathy at the age of three months. Patient 2 had neonatal citrullinaemia with a similar clinical course. At the age of 3, considerable delay of psychomotor development was present. Liver transplantation was declined by the parents. In both children, cryopreserved allogeneic hepatocytes were infused over a 4F Hickman catheter in the superior mesenteric vein. In patient 1, HCT was well tolerated. Because patient 2 developed severe transient neurological symptoms after 50% of the planned dose, her treatment was discontinued. Urea cycle function was assessed in vivo using stable isotope techniques before (patient 2 only) and after LCT.
Results: After an observation period of 2–4 months, both children are stable without metabolic crises. Preliminary results indicate that glutamine transfer into urea considerably increased after LCT in patient 2, reflecting improved urea cycle function. Pharmacological and dietary treatment has remained unchanged so far.
Conclusions: LCT was technically feasible in both patients. However, in one child transient neurological complications demanded discontinuation of the therapy. For the first time, functional *in vivo* detection of transplanted liver cells was possible by means of stable isotope techniques.

331-P

HIGHLY VARIABLE CLINICAL PHENOTYPE OF CPS1-DEFICIENCY IN ONE FAMILY

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Background: Carbamylphosphate synthetase I (CPS1) deficiency is a rare autosomal recessive urea cycle disorder often resulting in neonatal hyperammonemia but onset in adulthood has also been reported. Neonatal onset is often associated with early death or a severe clinical course thought to be the result of the near loss of enzyme function.
Methods: Described here are two individuals from the same family presenting with neonatal hyperammonemia (grandson) and with the first hyperammonemic episode at age 55 (grandfather), respectively. CPS1 transcript analysis using RNA from cultured fibroblasts was performed in six overlapping fragments. Cloning experiments were added in order to better understand the clinical variability.
Results: Both patients carried the mutations c.3558+G>C and c.4101 +2T>C on each one allele. Cloning revealed that the splice site mutation c.4101+2T>C can lead to either a deletion of exon 34 or an insertion of 42 bp in the transcript due to the activation of a cryptic donor splice site within intron 34 (c.4101+42) that yielded a high splice score.
Conclusions: CPS1 deficiency can present with a wide clinical presentation despite identical genotypes at the disease locus. This presentation demonstrates that in CPS1 deficiency a molecular study alone does not entirely forecast the overall prognosis.

332-P

mRNA ANALYSIS IMPROVES EFFICACY OF MOLECULAR GENETIC ANALYSIS IN OTC DEFICIENCY

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Background: The most common urea cycle defect is the deficiency of ornithine transcarbamylase (OTC). So far, 341 disease causing mutations of the *OTC* gene have been described. However, 20% of patients with enzymatically proven OTC deficiency do not show any mutation when exonwise sequencing of the *OTC* gene in genomic DNA is performed.
Methods: Four consecutive OTC patients in whom standard genomic DNA analysis of the *OTC* gene had not revealed any mutation were investigated by analysis of liver derived mRNA. Liver tissue was obtained by needle aspiration or by open biopsy.
Results: In all four patients complex novel rearrangements of the *OTC* transcript (2 insertions and 2 deletions) were revealed.
Conclusions: Analysis of liver *OTC* mRNA is a suitable tool to resolve the genotype of patients with a strong suspicion of OTC deficiency but normal sequencing of coding exons and flanking intronic regions. Liver tissue sampling by needle aspiration allows for both enzymatic and RNA based analysis of OTC deficiency.

333-P

PLASMA CITRULLINE IS RARELY LOW EVEN DURING ACUTE PRESENTATION IN MILD, LATE ONSET ORNITHINE TRANSCARBAMYLASE DEFICIENCY

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Ornithine transcarbamylase deficiency (OTC) (OMIM 311250) is an X-linked disorder, the commonest urea cycle defect (UCD). The condition usually affects males although a significant number of heterozygous females show symptoms. Classical disease presents around 24–48 h with lethargy, vomiting and rapid progression to encephalopathy and death if untreated. Milder, late onset patients are well described, presentation precipitated by infection, surgery, valproate therapy or increased protein intake.

All UCD present biochemically with increased ammonia and glutamine. For differential diagnosis plasma amino acid and urinary orotate are used, defects post carbamoyl phosphate synthetase resulting in orotic aciduria and neonatal-onset OTC cases having low and often undetectable plasma citrulline. We have reviewed our plasma citrulline data in presenting samples taken from late onset OTC patients and report our results.

Nine male patients were included in the study. Age at presentation was 6 months to 42 years. All had increased orotate excretion. Plasma glutamine ranged from 760 to 3527 μmol/L (reference range 385–862), citrulline was between 5 and 61 μmol/L (reference 10–45) with 3 results below, 3 within and 3 above the reference range.

We conclude a normal or even elevated plasma citrulline at presentation does not exclude OTC deficiency in late onset patients.

334-A

UNEXPECTED DEATH OF A CASE WITH ARGININOSUCCINATE LYASE DEFICIENCY

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Background: Argininosuccinate lyase deficiency (ASL) (McKusick 207900) is a rare autosomal recessive disorder affecting the urea cycle. The cardinal symptom in the neonatal form is progressive hyperammonemia, which is often life-threatening. However, clinical symptoms in the late onset form are quite heterogeneous: neuromotor developmental delay, hepatomegaly, skin involvement and seizures.
Case report: We reported a three-year-old boy presented with vomiting and normal neuromotor development. On his physical examination weight: 14 kg (50 centile), height 97 cm (90 centile) and no visseromegaly was found. Metabolic screening of the patient after a protein challenge revealed hyperammonemia with respiratory alkalosis and a high citrulline and argininosuccinate, but normal arginine levels. The urine orotic acid level was slightly elevated. Argininosuccinic acid lyase was assayed in cultured fibroblasts, providing the definitive biochemical diagnosis. The patient was excellent managed by protein restriction and sodium benzoate for six months. Unfortunately, during the attack of moderate dehydration and slight hyperammonemia, the patient had a seizure and died from sudden cardiac arrest. He did not develop hepatic dysfunction or coma.
Conclusion: The data of death after episodes of coma and hyperammonemia are well-known, but the sudden death is unexpected in urea cycle defects.

335-P

HYPERORNITHINAEMIA, HYPERAMMONAEMIA, HOMOCITRULLINURIA SYNDROME (HHH) PRESENTING WITH ACUTE FULMINANT HEPATIC FAILURE

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We report on two Aboriginal patients with the hyperornithinaemia, hyperammonaemia, homocitrullinuria (HHH) syndrome. Both presented with acute hepatic failure with severe hyper-transaminasaemia and coagulopathy prompting evaluation for emergent liver transplantation.. The diagnosis of HHH syndrome was established based on the presence of typical metabolic abnormalities. A protein-restricted diet and L-arginine or L-citrulline supplementation were immediately started with rapid normalization of liver function tests and other biochemical abnormalities. Molecular analysis of the *SLC25A15* gene showed that the two patients were homozygous for the common French Canadian mutation (F188Δ). The diagnosis of HHH syndrome should be considered in patients with unexplained fulminant hepatic failure. There does not appear to be genotype-phenotype correlation for this presentation as the only other reported patient presenting with this picture had two different point mutations. Early identification and prompt treatment of these patients is crucial to avoid liver transplantation and can be life saving.

336-P

HHH SYNDROME: CLINICAL AND MOLECULAR CHARACTERISTICS OF 12 NEW CASES

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Background: The hyperornithinemia–hyperammonemia–homocitrullinuria (HHH) syndrome is an AR disorder caused by mutations in *SLC25A15* gene, resulting in the defective activity of a mitochondrial carrier that transports ornithine across the inner mitochondrial membrane. The characteristic clinical signs include episodes of hyperammonemia with lethargy/coma, liver dysfunction, coagulation abnormalities, pyramidal dysfunction, seizures and developmental delay/mental retardation.
Objective and results: We report the clinical and molecular characteristic of 12 new HHH patients. Symptoms started in 5 patients in neonatal period, in 5 patients in infancy, in 1 patient in childhood and in 1 patient in adulthood; 8 experienced lethargy/coma, 5 had moderate/severe liver dysfunction, 6 had moderate/severe coagulopathy (<factor VII and X). Only one patient died at 2 months of age before establishing the diagnosis. At follow-up, 7 patients presented pyramidal signs, and 6 had mild/severe MR. Molecular analysis showed a total 12 mutations in SLC25A15 gene of which 9 were novel. Mutations included 2 nonsense (K245X, R179X), 4 missense (M37R, L71Q, T272I, G27R) and 6 frameshift variants, and were not correlated with the clinical signs. **Conclusions**: Our study proposes that the mutations in *SLC25A15* gene are more common than previously believed and the array of variations is wide. Besides the known F188del in French Canadian no other hotspot mutation are usually detected. At follow-up, spastic paraplegia, rare occurrence of metabolic relapses while on treatment and low mortality rate are the characteristic findings in HHH syndrome. Interestingly, acute liver failure and severe coagulopathy were the presenting symptoms in one patient.

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EPILEPSY STARTING 7 YEARS AFTER SUCCESSFUL LIVER TRANSPLANTATION IN A GIRL WITH ARGININOSUCCINATE LYASE DEFICIENCY: MRI AND MRS FINDINGS

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Background: Argininosuccinate lyase (ASL) deficiency is an autosomal recessive urea cycle disorder often presenting with acute encephalopathy in the neonatal period. Although ASL is mainly expressed in liver, it is also present in other organs, like the brain. It is unclear whether late-onset progressive neuropsychiatric symptoms in certain patients with ASL deficiency are related to long-term exposure to elevated systemic ammonia or to the cerebral deficiency of ASL.
Case report: The patient, an 11-year-old girl, developped hyperammonaemic encephalopathy in the neonatal period, leading to the diagnosis of ASL deficiency. Because of insufficient metabolic control with protein restriction, liver transplantation was performed at age 3 years, resulting in excellent metabolic control. At age 10, she had a first complex partial seizure, recurring twice in the following 9 months. EEG showed left hemispheric epileptiform acitvity. MRI showed mild periventricular leukomalacia. MRS showed an elevated ratio of Cho/Cr in white matter, a nonspecific marker of myelin turnover, but no elevation of the Glu/Gln peak. Treatment with lamotrigine resulted in seizure freedom.
Conclusions: Although the epilepsy in our patient can be considered a late manifestation of cerebral damage acquired before liver transplantation, neuro-imaging showed only mild white matter changes and no cortical lesions. Alternatively, epilepsy may be related to cerebral deficiency of ASL. MRS showed no arguments for elevated brain ammonia, indicating that brain ASL is not necessary for ammonia detoxification, but may have another function, possibly related to late-onset neurological symptoms.

338-P

LIVER TRANSPLANTATION CURES BIOCHEMICAL DEFECTS IN A BOY WITH HYPERAMMONEMIA–HYPERORNITHINEMIA–HOMOCITRULLINURIA (HHH SYNDROME)

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We describe a boy with HHH syndrome who received a liver transplantation at the age of seven. He presented at age four weeks in a hyperammonemic coma (ammonia 2300 μM, glutamine 1355 μM, ornithine 616 μM, and arginine 32 μM). Urinary analysis showed increased orotic acid and homocitrulline.

The diagnosis of HHH was confirmed by mutation analysis of the *SLC25A15* gene. Treatment with intravenous benzoate, phenylacetate and L-arginine resulted in rapid normalization of the serum ammonia levels in less than 24 h, but cerebral edema, coma and convulsions lasted for 5 days before recovery. Follow-up showed a major delay in psychomotor development, and a hemiparesis. However, since the age of two, his metabolic disease became more difficult to control with persistent mild hyperammonemia (typical 200–300 μM), poor weight gain and occasional metabolic decompensations requiring repeated hospitalizations. Liver transplantation was performed to improve metabolic control thus avoiding recurrent hospitalization. His ammonemia normalized within the first day following transplantation, and remained normal even though protein intake was normalized from day 10 on. Liver enzymes and serum amino acid levels normalized completely.He is currently one year post transplantation. The parents note an improved mood and concentration.

This is the first report of liver transplantation in a patient with HHH. Liver transplantation adds to the therapeutic possibilities particularly in patients in whom metabolic control is difficult to maintain, requiring a very strict protein restricted diet. This will facilitate effective therapy and prevent secondary neurological damage.

339-P

DIETARY TREATMENT OF HYPERTRIGLYCERIDEMIA OF A PATIENT WITH ADULT CITRULLINEMIA TYPE 2 AND PERITONEAL DIALYSIS

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Introduction: A 14-year-old Kurdish girl from consanguineaous parents is known with neglected meningomyelocele (L4/L5), chronic renal failure, and treated with hemo-(HD) in 2005 and peritoneal(PD) dialysis since 2006. In November 2005 she had incidents of nausea, intermittent hyperammonia, headache, disorikntation and convulsions. At HD triglycerides (TG) were 7 mmol/L. During incidents; ammonia 187 mmol/L, cholesterol 20 mmol/L and TG 75 mmol/L were found. Citrulline (above 800 mmol) and threonine/serine ratio (4) were abnormal. Citrullinemia type 2 was diagnosed and the mutation was demonstrated (*SCL25A13* gene).

Treatment: The initial diet was proteon/potassium/fluid restricted. The patient had an aversion of sweets and preferred fat food. After diagnosis the initial diet was combined with a (ga)lactose free, fat restriced (20 en%), MCT enriched diet. To lower the TG levels under 10 mmol/l a carbohydrate restriction (38 en%) was introduced.

After HD failure PD was started. A high carbohydrate uptake from the PD fluid occured. In reaction TG rose up to 27 mmol/L. Therefore the diet changed ketogenic (60 en% fat, 25 en% carb, 15 en% prot). Dialysis fluid was changed into combination of Nutrineal/glucose 2.27/ ico-dextrine. Citrullinemia related medication; arginine HCl 10% 40 ml, L-aspartate 6000 mg, modalim 25 mg, simvastatine 10 mg was given.
Conclusion: The combination of dialysis and metabolic disease makes the diet complex and difficult in daily practice. Despite lack of appetite (of which special tube feeding is given at night to complete needs of energy and protein) the patient is in stable condition and metabolic control.

340-P

CITRIN DEFICIENCY: PRESENTATION, TREATMENT AND MUTATIONS IN NORTH AMERICA

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Case 1: Elevated citrulline on urinary NBS. Plasma amino acids: Isolated elevated citrulline. No ASA. Initial Rx: Protein intake restricted. Testing: ASS activity: Deficient; ASS Gene sequencing: no mutations. Clinical course, more consistent with citrin deficiency, dominated by significant hepatic dysfunction, recurrent hypoglycemia without elevations in ammonia.

Based on our previous experience a high protein, low carbohydrate diet was instituted. Hepatic dysfunction immediately resolved at approximately one year of age. Clinical sequencing revealed novel heterozygous mutations: R43X and R355X.
Case 2: Elevated citrulline on urinary NBS. PAA: Citrin profile. Citrin sequencing: R43X and R355X. A high protein, low carbohydrate diet was commenced at 6 months of age. Poor compliance with Rx has lead to persisting failure to thrive.
Case 3: Elevated citrulline (350 μmolar) with borderline elevation in tyrosine on MS/MS bloodspot screening. Reflex plasma amino acids at 3 weeks showed a citrulline of 850 μmol with elevations in tyrosine, methionine, threonine to serine ratio and arginine. She had elevated bilirubin and prolonged coagulation time. The liver failure has responded to a high protein low carbohydrate diet.
Discussion: These cases illustrate the importance of considering Citrin deficiency in patients of all ethnicities with elevated citrulline. Similarly, they illustrate the importance of whole gene sequencing when a disorder is suspected outside of the population where common mutations have been described. The availability of dietary therapy for this condition, and the significant worsening seen if protein is restricted, make it vital to distinguish this condition from argininosuccinate synthetase deficiency.

341-P

SERUM FREE CARNITINE LEVELS ARE HIGH IN NEONATAL INTRAHEPATIC CHOLESTASIS CAUSED BY CITRIN DEFICIENCY (NICCD)

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Background: Citrin deficiency causes two age-dependent phenotypes, namely CTLN2 in adults and NICCD in infants. In this study we measure serum levels of free and acyl carnitine and the mechanism of fatty liver, one of distinctive characteristics of NICCD, is considered.
Patients and Methods: Serum levels of free and acyl carnitine of 10 patients with NICCD, whose diagnoses were confirmed by gene analyses, were measured by a fluorometric method using carnitine dehydrogenase. Eight of 10 visited our hospital because of abnormalities in newborn screening.
Results and discussion: The mean free carnitine in NICCD at the initial visit (58.7 ± 7.5 μmol/L) was higher than that in control neonates (22.6 ± 8.6), and that in screen-positive patients (66.0 ± 7.2) was significantly higher. The mean acyl carnitine (13.8 ± 4.5) was almost the same. In NICCD, the malate-citrate shuttle is compensatory hyperactive, which becomes a cause of increased fatty acid synthesis. Carnitine palmitoyltransferase I (CPT I) is the key enzyme in fatty acid oxidation and increased serum free carnitine is a common finding in CPT I deficiency. We speculate that both of increased fatty acid synthesis and decreased fatty acid oxidation are the cause of fatty liver in NICCD. Not more than half of patients with NICCD are detected by newborn screening. A more efficient method to detect NICCD would be measure serum free carnitine level.
Conclusion: In NICCD, both of increased fatty acid synthesis and decreased fatty acid oxidation would be the cause of fatty liver.

342-P

INTRACELLULAR TRANSPORT OF BILE ACID INTERMEDIATES ACROSS RAT LIVER PEROXISOMAL MEMBRANE

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The side-chain cleavage of 3a,7a,12a-tri- and 3a,7a-dihydroxy-5b-cholestan-26-oic-acids (THCA and DHCA) and conversion of the primary bile acids cholic and chenodeoxycholic acid (CA, CDCA) thus generated to glycine- and taurine-conjugates respectively, is catalized by peroxisomal enzymes. In the present study we have investigated the transport of the bile acid intermediates across the peroxisomal membrane, using highly purified hepatic peroxisomes isolated from rats as well as *ALDP*$^{-/-}$ mice and ESI-MS/MS to monitor the formation of the conjugates.

Conjugates were only formed incubating rat hepatic peroxisomes with THCA- and DHCA-CoA, suggesting that the CoA esters are preferentially imported. Product formation was clearly dependent on both the concentrations of substrates and peroxisome proteins, yet did not require ATP/Mg^{2+}, and proved to be inhibited by BSA. Biosynthesis of conjugates was also inhibited, pre-treating the organelles with either protease K, histone or Triton X-100. Last but not least, lower rates of conjugates were formed by peroxisomes from ALDP$^{-/-}$ mice or when rat hepatic peroxisomes were incubated with both the CoA esters of bile acid intermediates and VLCFA. In contrast, β-oxidation activities proved to be unaffected in all the experiments.

Taken together, these findings provide evidence that bile acid intermediates and VLCFA compete for a common 'transporter' across the peroxisome membrane with the ALD protein involved in the transport.

343-O

GENETIC CLASSIFICATION OF CELLS FROM PATIENTS WITH A PEROXISOME BIOGENESIS DISORDER

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Background: The peroxisome biogenesis disorders (PBDs), including Zellweger syndrome, NALD and IRD; comprise a group of severe, often lethal, inherited multi-systemic disorders. They can be caused by mutations in any of at least 12 different PEX genes. Cells of PBD patients lack peroxisomes and show marked aberrations in metabolic pathways that involve functional peroxisomes. To identify the defective PEX genes, we set out to assign all PBD cell lines from our large laboratory cell collection to different genetic complementation groups (CGs).
Method: Skin fibroblasts from PBD patients were assigned to CGs using the traditional polyethylene glycol (PEG) fusion complementation assay and a newly developed PEX transfection assay. The latter involves co-transfection of wild type PEX cDNAs with the peroxisomal reporter protein GFP-SKL into PBD cells with an unknown PEX gene defect. Two days after transfection, cells are examined by fluorescent microscopy for the localization of GFP-SKL.
Results: We assigned more than 500 different PBD cell lines to 14 different CGs. Interestingly, two patient PBD cell lines could not be assigned to any of the 12 known CGs, but were able to complement each other upon PEG-induced fusion. We are currently searching for candidate PEX genes that may be defective in these two cell lines.
Conclusions: The newly developed PEX transfection assay allows for rapid identification of PEX genes defective in PBD patients, which is an important step in the diagnostic workup of such patients and allows for the identification of patients with novel PEX gene defects.

344-P

URINE ACYLCARNITINE ANALYSIS BY ESI-MS/MS: A NEW SCREENING METHOD FOR PEROXISOMAL DISORDERS

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Background: We have previously shown that patients with peroxisomal biogenesis disorders (PBDs) show an abnormal profile of circulating long-chain dicarboxylic C16- and C18-carnitines (i.e. hexadecanedioyl- and octadecanedioyl-carnitine), and very long-chain C24- and C26-acylcarnitines (i.e. lignoceroyl- and cerotoyl-carnitine) as detected by ESI-MS/MS (Pediatr Res 2003). Accordingly, we have now tested whether similar abnormalities were also detectable in urine.
Methods: Urine spot morning samples (3.5 μl) were studied from 7 patients with PBDs, from 2 patients. with D-bifunctional protein deficiency (D-BP), 14 with X-ALD, and 130 healthy controls. Samples were extracted with methanol, analysed as butylesters by ESI-MS/MS using multiple reactions monitoring (MRM) method and quantified using labelled [2H9]-hexadecanedioyl-carnitine and [2H4]-cerotoyl-carnitine as internal standards. Acylcarnitine values are expressed as μmol/mmol creatinine. Acylcarnitine levels between groups were compared by the Mann–Whitney test.
Results: In PBDs, the urinary excretion of both long-chain dicarboxylic carnitines [C16- (median 0.62; controls <0.03) and C18- (median 0.28; controls <0.03)] and very long-chain acylcarnitines [C24- (median 0.05; controls <0.03) and C26- (median 0.02; controls = undetectable)] were significantly elevated compared to controls ($p<0.0001$). Also in D-BP, the excretion of long-chain dicarboxylic carnitines was significantly raised, whereas very long-chain acylcarnitines were elevated only in one of the two samples studied. Urine acylcarnitines were normal in all X-ALD samples.
Conclusions: The ESI-MS/MS quantification of urinary long-chain dicarboxylic carnitines represents a new simple, rapid and reliable screening method to identify PBDs and D-BP deficency.

345-P

A NOVEL APPROACH FOR QUANTIFICATION OF PRISTANIC, PHYTANIC, C22:0, C24:0 AND C26:0 BY UPLC-MS/MS FOR THE DIAGNOSIS OF PEROXISOMAL DISORDERS

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Background: Peroxisomal disorders (PDs) are a group of congenital diseases caused by defective peroxisomes. These organelles are mainly involved in lipids metabolism and a progressive multisystem disease often occurs due to their dysfunction. Quantitative analysis of pristanic, phytanic, C22:0, C24:0 and C26:0 (VLCFAs) by GC-MS is used for the diagnosis of PDs in the majority of biochemical genetics labs, however, it is rather demanding with tedious sample pretreatment and long chromatographic times. In this work, VLCFAs were quantified after derivatization with 4-[2-(N,N-dimethylamino)ethylaminosulfonyl]-7-(2-aminoethylamino)-2,1,3-benzoxadiazole (DAABD-AE), a recently developed labeling reagent for carboxylic acids with excellent mass spectrometric characteristics (1). **Methods:** Plasma (20 µl) was heated with acetonitrile-HCl (1000C, 1 h), extracted with hexane and treated with DAABD-AE (45 min, 65°C). The resultant reaction mixture was analyzed by UPLC-ESI-MS/MS in the positive-ion mode using MRM with a turnaround time of 5 min. **Results:** Calibration curves were linear in a range covering physiological and pathological levels for all analytes investigated. At low and high concentrations, inter-day (n = 12) and intra-day (n = 10) variations were less than 9.2%. Reference ranges obtained (n = 250) were in excellent agreement with literature. The method was applied retrospectively for the analysis of plasma samples from patients with established PDs (n = 60). **Conclusions:** This simple, specific and sensitive UPLC-MS/MS for VLCFAs is an attractive alternative for the classical GC/MS methods. The assay requires no special precautions and no interference was observed in all the samples analyzed.

(1) Al-Dirbashi OY, Santa T, Al-Qahtani K, Al-Amoudi M, Rashed MS. Rapid Comm. Mass Spec. In press.

346-P

CHARACTERISATION OF ZELLWEGER SYNDROME PATIENTS WITH A DEFECT IN THE *PEX5* GENE

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Background: Proteins destined for the peroxisomal matrix are targeted by virtue of PTS1 or a PTS2 signal. In humans, both import pathways rely on a cytosolic receptor protein encoded by the *PEX5* gene. Transcription of *PEX5* results in two splice forms encoding the two protein variants *PEX5S* and *PEX5L*. *PEX5*S is exclusively involved in PTS1 protein import, whereas *PEX5L* also mediates the import of PTS2 proteins. Complementation testing with cells from Zellweger Syndrome patients identified 10 different cell lines with a defect in *PEX5*. The aim of this study was to characterize these cell lines at a biochemical and genetic level.

Method: Cultured patient skin fibroblasts were analyzed for VLCFA concentrations, peroxisomal β-and α-oxidation and DHAPAT activity, and used for immunoblot analysis of thiolase and immunofuorescence microscopy with antibodies against peroxisomal catalase and ALDP. Mutation analysis of the *PEX5* gene was performed with gDNA and cDNA. Import of PTS1 and PTS2 proteins was assessed by transfection of cell lines with a GFP protein fused with either a PTS1 or a PTS2 signal.

Results and **Conclusions:** All patient cells showed a severe biochemical phenotype in line with the original clinical diagnosis of Zellweger Syndrome. Six patient cell lines showed a defect in both PTS1 and PTS2 protein import. The other four cell lines only showed a defect in PTS1 protein import. Patient cells with the isolated PTS1 import defect all had missense mutations affecting amino acids not located in domains previously shown to be involved in PTS2 protein import.

347-P

RATIONAL DIAGNOSTIC PROCEDURE FOR ZELLWEGER SYNDROME (ZS) AND OTHER PEROXISOMAL BIOGENESIS DEFECTS (PBDs)

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Objective: PBDs are clinically and genetically heterogeneous disease entities. The Zellweger spectrum comprises a clinical continuum from ZS as the most severe form, over neonatal adrenoleukodystrophy to infantile Morbus Refsum as the mildest variant.

Measuring plasma very long chain fatty acids (VLCFA) is a well-established screening, when clinical symptoms like floppy infant, failure to thrive, hepatomegaly, renal cysts as well as dysmorphic features suggest a PBD. Determination of plasmalogen concentration in erythrocytes then allows distinguishing between the PBD and the peroxisomal beta-oxidation defect. If VLCFAs are elevated and plasmalogen biosynthesis is impaired, diagnosis of the PBD is most likely. So far, 13 different PBD disease genes (PEX genes) are known. Identification of the affected PEX gene and consecutive mutation analysis is indispensable for the diagnosis as well as for prediction of the clinical course.

Methods and **Results**: Since defect in any of 13 PEX genes can be responsible for PBD, a rational diagnosis is necessary to clarify the primary genetic defect in a given patient. Thus, we have developed a diagnostic flow-chart applying cell biology and molecular genetic methods to identify the patient mutation rapidly and cost-effectively. After exclusion of the two most common mutations G843D and I700YfsX42 in the *PEX1* gene, we recommend a skin biopsy to culture fibroblasts. We then transfect the cultured patient fibroblasts with all known human PEX genes and screen for restoration of peroxisomal biogenesis. The PEX gene recovering peroxisomal function will be sequenced to define the exact genetic defect.

348-P

NON-INFORMATIVE FIBROBLAST ANALYSES IN ZELLWEGER SPECTRUM DISORDER PATIENTS: A RISK TO PRENATAL TESTING

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In general patients with Zellweger spectrum disorder (ZSD) have elevated plasma very long chain fatty acids (VLCFA), bile acid intermediates and pipecolic acid, and deficient erythrocyte plasmalogens. In the majority of ZSD patients defective peroxisome metabolism and assembly can be corroborated by analyzing cultured fibroblasts. However, cultured cells from a subset of patients do not express the expected abnormalities. Previously we have referred to this disparity as type 1 peroxisome mosaicism (Steinberg et al 2006 BBA 1763:1733-1748). Confirmation of the diagnosis still can be made by raising the culture temperature to 400°C (Gootjes et al., Human Mutation. 2004;24:130–9) or by molecular analysis.

Although type 1 mosaicism is documented in the literature, no one has previously reported its prevalence. To assess the proportion of patients with type 1 mosaicism we reviewed the fibroblast results for samples received in the last 7 years from patients with a presumptive ZSD diagnosis. Of 159 cases, 5 patients (3.1%) had both normal fibroblast VLCFA and plasmalogen synthesis. Molecular analysis in all 5 patients identified PEX gene mutations (2 *PEX10* and 1 each of *PEX6*, *PEX12* and *PEX26*) consistent with ZSD. Thus, we estimate that about 3% of ZSD patients do not express defects in cultured cells using standard culture conditions; if not recognized in the proband then this could lead to error in prenatal diagnosis.

349-P

CILIATED BILE DUCT EPITHELIUM IN THE LIVER OF A ZELLWEGER PATIENT

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Mutations in cilium proteins polycystin 1 and 2 are linked to polycystic diseases in kidney and liver. In rats bile duct cells possess a single cilium; its mechanostimulation by bile flow increases intracellular Ca++ and lowers cAMP that drives HCO3 secretion (Masyuk, 2006). In contrast human liver has no ciliated bile ducts, except for a single report (Yoshino, 1979) not confirmed by others.

We report the presence of multiple cilia on intralobular bile duct cells in the liver of a 1 month-old girl born to non-consanguinous W-European parents. Her head circumference was below the 3rd P for her age and she showed minor facial dysmorphia, major hypotonia, increased liver enzymes and hypoglycemia. A Zellweger syndrome was suggested by strongly increased VLCFA and cortical renal cysts. Further work-up revealed abnormal ABR with a threshold of 80 db. The baby died at 3.5 months in status epilepticus.

Standard microscopy of the liver was unremarkable, but immunolocalisation of catalase and AGT showed staining in the parenchymal cytoplasm instead of in granules. By electron microscopy no peroxisome-like organelles were found; macrophage lysosomes contained typical but small trilamellar inclusions, and insoluble lipid. The lumen of 9/11 interlobular bile ducts in different blocks of a surgical biopsy sample demonstrated multiple cilia in addition to normal microvilli. Cilia were incomplete, for ex. single tubuli instead of doublets. In bile ducts of 5 out of 6 other Zellweger livers we did not observe cilia. We propose that ciliated cells reflect a partial transdifferentiation likely related to specific mutations.

350-P

TREATING PEROXISOMAL BIOGENESIS DEFECTS BEYOND THE NEONATAL PERIOD: A CASE FOR AGGRESSIVE TREATMENT

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A 9 and half-month-old girl presented in 2002 with failure to thrive, elevated liver enzymes, hypotonia, nystagmus and developmental delay. She did not orient to sounds and had poor visual regard. Developmental skills were in the 4–5 month range. Her examination was significant for a very large anterior fontanelle, hepatomegaly, nystagmus with poor fixation and moderate diffuse hypotonia. Her biochemical profile was suggestive of a peroxisomal biogenesis defect which was confirmed by fibroblast studies. An aggressive treatment regimen with docohexanoic acid (DHA), normalization of carnitine levels, bile acids, fat soluble vitamins and low phytanic diet was initiated along with developmental services. Within months, she exhibited an improvement in vision, tone and development. She was fitted for bilateral hearing aids and glasses.

At 5 years of age, she is able to ride a tricycle and scooter. She enjoys drawing, cutting and dressing herself. She has 75–100 words which she combines into phrases and also uses 60 signs. She can follow multi-step commands and can count objects. Her night vision remains poor and she has decreased peripheral vision. There has been near normalization of liver enzymes. Her adrenal function has remained normal.

This case demonstrates the potential benefits of aggressive treatment of peroxisomal biogenesis defects diagnosed beyond the neonatal period. A multidisciplinary approach to treatment significantly improves outcome.

351-O

DISCOVERY OF THE PEROXISOMAL PROTEASE RESPONSIBLE FOR THE PROCESSING OF PEROXISOMAL PROTEINS CARRYING A PEROXISOMAL IMPORT SIGNAL TYPE 2

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Background: Proteins destined for the peroxisomal matrix are targeted to peroxisomes by virtue of peroxisomal import signals (PTS). In mammals, the N-terminal PTS type 2 (PTS2) is found in peroxisomal 3-oxoacyl-CoA thiolase (pTH1), alkylglycerone phosphate synthase (AGPS), and phytanoyl-CoA hydroxylase (PhyH). The N-terminal PTS2 presequence is cleaved off after import into peroxisomes via the proteolytic activity of a peroxisomal protease.
Methods: Subcellular localization of peroxisomal proteins was determined using fluorescence microscopy and organellar fractionation in sucrose gradient from mouse tissue homogenates. Proteolytic activity was measured in fibroblasts and ascertained using western blot analysis.
Results: Using the database containing the genome of the plant Arabidopsis thaliana, we underwent a search for new peroxisomal proteins. Based on motif analysis we found three proteins with protease-like domains. One of these protease-like proteins had homologues in other species and was chosen for further studies. We cloned the mouse Tysnd1 gene and found that the encoded protein (PPP) was imported into peroxisomes in a PEX5-dependent manner. Using a patient cell line with defective PTS1 mediated import, but normal PTS2 mediated import we were able to determine that in vivo PPP cleaved both AGPS and pTH1 to their mature forms, albeit with different efficiencies.
Conclusions: We have identified PPP as a new peroxisomal protein belonging to the serine family of proteases and have characterized it as the protease responsible for the intra-peroxisomal cleavage of the PTS2 presequence of PTS2-containing proteins. Further studies will reveal the role of the cleavage process for the functioning of PTS2-containing proteins.

352-P

X-LINKED ADRENOLEUKODYSTROPHY IN ARGENTINA. IDENTIFICATION OF NOVEL, KNOWN MUTATIONS AND POLYMORPHYSMS IN *ABCD1* GENE

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Background: X-linked adrenoleukodystrophy (X-ALD) is a neurodegenerative disorder, characterized by an increase of very long-chain fatty acids in serum. More than 900 mutations have been reported in the *ABCD1* gene, which has 10 exons that encodes a peroxisomal ABC transporter protein.
Objective: From a total of 22 patients, we analyzed the genomic variations in the *ABCD1* gene in 9 unrelated probands (8 from Argentina and 1 from Italy).
Methods: It was designed 6 primers for 1, 4, 6 and 10 exons of this gene. PCR products distinguished by single strand conformation analysis were subjected to direct sequencing. **Results**: We identified 2 novel and 1 known mutations that allowed us the molecular characterization of 4/9 patients (2 with childhood cerebral form and 2 with adrenomyeloneuropathy; intra-familiar phenotype variability was observed). The new mutations identified were an insertion in exon 1 (c.852853insACTC), that cause a frameshift in Serine 284 (p.Ser284fs); a missense mutation in exon 4 (c.1259A>C, p.His420Pro). A known missense mutation (c.2006A>G, p.His669Arg in exon 10) was detected in 2 patients. In exon 6 none patients had mutations. Moreover, 4 polymorphisms were observed: 1 frequent at world level (c.1548G>A, exon 6 $n = 1$), 1 rare (c.2019C>T, exon 10 n=1) and 2 intronic changes (one novel, IVS6+14T>A $n = 2$ and the other IVS9-32C>T as unique but frequent in our population $n = 6$).
Conclusions: These findings expand the recognition of this disorder and its variants in Argentina, besides of the molecular analysis that provides assessments for female carriers of families on risk.

353-O

PEROXISOME-MITOCHONDRIA CROSS-TALK AND OXIDATIVE STRESS UNDERLYING NEURODEGENERATION IN X-ALD

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X-linked adrenoleukodystrophy (X-ALD) is a neurometabolic syndrome and the most frequent inherited monogenic demyelinating disease (minimal incidence 1:17 000). X-ALD leads to death in boys due to cerebral demyelination or to motor disability in adults, due to spinal cord and peripheral nerve degeneration (adrenomyeloneuropathy or AMN). The gene mutated in the disease (*ABCD1*) is a peroxisomal ATP-binding transporter of very-long-chain-fatty acids (VLCFAs), whose accumulation in plasma and tissues is the hallmark of the disease. We have generated and characterized mouse models for X-ALD, by classical knockout of the *ABCD1* gene. These mice exhibit a late-onset phenotype closely related to AMN patients, with neurodegenerative features (excitotoxicity, microgliosis, axonal degeneration, slower nerve conduction velocity and psychomotor impairment), that begin at 15 months of age[1–3]. The pathogenesis of X-ALD is largely unknown, so are the mechanisms of toxicity through VLCFAs. Using microarrays, Q-PCR and Western Blots of mouse spinal cords, we have identified a dysregulation of oxidative stress routes and mitochondria depletion as early events in the pathogenesis (3.5 months of age). Treatment with VLCFAs of fibroblasts and neuronal cultures induces ROS generation and decrease of mitochondria membrane potential. Ex-vivo organotypic spinal cord slice cultures recapitulate closely the pathogenic events seen in spinal cords, and will constitute a powerful screening tool for therapeutic agents, and for deciphering molecular cues underlying neuro-degeneration in X-ALD.

[1]Pujol et al, Hum Mol Genet. 2002;11(5):499–505.
[2]Pujol et al, Hum Mol Genet. 2004;13(23):2997–3006.
[3]Ferrer et al, Hum Mol Genet. 2005;14(23):3565–77.

354-O

HOMO- AND HETERODIMERIZATION OF THE HUMAN ADRENOLEUKODYSTROPHY PROTEIN (ALDP; ABCD1) IN THE PEROXISOME MEMBRANE

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Adrenoleukodystrophy protein (ALDP) and the 70-kDa peroxisomal membrane protein (PMP70) are half ATP-binding cassette (ABC) transporters in the mammalian peroxisome membrane. Mutations in the gene encoding ALDP result in a devastating neurodegenerative disorder, X-linked adrenoleukodystrophy (X-ALD) that is associated with elevated levels of very long chain fatty acids due to impaired peroxisomal β-oxidation. The interactions of peroxisomal ABC transporters and their functions in disease pathogenesis are poorly understood. Studies on ABC transporters revealed that half transporters have to dimerize to gain functionality. So far, conflicting observations are described for ALDP. By the use of in vitro methods on the one hand, it was shown that ALDP homodimerizes as well as heterodimerizes with PMP70 and ALDR, while on the other hand, it was demonstrated that ALDP and PMP70 exclusively homodimerize. To circumvent problems of artificial interactions due to biochemical sample preparation in vitro, we investigated protein-protein interaction of ALDP and PMP70 in its physiological environment by FRET microscopy in intact living cells. The statistical relevance of FRET data was determined in two ways using probability distribution shift analysis and Kolmogorov-Smirnov statistics. We demonstrate in vivo that ALDP and PMP70 form homodimers as well as ALDP/PMP70 heterodimers. Using C-terminal deletions of ALDP, we demonstrate that the last 87 C-terminal amino acids harbor the most important protein domain mediating these interactions, and that the N-terminal transmembrane region of ALDP has an additional stabilization effect on ALDP homodimers. Loss of ALDP homo- or heterodimerization is highly relevant for understanding the disease mechanisms of X-ALD.

355-P

LIPOPEROXIDATION IS INDUCED IN FEMALE CARRIERS OF X-LINKED ADRENOLEUKODYSTROPHY

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Background: X-linked adrenoleukodystrophy (X-ALD) is an X-chromosome linked severe demyelinating disease that is biochemically characterized by the accumulation of very long chain fatty acids (VLCFA), particularly hexacosanoic and tetracosanoic acids in different tissues and in biological fluids. The clinical characterization of this disease is due to a central and peripheral demyelination, as well as adrenal insufficiency. A considerable number of heterozygote (HTZ) for X-ALD develops neurological symptoms like spinal cord involvement resembling milder forms of adrenomyeloneuropathy. The mechanisms of brain damage in hemizygotes and heterozygotes X-ALD patients are poorly understood. Considering that oxidative stress was involved in various neurodegenerative disorders and that in a previous study we showed evidence that oxidative stress is probably involved in the pathophysiology of X-ALD symptomatic patients, in the present study we evaluated oxidative stress parameters.
Methods. Thiobarbituric acid reactive substances (TBA-RS) and total antioxidant reactivity (TAR) was measured in plasma of HTZ individuals.
Results. It was observed that female carriers present a significant increase of plasma TBA-RS measurement, indicating a stimulation of lipoperoxidation, as well as a decrease of plasma TAR, reflecting a deficient capacity to rapidly handle an increase of reactive species.
Conclusions. These results indicate that oxidative stress is involved in the pathophysiology of heterozygotes X-ALD individuals.

356-P

LONG-TERM MONITORING OF UNSATURATED AND SATURATED VLCFA PROFILE SERUM IN HEMIZYGOTES AND HETEROZYGOTES WITH X-ALD, TREATED WITH LORENZO OIL

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Background: Elevation of VLCFA (C26:0, C24:0) levels in serum in patients with X-ALD is believed as one of the pathogenic factor in this disorder. The VLCFA measurement is a main diagnostic marker for X-ALD besides the clinical status of the patient. One of the recommended methods of treatment is administration of the diet and Lorenzo oil (LO). The aim of this work was to analyze and estimate the VLCFA profile and unsaturated acids (erucic C22:1; nervonic C24:1) in patients with X-ALD treated with diet and LO.
Methods: Serum samples (55) from 6 hemizygotes and from 1 heterozygote treated with diet and LO from 1 to 6 years were analyzed. The measurements of unsaturated and saturated VLCFA were performed by gas chromatography method, every 2 to 8 months.
Results: The serum levels of saturated VLCFA in all patients after two months of treatment decreased to the normal value. We observed the increase of C22:1 level about 500%, and of C24:1 up to 450% comparing with the previous values before the treatment began.
Conclusions: Long term diet and LO administration cause the decreasing of VLCFA (C26:0, C24:0) levels but leads to the high accumulation of unsaturated fatty acids C22:1and C24:1. The influence of high concentration of these fatty acids on X-ALD clinical course is unknown.

357-P

VLCFA LEVELS IN SERUM IN PATIENTS WITH X-ALD AFTER THE BONE MARROW TRANSPLANTATION

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Background: Elevated accumulation of VLCFA (C26:0, C24:0) is a main marker for X-ALD patients identification. High concentration of these fatty acids in tissues and serum seems to be the pathogenic factor in these severe demyelinization disease. Bone marrow transplantation is one of the therapeutic proposals for affected boys.
Methods: We measured the serum VLCFA levels in 5 X-ALD transplanted patients (mean age 8.6 years), in one the BMT was profound twice. Two of them were transplanted in symptomatic and advanced stage of the disease. Three were transplanted before the symptoms appeared. All of the patients, but one, have transplanted from family donors.
Results: Our results show that the VLCFA accumulation measured as a ratio C26:0/C22:0 and C24:0/C22:0 8 months after a successful BMT in 4 recipients were on heterozygotes levels (mean $\pm$ SD; 0.024 ± 0.010; 1.254 ± 0.110), in one remained stable from 6 years. In patients in whom BMT was not successful – VLCFA were on hemizygotes levels.

358-P

HEMATOPOIETIC STEM CELL TRANSPLANTATION IN X-LINKED ADRENOLEUKODYSTROPHY: FATTY ACIDS IN BLOOD AS MARKERS OF PEROXISOMAL FUNCTION RESTORATION

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X-linked adrenoleukodystrophy (XALD) is a neurometabolic disease with a decreased ability to degrade very long chain fatty acids (VLCFA) and significant phenotypic variation. Hematopoietic stem cell transplantation (HSCT) constitutes the only curative approach able to prevent the progression of childhood cerebral XALD (CCALD).
Objective: To study the effect of HSCT on the fatty acid (FA) profile in plasma and mononuclear cells (MNC) as a marker of peroxisomal function restoration.
Patients: ALD1 developed severe acute graft-versus-host disease and died 6 months after HSCT; ALD2 died one year after HSCT due to XALD progression; ALD3 is still alive after 6 months of HSCT, with slow neurological recovery and reduction of cerebral MRI demyelization. ALD2 and ALD3 had complete chimerism in peripheral blood and ALD1 only 80%.
Results: After HSCT: (a) All patients presented an increase of total FAs in plasma, but not in MNC; (b) the C26:0/C22:0 ratio in plasma was variable, but decreased in MNC; (c) in ALD3 docosahexaenoic acid (C22:6w3) increased in plasma and MNC; in ALD1 and ALD2 remained invariable in serum and lowered in MNC.
Conclusion: After HSCT, VLCFAs in MNC, can be useful as markers of the peroxisomal function restoration better than in plasma. In addition, an increase of C22:6w3 can indicate a good prognosis in the XALD evolution, due to its inflammatory negative modulator effect.

359-P

REFSUM DISEASE IN A YOUNG GIRL: EFFECTIVENESS OF DIET AND LIPID APHERESIS

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Refsum disease leads to retinopathy, polyneuropathy, ataxia, and deafness. It is caused by a deficient peroxisomal breakdown of phytanic acid (PA). Patients accumulate large amounts of toxic PA from nutritional sources in their tissues. Dietary avoidance of PA can reduce the tissue load. Lipid apheresis has been used in adults to remove PA.

Ophthalmological examination of a 14-year-old girl with night blindness revealed retinopathy and anosmia, a constellation suspicious of Refsum disease. This diagnosis was confirmed by elevated plasma PA and by demonstration of homozygosity for a novel mutation of the PA hydroxylase gene (*PHYH* p.A277E).

By institution of a PA-restricted diet PA plasma levels could be reduced from 8% of total fatty acids (normal $<0.05\%$) to 5% in 18 months. As body fat represents a large reservoir of PA, a danger of PA toxicity may persist for a long time, even with strict dietary compliance. We therefore instituted monthly lipid apheresis for a period of 2 and half years, using extracorporeal veno-venous membrane differential filtration, a procedure well tolerated and later substituted by heparin-induced LDL precipitation. Both apheresis procedures appeared effective. The cumulative removal of PA was 2.500 mg. PA plasma levels could be further decreased to below 3%, a probably non-hazardous range, so that therapy was reduced to diet alone. During a 5-year follow-up, ophthalmological findings as well as signs of a minor demyelinating polyneuropathy have stabilized completely.
Conclusion: We advocate early recognition of Refsum disease, as adequate treatment can prevent devastating neurological consequences in young persons.

360-O

ALDH10 – THE ALDEHYDE DEHYDROGENASE DEFICIENT IN SJOGREN LARSON SYNDROME – PLAYS A KEY ROLE IN THE OMEGA-OXIDATION OF VERY-LONG-CHAIN FATTY ACIDS: IMPLICATIONS FOR BOTH X-LINKED ADRENOLEUKODYSTROPHY AND SJOGREN LARSON SYNDROME

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Background: In search of therapeutic strategies for X-ALD we are studying different metabolic pathways including the chain elongation and omega-oxidation of very-long-chain fatty acids, whose up- or down regulation, may lower VLCFA levels *in vivo* in X-ALD patients. Our earlier work has shown that VLCFAs like C24:0 and C26:0 do undergo omega-oxidation with CYP4F2 and CYP4F3B, catalyzing the first reaction. The aim of this work was to further characterize the VLCFA omega-oxidation pathway.
Methods: Total homogenates as well as microsomal and cytosolic fractions of human liver were incubated with different omega-OH fatty acids, followed by analysis of the products formed by tandem-mass spectrometry.
Results: Our results show that omega-OH-VLCFAs like omega-OH-C22:0 and omega-OH-C26:0 can be omega-oxidized to the corresponding dicarboxylic acid via two routes, one involving two cytochrome P450s (CYP450s), and the other mediated by alcohol dehydrogenase and aldehyde dehydrogenase, respectively. The latter route predominates in human liver (microsomes). The two routes were further characterized using specific inhibitors. Subsequent studies of the two routes revealed that ALDH10, the enzyme deficient in Sjogren Larson syndrome (SLS) plays a key role in the NAD-dependent route.
Conclusion: We have now resolved the mechanism of omega-oxidation of VLCFAs including the enzymes involved. The finding that ALDH10 is involved in VLCFA omega-oxidation may well have implications for both X-ALD and SLS.

362-P

SUBCLINICAL CHANGES IN THE JUVENILE CRYSTALLINE MACULAR DYSTROPHY IN SJÖGREN-LARSSON SYNDROME DETECTED BY OPTICAL COHERENCE TOMOGRAPHY

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Introduction: Sjögren-Larsson syndrome (SLS) is an autosomal recessive neurocutaneous disorder due to fatty aldehyde dehydrogenase deficiency. A crystalline retinopathy is one of its peculiar clinical features. We aimed to study the morphologic changes in the macula by optical coherence tomography (OCT) in patients with SLS.
Methods: Fourteen patients, mean age 14.6 (range 3–24) years, with biochemically and genetically proven SLS underwent full clinical ophthalmologic examination and OCT investigation. OCT scanning was performed using the macular thickness map protocol of Stratus OCT. The results were compared to earlier obtained cerebral magnetic resonance (MR) imaging and spectroscopy studies of the same patients.
Results: Besides clinically visible perimacular crystalline deposits in all eyes of all patients, macular morphology and reflectivity were significantly abnormal on OCT. We found focal hyper-reflectivities in all eyes within the perimacular ganglion cell layer and the inner plexiform layer, corresponding to the clinical localization of retinal crystals. More interestingly, a cystic foveal degeneration on OCT was present in the majority of patients with SLS, varying from multiple microcysts to cystic foveal atrophy. In general, patients who were severely affected on OCT showed the most prominent abnormalities on previously performed cerebral MR spectroscopy studies. OCT proved to be an interesting technique for the study of patients with neurodegenerative and metabolic disorders with involvement of the eyes.

363-P

SJÖGREN-LARSSON SYNDROME: MOTOR PERFORMANCE AND EVERYDAY FUNCTIONING

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Introduction: Sjögren-Larsson syndrome (SLS) is an autosomal recessive disorder due to fatty aldehyde dehydrogenase deficiency, characterized by spasticity, mental retardation and ichthyosis. We aimed to study the level of motor performance and everyday functioning in patients with SLS, since these data are missing in the literature.
Methods: Nine female and eight male patients with biochemically and genetically proven SLS were investigated. Age ranged from 1 to 35 years. Data were obtained by structured interview with parents and children or young adults with SLS, a questionnaire by telephone interview, and physical examination. Motor performance was measured by the Gross Motor Function Measure, everyday functioning by Pediatric Evaluation of Disability Inventory and the Vineland Adaptive Behaviour Scale.
Results: In most patients spasticity was bilaterally present in hamstrings, adductors of the hips and gastrocnemic muscles; all participants above the age of 7 years had contractures in the lower extremities. Limitations were present in all gross motor activities, except for lying and rolling. Walking was very difficult or impossible for most children and adolescents. Developmental ages were far below calender ages. Although some patients can reach a certain level of independency, most of them have activity limitations and restrictions in their participation in society.

364-O

HEPARAN SULFATE OLIGOSSACCHARIDES ACTIVATE MICROGLIA BY SIGNALLING THROUGH TOLL-LIKE RECEPTOR 4 AND MYD88 ADAPTOR PROTEIN

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In mucopolysaccharidosis type IIIB (MPSIIIB), alpha-N-acetylglucosaminidase deficiency results in heparan sulfate oligosaccharides (HS oligo) accumulation in tissue, neurodegeneration and neuroinflammation. We investigated to which extend HS oligo accumulation causes microglia activation *in vitro* and neuroinflammation in vivo. HS oligo fractions purified from MPSIIIB patient urines were characterized by tandem mass spectrometry. We showed these fractions activate normal mouse microglia, inducing morphological changes, increased expression of CD11b and ED1, release of TNF-alpha and IL-6 and increased amounts of TNFalpha, IL-6, IL-1β and MIP-1alpha mRNAs. Normal bovine HS, synthetic heparin and GAG purified from normal urines were ineffective. These effects persisted in the presence of polymyxin B, a drug which inhibits endotoxin effects. Similar experiments performed using microglia isolated from TLR4 or MyD88 deficient mice showed reduced activation. Consistently with *in vitro* data, staining of brain section showed activated microglia and amounts of TNFalpha, IL-6, IL-1β and MIP-1alpha mRNAs increased with age. After correction of the enzyme defect and HS accumulation by gene therapy, TNFalpha, IL-6, IL-1β, MIP-1alpha mRNAs were equivalent to normal levels at 8 months. In MPSIIIB mice cross-bred with TLR4 or MyD88 KO mice, amounts of TNFalpha, IL-6, IL-1β and MIP-1alpha mRNAs were much lower in double mutant mice than in MPSIIIB mice at 10 days, slightly lower at 3 months and equivalent at 8 months. These results indicated that neuroinflammation was delayed but still present in MPSIIIB deficient for TLR4 or MyD88, suggesting that HS oligo induce neuroinflammation not only through this pathway but also through additional mechanisms.

365-O

CALPAIN CLEAVAGE OF CRMP1 IN BRAIN OF MPSIIIB MOUSE MODEL

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Background: Mucopolysaccharidosis type IIIB (MPSIIIB) is a lysosomal disorder characterized by untreatable progressive neurological involvement. It results from a defect of alpha-N-acetylglucosaminidase required for heparan sulphate (HS) catabolism. Pathophysiology is only partially elucidated but recently, an alteration of the brain expression of the fibroblast growth factor (FGF) family members has been shown. Collapsin response mediator protein-1 (CRMP-1) is a downstream target of the FGF signalling pathway implicated in neurite outgrowth and adult brain plasticity. Thus, considering the close relationship between HS, FGF and CRMP-1, we have hypothesized that expression of CRMP-1 could be altered in our model.
Methods: Expression of CRMP-1 was explored by RT-PCR and western blot in MPSIIIB and control mouse cortex. Specific CRMP-1 cleavage by calpain was explored *in vitro*. Calpain activation in MPSIIIB mouse cortex was studied by proteolytic cleavage of alpha2-spectrine.
Results: CRMP-1 mRNA levels remained unchanged. A proteolytic cleavage of CRMP-1 was observed. We showed that calpaine was activated in MPSIIIB brain and that CRMP-1 is a calpain substrate.
Conclusions: Calpain activation is well known in neuropathology and recently calpain cleavage of CRMP was described in models of traumatic brain injury and neurotoxicity. In MPSIIIB, calpain activation could be a consequence of intracellular calcium overload due to secondary brain gangliosides accumulation. These findings suggest potential targets for treating neuropathology in MPSIIIB.

366-P

WHEN THE LYSOSOME MEETS THE MITOCHONDRION

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Background: Niemann–Pick disease, type C (NPC) is an autosomal recessive neurodegenerative disease caused by mutations in the NPC1 or NPC2 genes. Mitocondrial dysfunction is reported in many neurodegenerative disorders, including Parkinson, Huntington, and Alzheimer diseases as well as many genetic disorders such as Prader Wılli syndrome, neuronal ceroid lipofuscinosis, etc
Methods and **Results**: We describe an infant who presented with the nonspecific symptoms of intractable seizures, hypotonia, and subsequent global developmental delay and regression. The MRI brain was suggestive of mitochondrial disease. He had low complex I activity on muscle biopsy and fibroblast culture, although the blood lactate and fibroblast lactate/pyruvate ratio was normal. The clinical and MRI findings and biochemistry suggested a mitochondrial Leigh-like disease. Extensive investigation did not reveal another cause of his neurological disease. He had no organomegaly, or history of cholestasis. At 7 years of age, he developed down gaze palsy. He was diagnosed to have NPC on fibroblast cholesterol esterification studies and filipin staining. *NPC1* gene analysis revealed homozygosity for c.1836 A-> C (E612D).
Conclusion: This case demonstrates that NPC patients may have secondary mitochondrial dysfunction. It also illustrates the diverse clinical presentation of even severe infantile NPC without hepatosplenomegaly and or cholestasis. The MRI findings, but not the clinical status, improved over time and this will be discussed. It is likely this condition is more common than previously thought. An easier biochemical screening and /or diagnostic test should be developed

367-P

NOVEL BIOMARKER OF A NEURODEGENERATIVE DISEASE: INCREASED EXPRESSION OF LYSOSOMAL ACID PHOSPHATASE IN CLN3-DEFECTIVE CELLS AND MOUSE BRAIN TISSUE

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Juvenile neuronal ceroid lipofuscinosis (JNCL) is a neurodegenerative disease of childhood which is characterized by progressive loss of neurons in brain and retina. Clinical features include visual loss, mental and motoric retardation, epilepsy and premature death. JNCL is caused by mutations in the *CLN3* gene which encodes a lysosomal membrane glycoprotein. We have studied the biosynthesis, proteolytic processing, turnover and mRNA expression of another lysosomal membrane protein, the lysosomal acid phosphatase LAP, in fibroblasts of JNCL patients, in *CLN3* knock-down cells, and in the brain of $Cln3^{-/-}$ mice. The expression of LAP was increased on mRNA and protein level accompanied by a reduced degradation of LAP in fibroblasts of JNCL patients. Knock-down of *CLN3* mRNA in HeLa cells also resulted in an increase of LAP expression. During early stages of postnatal life the enzymatic activities of LAP were significantly elevated in the brain of $Cln3^{-/-}$ mice which was not due to increased transcriptional activity. Histochemical localization studies revealed an increased LAP staining intensity in neurons of layers I-II and III-IV of the cerebral cortex in $Cln3^{-/-}$ mice. Additionally the expression of another lysosomal membrane protein LAMP-2 was increased in all brain areas of $Cln3^{-/-}$ mice. The findings of *CLN3*-dependent expression of LAP and probably other lysosomal membrane proteins such as LAMP-2 may represent the identification of potential biomarkers.

368-P

C-TERMINAL PRENYLATION OF THE CLN3 MEMBRANE GLYCOPROTEIN IS REQUIRED FOR EFFICIENT ENDOSOMAL SORTING TO LYSOSOMES

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Juvenile neuronal ceroid lipofuscinosis is a neurodegenerative disorder of childhood caused by defects in the polytopic CLN3 membrane glycoprotein. CLN3 is localized in late endosomes/lysosomes in transfected non-neuronal and neuronal cells, and in early endosomes along neuronal processes. Previous studies identified two cytosolic signal structures contributing to lysosomal targeting. We have analyzed the role of glycosylation and the C-terminal CAAX motif in lysosomal transport of CLN3 in non-neuronal and neuronal cells. Mutational analysis revealed that in COS7 cells CLN3 is glycosylated at asparagine residues 71 and 85. Both partially and non-glycosylated CLN3 was transported correctly to lysosomes. Mevalonate incorporation and farnesyltransferase inhibitor studies indicate that CLN3 is prenylated most likely at cysteine 435. Substitution of cysteine 435 reduced the steady state level of CLN3 in lysosomes most likely due to impaired sorting in early endosomes, particularly in neuronal cells. Additionally, the cell surface expression of CLN3 was increased in the presence of farnesyltransferase inhibitors. Alteration of the spacing between the transmembrane domain and the CAAX motif or the substitution of the entire C-terminal domain of CLN3 by cytoplasmic tails of mannose 6-phosphate receptors have demonstrated the importance of the C-terminal domain of proper length and composition for exit of the endoplasmic reticulum. The data suggest that different signal structures localized in the second cytoplasmic loop and the C-terminal domain of CLN3 are required for efficient lysosomal sorting in distinct subcellular compartments.

369-P

WITHDRAWN

370-P

ANALYSIS OF ENDOCYTOSIS IN CLN6-DEFICIENT NCLF-MICE

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Defects in the gene *CLN6* cause a variant of late infantile neuronal ceroid lipofuscinosis, which is characterised by lysosomal storage of proteins and lipids, appearance of autophagosomes, neurodegeranation and early death. The gene encodes a polytopic ER-resident membrane protein of unknown function. Expression analysis of *CLN6* exhibiting mutations identified in CLN6 patients revealed strongly reduced half lifes of the mutants. The naturally occurring nclf-mouse model resembles genetically and clinically the human disease. Gene-array analysis of cerebral cortex tissues of 4 weeks old nclf-mice revealed, that genes encoding for the alpha subunit of the adapter protein AP-2 complex and epsin1 were down regulated. Both adapters are important for receptor-mediated endocytosis. RT-PCR and Western-blotting of cultured hippocampal neurons of nclf-mice confirmed the strong reduction of AP-2 and epsin expression. Immunohistochemical staining has demonstrated that AP-2 appears to be lost specifically in the hippocampal CA3 region and in the granular cell layer of the cerebellum. In contrast, epsin staining was reduced in the Purkinje cells of the cerebellum. The functional significance for receptor-mediated endocytosis of ligands in cultured neurons is under investigation. These results suggest that defects in *CLN6* impair secondary endocytic processes important for survive and function of specific populations of neurons.

371-O

DOWNREGULATION OF GABA A ALPHA2 RECEPTOR IN THE MOUSE MODEL OF NEURODEGENERATIVE LYSOSOMAL STORAGE DISORDER CLN6

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CLN6 is a polytopic membrane protein of unknown function resident in the endoplasmic reticulum (ER). Mutant CLN6 causes the late infantile form of lysosomal storage disorder neuronal ceroid lipofuscinosis. Nclf-mice represent a naturally occurring animal model for CLN6 disease. To analyze genes whitch were altered in expression in early stages of the disease a gene-array was performed using cortical brain tissue of 4 week-old nclf-mice. Among 63 and 72 up and downregulated genes respectively, the mRNA level of the GABA receptor subunit alpha2 was strongly reduced in nclf-mice. These data were confirmed by RT-PCR and Western-blotting of cultured hippocampal neurons of nclf-mice. Second, the amounts of the GABA receptor anchorage protein gephyrin was reduced. Overexpression of gephyrin in hippocampal neurons of nclf-mice results in an altered localization in lysosomal/autophagosomal like clusters, suggesting that the transport of GABA receptors to the synapses may be affected. The expression of GABA receptor subunits alpha1 and 3 appear to be unchanged in nclf-neurons. Histochemical stainings showed a loss of immunoreactivity for GABA α2 receptor in Purkinje-cells of the cerebellum and a week staining in neuronal extensions in the hippocampal pyramidial cell layer. To investigate whether the loss of alpha2 subunit effects total GABA binding in specific brain regions of nclf-mice, [3H]muscimol autoradiography was achieved. Electrophysiological analysis of GABA-mediated currents in cultured hippocampal neurons and cerebellar Purkinje-cells in brain slices from 4 weeks old nclf-mice demonstrated that at other GABA receptor subunits can compensate the loss of alpha2 subunit at this timepoint.

372-O

THE CLN10 SUBTYPE OF NEURONAL CEROID LIPOFUSCINOSIS IS CAUSED BY MUTATIONS IN THE *CTSD* GENE AND IS ASSOCIATED WITH A VARIABLE AGE OF ONSET

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We have recently described a novel type of neuronal ceroid lipofuscinosis (NCL), CLN10, which is caused by cathepsin D (CTSD) deficiency. One affected child developed blindness before the age of 5 and later progressive psychomotor disability. In the patient's fibroblasts CTSD activity was reduced to about 8% of controls. Electron microscopic investigation of the skin biopsy revealed granular deposits in the Schwann cells. Two missense mutations, p.Phe229Ile and p. Trp383Cys, in the *CTSD* gene were identified and expressed in CTSD negative mouse fibroblasts to further characterize their functional consequences.

A natural animal model of CLN10 occurs in American Bulldogs and is associated with a 36% residual CTSD activity. We therefore screened adult NCL patients for reduced CTSD activities using a sensitive peptide assay. Two siblings showed a 50% reduction in activity and amount of CTSD in their fibroblasts. In both individuals the homozygous deletion Lys331 was identified in the *CTSD* gene. The two patients first suffered from visual disturbances at their teens, later showed motor difficulties at adolescence and eventually developed dementia and psychiatric symptoms in their late twenties.

Since congenital NCL is allelic to CLN10 our findings indicate that there is a direct correlation between the level of residual activity and the clinical phenotype in those NCL forms that are caused by CTSD deficiency. Mutations in the CTSD gene can be associated with NCL of any age of onset and CTSD activity should be assessed routinely or whenever a granular pattern of storage material is detected under EM.

373-O

GIANT LYSOSOMES OF CHEDIAK-HIGASHI DISEASE: COMPOSITION OF AUTOFLUORESCENT AGGREGATES AND EFFECT OF TRANSFECTED *ARL8B*

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Background: Human Chediak–Higashi syndrome (CHS) is characterized by oculocutaneous albinism, a bleeding tendency, severe infections and the occurrence of 'giant' organelles of common origin; i.e. lysosomes, melanosomes, and platelet dense bodies. The mutated gene encodes a 3801 amino acid protein, LYST, whose function is unknown. The beige strain of mice with mutations in bg the mouse homolog of LYST mimics the giant lysosomes of CHS. We recently showed the bg liver lysosomal membrane contains higher amounts of endoplasmic reticulum proteins compared to normal.

Methods/**Results**: We have now isolated from the luminal compartment of 5–6-month-old mice an insoluble protein aggregate that displayed ceroid-like autofluorescence with multiple excitation/emission maxima. In this aggregate we identified 144 mature lysosomal, ER (particularly BiP), mitochondrial, peroxisomal, and cytosolic proteins. LC3 conversion was normal suggesting that these proteins are not acquired due to hyper-autophagy. Arl8b is a cytosolic Arf protein one role of which is to maintain the integrity of organelle structure. It is activated by binding GTP, binds specifically to lysosomes but becomes inactivated and is released when GTP is converted to GDP. Transfection of CHS fibroblasts with mutated, constitutively active GTP-bound Arl8b results in shrinkage of giant lysosomes to normal size in CHS fibroblasts while transfection of the constitutively-inactive (GDP-bound) or wild type forms of Arl8b do not.

Conclusions. Our results suggest that the LYST/ bg mutations affect both intraluminal and intramembrane protein turnover and that overexpression of activated Arl8b partially corrects these defects.

Supported by CIHR

374-P

ACCUMULATION OF BIS(MONOACYLGLYCERO) PHOSPHATE/ LYSOBISPHOSPHATIDIC ACID IN MICE DEFICIENT FOR THE LYSOSOMAL PROTEASE CATHEPSIN D

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Background: Neuronal ceroid lipofuscinoses (NCL) are a group of severe neurodegenerative lysosomal storage disorders. Currently nine NCL genes have been identified encoding soluble lysosomal proteins (e.g. cathepsin D, CtsD) or membrane proteins (e.g. CLN6) of unknown function. Similar clinical symptoms and pathological findings in different NCLs, however, suggest common disregulated functional pathways. Whereas in many types of MPS disease storage of glycosphingolipids, most notably GM2 and GM3 gangliosides, accompanied by sequestration of free cholesterol has been documented, little is known on the role of sphingolipids in NCL. **Methods**: Murine models of cathepsin D (Ctsd) and CLN6-deficiency, closely resembling human diseases, were used to investigate the expression of phospho- and glycosphingolipids in brains and cultured brain cells by HPTLC, mass spectrometry, immunofluorescence microscopy, TLC-immunostaining, or immunohistochemistry. **Results**: Both in $Ctsd^{-/-}$ mouse and CLN6-defective mouse an increase in GM2 and GM3 gangliosides were found. Among neutral lipids galactosylceramides are reduced in brain tissue of $Ctsd^{-/-}$ mice and numerous significant increases were found in the proportions of polyunsaturated phosphatidylcholine and phosphatidylethanolamine species. Unexpectedly, there was a marked elevation in the unusual phospholipid bis(monoacylglycero)phosphate/lysobisphosphatidic acid (BMP/LBPA) in $Ctsd^{-/-}$ brain and in endosomes/lysosomes of cultured primary brain cells. No differences were found in cholesterol content. **Conclusion**: Given that BMP/LBPA is required for protein sorting in late endosomes and for degradation of glycosphingolipids at the internal endosomal/lysosomal membrane, its accumulation suggests the presence of defects in the composition, trafficking, and/or recycling of membrane components and thus possible new mechanisms to explain neuronal dysfunction in NCL disorders.

375-P

SIMULTANEOUS QUANTIFICATION OF CERAMIDE, GLUCOSYLCERAMIDE, AND CERAMIDE TRIHEXOSIDE IN BIOLOGICAL SAMPLES

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Background: Simple, reproducible assays are needed for the quantification of sphingolipids, ceramide, and sphingoid bases. We developed an HPLC method to quantify these compounds in biological samples. **Method**: After addition of sphinganine as internal standard we extracted lipids from plasma. Ceramide and glycosphingolipids were deacylated by microwave-assisted hydrolysis in methanolic NaOH, followed by derivatisation of the liberated amino-group with o-phtalaldehyde. The derivatized sphingoid bases and lyso-glycosphingolipids were separated and quantified using reverse phase HPLC. **Results**: We established optimal conditions for the complete deacylation of ceramide and neutral glycosphingolipids without decomposition. The limit of detection is 20 fmoles. In practice the limit of quantification is 2 pmol. Plasma glucosylceramide levels are on average 3-fold increased in gaucher patients (median Gaucher plasma 17.5, range 4.7–54.5 nmol/ml (n = 50); median control plasma 5.8, range 3.7–7.5 nmol/ml (n = 30). Enzyme replacement and substrate deprivation therapy both result in a decrease in plasma glucosylceramide levels 6 months. Plasma ceramide trihexoside are on average 3-fold increased in hemizygous Fabry patients (median Fabry plasma 7.0, range 3.1–9.7 nmol/ml (n = 16); median control plasma 1.9, range 0.8–3.3.6 nmol/ml (n = 22). In heterozygote Fabry patients plasma ceramide trihexoside levels did not differ from controls. Enzyme replacement therapy resulted in a decrease in plasma ceramide trihexoside level in hemizygous Fabry patients. **Conclusions**: HPLC enables quantification of ceramide, glucosylceramide, and ceramide trihexoside. The method is useful for the follow up of Gaucher patients and Fabry patients on therapy. The method can also be used in the diagnosis of saposin C deficient Gaucher patients.

376-P

COMPARISON BETWEEN THE BIOCHEMICAL PROPERTIES OF PLASMA CHITOTRIOSIDASE FROM NORMAL INDIVIDUALS AND FROM PATIENTS WITH GAUCHER DISEASE, GM1-GANGLIOSIDOSIS, KRABBE DISEASE AND HETEROZYGOTES FOR GAUCHER DISEASE

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Chitotriosidase (CT) is a fully active chitinase synthesized by activated macrophages. This human chitinase belongs to the family of 18 glycosyl hydrolase, being an endoglucosaminidase that cleaves and shows transglycosylation activity towards chitin, a polymer of N-acetyl-d-glucosamine.
Objectives: The aim of the present work was to establish the range of CT activity in normal individuals (controls), patients with Gaucher disease (GD), GM1-gangliosidosis (GM1), Krabbe disease (KD) and heterozygotes for Gaucher disease (HG). The kinetics of the enzyme in the five groups was also investigated.
Methods: Plasma CT activity, as well as Km, Vmax, optimum pH and thermal stability of the enzyme was determined in plasma of controls, GM1, KD, GD and HG subjects.
Results: CT activity in GD, GM1 and KD patients was, respectively, around 600-fold, 15-fold and 12-fold greater than in normal individuals. There was no significant difference between CT activity in the HG and the control group. We also demonstrated that all CT kinetic parameters evaluated (optimum pH, Km, Vmax, thermal stability) in plasma of GD, KD and GM1 patients were significantly different from those of normal individuals. Regarding to thermal stability, our results show that CT activity in the control group was more stable than in the other groups.
Conclusions: Based on the differences found in the biochemical parameters studied, we presume that the parameters analyzed may be useful in the diagnosis of the lysosomal storage diseases.

377-A

IMPROVING CARE OF THE DYING CHILDREN WITH LYSOSOMAL NEURODEGENERATIVE DISEASES

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Lysosomal diseases with primary involvment of central nervous system are untreatable and inevitably lead to death. A variety of experimental treatment have been proposed, but without influence on long-term outcome.
Case 1: First symptoms of the girl with Tay-Sachs disease started at the age of 6 months with hypotonia, poor feeding, lethargy, seizures and developmental regression. Clinical state gradually worsening with progression to deafness, visual impairment and spasticity. Medical treatment was aimed at controlling seizures and dehydratation during recurrent infections. After frequent hospitalizations and when risk of dying was high, all responsibilities for health care was transferred to specialists for home palliative care (total 88 days, 8 visits with modification of antiepileptic treatment, application of nasogastric tube, oxygenotherapy, sucking action, inhalation). She died at home at the age of 3 years and 6 months.
Case 2: In a boy with late infantile ceroid lipofuscinosis devastating clinical features started at the age of 4 years with gait disturbances, frequent falls, seizures, blindness and dementia. Combined medical and hospice home care was established at the age of 11 years (total 18 months, 13 visits with supply material facility, education, psychosocial support). Boy died at home during sleep at the age of 12 years and 6 months.

In both cases decision to stay at home with dying children parents do not regret.

In **conclusion**, we recommend professional palliative home care as an alternative approach in cases of fatal lysosomal disorders, if no treatment is known to be effective.

378-A

THE FIRST RESULTS OF 18 MONTHS EXPERIENCE WITH LYSOSOMAL STORAGE DISEASE

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Lysosomal storage diseases (LSDs) characterized by defects in lysosomal enzymes are an important group of inherited metabolic diseases and they are commonly autosomal recessive. In recent years, there are some important progresses in the treatment like enzyme replacement, substrate reduction and gene therapy which are very hopeful for patients. Also the early diagnosis increases the success of the treatment. In Turkey the inherited metabolic diseases have a considerably high ratio due to the high frequency of consanguineous marriages (21%). In our study we intend to present the first results on lysosomal enzyme activities of our laboratory.

Lysosomal enzyme activities are analyzed in 330 patients refered to our laboratory as suspected of LSDs between September 2005 and March 2007. Enzyme activities were determined by fluorometric method using 4-methylumbelliferryl (4 MU) substrates and spectrophotometric method using p-nitrocatecholsulphate.

Seventy two of 330 suspected patients are identified as patients with LSD. There are 23 Gaucher disease, 19 Niemann–Pick type A/B, 8 mucopolysaccharidoses (MPS) type I, 10 MPS type II, 5 MPS type VI, 1 MPS type VII, 3 Fabry disease, 2 a-fucosidosis and 1 GM1 gangliosidosis in 72 patient with LSD.

As a **conclusion** the inherited metabolic diseases including LSDs have a very high ratio due to consanguineous marriage in our country. Also the early diagnosis of this kind of disorders will increase the success of the treatment.

379-P

MUCOPOLYSACCHARIDOSES IN BRAZIL: WHAT HAPPENS FROM BIRTH UNTIL BIOCHEMICAL DIAGNOSIS?

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The delay on diagnosis of MPS diseases can prevent patients and families to benefit from the therapeutic and preventive alternatives available. To better understand the causes of this delay, we investigated the path followed by Brazilian patients with MPS from birth until diagnosis. An interview was performed with patient's parents or guardians with subsequent review of medical records. A total of 113 patients with MPS, from 97 families, were included (18 MPS I, 43 MPS II, 2 MPS IIIA, 3 MPS IIIB, 1 MPS IIIC, 15 MPS IVA, 1 MPS IVB, 29 MPS VI, 1 MPS VII). The median of age at onset of signs/symptoms was 18 months (MPS I: 18, MPS II: 24, MPS IVA: 8, MPS VI: 8). Skeletal abnormalities (MPS IV and MPS VI), joint contractures (MPS II) and facial typical features (MPS I) were the first signs/symptoms most frequently reported. Many patients were admitted to hospitals and submitted to surgeries (a high-risk procedure in these patients) before diagnosis was established. The median age at diagnosis was 76 months (MPS I: 75, MPS II: 95, MPS IVA: 75, MPS VI: 52). Considering the whole group, there was a 4.8 years delay from beginning of signs/symptoms until diagnosis of MPS. Considering that treatment is available for some cases and that early intervention is likely to improve the natural history of the disease, efforts should be made to minimize this delay. We believe that this situation can be improved through health professional's education and expanded access to diagnostic protocols.

380-P

LABORATORY DIAGNOSIS OF MUCOPOLYSACCHARIDOSIS IN EGYPT

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MPSs share many clinical features, urinary glycosaminoglycans are useful for establishing the likely diagnosis. Each MPS type is associated with an enzymatic deficiency.

Aim of the work: the laboratory diagnosis of MPSs and the prevalence of each type in Egypt.

Materials and methods: The study included 579 patients referred to Biochemical Genetics Laboratory, National Research Centre, Cairo, Egypt, for the diagnosis of MPS. Quantitation of urinary GAGS. Cases with high concentration ($>$ 15 mg/mmol creatinine) were subjected to electrophoretic separation of the GAGs. The activities of the specific enzymes were fluourometrically assayed.

Results: 308 (53%) cases had high concentration of urinary GAGS. 149 (25.7% of the total 579 and 48% of the 308 cases) cases proved to have MPS and five cases (0.86% of the total and 1.6% of the 308 patients) proved to have mucolipidosis.

Enzyme assay of 149 cases revealed the following: 50 cases type I (33.6%), 20 cases type II (13.4%), 11 cases type III (7.4%),18 cases type IV (12%),and 50 cases type VI (33.6%).

Conclusion: Quantitative determination of urinary GAGS is a simple to select cases for electrophoretic separation. Enzyme assay is mandatory to confirm the MPS type. Two thirds of patients were either type I or VI.

381-A

IMPROVING THE DIAGNOSIS OF MUCOPOLYSACCHARIDOSIS IN BRAZIL: A REPORT FROM THE MPS-BRAZIL NETWORK

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Purpose: To present the results of the first 34 months of operation of the MPS-BRAZIL network, a collaborative initiative joining centers from different Brazilian regions to improve the diagnosis and management of MPS diseases in the country.

Methods: The coordinating center, located at MGS/HCPA in Porto Alegre, provides the information on the management and makes available the tests needed for the diagnosis.

Results: (1) During this period, 455 Brazilian patients suspected of having MPS were investigated; the diagnosis of MPS was confirmed in 275/455 patients; (2) MPS I was confirmed in 65/275 patients (mean age at diagnosis: 6 years); (3) MPS II was confirmed in 86/275 patients (mean age at diagnosis: 7 years 9 months); (4) MPS III was confirmed in 35/275 patients (mean age at diagnosis: 7 years 9 months); (5) MPS IV was confirmed in 21/275 patients (mean age at diagnosis: 11 years 9 months): (6) MPS VI was confirmed in 63/275 patients (mean age at diagnosis: 7 years 5 months); (7) MPS VII was confirmed in 5/275 patients (mean age at diagnosis: 4 years 10 months).

Conclusions: MPS II, I and VI seem to be the most frequent types of MPS in Brazil, and MPS III seems to be underdiagnosed. There seems to be a difference in regional distribution of MPS, since MPS I is more common in the S and SE regions, while MPS VI seems to be less frequent in the S region. Mean age at diagnosis was found to be high in all the MPS types.

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382-P

SEPARATION OF SULFATED URINARY GLYCOSAMINOGLYCANS BY HIGH-RESOLUTION ELECTROPHORESIS FOR ISOTYPING OF MUCOPOLYSACCHARIDOSIS IN MALAYSIA

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Mucopolysaccharidoses (MPS) developed as a result of deficiency of one of the 11 lysosomal enzymes which involved in degradation of glycosaminoglycans (GAGs), resulting in accumulation of GAGs in cells and excess excretion in urine. Measurement of GAGs content in urine is therefore generally used as screening procedure for MPS while separation of GAGs for isotyping is done by high-resolution electrophoresis (HRE)

In Malaysia, the method for detection of urinary GAGs uses dimethyl methylene blue (DMB) for quantitation of urinary GAGs. We have established High resolution electrophoresis for the separation of sulfated GAGs. Out of 349 urine patients' samples analyzed, 30 patients have abnormal HRE patterns. Five patients have elevated urinary GAG and show the presence of dermatan sulfate (DS) and heparan sulfate (HS) suggesting MPS I or II where 3 patients were confirmed to have MPS II. 11 patients have elevated urinary GAGs and elevation of HS suggesting MPS III, and 2 patients were confirmed to have MPS III. 8 patients have normal urinary GAGs with elevation of HS. Since these patients have abnormal features suggestive of storage disorders, milder type of MPS III or glycophingolipidosis has to be excluded. 4 patients have borderline increase of urinary GAG with the presence of keratan sulfate, suggesting MPS IV. One patient show elevation of DS and this may suggest MPS VI. One patient has clinical features of MPS Type II but with borderline increased of GAG and HRE showed trace amount of DS and HS was confirmed to have mucolipidosis II.

383-P

SCREENING FOR FABRY DISEASE, POMPE DISEASE AND OTHER LYSOSOMES DISORDERS BY TANDEM MASS SPECTROMETRY (HPLC-MS/MS)

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Fabry disease and Pompe disease may now by treated by enzyme replacement therapy.

Methods: We have used HPLC-MS/MS as a powerful tool for analysis of key metabolites in inborn errors of lysosomal metabolism. Sample preparation is easy. Metabolites were first separated by a C18 reversed phase column and quantified in multiple reaction monitoring mode by using C17 GL-3 as an internal standard for example in Fabry disease. Preparation of oligosaccharides is with derivatization. **Results**: Globotriaosylceramides (GL-3) in Fabry disease are screened in urine and plasma. In the following diseases screening is done in urine. Key metabolites in urine are maltotetraose (Hex-4) in Pompe disease, mannose-N-acetyl-glucosamine (Man-GlcNAc) in mannosidosis, fucose-N-acetyl-glucosamine (Fuc-GlcNAc) in fucosidosis, N-acetyl-glucosaminylasparagine (N-Asn) in aspartylglucosaminura, sialic acid (SA) in Salla disease and Infantile Sialic Acid Storage disease (ISSD). Concentration (in mmol/mol Crea) of key metabolites in control patients (H: healthy) vs. patients with lysomsomal disorders (I: ill) are listed with the appropriate key metabolites: Man-GlcNAc (H: 0.05 vs I: 250); FucGlcNAc (H: nd vs I: 110), Hex-4 (H:0.0.1–11 vs I: 130–360) and SA (H: 40 vs I: 800–1200). Analyzing healthy patients and patients with lysosomal disorders this new method offers enough sensitivity and precision for screening purposes. **Discussion:** The presented methods offer short total run time in combination with rapid and easy sample preparation. This is an economic method in screening for lysosomal disorders and a possible tool in effective therapy monitoring, too.

The study is supported in part by Genzyme Germany.

384-P

NEONATAL SCREENING FOR POMPE DISEASE: A TWO-TIER SCREENING TEST

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Background: Pompe disease is caused by the deficiency of acid alpha-glucosidase (GAA). Recombinant human GAA has been used to treat infantile-onset Pompe disease (IOPD), resulting in prolonged survival, reversal of cardiomyopathy, and growth and motor gains, although not all patients achieve ambulation. Best motor outcomes are reached when recombinant human GAA treatment is initiated early.

Methods: A neonatal screening pilot program for Pompe disease was started in Oct. 2005 at National Taiwan University Hospital (NTUH). Blood spot GAA activities were measured on regular dry blood cards for babies three days after birth. The methods employed 4-MU-glucoside as the substrate, and acarbose as an inhibitor of maltase-glucoamylase (MGA). The screening test included a first tier assay for GAA activity. For 10 to 15% of newborns with a decreased activity, the second tier assay which included both GAA activity and ratio between neutral maltase and GAA activity will select those who need a second dry blood filter.

Results: This two-tier assay is necessary because normal individuals with low GAA activity are not rare. Currently, more than 130 000 newborns have been screened and the incidence for IOPD is close to 1 in 40 000. No false negative has been encountered. All classical IOPD cases were detected before 1 month of age.

Conclusions: The result from this pilot program suggests that neonatal screening for Pompe disease is feasible and would be helpful in its early diagnosis.

385-P

AN IMPROVED DRIED BLOOD SPOT SCREENING METHOD FOR GAUCHER DISEASE

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Introduction: Gaucher disease is characterized by a deficiency of the lysosomal enzyme glucocerebrosidase. Many patients are misdiagnosed or remain undiagnosed. A simple screening method would increase detection rate and allow for early implementation of therapy when needed to prevent the serious complications of Gaucher disease. We developed a rapid and reliable screening assay for measuring glucocerebrosidase activity in dried blood spots (DBS) based on the method of Chamoles et al. [Clin. Chem. (2002)317:191].

Methods: A fluorescent assay was developed using the substrate 4-methylumbelliferyl-beta-D-glucopyranoside and conduritol B epoxide (CBE), an irreversible inhibitor of glucocerebrosidase. The difference in activity with and without CBE is used to distinguish glucocerebrosidase activity from that of other beta-glucosidase isoenzymes.

Results: We measured glucocerebrosidase activity in DBS samples from 43 untreated Gaucher disease patients and 153 normal adults. Activity in the Gaucher disease samples ranged from below the limit of detection to 4.4 pmol/(punch*h) with a mean of 1.6 pmol/(punch*h). Activity in the normal samples ranged from 5.6 to 34.7 pmol/(punch*h) with a mean of 10.9 pmol/(punch*h).

Conclusions: These results demonstrate that the assay was sensitive enough to differentiate DBS from patients with Gaucher disease from normal controls. The DBS assay's speed, throughput, and low cost make it an ideal method to screen for Gaucher disease. The applicability of this method for diagnosing Gaucher disease remains to be determined.

386-P

CILIARY FUNCTION IN CHILDREN WITH MUCOPOLYSACCHARIDOSIS

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A common finding in patients with mucopolysaccharidosis (MPS) is dyscrinia affecting the airways, resulting in symptoms like rattling breath sounds and diseases like recurrent bronchitis. Reason for this could be the adverse midface anatomy as well as deposition of mucopolysaccharides in different tissues along the airway with changing flow from laminar to turbulent.

With deposition of material in basal membrane of the epithelium of the airways, ciliary beating could be impaired as well – similar to secundary ciliary dyskinesia of other causes (e.g. smoking). To prove this theory, we determined the ciliary beating frequency in MPS patients of different types.

Samples were taken from nasal airway with a soft brush, stored in medium and analyzed immediately and after 24 h of incubation. Under a phase contrast microscope placed on a solid swinging table to exclude vibrations mimikring ciliary movement first of all we described the appearance of the epithelial layer and estimated movement of cells. Then the ciliary beat frequency (CBF) was detected by light absorption changes, that were automatically processed, the medium ciliary beat frequency was then determined.

In all our patients except one there was no abnormal ciliary beat frequency detectable. We think, that ciliary dyskinesia is not a cause for dyscrinia in MPS-patients.

387-P

NEUROLOGICAL EXAMINATIONS AND CLINICAL MANIFESTATIONS IN MPS I AS REPORTED IN THE MPS I REGISTRY

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Background: Mucopolysaccharidosis type 1 (MPS1) is a multisystemic disease with broad phenotypic expression. CNS involvement can include headaches, hydrocephaly, neurocognitive impairment, and spinal cord compression due to bone dysplasia and thickening of the meninges.
Methods: As of January 2007, the MPS1 registry contained data from 585 MPSI patients, of which 55%, 23%, and 11% were the Hurler, Hurler-Scheie, and Scheie phenotypes, respectively. To better understand monitoring of CNS involvement in MPS1, registry data were collected on neurological symptoms and exams regardless of treatment, phenotype, and age.
Results: Brain MRI/CT or spine MRI was performed at least once in 79% and 57% of patients, respectively. Overall, 14% and 29% of patients were reported as never having brain imaging or spine MRI, respectively, and in 7% and 14% of cases, data were missing or inconsistent.

Neurocognitive impairment was reported for 52% of patients with responses (mean age at first report: 2.6 years, n = 247), and 15% reported myelopathy (mean age at diagnosis: 9.8 years; median age 6.0, range 0-40.3, n = 70).

Of the 77 patients reporting myelopathy, the most frequently reported vertebral level for compression was 'cervical' (28 patients); 20–25% of patients reported other vertebral levels (lumbar, cranio-cervical, and thoracic). Surgical spinal cord decompression was reported in 8% of patients overall, and in 51% (n = 39) of patients with myelopathy.
Conclusion: Neurocognitive impairment and myelopathy first manifest at very different ages. Spinal cord compression might be under-recognized as nearly 30% of the patients did not have a spine MRI. Surgical spinal cord decompression is uncommon.

388-P

HIGH INCIDENCE OF PROPTOSIS IN MUCOPOLYSACCHARIDOSIS TYPE 1 (MPS I) CORRELATING WITH ORBIT DEFORMITY AND HYDROCEPHALUS

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Background/Objective: MPS I accumulation of glycosaminoglycans (GAGs) causes multisystemic tissue damage. Proptosis is one of the ophthalmologic complications. We studied proptosis in relation to hydrocephalus and bone deformity of the skull.
Methods: We retrospectively evaluated MRI and CT-scans of the brain of 10 MPS I patients with different age and phenotype. The presence of proptosis and hydrocephalus was assessed. The thickness of the orbital walls, the mid frontal, frontal lateral, occipital and temporal bone were measured and compared to age matched controls.
Results: Proptosis was observed in 4 out of 7 patients with the severe Hurler phenotype. In two patients, proptosis was caused by thickening of the lateral orbit wall, in one patient by high pressure hydrocephalus and in one patient by a combination of both. The thickness of the mid frontal and occipital bone was significantly increased in older patients with the severe Hurler phenotype as compared to controls. In one patient the high pressure hydrocephalus was concomitant with thinning of the skull, followed by thickening after ventriculoperitoneal shunting.
Conclusion: This study demonstrates the clinical importance of both proptosis and skull thickness for the recognition of high pressure hydrocephalus in MPS I. Proptosis is an important ophthalmologic feature in the severe phenotype of MPS I and may be caused by either deformation of the orbit related to extensive thickening of skull bones, as well as by high pressure hydrocephalus. For early recognition timely interventions including imaging of the skull and the orbit is recommended.

389-P

MAGNETIC RESONANCE IMAGING OF CERVICAL SPINE ANOMALIES IN ADULT MUCOPOLYSACCHARIDOSIS TYPE I PATIENTS

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Backgound: Mucopolysaccharidosis type I (MPS I) is an autosomal recessive lysosomal storage disease in which deficiency of the enzyme α-L-iduronidase results in harmful progressive accumulation of glycosaminoglycans in lysosomes in a variety of tissues, including bone and connective tissue. Cervical spine abnormalities have been reported in MPS I patients, particularly in patients with the severe phenotype of MPS I, i.e. Hurler syndrome.
Methods: We analyzed cervical spine anomalies in 7 adult MPS I patients with the attenuated phenotype, using Magnetic Resonance Imaging (MRI). 4 of these 7 patients are presently receiving weekly enzyme replacement therapy (ERT) with recombinant human α-L-iduronidase at a dose of 100 U/kg body weight.
Results: All studied patients have cervical spine abnormalities in varying degrees of severity when examined by MRI. In all patients MRI showed thickening of the dura mater, a narrowed foramen magnum and a straightened cervical lordosis. In 4 patients MRI showed signs of degenerative bone structure, although in contrast to patients with the severe phenotype, in no patient in this study, evidence of odontoid dysplasia or atlanto-axial dysplasia was found. Most notably, however, 2 out of our 7 MPS I patients had MRI evidence of spinal cord compression.
Conclusions. We advise inclusion of standardized clinical and radiological analysis on this aspect, including MRI of the cervical spine, to be part of the structured follow up of this specific category of MPS I patients, irrespective of treatment modality.

390-P

MEASUREMENT OF THE FUNCTIONAL CAPACITY IN PATIENTS WITH MPS1

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Background: In MPS1 patients under ERT (enzyme replacement therapy) there are functional subjective changes reported by the patients in short periods of time that can not be determined by degrees of restriction in joint motion. Recently,a new test to measure the physical performance of MPS1 patients was published[1].
Objetive: To measure functional capacity (FC) of MPS1 patients before and after ERT. To obtain a variable to quantify the range of deviation from percentile 50th for FC, form a reference population.
Patients and Methods: Three female Hurler-Scheie (8–16-years-old) patients were evaluated. They are on ERT (from 5 to 14 months). FC was evaluated before ERT (time1) and 6 month later (time2). The test include 8 functional tasks (fine grasp, pullover shirt, putting on pants, donning backpack, stand to squat and return (× 5 times), floor to stand, shuttle run and stand and reach.). The outcome is the time in seconds to perform each of them. A total score is obtained (0–100). A Z score for age was calculated through the following formula: (patient total score – media ref. population)/sd.
Results: The total score and Z score (Standard deviation) at time 1 for each patient were 63.3 (–5.1); 76.37 (–7.8); 51.77 (–18). In Case 2, time 2 was 84.39 (–4.8). Time 2, in cases 1 and 3, will be evaluated in June and data reported at Congress.
Conclusion: Z score gives additional information to total score. It is possible to measure the improvement of the FC referred by the patient when compare z score time 1 and time 2.

[1]Haley SM. Dev Med Child Neurol. 2006;48(7):576–81

391-P

CARDIAC INVOLVEMENT IN ADULTS WITH THE ATTENUATED FORM OF MUCOPOLYSACCHARIDOSIS TYPE I

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Background: Mucopolysaccharidosis type I is a lysosomal storage disease caused by a deficiency of the enzyme α-L-iduronidase, resulting in incomplete stepwise breakdown of glycosaminoglycans (GAGs). This deficiency leads to accumulation of GAGs in many tissues. Cardiac involvement has been evaluated mainly in the severe phenotype of Mucopolysaccharidosis type I (MPS I), i.e. Hurler syndrome. Cardiac anomalies in the attenuated form of MPS I are less well known.
Methods: Cardiac function and anomalies were evaluated in 9 adults with the attenuated phenotype of MPS I. All patients underwent electrocardiography, Holter monitoring, blood pressure measurement and two-dimensional echocardiography including tissue Doppler imaging (TDI).
Results: All patients had mild to moderate aortic and mitral valve regurgitation and six patients had mild to moderate tricuspid valve regurgitation. Aortic, mitral and tricuspid valve thickening was noted in respectively 5, 4 and 2 patients. Moderate mitral and aortic valve stenosis was noted in respectively 1 and 2 patients. All evaluated patients had abnormal mean systolic and mean early diastolic mitral annular velocities, compared to normal control subjects. The systolic and diastolic aortic diameters were significantly increased in the MPS I patients. The calculated mean aortic stiffness index was increased in all MPS I patients as well.
Conclusions: Cardiac anomalies are found in patients with the attenuated phenotype of MPS I. The evaluation of cardiac anomalies by noninvasive methods provides fast and accurate data and forms a suitable way to follow any progression or effects of therapy.

392-P

EARLY PRESENTATION AND DIAGNOSIS OF HUNTER SYNDROME: NEW INSIGHTS FROM HOS – THE HUNTER OUTCOME SURVEY

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Background and Aims: HOS is a global survey of patients with Hunter syndrome (mucopolysaccharidosis type II) designed to enhance our understanding of the natural history of the disease and to monitor the safety and efficacy of enzyme replacement therapy. The current study aimed to assess the early clinical manifestations of Hunter syndrome prior to diagnosis.
Patients and methods: Using data from the HOS database, a retrospective analysis was carried out to examine the clinical features of patients who were later diagnosed with Hunter syndrome.
Results: In a cohort of 216 patients with Hunter syndrome, the most common clinical symptoms before diagnosis were characteristic facial features (96%), enlarged spleen/liver (87%), hernia (75%), otitis (70%), enlarged tonsils/adenoids (67%) and nasal obstruction (30%). Median age at diagnosis was 3.5 years (10th–90th percentile, 0.7–12.0 years). The earliest clinical manifestations of were otitis (1.7 years), hernia (1.8 years) and nasal obstruction (2.5 years). Facial features characteristic of Hunter syndrome, enlarged tonsils/adenoids and enlarged liver/spleen were observed at median ages of 2.8, 3.4 and 3.5 years, respectively.
Conclusions: This analysis reveals that at least 70% of patients with Hunter syndrome exhibit otitis or hernia in the first 2 years of life. Increased awareness of these early symptoms may aid diagnosis and allow prompt therapeutic intervention in order to halt or slow the natural progression of Hunter syndrome in affected patients.

393-P

HUNTER SYNDROME IN A GIRL: CASE REPORT

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Introduction: Mucopolysaccharidosis type II (MPS II, OMIM 309900), also called Hunter syndrome, is an X linked recessive disease resulting from deficiency of the lysosomal enzyme iduronate-2-sulphatase (IDS). The enzyme deficiency leads to the accumulation of heparan sulfate and dermatan sulfate in lysosomes. The frequency of Hunter syndrome is estimated at 1 in 100 000 live births. MPS II occurs predominantly in males; disorder in females may be the result of an X chromosome anomaly or homozygosity for the mutated gene. The most frequent cause of disease is the result of skewed X chromosome inactivation. Clinical features include coarse features, bone and joint dysplasia, hepatosplenomegaly, neurological abnormalities.
Case Report: Our patient presented at the age of 3 years with coarse facial features, enlarged tongue, stiffness of joints, 'claw-like' hands, hypertrichosis, hoarse voice, umbilical hernia, hepatosplenomegaly. Hearing was slightly decreased, no corneal clouding. She had delay of speech, runny noses, colds. Her weight and height were at 97th centile. She had high level of GAGs (4 times above normal value) in urine and increased dermatan sulphate and heparan sulphate excretion. A marked deficiency of IDS activity in leukocytes confirmed the diagnosis. She has normal karyotype, but skewed X chromosome inactivation was found. Molecular studies are not finished.

394-P

CORRELATION BETWEEN GENOTYPE AND PHENOTYPE IN DIFFERENT TYPES MPS ON RUSSIAN POPULATION

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Background: Correlation between genotype and phenotypes in MPS is often discussed. However, compared data on the correlation in different types MPS is limited because of the small number of patients.
Objectives: In our study we have analyzed the genotype/phenotypes correlation in large group of MPS patients on Russian population
Methods: Genotypes were detected by PCR, SSCP and direct sequencing
Results: Among 160 in 146 patients with MPS at age 1–52 years DNA analysis on PCR suitable exons was carried out. Novel mutations were revealed in 51 of 127. The most mutations were Q70X9 (52.6%), mutation W402X (6.1%), Q380R (4.6%) and one mutation de novo del 161bp(1875-1891)+ins 3bp(ACA) in *IDUA* gene exon 14. The other mutations types were nearly 3%. Patients MPSI with mutation Q70X/Q70X and 070X/W402X had the severe phenotypes.

Among 63 patients with MPSII (54 DNA analysis) was established 23 novel mutations. Three patients with A85S mutations had a mild clinical symptoms. In 10 of 11 patients with MPSIII (Sanfilippo A) was tested genotypes and it was revealed one novel mutation – del 1080C. Mutations frequency of R74C was 54.5%, mutation K245H – 13.6%. All of the patients with MPSIII had severe clinical symptoms. In 13 of 15 patients with syndrome Maroteaux-Lamy (MPSVI) genotype was examined. Most mutations were novel, of them the most mutation-R152W. The carrier of that mutation had relative mild clinical symptoms.
Conclusions: These findings will be able provide further insight into the possible ways for adequate treatment patients with muco-polysaccharidosis.

395-P

STUDY ON THE NATURAL COURSE OF MUCOPOLYSACCHARIDOSIS TYPE IIIA

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Background: Mucopolysaccharidosis type IIIA (MPS IIIA, Sanfilippo syndrome) is caused by a deficiency of lysosomal N-sulfoglucosamine sulfohydrolase and leads to a defective degradation of the glycosaminoglycan heparan sulfate. The onset and progression of the disease is highly variable.
Methods: The natural course of the disease was assessed in 71 MPS IIIA patients using a questionnaire and a 'Four-Point Scoring System' (FPSS).
Results: First symptoms of disease were observed on average at seven months of age. Speech and motor development were delayed in 66.2 and 33.9% of patients, respectively. The median age at diagnosis was 4.5 years (SD = 2.6, R = 0.25–13.8). The onset of regression in speech, motor and cognitive function was observed at an average age of 3.3 years (R = 2.8–4.1). The loss of all three assessed abilities was observed at an average age of 12.5 years (R = 8.0–26.5). Speech was lost before motor and cognitive functions. In a small group of patients older than 12.5 years (9.9%) speech, motor and cognitive skills were partially preserved up to a maximum age of 23.8 years.
Conclusion: This is the first systematic and comprehensive study on the natural course of MPS IIIA. The FPSS may be used to classify patients into groups with a rapid or slower course of the disease. This may have an important impact on parental counselling as well as therapeutic interventions.

396-O

CLINICAL COURSE AND MOLECULAR ANALYSIS OF 29 DUTCH PATIENTS WITH SANFILIPPO TYPE C (MPSIIIC) DISEASE

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Background: Mucopolysaccharidosis IIIC (MPSIIIC, Sanfilippo C syndrome) is a lysosomal storage disorder caused by deficiency of lysosomal acetyl-CoA:alpha-glucosaminide N-acetyltransferase (HGSNAT). **Methods**: We performed a clinical study on 25 Dutch MPSIIIC patients and determined causative mutations in the recently identified *HGSNAT* gene. **Results**: Psychomotor development was reported normal in all patients during the first year of life. First clinical signs were usually noted between 1 and 6 years (mean 3.5 years), and consisted of delayed psychomotor development and behavioural problems. Other symptoms included sleeping and hearing problems, recurrent infections, diarrhoea and epilepsy. Two sisters had attenuated disease and did not have symptoms until the third decade. Mean age of death was 34 years (range 25–48). Molecular analysis revealed mutations in both alleles for all patients except one. Altogether 14 different mutations were found: two splice site mutations, one frame shift mutation due to an insertion, 3 nonsense mutations and 8 missense mutations. Two mutations, p.R372C and p.S546F, were frequent among probands of Dutch origin representing 22.0 and 29.3%, respectively, of the mutant alleles. **Conclusions**: This study demonstrates that MPSIIIC has a milder course than previously reported and that both severity and clinical course are highly variable even between sibs complicating prediction of the clinical phenotype for individual patients. A clear phenotype-genotype correlation could not be established, except that the mutations p.G290R and p.S567C were only found in two sisters with late-onset disease and presumably convey a mild phenotype.

*Authors 1 and 2 contributed equally to this work.

397-P

CHLORAL HYDRATE USE IN CHILDREN WITH SANFILIPO; GOOD OR BAD?

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Background: In children with Sanfilippo syndrome, the central nervous system involvement is the predominant component as manifested by abnormal brain imaging and electroencephalograms. Clinically they have delayed developmental milestones with regression and seizures. Does the underlying brain pathology make them more at risk of the effects of sedative medication?
Methods: Case study of two children with Sandfilippo syndrome. Given routine doses of chloral hydrate to settle periods of agitation while on the ward. Both became encephalopthic with glasgow coma scales 8–10. Both had abnormal electroencephalograms.
Results: Investigative tests ruled out a possible infectious cause. Both children showed rapid improvement in their Glasgow coma scores within 24 h of discontinuing chloral hydrate medication.
Conclusion: The question we ask is if the underlying encephalopathy in children with Sanfilippo syndrome as manifested by abnormal electroencephalograms and brain imaging make them more sensitive to the sedative and hypnotic effects of chloral hydrate especially during periods of illness. In view of the observed rapid improvement once the sedative medication was discontinued we recommend using alternative medication or lower than recommended doses.

398-A

A HIGH FREQUENCY OF MORQUIO'S SYNDROME TYPE A IN A GENETIC ISOLATE IN AN IMMIGRANT POPULATION

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Morquio's Syndrome type A (MPS4A) is a mucopolysaccharidosis due to a defect in the lysosomal enzyme galactosamine-6-sulphatase. The exact frequency of this condition is not known but estimates in different countries vary from 1/216 412 to 1/640 000. The West Midlands is an ethnically mixed region of the UK with a total population of 5.3 million and a birth rate of 70 000 per annum. The laboratory at Birmingham Childrens Hospital screens for mucopolysaccharidoses in this region. Between 1970 and 2006 we diagnosed 40 cases giving a disease frequency of 1/63 000. 26 (65%) of these were ultimately of Pakistani origin which is far higher than the frequency of this group in the West Midlands (2.9%).

In the 16 patients where mutation analysis was performed the majority (81%) were homozygous for a specific novel mutation (c.347G>T p.Gly 116 Val.). These were from a community that practised first cousin marriages and originally came from the Saleh Khana area of Northern West Pakistan. The clinical phenotype is homogeneous with males inexplicably more severely affected than females. The high frequency of MPS4A in this population is explicable by a combination of founder effect and a high level of consanguinity. As a consequence this has lead to our region having the highest reported frequency of MPS4A with an associated increase in the resources required for treatment, carrier testing and antenatal diagnosis.

399-A

UNILATERAL LIMB PAIN AN UNSUSPECTED CAUSE OF MORQUIO TYPE IVB (MPS IVB)

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Background: Acid β-D-galactosidase (β-gal, EC3.2.1.23) deficiency can result in two clinically different autosomal recessive lysosomal disorders, GM1 gangliosidosis (MIM#230500) and Morquio disease type B (MPS IVB, MIM#253010), a mucopolysaccharidosis without neurological symptoms but with skeletal changes and keratansulfaturia. A common mutation (W273L) found in Morquio B patients is useful for the prediction of the Morquio B phenotype in patients with β-gal deficiency. **Objective**: A six-year old girl with MPS IVB is described with the objective of highlighting (1) the mild clinical features causing considerable delay in diagnosis, (2) the absence of keratansulfaturia, (3) the important role of mutation analysis in diagnosis. **Case report**: The proband, the second offspring of unrelated parents, had unilateral limb pain from the age of 5. After examination by 3 orthopedic specialists, 2 of whom found her normal, she was referred by the third with a diagnosis of spondyloepiphysial dysplasia. Clinical assessment revealed a body height <3–5th PC, normal intelligence, mild facial dysmorphy and mild skeletal abnormalities (lordosis, scoliosis). Spinal X-rays revealed ovoid vertebrae with beaking, raising suspicion for a MPS. MPS electrophoresis was normal but minimal β-gal activity and homozygosity for W273L confirmed MPS IVB. Her parents and younger sibling were heterozygous for W273L. Prenatal diagnosis of a further pregnancy revealed a heterozygous fetus.
Conclusions: There is need for increased awareness of the mild phenotypes of some patients with Morquio's disease. Keratansulfaturia may be absent in MPS IVB. Mutational analysis is a useful tool for differentiation of the two entities of β-gal deficiency.

400-P

ADULT PATIENTS WITH MUCOPOLYSACCHARIDOSIS VI

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Background: A characteristic feature of MPS-disorders is their impressive heterogeneity. The same enzyme deficiency can lead to a severe infantile or a slowly progressive disease. Adult patients are often seen as mildly affected.
Methods: We presently treat 13 adult patients with MPS VI. Before starting enzyme replacement therapy (ERT) all patients underwent an extensive baseline evaluation including clinical, ophthalmological, audiometric, pulmonological, cardiological and orthopaedic examination and a 6-minute-walk-test.
Results: Patients were 15 to 39 years old (median 24), 6 male and 7 female. Adult height ranged between 100 and 160 cm (median 148). Organomegaly was diagnosed in 11/13. 6/13 suffered from hearing impairment, 12/13 from visual impairment, 11/13 showed corneal clouding. Forced vital capacity varied from 0.57 to 3.61 l (median 1,7). In our cardiological evaluation all patients showed abnormalities of cardiac valves, in 3 of them we diagnosed a cardiomegaly. ECG was abnormal in 7/13. 10 of our patients showed a reduced passive shoulder flexion. A 6-min-walk test was done in 12/13 patients, walk-distance varied between 9 and 495 meter (median 390). In 11/13 a severe medullar stenosis of cervical spine was diagnosed.
Conclusions: Our results underscore the relevant burden of illness even in adult patients with so-called 'mild' type-disease. Neurological complications in our patients were underestimated – spinal cord compression is a significant sign that cannot be treated by ERT so far. We recommend regular clinical and neurological investigations also in adult MPS VI patients to prevent irreversible damage.

401-P

MUTATION ANALYSIS OF *GNPTAB* GENE IN 24 JAPANESE MUCOLIPIDOSIS II AND III PATIENTS

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Background: Mucolipidosis type II and type III are autosomal recessive diseases caused by a deficiency of the alpha and beta subunit of the enzyme N-acetylglucosamine-1-phosphotransferase encoded by GNPTAB gene. In patients, targeting of lysosomal enzymes to the lysosome is impaired and lysosomal enzymes are present at elevated levels in the serum and body fluids. Incidence is higher in Japan than Western countries. We analyzed mutations in Japanese mucolipidosis II and III patients and compared with clinical phenotypes. **Methods**: Full length of cDNAs were amplified by RT-PCR technique from mRNAs which were obtained from patients' lymphocytes or skin fibroblasts. DNA sequence were carried out using an ABIPRISM Dye Terminator Cycle Sequencing kit, with 6 forward and 6 reverse primers. Each mutations were confirmed by DNA sequence with genomic DNA. We compared clinical phenotypes and obtained genotypes. **Results**: Previously reported and new mutations were detected, including nonsense mutation (R1189X), missense mutations (F374L, N1153S), frame shift (del67bases in Exon1, 324-325delAG, 2089insC, 2541-2544delA) and abnormal splicing (duplication of Exon2, deletion of Exon3-8, deletion of Exon11). Nonsense mutation R1189X allele frequency was 32/48. **Conclusions**: It is speculated that patients with R1189X in homozygote show clinically severe phenotype, however, patients with compound heterozygote mutations with missense mutation show relatively mild clinical form. In Japanese, allele frequency of R1189X is almost 67%, which is higher than previous reports of other region.

402-P

MUTATIONAL ANALYSIS OF THE *GNPTG* GENE IN PATIENTS WITH MUCOLIPIDOSIS III

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Mucolipidosis II (MLII; I-cell disease) and mucolipidosis III (MLIII; pseudo-Hurler polydystrophy) are rare autosomal recessive lysosomal storage diseases. MLII and MLIII are caused by abnormal trafficking and subcellular localization of lysosomal enzymes due to defects in the GlcNAc-1-phosphotransferase. This enzyme catalyses the formation of the mannose-6-phosphate marker on lysosomal enzymes and is composed of three subunits (alpha, beta, and gamma) encoded by two different genes, *GNPTA* and *GNPTG*. Mutations in the *GNPTA* are thought to be responsible for the severe MLII form whereas *GNPTG* appears to be defective in MLIII patients exhibiting a milder phenotype. However, there are reports that clinically diagnosed MLIII patients exhibit mutations in the *GNPTA* gene indicating that other genes can be linked to MLIII. We have analysed the *GNPTG* gene in 11 patients clinically and enzymatically diagnosed as MLIII patients. One known missense mutation and six novel mutations, four homozygous deletions and two heterozygous substitutions have been identified. It is likely that in two patients lacking defects in the *GNPTG* gene the *GNPTA* gene is mutated. Expression analyses might give insights into the effects of *GNPTG* mutation on stability, localization and function of the GlcNAc-1-phosphotransferase.

403-P

SIX CASES OF MUCOLIPIDOSES II AND III: RANGE OF CLINICAL SEVERITY AND PREVIOUSLY NOT DESCRIBED SYMPTOMS

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Objective: To evaluate and compare clinical phenotypes of six Lithuanian cases of mucolipidoses II and III. In all cases lysosomal enzymes activity in serum was 10–13 times increased. **Results**: Patients 1 and 2 presented with severe phenotype of prenatal onset: hypotrophy, coarse facies, striking gingival hyperplasia, skeletal dysplasia and multiple contractures were noted at birth. Severe osteoporosis, metaphyseal flaring, widening of ribs, massive periostoses and severe deformations of long bones after multiple intrauterine bone fractures in patient 1 were found in X-rays. Patient 1 succumbed to cardiorespiratory failure at 2 months of age, patient 2 progressed with hepatomegaly, craniosynostosis, feeding difficulties and kidney stones. Multiple episodes of hypoglycemia and metabolic acidosis were observed in patient 2 in neonatal period. Patients 3 and 4 were slightly mentally retarded siblings with coarse facies, hepatomegaly, severe skeletal dysplasia, contractures of major joints, complex valvular cardiac defects and hypertrophic cardiomyopathy progressing to cardiac failure. Patient 5 presented at the age of 5 years with macrocephaly, hydrocephaly, inguinal hernia, frequent respiratory infections, contractures of major joints, severe skeletal dysplasia and normal mental development. Patient 6 presented at the age of 7 years with kyphosis, mild mental retardation, contractures of minor and major joints, facial coarseness and mild aortic valve stenosis. **Conclusions**: Having the same etiological basis mucolipidoses II and III present with a continuous range of clinical severity. Several previously not described symptoms were found in our patients including multiple intrauterine bone fractures, episodes of hypoglycemia and metabolic acidosis in neonatal period and kidney stones.

404-P

THE FREQUENCY OF CLASSICAL LATE INFANTILE NEURONAL CEROID LIPOFUSCINOSIS (cLINCL) IN RUSSIA

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Classical late infantile neuronal ceroid lipofuscinosis (cLINCL, CLN 2; McKusick 204500) is autosomal recessive disorder caused by a deficiency of a lysosomal enzyme, tripeptidyl peptidase 1 (TPP 1), encoded by gene *CLN2*. This is one of the commonest inherited neurodegenerative disorders in Europe; nonetheless the frequency of cLINCL in the Russia is unknown yet.
Methods: The biochemical diagnosis based upon assay activity in homogenate of leucocytes of tripeptidyl peptidase 1 (TPP1) with AMC-substrate. Direct sequencing of the *CLN2* gene had been conducted in all patients. Mutation pR208X was detected by allele-specific amplification.
Results: Between 1999 and 2007 we have diagnosed 24 patients with TPP1 deficiency by biochemical method. Genotype analysis of these individuals have revealed predominance of pR208X (35/48 alleles) and IVS2+5G>C (4/48 alleles) mutations (72.9% and 8.3%, accordingly). To determine a frequency of carrier cLINCL, we have screened 2000 dried blood spots from healthy newborns by allele-specific PCR for pR208X mutation. We have founded a heterozygous pR208X mutation in 8 out of 2000 newborns.
Conclusions: The incidence of cLINCL is 1 per 250 000 live births, and the carrier frequency is 1:250 in Russian population.

405-O

MOLECULAR ANALYSIS OF THE GLCNAC-1-PHOSPHOTRANSFERASE

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Mucolipidosis II (ML II; I-cell disease) and mucolipidosis III (ML III) are autosomal recessively lysosomal disorders caused by defective GlcNac-1-phosphotransferase. GlcNac-1-phosphotransferase and a phosphodiesterase catalyse the formation of mannose-6-phosphate (M6P) recognition marker required for transport of soluble lysosomal enzymes. Lysosomal enzymes lacking M6P residues were missorted and failed to reach lysosomes. GlcNac-1-phosphotransferase is a multimeric enzyme complex composed of three subunits ($\alpha 2\beta 2\gamma 2$) that are products of two genes: *GNPTAB* and *GNPTG*, encoding for the α/β subunits and for the γ subunit, respectively. Here we analyzed the proteolytic processing, glycosylation, assembly and half-life of *GNPTAB* expressed in COS7 cells and fibroblasts of ML II patients. By quantitative real-time PCR we demonstrated that retroviral re-expression of *GNPTAB* in fibroblasts of ML II patients affected the mRNA level of several genes involved in biogenesis of lysosomes, such as phosphodiesterase, cathepsin D and L. These data suggest that the amounts of lysosomal enzymes present in lysosomes might regulate transcriptional activity of components of the lysosomal degradation pathway.

406-P

AN ONLINE NCL PATIENT DATABASE TO STUDY THE CLINICAL COURSE OF NCL – A TOOL FOR THE EVALUATION OF EXPERIMENTAL THERAPIES

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Ten different genetic forms of neuronal ceroid lipofuscinoses (NCL) have been described to date. They are clinically categorized in NCL forms with congenital, infantile, late infantile, juvenile and adult age of onset. The clinical course and its variability in the different NCL forms is still widely unknown, which makes the evaluation of therapies (experimental and traditional) difficult. Older studies included data of patients without genetic diagnosis. Due to a high genotype-phenotype variability in NCL, these older data must be used with care. With regard to the increasing number of experimental therapy studies, a precise description of the clinical course and its variability in different NCL forms is urgently needed in order to be able to evaluate the effects of such therapies.

To achieve this goal, we have established an online database containing data of almost 500 German NCL patients. Included are static (invariable) data such as the genetic diagnosis and dynamic data (related to the patient's age) such as clinical scoring and MRI results. We would like to present a first evaluation of the data from German patients. In addition we offer the use of this database to the entire international NCL research community. This database is accessible online and password protected. Data can be entered by the researchers themselves. Patients' codes can only be identified by the person entering their data. All patient data are anonymous and can be used by all researchers in the NCL field.

407-P

MUTATION SCREENING OF HIGH-RISK PATIENTS WITH NEURONAL CEROID LIPOFUSCINOSES-A CONVENIENT WAY OF DIAGNOSIS?

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Introduction: Neuronal ceroid lipofuscinoses (NCLs) are a large group of autosomal recessive lysosomal storage disorders and constitute the most common class of neurodegenerative disease in children. However definitive diagnosis is a challenge and involves complex biochemical tests which may not be available locally. The typical mutations causing the common types of NCLs have been identified and selective genetic testing of patients with distinct clinical features of NCLs may represent a more simple and quicker means of diagnosis.
Patients and methods: 7 children (age 6 months to 13 years) with common clinical features (microcephaly, developmental delay, neuroregression, behavior changes, seizures, EEG changes and MRI changes of cerebral atrophy) – all suggestive of NCLs – were selected for DNA testing. Genomic DNA was extracted from EDTA blood sample and amplified by PCR. DNA sequences were analyzed to detect the presence of the common mutations in the *CLN 1*, *2* and *3* genes.
Results- One patient carries a homozygous mutation in *CLN2* exon 12 (G514R5837A>T. Another patient carries a compound heterozygous mutation in *CLN3* gene (IVSII-3C>T) and in *CLN2* gene (G514R 5837 A>T in exon 12). A third patient carries a heterozygous mutation in *CLN1* gene (IVSIII-18). The other 4 patients carry no mutation
Conclusion: In selected cases with typical clinical features of NCLs DNA testing, where available, is a useful tool for diagnosis. It is more patient friendly and a reasonable alternative to complex biochemical tests involving skin biopsy and enzyme studies especially if these tests are not locally available.

408-P

GENETIC DIAGNOSIS IN GAUCHER DISEASE

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Gaucher disease is the most common lysosomal storage disease with a high prevalence in the Ashkenazi Jewish populations but is also present in other population. Type 1 Gaucher disease, which is the most common form, has no associated neurological manifestation. The most common finding in patients with type 1 Gaucher disease are organomegaly, cytopenia and skeletal involvement.

Gaucher disease results from the inherited deficiency of the enzyme glucocerobrosidase although >100 mutations in the gene for human glucocerebrosidase have been described; most genotype-phenotype studies have focused upon screening for a few common mutation. We used several approaches-including direct sequencing, restriction digestions and amplification refraction mutation system (ARMs) and sequencing. We checked 7 common mutations in the exon 8–11 (N370S – L444P – 84GG – IVS2+1 – del 55 bp – 1226G – 1297 T), N370S is a frequent mutation and associated exclusively with no neurological presentation of the disease. Prenatal diagnosis can be made for all types of Gaucher disease using chronic villi and amniotic fluid cells. It is hoped recent success in cloning the B-glucosidase gene will lead to effective therapy in the future.

409-P

EARLY INVOLVEMENT OF CORPUS CALLOSUM IN LATE INFANTILE FORM OF METACHROMATIC LEUKODYSTROPHY

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Background: Metachromatic leukodystrophy (MLD) is characterised by progressive loss of motor and cognitive functions. Brain MRI identifies white matter lesions and atrophy. There is no cure for MLD. In pre- or early symptomatic late infantile forms, haematopoietic stem cell transplantation may stabilize motor and neurocognitive functions. Recognition of early stages of the disease is crucial to propose transplantation.
Case Report: A 21-month-old boy presented with gait disturbances. His development was normal up to the age of 12 months. Afterwards he made no further progress and stopped walking. Clinical exam revealed slight axial hypotonia with increased deep tendons reflexes. Brain MRI showed non-specific mild signal changes in the cerebral white matter. However, the genu of the corpus callosum was also involved, which was a striking finding. Diagnosis of MLD was confirmed by measuring leukocyte arylsulfatase A activity, urinary sulfatides, and by mutation analysis. MRI performed 3 months later, just before cord blood transplantation, showed an increase of the white matter abnormalities with high signal in the periventricular white matter with involvement of the entire corpus callosum and sparing of the U-fibers.
Conclusion: This case underlines that corpus callosum involvement appears early in the course of MLD and could help the physician to establish the diagnosis.

410-P

METACHROMATIC LEUKODYSTROPHY (MLD): ATYPICAL CLINICAL AND BIOCHEMICAL JUVENILE PRESENTATION

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In metachromatic leukodystrophy (MLD) due to deficient arylsulfatase A (ARSA) lysosomal storage of sulfatide and other sulfated glycolipids leads to progressive demyelination in the central and peripheral nervous systems.

We describe a 5-year-old male of non-consanguineous parents, who presented with a slowly progressive pseudoparalytic gait. Parents had noted stumbleness since his third. Intelligence and behavior were normal. Neurological examination showed normal strength, diminished symmetrical deep tendon knee and ankle reflexes and a bilateral Babinski. MRI showed slightly abnormal periventricular white matter on T2 weighted images. Differential diagnosis was X-ALD, globoid cell leukodystrophy or MLD. Because of possible stem cells transplantation (SCT) rapid diagnostic work-up was necessary. Plasma VLCFAs showed increased C26/C22 ratios: 0.117 (normal <0.025), but a second sample showed normal results. On molecular level X-ALD was excluded.

In leukocytes galactocerebrosidase activity was normal, whereas ARSA showed activity of 33 nmol/h/mg protein (range: 55–285) with 26% residual enzyme activity (relatively high compared to other MLD patients). Two pathological mutations c.245C>T and c.1144G>A were found and urinary sulfatide was elevated so that ARSA pseudo deficiency was ruled out, substantiating the enzyme deficiency. SCT could be performed at short notice resulting in normalization of ARSA in peripheral leukocytes.

Many conclusions can be drawn from this case. When SCT is a therapeutic option, fast diagnosis is of utmost importance but may be hampered by biochemically confusing results necessitating even the rather time consuming measurement of urinary sulfatide as even gene mutations can not exclude the possibility of pseudo deficiency.

411-P

CHARACTERIZATION OF NEURONAL CEROID LIPOFUSCINOSES IN ARGENTINA

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Background: The neuronal ceroid lipofuscinoses (NCL) are inherited neurodegenerative diseases of all ages, with autosomal recessive forms caused by mutations in eight known genes and a dominant adult form whose gene is yet to be identified. The histopathological hallmark is cerebral and extra-cerebral accumulation of ceroid lipofuscin-like bodies. The aim of this work was to perform a retrospective and prospective study of 103 suspected NCL patients. **Methods**: The clinical, morphological, and enzymatic phenotypes, and the genotypes were assessed. The strategy was: (1) update the clinical diagnoses; (2) evaluate palmitoyl-protein-thioesterase 1 (CLN1p/PPT1) and tripeptidyl-peptidase-I (CLN2/TPP-I) activities, which are deficient in CLN1 and CLN2 types of NCL respectively; (3) search for vacuolated lymphocytes (CLN3 type); (4) screen for ceroid lipofuscin-like bodies using electronic microscopy (EM); (5) analyse DNA changes. **Results**: A diagnosis of NCL was confirmed in 17/103 patients. Nine were shown to have an enzyme deficiency ($n = 2$ for CLN1p/PPT, $n = 7$ for CLN2p/TPP-I). Four patients had vacuolated lymphocytes and displayed the most common *CLN3* mutation (c.462.677del), with three being homozygous and one heterozygous. The remaining four patients showed ceroid lipofuscin-like bodies, with two having mutations in *CLN6*, and two in *CLN5*. **Conclusions**: The DNA analysis rendered a total of 20 changes, consisting of four previously reported mutations, four new mutations, five new missense and four new intronic changes with validation in progress, and three polymorphisms. A mutation search is underway for a further 15 patients with positive EM. The confirmed diagnosis to date of 17 patients validates the applied diagnostic strategy for NCL.

412-P

ACUTE PANCREATITIS IN A CHILD WITH LATE-INFANTILE METACHROMATIC LEUKODYSTROPHY

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Background: Metachromatic leukodystrophy (MLD, OMIM 250100) is an autosomal recessively inherited inborn error of metabolism (IEM) affecting the lysosomal enzyme arylsulfatase A (ARSA). The clinical manifestations of MLD include dementia, ataxia, spasticity, seizures and a demyelinating peripheral neuropathy. Extraneuronal deposition of sulfatide in MLD occurs in the epithelial cells of the renal tubules and the wall of the gallbladder (Heier et al., 1983). Hepatobiliary disease (HBD) has been well described as a complication of MLD, generally presenting with gallbladder polyposis or gallstones.

Case Report: We present the case of a 3.5-year-old boy with late-infantile MLD who developed acute pancreatitis, presenting with vomiting, diarrhoea and acute epigastric pain. The diagnosis of pancreatitis was confirmed by a significantly elevated lipase of 4751 U/L (reference range <204). Serial liver function tests showed persistent mild elevation in the gamma glutamyl transferase (572 IU/L, reference 0-40) and alanine aminotransferase (210 IU/L, reference range, <55). The pancreatitis resolved clinically and biochemically in 48–72 h after treatment with analgesia and bowel rest. Parenteral nutrition was not required.

Conclusion: To our knowledge this is only the second report of acute pancreatitis in MLD. Acute and chronic pancreatitis are uncommon but recognised complications of numerous inborn errors of metabolism (IEM). The clinical features of MLD are dominated by the neurological phenotype. However, this case highlights the need for the metabolic physician to consider pancreatitis and hepatobiliary disease in patients with MLD with acute abdominal pain.

413-P

TWO NOVEL MUTATIONS IN THE *ARSA* GENE, p.G293C AND g.445.446insG, IN LATE INFANTILE METACHROMATIC LEUKODYSTROPHY PATIENTS FROM POLAND

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Metachromatic leukodystrophy (MLD) is a severe neurodegenerative metabolic disorder caused by deficient activity of arylsulfatase A (ARSA). Mutation screening with PCR-RFLP method and DNA sequence analysis on ABI PRISM 377 were performed in two late infantile MLD patients. Two novel mutations were found: p.G293C in the fifth exon and the insertion of one guanine at nt 445 in the second exon of the *ARSA* gene. Both patients were compound heterozygotes for the novel mutations and two previously described mutations c.1204+1G>A and p.E382K, respectively. The presence of each novel mutation was confirmed by demonstrating the transmission from a parent and neither of these mutations was found in a PCR-RFLP based screening in 50 healthy volunteers.

p.G293C is a missense mutation which changes the uncharged neutral glycine with aliphatic chain to uncharged neutral cysteine containing SH group. It is located in a loop between beta-sheet structure B11 and helix F, close to the surface of the ARSA molecule and away from the active centre.

Mutation g.445446insG results in the frame shift and a premature stop codon, which most probably causes the synthesis of a truncated 130 aminoacids long ARSA polypeptide.

The already known c.1204+1G>A and p.E382K mutations were described in late infantile and juvenile patients, and are classified as *ARSA*-null mutations. It can be speculated that the two novel MLD causing mutations lead to the lack or very low residual ARSA activity and in consequence to the severe form of MLD with typical signs of progressive demyelinating process, observed in presented patients.

414-P

FABRY DISEASE PATIENTS AND FABRY MICE HAVE DECREASED NUMBERS OF NKT CELLS

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Background/Objectives: Fabry disease, an X-linked sphingolipidosis, results from the defective activity of the lysosomal enzyme α-galactosidase A, leading to the accumulation of sphingolipids, namely globotriaosylceramide (Gb3) and digalactosylceramide, primarily in vascular endothelium. Considering: (1) that Gaucher disease (GD) patients have expansions of Valpha24+CD4+ T cells (iNKT), akin to up-regulation of CD1d and MHC-class II expression on monocytes (Balreira et al., 2005); and (2) that the Gb3 isomer (iGb3) is a putative endogenous ligand for human (Valpha24+) and mice (Valpha14+) iNKT cells, we performed a similar study in Fabry patients and Fabry mouse knockout mice.
Methods: Peripheral blood leukocytes were isolated from Fabry patients under ERT and healthy controls. Intrahepatic and splenic mononuclear cells were isolated from Fabry and control mice. iNKT cells and expression of MHC molecules were studied by flow cytometry.
Results: While Fabry patients have a decreased percentage of Valpha24 +CD8+ T (0.3 ± 0.1, $p = 0.005$) cells and up-regulation of MHC class II molecules (252.5 ± 81.5, $p = 0.05$) on monocytes, Fabry mouse showed a decreased number of hepatic Valpha14+CD3+ T cells (16.82 ± 1.80, $p = 0.010$).
Conclusions: In contrast to GD patients, Fabry patients have lower number of Valpha24+CD8+ T cells than controls and no anomalies in CD1d expression. However, like in GD, Fabry patients have up-regulation of MHC class II expression. The results obtained with Fabry mouse are in agreement with the recent results obtained by others (Gadola et al., 2006), reinforcing the view that mice and human respond differently to sphingolipidosis.

415-P

FABRY DISEASE: PERIPHERAL NERVOUS SYSTEM AND BEHAVIOURAL CHARACTERISATION OF THE FABRY KNOCKOUT MOUSE

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Background: Fabry disease is an X-linked inherited disorder of glycolipid metabolism resulting from deficient activity of the lysosomal enzyme, α-galactosidase A. Glycosphingolipids, predominantly globotriaosylceramide, accumulates in several tissues. Fabry patients have intermittent lancinating pain for years suggesting the involvement of the peripheral nervous system (PNS). To the date the Fabry mouse was not used to study the PNS and/or behaviour.
Methods: Three ages were studied; 12, 24 and 48 weeks. We characterised the behaviour of the mice by subjecting them to a primary behavioural screen (SHIRPA protocol). Motor nerve conduction velocity (MNCV) was recorded. Morphological and ultrastructural studies in myelin of sciatic nerves (SN) were performed examining cross sections by light and electron microscope. Evaluations were done by comparing C57BL/6 and Fabry mice.
Results: When screening for the SHIRPA protocol it was found: increased body weight, decreased in locomotor activity and increased in righting reflex for the Fabry mice for all ages. MNCV was slightly delayed in the 24 week Fabry male group. No differences were found in the mean of g-ratios of myelinated axons, although there was a trend toward thinner myelin sheaths in SN of Fabry mice for small diameters axons. Unmyelinated fibres were reduced for Fabry males group at 24 weeks old. SN of Fabry mice revealed inclusions of lamelleted bodies, infiltration of macrophages and higher number of myelinated fibres going under degeneration.
Conclusions: Our data seems to be in agreement to that found for humans: small myelinated and unmyelinated fibres are preferentially lost in the Fabry mouse.

416-P

THE CANADIAN FABRY DISEASE INITIATIVE (CFDI)

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Background: Clinical trials of enzyme replacement therapy (ERT) for lysosomal storage disorders (LSDs) have been hampered by low disease prevalence, leading to trials of short duration, small numbers, and which often rely on the use of surrogate endpoints. Observational registries exist but data collection is incomplete and participation in such registries is optional, posing potential recruitment bias. The CFDI is a unique project to provide infrastructure both for patient care and research.
Methods: The CFDI is a national program for all Canadian patients with Fabry disease (FD). Patients will be triaged to 1 of 5 geographic sites and 1 of 3 study cohorts: 1a: patients previously treated with ERT whose treatment will be maintained; 1b: ERT naove patients meeting Canadian ERT guidelines with randomization to either Agalsidase-α or Agalsidase-β; Natural history: Patients not meeting Canadian ERT guidelines who will be followed for FD-associated complications and use of supportive therapies. Patients who do not enrol in the CFDI will not be eligible for publicly funded reimbursement of ERT. We expect to enrol 200 patients with a minimum 3 year follow-up.
Results: The CFDI has several strengths: (1) Decreased recruitment bias since all Canadian patients with Fabry disease are eligible to participate, (2) A small number of centers of excellence to enhance uniformity of patient care and data collection, (3) data on supportive therapies used in FD, (4) Data on patients receiving treatment with both currently approved ERT therapies.
Conclusions: The CFDI is a unique Canadian project contributing evidence-based management of FD.

417-P

THE CLINICAL ROLE OF THE PULVINAR SIGN IN FABRY DISEASE

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Background: Fabry disease is a lysosomal storage disorder caused by deficiency of the activity of alpha-galactosidase A. CNS involvement, including stroke, occurs in a significant proportion of patients. Brain MRI abnormalities are frequent and involve mostly the white matter and the posterior region of the thalamus (pulvinar). Hyperintensity of bilateral pulvinar in T1-weighted images (the pulvinar sign) has been reported to be a pathognomonic imaging sign of the disease. We investigated the frequency and the clinical correlations between the pulvinar sign and renal, cardiac and cerebrovascular manifestations.
Methods: A total number of 36 Fabry patients (16 males, 20 females) were studied at the Bichat Hospital in Paris and at the University Hospital in Padova. Brain MRI of each patient included T1 and T2-weighted images, FLAIR sequences, and in some cases diffusion weighted images.
Results: Bilateral hyperintensity in the pulvinar on T1 images was found in 5 males, but not in females (13% of the total patients examined; 31% of the male patients). Seven patients had at least one stroke (territorial or lacunar). No correlation was detected between neuroradiological stroke and the pulvinar sign. All patients with the pulvinar sign had hypertrophic cardiomyopathy. Four out of five patients with the pulvinar sign had a severe renal involvement (dialysis or kidney transplantation).
Conclusions: Our data support a gender distribution for the pulvinar sign, which was present only in male Fabry patients. Furthermore, our study indicates a strong association between the pulvinar sign and cardiac and severe renal involvement in Fabry disease.

418-P

THE UNDER DIAGNOSIS OF SIGNIFICANT DEPRESSION IN FABRY DISEASE – A UNITED KINGDOM SURVEY

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Background: Before now no large study of depression exists in the adult Fabry population. This study examined the adult Fabry population in the United Kingdom to describe prevalence, extent of diagnosis, and associated factors for depression.
Methods: Postal questionnaires were sent from four clinics to 296 FD patients with reminders at week 3. A response rate of 62% (n = 184, 74 males; 110 females) formed the data set. Questionnaires collected demographic and Fabry-specific information. Depression status was assessed using the Centre for Epidemiological Studies depression scale (CES-D).
Results: The prevalence of depression was 46%, of which 28% indicate severe clinical depression. This is significantly higher than previously reported in the Fabry population, but comparable to other chronic diseases such as multiple sclerosis. Unlike the normal population, males with FD report a higher prevalence of severe depression than females (36% males; 22% females).

In logistic regression models, symptom interference on individuals' lives (particularly acroparesthesia or anhidrosis) showed the largest odds of being depressed. Like the general population, relationship and financial status proved strong predictors of depression. Gender, age, UK location, children with Fabry, receiving enzyme replacement therapy, education and employment status bore no statistically significant impact on depression.

Depression in this group is enormously under-diagnosed and under-treated (88% mild-moderate depression and 72% severe depression remain undiagnosed).
Conclusion: Diagnosis and treatment of depression can significantly improve an individual's quality of life, management of their disease and position within society. Increased assessment of mental state as part of routine clinical management is encouraged.

419-P

FABRY DISEASE AND DEPRESSION – YET ANOTHER SYMPTOM

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Background: Fabry disease (FD) is an inherited lysosomal storage disease with onset in childhood and reduced life expectancy. The mean time between first symptoms and diagnosis is reported to be ~13 years. Pain is one of the major symptoms, leading to sleep disturbances, decreased activity levels and disturbed social relations. Each of these symptoms alone is likely to be related to with depression. However, systematic investigations on this relation are lacking.
Objective: To evaluate the prevalence and impact of depression in a large cohort of patients with FD.
Methods: Members of the German FD patient organisation were asked to answer the WHO-5-questionnaire, a validated instrument for screening of depression. Another five questions covered their personal situation. **Results**: So far, 29 patients answered the questionnaires (14 ♀, 15 ♂). Mean age was 44.2 years (range 18–67 years). 93.1% of the patients received enzyme replacement therapy. The overall prevalence for depression in this cohort was 58.6% (females 73.3%, males 40.0%). Of those patients with a questionnaire result indicative for depression (n = 17) only four are on treatment by a psychiatric specialist, and 8/16 patients used antidepressive drugs.
Conclusions: (1) Patients with FD are at high risk of developing depressive symptoms. (2) The minority of patients with such disturbances is sufficiently treated by a psychiatric specialist.

420-P

DEVELOPMENT OF AGE-SPECIFIC PAEDIATRIC HEALTH AND PAIN-RELATED QUESTIONNAIRES FOR FABRY DISEASE

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Background: Fabry disease (FD) is an X-linked lysosomal storage disorder with symptoms starting in early childhood. Questionnaires to examine the burden of disease in paediatric patients with FD do not exist. We have therefore developed age-specific questionnaires to assess the effects of FD and the response to treatment.
Methods: A 28-item questionnaire was developed for children aged 4–7, 8–12 and 13–18 years. The questions explore disease-specific symptoms, such as hypohidrosis, heat/cold intolerance, gastrointestinal disorders, tinnitus, fatigue and pain/acroparaesthesia. Phrasing of the questions was adjusted to be appropriate for each age group, and the questions translated for use in all countries taking part in FOS – the Fabry Outcome Survey. Answers to questions concerning the presence/frequency of symptoms are based on a five-point scale to allow changes to be monitored over time.
Results: Initial results from 26 girls and 23 boys showed that patients mainly experience pain when hot and fatigue when cold. Bloating after meals was reported frequently by older patients. Diarrhoea was reported in all age groups, but especially in younger children. Tinnitus was commonly noted in adolescents. Pain crises when hot or associated with fever or physical exercise were reported from 4 years of age. All patients experienced excessive fatigue when participating in sporting activities. Acroparaesthesia was reported in over 50% of adolescents.
Conclusions: This new questionnaire is a valuable tool for clinicians treating paediatric patients with FD. It will also be useful for evaluating treatment benefits in children with this progressive and life-threatening disease.

421-P

MUTATION SPECTRUM AND INCIDENCE OF DE NOVO MUTATION OF JAPANESE FABRY PATIENTS

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Objective: Fabry disease is an X-linked lysosomal disorder resulting from mutations in α-galactosidase A (α-GalA) gene. The only way to diagnose female heterozygotes correctly is gene analysis because enzyme analysis is not reliable to diagnose heterozygotes. In X-licked disease such as Duchenne muscular dystrophy, de novo mutation is common. To elucidate the incidence of de novo mutation in Fabry disease, we carried out gene analysis of Japanese Fabry patients and their family member.
Material and Method: We performed mutation analysis among 105 Japanese cases from 60 families suspected as Fabry disease from clinical symptoms or family history (52 males, 53 females). Each exon with flanking intronic sequence of α-GalA gene was amplified by PCR from the patients genomic DNA and sequenced.
Result: Gene mutations were found in 48 out of 52 male patients who diagnosed by enzyme analysis and in 40 out of 53 females who were suspected as Fabry disease from clinical symptoms or family history. Forty-five different mutations were detected form 55 families (24 missense mutations, 5 nonsense mutations, 7 small deletions with frame shift, 2 small deletions in frame, 6 splicing defects and 1 complex mutation). Five families have no mutation in coding exons. Eleven patients were diagnosed as Fabry disease without family history (7 males, 4 females). De novo mutation was found in 3 male patients and 1 female heterozygote.
Conclusion: The mothers of male patients are not necessarily heterozygotes because some patients are de novo cases. Physicians should diagnose heterozygotes carefully based on mutation analysis.

422-P

FRENCH OBSERVATOIRE ON GAUCHER DISEASE (FROG) RESULTS ON 107 PATIENTS

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Introduction: Gaucher disease (GD) is a rare disease with a very heterogeneous presentation. This French national prospective registry is an epidemiological study which objectives are to describe the clinical aspects of adult GD patients and their current management with focus on co-morbidities and impact on the quality of life.
Patients and method: Clinical data of adults GD patients were collected during a routine visit; No additional tests were performed. A specific CRF was designed for guidance of the physician as an educational tool.
Results: From May 2005 to September 2006, 105 type 1 GD patients and 2 type 3 GD patients (49 men and 58 women, mean age 45 ± 14 years, were included in 45 centres. Mean age at first signs was 21.8 ± 13.7 years with a GD diagnosis 5.0 ± 5.5 years after. Physical asthenia, psychic asthenia and muscular fatigue were found in 53, 33 and 32% of the patients respectively. History rheumatologic manifestations occurred in 85%, at least one neurological sign in 56%, depression in 21% and gammopathy in 15% of the patients. 36% needed analgesic medications regularly (68%) or daily (32%). 76 patients received GD specific treatment. Each dimension of the quality of life SF36 questionnaire was impaired in comparison with standard population.
Conclusion: This French GD population presents with very frequent co-morbidities that impair quality of life. These findings suggest the need for a multidisciplinary follow up of GD patients.

423-P

BONE DISEASE IN M GAUCHER: LABORATORY FINDINGS, BONE DENSITY AND 3-TESLA MRT IMAGING

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Introduction: Bone manifestations are frequent findings in adult patients with Gaucher's disease. Information about skeletal involvement is critical for treatment and prognosis of patients with this disease. Besides technically challenging and not widely available techniques as quantitative chemical shift imaging (QCSI), magnetic resonance imaging (MRI) is a standard technique for diagnosing bone disease in this disease. The value of laboratory tests, bone density and the possible advantage of new MRI techniques are not clear.
Methods: Patients with Gaucher's disease presenting in the adult metabolic clinic were clinically examined, subjected to laboratory tests, Dual-energy X-ray-absorption (DEXA) and 3-Tesla-MRI using a body array coil.
Results: 11 subjects (age 23–40, 6 females 5 males) were examined, 5 of them receiving enzyme replacement therapy. Bone specific alkaline phosphatase and osteocalcin were not significantly in affected. Plasma vitamin D was decreased in patients being lowest in untreated, but close to normal in treated patients (normal: 30–68 5 g/L; no therapy: 15 ± 7.8 5 g/L; therapy: 32 ± 15 5g/L). Parathyroid hormone was increased without therapy, but normal in treated patients. Age corrected bone density (Z-scores) was decreased in untreated patients (lumbar spine: –0.7; femur –0.5), but not significantly affected in treated patients. Besides typical bone manifestations of Gaucher's disease, 3-Tesla-MRI showed that the vertebra disc ratio (VDR) was lower in patients without therapy (0.9 ± 0.5) than in treated patients (1.4 ± 0.5; normal VDR: > 1.68).
Conclusion: The laboratory changes could be due to different intestinal vitamin absorption. 3-Tesla-MRI showed that VDR is a sensitive parameter for monitoring bone dieasease in M. Gaucher.

424-P

GUIDELINES FOR THE ASSESSMENT AND MONITORING OF BONE DISEASE IN CHILDREN WITH GAUCHER DISEASE

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Background: Gaucher disease (GD)-related bone disease puts children at risk for developing irreversible complications and interferes with the achievement of optimal bone mass. Appropriate disease management includes an initial assessment and ongoing monitoring of the bone marrow and mineral components, and guidelines addressing the clinical and technical challenges of these evaluations are needed. **Methods**: A working group met in October 2006 to develop evidence- and consensus-based guidelines to facilitate the assessment and monitoring of bone disease in children with GD. **Results**: Magnetic resonance imaging (MRI) and dual-energy X-ray absorptiometry (DXA) are recommended for the evaluation of bone marrow infiltration and bone mineral density, respectively. Assessments should be performed at centers with expertise in children. MRI measurements (T1-weighted and STIR sequences) of the femur, pelvis and spine should be made at baseline and at least every two years thereafter, preferably annually. DXA measurements of the lumbar spine, proximal femur, and entire body should be made at baseline and annually thereafter, with serial scans performed on the same DXA machine. DXA measurements must be analyzed with the appropriate pediatric software option and reported as Z-scores with respect to the best available databases of age-matched controls. Plain radiography has a limited role in routine assessment of pediatric GD, but can be useful under certain circumstances. **Conclusions**: These guidelines will facilitate the assessment of GD-related bone disease needed to tailor clinical management in order to achieve the therapeutic goals of eliminating bone marrow infiltration and achieving optimal bone density in children with GD.

425-P

BONE MANIFESTATIONS IN GAUCHER DISEASE. DATA FROM THE FIRST 107 OF THE FRENCH OBSERVATOIRE ON GAUCHER DISEASE (FROG)

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Background/Objectives: Gaucher disease (GD) is a rare disease with a heterogeneous clinical presentation. A French national prospective registry was implemented to describe the clinical features of adult GD patients with focus on bone manifestations and their impact on patients'quality of life. **Methods**: Clinical data were collected during a routine visit. No additional tests were performed. A specific case report form was designed to guide the physicians. **Results:** From May 2005 to September 2006, 107 patients were included. Mean age (SD) was 45 (14) years (54% female). Early diagnosis (< 1 year after the first signs) was less frequent when first signs were skeletal manifestations (25% vs 47%). 85% of patients had a history of skeletal manifestations: osteonecrosis 30%, bone infarction 28%, bone crisis 21%, peripheral fracture18% and vertebral fracture 14%. 17% of patients had undergone joint replacement at a mean age (SD) of 40 (10) years. 57 (53%) patients complained of pain (severe with VAS > 6 cm in 18%) and 38 (67%) of them were taking analgesics drugs on a regular basis (daily in 32%). MRI showed bone marrow infiltration in 54% of patients. Of the 64 patients with bone mineral density (BMD) data available, 44% had osteopenia, 18% had osteoporosis. **Conclusion:** Skeletal manifestations are frequent in adult GD patients and have major impact on patients'quality of life. A multidisciplinary team approach including a rheumatologist seems essential for optimizing patients' care.

426-P

MONOCLONAL GAMMOPATHY ASSOCIATED WITH GAUCHER DISEASE. REPORT OF 16 CASES

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Background/Objective: Gaucher disease (GD) is frequently associated with polyclonal (PG) and monoclonal gammopathy (MG). The objective of our study was to determine the frequency and the presentation of MG in GD patients.
Methods: The FRench Observatoire on Gaucher disease (FROG) is a prospective epidemiological study on adult GD patients involving 45 French centres. In patients with suspected MG specific assessments were done: immunofixation, dosage of immunoglobulins, bone marrow aspiration.
Results: From March 2005 to September 2006 105 GD type 1 and 2 GD type 3 patients were included. Sixteen cases of MG (15%) were observed (10 males, 6 females). Mean age of patients with MG (60.7 ± 8.5 years) was significantly higher ($p < 0.0001$) than those without MG (42.5 ± 12.8 years). Thirteen cases (12.9%) were classified as monoclonal gammapathy of undetermined significance (MGUS) and 3 (2.8%) as B cell malignancies: 1 multiple myeloma, 1 chronic lymphocytic leukaemia, 1 non-Hodgkin lymphoma.

Immunochemical typing revealed 11 monoclonal IgG, 2 monoclonal IgM, 1 biclonal (IgG+IgA) and 1 triclonal (IgG+IgA+IgM); 11 cases were kappa and 4 lambda gammopathy; one result missing. Among the 13 patients receiving treatment for GD, we observed 7 cases of decrease and 6 cases of stabilisation of Ig levels.
Conclusion: Our study confirms the high prevalence of gammopathies among GD patients. Like in the general population, increase of frequency of gammopathies seems to be related with increased age. Risk of B cell malignancy needs specific follow up. GD treatment can eventually reduce the level of IgG.

427-P

PREVALENCE OF POLYNEUROPATHY IN ADULT TYPE 1 GAUCHER DISEASE (GD1): A MULTINATIONAL PROSPECTIVE OBSERVATIONAL STUDY

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Background: GD1 has traditionally been categorized as non-neuronopathic. However, some cases of polyneuropathy (PNP) have been reported and also in patients exposed to miglustat. Since there is no definite explanation, a multinational (7 countries, 8 centres) prospective, observational, study has been set up to establish the prevalence and incidence of PNP in GD1. **Methods**: This study was set up under the auspices of the European Working Group on Gaucher Disease. Diagnosis of PNP was based on compatible neurological signs and/or symptoms and abnormal electrodiagnostic studies. A standardised protocol has been used in all centres. An independent central assessor adjudicated PNP diagnosis. Secondary endpoints include 2-year incidence of PNP and other parameters (neuropsychological status, organ involvement, skeletal manifestations, laboratory measurements and quality of life). **Results**: 103 GD1 patients were enrolled; either untreated ($n = 17$) or treated by enzyme replacement therapy ($n = 86$). Mean age $\pm$ SD was 42.6 ± 14.5 years (53% female). Eleven patients were diagnosed with sensory or sensory/motor axonal PNP (10.7%, 95% CI = 5.5–18.3%). This prevalence is significantly higher than in the general population (0.12 to 3.6%).[1,2]. Patients with PNP were older than those without PNP (mean $\pm$ SD: 61.1 ± 10.3 vs. 40.4 ± 13.4, respectively). Further investigations will focus on the relation with disease severity, and other factors associated with PNP. **Conclusions**: These findings prompt awareness of this new co-morbidity in GD1, and suggest that careful questioning and, in case of suspected PNP, further examination by an experienced neurologist is needed.

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428-P

VERY OLD GAUCHER TYPE 3

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Introduction: Gaucher disease is a lisosomic disease caused by a deficiency of the glucocerebrosidase. There are three clinical phenotypes: type 1, not neuronopathic, type 2, acute neuropathic and type 3, chronic neuropathic. In type 3 the neurological symptoms are predominant although they are combined with weaker systemic symptoms.
Clinical case: A sixty-seven year-old patient with symptoms since she was sixty years-old: unbalanced walk with frequent falls and motor difficulties in the arms. She is the child of consanguineous parents and her brother died at the age of 18 of a possible neurological disease. Neurological exam: slight cognitive deficit, cerebellar dysarthria, negative cephalic exuberant tremor, severe changes of eye movements without superior verticality, ocular apraxy and limitation of the horizontality to the right, tetraparesy, myoclony of the torso and limbs, predominantly rest tremor, dysmetry and bilateral dissynergy, spasticity of the legs, without any balance of the torso (walks helped by another person). Additional exams: cranium and cervical MRI: cerebellar atrophy, X-ray of the long bones, hemogram, biochemistry, abdominal ultra sound do not show changes. The determination of the glucocerebrosidase in leucocytes: 1.0 nmol/h/mg protein (N of 2.8–19) and fibroblasts: 15.0 nmol/h/mg protein (N of 103–552) is compatible with the diagnosis of Gaucher disease.
Discussion/conclusion: Type 3 Gaucher disease is rare. There are approximately registered 250 cases in the world. The peculiarity of this clinical case is the fact that it started extremely late in life and that it did not have any systemic symptoms.

429-P

RED BLOOD CELL PLASMALOGEN LEVELS IN GAUCHER DISEASE. THE EFFECT OF ERT

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Background/Objectives: Plasmalogens are unique phospholipids characterized by the presence of a vinyl ether bond at the sn-1 position. Their suggested functions include protection against oxidative stress, participation in signal transduction, membrane fusion events, cholesterol transport and membrane trafficking, processes known to be disturbed in sphingolipidoses. We report on red blood cell membrane plasmalogen levels in Gaucher disease (GD) patients on diagnosis and one year after ERT. **Patients/Methods**: A total of 16 Gaucher disease patients were studied. Plasmalogen levels were measured by gas chromatography in lipid extracts of erythrocytes as their dimethylacetal (DMA) derivatives. Their relative amount was estimated as the% ratio between C18:0DMA and Me-stearate, as well as C16:0DMA and Me-palmitate. Chitotriosidase plasma activity was assayed by the method of Hollak et al. The results were analysed by non parametric tests. **Results**: On diagnosis statistically significant lower levels of C16:0DMA/C16:0 and C18:0DMA/C18:0 were observed in GD compared to normal individuals both ($p < 0.001$). Furthermore, a statistically significant negative correlation between plasmalogen levels and chitotriosidase activity was observed in GD patients, before ERT. ERT resulted in a significant rise of plasmalogen levels and fall of chitotriosidase activity levels ($p = 0.001$, $p = 0.004$ and $p < 0.002$). However in GD C18:0DMA/C18:0 were still significantly lower than in normals and the negative correlation to chitotriosidase levels persisted. **Conclusions**: Reduced plasmalogen levels that correlate negatively to total disease burden, as expressed by chitotriosidase activity, and are ameliorated by ERT is observed in Gaucher disease.

430-P

INVESTIGATION OF 10 COMMON MUTATIONS IN TURKISH GAUCHER PATIENTS BY USE OF THE NANOCHIP MICROELECTRONIC ARRAY TECHNOLOGY

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Electronic deoxyribonucleic acid microarray technique has been used for the rapid molecular investigations of genetic diseases in large group of patients with a high accuracy in the recent years. Gaucher disease is caused by the genetically determined deficiency of the lysosomal enzyme acid beta-glucosidase. About 200 mutations have been described for glucocerebrosidase gene so far. We have investigated 10 common mutations in 28 enzymatically proven Turkish Gaucher patients by use of electronic deoxyribonucleic acid microarray technique.

Methods: Twenty-eight Turkish Gaucher patients (20 female and 8 male, age range: 1.5–52 years, median 8 years) who were enzymatically proven to have Gaucher disease were included in the study. A microarray chip including 10 common mutations which were previously reported in Gaucher patients was designed and 56 disease causing alleles in all enzymatically proven Turkish glucocerebrosidase deficient patients were investigated.

Results: By using the 'Gaucher Chip' of 10 common mutations, 45 of the 56 disease causing alleles (80.35%) were determined. None of the 10 mutations were found in three of the patients. The most common mutation was noted to be N370S (54% of the alleles) followed by L444P, D409H (14% of alleles each) and IVS2 (6% of the alleles) respectively. None of the point mutations R463C, R496H, V394L and neither the insertion c.84-85insG was noticed in any of the patients.

Conclusions: It was noted that investigation of common mutations in Gaucher disease with electronic deoxyribonucleic acid microarray technique provides rapid and reliable results with a high coverage rate.

431-P

NONMODIFIED siRNA LEADS TO DOWNREGULATION OF ALPHA-GLUCOSIDASE: TOWARDS A MOUSE MODEL FOR M POMPE

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Pompe disease is a rare autosomal recessive lysosomal storage disease caused by the deficiency of acid-alpha-glucosidase (GAA) which is responsible for degradation of glycogen into glucose. Currently, the most promising treatment for Pompe disease is enzyme replacement therapy (ERT). However, the ineffecient uptake and monitoring of the recombinant GAA in the cells is a major drawback. Blood analysis instead of invasive muscle biopsies would ease the monitoring of the effect of the current therapy and future therapies. Therefore, the generation of a GAA-deficient mouse model has been initiated using knock-down (KD) technology. This technology is characterized by random integration of siRNA directed against the genomic DNA sequence coding for the *GAA* gene. Three siRNAs are developed: mouse *GAA* (siRNA1), mouse-human *GAA* (siRNA2) and human *GAA* (siRNA3).

In 3T3 (mouse fibroblasts) both siRNA1 and siRNA 2 (1 5g complexed with the amphile SAINT) downregulate *GAA* expression with 45% and 64%, respectively. As expected, siRNA3 does not cause any downregulation.

Increasing amounts of siRNA1 or siRNA2, demonstrate increasing downregulation of *GAA* expression during siRNA-fection experiments in 3T3. In human skin fibroblasts analysis of the long-term effect of siRNA2 and siRNA3 show optimal downregulation ($\sim$80%) of *GAA* expression at day 12 after fection. At this moment cloning of the sequence of siRNA1 in a siRNA producing vector takes place.

The initial results demonstrate that the developed siRNAs are able to downregulate *GAA* expression in both mouse 3T3 and human skin fibroblasts and are very promising for generating a KD model for Pompe disease.

432-P

OBJECTIVELY MEASURING ATAXIA IN NEURONOPATHIC GAUCHER DISEASE

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Background: Neuronopathic Gaucher disease (NGD) is one of the three recognized subtypes of Gaucher disease, an inherited deficiency of lysosomal glucocerebrosidase. Ataxia is one of the many presenting neurological manifestations. An objective measurement of ataxia could offer a quantified measurement of disease severity.

Method: Four girls with NGD, all L444P homozygote were assessed using a Severity Scoring Tool (SST) and GAITRite. SST is disease specific, and developed to objectively quantify disease severity including ataxia1. GAITRite is a pressure-sensing matt, with sensors recording the imprint of each footfall2. This allows for the temporal parameters of gait to be measured. The girls were 8–22 years old, 3 were on enzyme replacement therapy and one post bone marrow transplant.

Results: Mean SST score was 7.1 (SD 2.2; range 0–33). The eldest two, scoring the highest score which is consistent with their clinical picture. GAITRite data for cadence, single support and double support times were examined (barefoot). The two youngest girls were consistently within the 3rd and 97th centile3 for all three variables. The eldest two were also within the normative centiles for single support, but were on the 97th centile for cadence, and outside the ranges for double support times.

Conclusion: Both assessment tools appear to demonstrate concordance in capturing disease severity. Further work on a larger sample size is needed but initial data is encouraging.

433-P

DEVELOPMENT OF A DISEASE SEVERITY SCORING SYSTEM FOR PATIENTS WITH POMPE DISEASE

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Background: A Disease Severity Scoring System (DS3) measures disease burden in patients. It consists of critical health domains, each described by relevant clinical assessment(s) quantified via reliable, feasible methods. DS3s are particularly useful in rare, heterogeneous diseases in which evaluating severity and prognosis is difficult. Properly configured, a DS3 provides inter- and intra-patient comparisons through time across critical organ systems. DS3 development is underway for Pompe disease, a rare, autosomal recessive, heterogenous, neuromuscular disorder. **Methods**: A panel of Pompe experts was assembled to identify critical Pompe disease health domains. A broader 'Delphi' physician group was consulted to capture standard medical practice(s) for severity measurement within each critical domain. Selected domains were: cardiac, respiratory, proximal muscle, physician reported outcomes and patient reported outcomes. Within each domain, 1–2 clinical assessments were identified. To test this preliminary model, 9 cases from the Pompe Registry representing a severity spectrum were scored. **Results** were compared to results from a blinded small expert group assessment of the cases using the Clinical Global Impression (CGI) Severity scale, yielding a 0.93 coefficient of correlation, indicating preliminary DS3 consistency with expert opinion, confirming DS3 validity, reliability and relevance. Validation will be completed by comparing DS3 results with expert 'Delphi group' opinion for multiple patient cases at multiple time points. **Conclusion**: Preliminary results indicate that the Pompe DS3 model will help standardize disease terminology and key clinical assessments to quantify disease severity. Ultimately this tool will evolve into a universal disease 'staging' system where specific medical interventions may be recommended.

434-P

THE POMPE REGISTRY: CENTRALIZED DATA COLLECTION TO TRACK THE NATURAL COURSE OF POMPE DISEASE

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Background: Pompe disease is a rare, progressive, and often fatal muscle disease that results from deficiency of acid alpha-glucosidase (GAA) that hydrolyzes lysosomal glycogen. This single gene disorder manifests clinically with a broad spectrum of ages at onset, rate of disease progression, and extent of organ involvement.
Methods: To better understand the natural course of Pompe disease, a global, observational Registry was developed to collect anonymous, longitudinal data on Pompe patients.
Preliminary Results: As of 11/11/2006, 265 patients from 18 countries have enrolled. The majority (69.1%) are of Caucasian ethnicity. 15.8% (42/265) of reported patients have infantile Pompe disease, typically with cardiomyopathy, profound skeletal and respiratory muscle weakness, and death within first year of life). The median age of the infantile diagnosis is 7.2 months. 68.3% of the reported patients have late-onset Pompe disease, typically without cardiac involvement but with progressive skeletal and respiratory muscle weakness and longer survival. The median age of those with late-onset diagnosis is 34.6 years. Age of onset was unknown in 15.8% of registry patients. The (median) time from recorded symptom onset to diagnosis is 3.6 months for infantile patients and 25.3 years for late-onset patients. Of the Pompe patients investigated for genotype, in 74.7% (56/75) the IVS1-13T>G mutation was found.
Conclusions: The Pompe Registry aims to increase understanding of this rare disorder and to improve patient management. Preliminary data show that the (median) range of time from symptom onset to diagnosis is similar to published literature, suggesting the need for greater disease awareness.

435-P

NONINVASIVE MUSCLE ASSESSMENT WITH MRI IN POMPE PATIENTS

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Background: Pompe disease is an inherited metabolic disorder leading to progredient storage of glycogen in lysosomes. In the most severe form children present with cardiomyopathy, muscular hypotonia and early respiratory failure. Untreated they die in the first year of life. Adult/juvenile patients present clinically with respiratory failure and/or skeletal muscular weakness. The first involved muscles are the paravertebral muscles and the iliopsoas.
Methods: We investigated in our juvenile/adult Pompe-patients the paravertebral and iliopsoal musculature with a 3-Tesla-MRI. In the T1-weighted-images we defined a semiquantitative score for the fatty degeneration of the muscles: 0 = normal muscle, 1 = less than 50% fatty degenerated, 2 = more than 50% fatty degenerated, 3 = totally fatty-degenerated/complete atrophic (maximum $2 \times 3 = 6$).We compared the MRI-score with the 6-min-walk-test and the functional-vital-capacity (FVC).
Results: The score range in our patient cohort ($n = 12$) was between 0 and 6 (mean: 3.2 ± 2.1). The MRI-score of the paravertebral musculature compared to the 6-min-walk-test is showing a significant correlation of 0.837 ($p = 0{,}01$). Instead of this there is no correlation to the FVC (0.136; $p = 0.69$).
Conclusions: With MRI you can examine the musculature without performing invasive muscle biopsies. Additionally it is possible to evaluate more and with normal biopsies not reachable muscles, especially the paravertebral musculature. This early and serious involvement of proximal muscles in Pompe-patients was confirmed by our MRI-studies.

With the MRI you can see the degree of degeneration of the muscles and in consequence the maximal possible gain under enzyme-replacement-therapy. So the MRI could be an objective parameter for the reconstitution of muscles.

436-A

CASE STUDY – BABY J. DIAGNOSIS: DOWN'S SYNDROME AND POMPE'S DISEASE

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Background: We report Baby J, who was born at home and was admitted to the neonatal intensive care unit at two days of age having collapsed. When extubated, epicanthic folds were observed. A diagnosis of Down's syndrome (trisomy 21) was confirmed. Pompe disease was detected shortly after birth, as there was a family history of two previous affected siblings, who both died in infancy (prenatal testing was not performed). Enzyme replacement therapy (ERT) (Myozyme) was commenced at the age of one month.
Discussion: To the best of our knowledge, this is the first time that Pompe disease and Down's syndrome have been diagnosed in the same patient. The ERT may result in significant residual burden, both in terms of health and quality of life. In addition, the effectiveness of ERT may be difficult to assess due to the hypotonia of both conditions.
Conclusion: Trisomy 21 was not considered to be a factor that should exclude the child from receiving ERT.

437-P

UNTREATED ACID MALTASE DEFICIENT PATIENTS HAVE VARIABLE IMPAIRMENT OF RESPIRATORY AND MUSCLE FUNCTION

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Background: Acid maltase deficiency (AMD) is due to lysosomal acid alpha-glucosidase deficiency, and leads to excessive glycogen accumulation. In adults, it usually presents as slowly progressive proximal myopathy with variable diaphragmatic involvement eventually causing respiratory failure. Recently, enzyme replacement therapy (ERT) has become available. Here we present baseline characteristics for 15 adult patients (10 males; 5 females) prior to starting ERT.
Methods: Assessments included cardiac and respiratory function tests, timed motor function tests, manual muscle testing (MMT), and sleep studies.
Results: Ages ranged from 15–75 years (mean 49.7). Ten used overnight ventilation; two also needed ventilatory support intermittently during day-time. Two were completely wheelchair dependent; two intermittently. Percentage drop in forced vital capacity from standing/sitting to supine ranged from 4.58–49.8% (mean 27.07%). 12 patients did 10 metre walks. Times ranged from 3.59–27 s (mean 12.43). 7 patients completed a 6-min walk test. Distance walked ranged from 210–480 metres (mean 272 metres). 2 patients completed a 3-min walk test. Distances walked: 69 and 143 metres. Time taken to stand from supine (4 patients) ranged from 3.91–48 s (mean 17.89). 8 patients (2 on overnight ventilation) had sleep studies. Mean desaturation events >4% below baseline were 16 (range 7–27). Lowest saturations ranges were 73–88% (mean 79%). MMT confirmed proximal myopathy. There were no significant correlations between motor or respiratory function and age.
Conclusions: Adult AMD patients have significant impairment of motor and respiratory function that varies considerably between patients. Careful longitudinal assessment will enable the proper evaluation of the impact of ERT.

438-P

DECREASED VENTILATOR USE IN A 27-YEAR-OLD MAN WITH LATE-ONSET POMPE DISEASE WITHIN ONE YEAR OF USING ALGLUCOSIDASE-ALFA

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We report a 27-year-old man with late-onset Pompe disease (acid alpha-glucosidase deficiency) with decreased ventilator use within 12 months of biweekly infusions of algucosidase-alfa (Myozyme) which have been sustained after 18 months of therapy.

The peak inspired flow rate (PIF) increased from 0.83 L/s (17% predicted) prior to the start of alglucosidase-alfa to 1.80 L/s (38% predicted) at 18 months. The peak cough flow rate (PCFR) doubled from 108 L/min to 200 L/min, maximum inspiratory pressure (MIP) tripled from –7 cm H20 to –22 cm H20 and the maximum expiratory pressure (MEP) doubled from 12 cm H20 to 25 cm H20 over the same time period. His ventilator use prior to the start of enzyme infusions was 24 h/day. Pressure control ventilation (PCV) with pressure support (PS) of 16 cm H20 and positive end expiratory pressure (PEEP) 4 cm H20. He has been weaned to pressure support ventilation (PSV) at a PS of 8 cm H20 and PEEP 4 cm H20. He only requires PCV with sleep. He can be off the ventilator for 2 h without breath stacking.

His right hand grip strength was 26 kg prior to the start of enzyme therapy and increased to 28 kg 15 months after therapy. There were no other measurable changes in his muscle strength.

Despite these modest changes in ventilator use, the patient reports improved quality of life owing to the ability to perform daily activities such as toileting and showering without needing the ventilator.

439-P

MOLECULAR GENETICS OF INFANTILE POMPE DISEASE IN ITALY

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Background: Glycogen Storage Disease Type II (GSDII) is an autosomal recessive disorder in which deficiency of acid alpha glucosidase (GAA) results in impaired glycogen degradation that accumulates within lysosomes. The *GAA* gene (MIM# 606800) localizes to human chromosome 17q25.2–25.3; the enzyme is synthesized as an inactive precursor of 110 kDa and then processed, in the lysosomal compartment, to the fully active forms of 76 and 70 kDa. Numerous mutations have been described up to date [http://www2.eur.nl/fgg/ch1/pompe]. **Methods:** We studied 37 unrelated patients affected by infantile Pompe disease coming from different parts of Italy. Genomic DNA was analyzed by PCR followed by automatic sequencing. RNA was extracted from patient fibroblasts, amplifyied by RT-PCR and sequenced. Functional characterization of novel missense mutation was performed by transient expression of wild-type and mutant proteins in a human *GAA* deficient cell line. **Results:** Among the 32 different alleles identified, nine were novel. Five single base substitutions were studied by functional expression assay: c.572A>G (p.Y191C); c.1124G>T (p.R375L); c.1202A>G (p.Q401R); c.1564C>G (p.P522A) and c.1796C>A (p.S599Y); none of the mutants tested expressed residual activity. The c.742delC deletion cause a shift in the reading frame introducing a premature stop codon that lead to a truncated protein p.L248PfsX20. Three mutant alleles were found in the intronic region (c.–32–3C>A, c.1075+13C>T, c.1636+5G>C) and might affect RNA processing. **Conclusions:** Mutational analysis of Italian infantile Pompe patients and the functional study of mutant alleles contribute to a better understanding of genotype-phenotype correlation and provide valuable insights into the molecular basis of the disease.

440-P

OXIDATIVE DAMAGE TO LIPIDS AND PROTEINS IN AN INFANT WITH NIEMANN–PICK DISEASE TYPE A WITH ASSOCIATED HYPERLIPIDAEMIA

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Background: Niemann–Pick disease (NPD) types A/B are lipid storage disorders caused by acid sphingomyelinase deficiency. Type A NPD is a neurodegenerative disease of infancy characterised by early and severe clinical presentation and short life expectancy. Lipid abnormalities may form part of the NPD phenotype and be associated with increased oxidative damage.

Patient: A 7-month male infant presented failure to thrive, vomits, axial hypotonia, hepatosplenomegaly, psychomotor retardation and cherry red maculae. Type A NPD diagnosis was confirmed by undetectable sphingomyelinase activity in leukocytes. Laboratory studies revealed elevated transaminases (AST 925 IU/L, ALT 411 IU/L), chitotriosidase (520 nmol/h/ml) and hypertriglyceridaemia (525 mg/dl).

Results: Lipid abnormalities persisted at 11 months of life despite dietetic intervention. Serum lipid profile showed dyslipoproteinaemia with severely low HDL-cholesterol (11.6 mg/dl), raised LDL-cholesterol (129.3 mg/dl) and hypertriglyceridaemia (341 mg/dl). Indicative lipid and protein oxidation markers measured in plasma demonstrated, compared with healthy controls, significantly raised malondialdehyde, the main end-product of lipid peroxidation, (1.5 vs. 0.4 nmol/ml), oxidized-LDL (110.8 vs. 43 U/L), advanced oxidation protein products (1.7 vs. 0.5 nmol/mg prot) and carbonylated proteins (2.8 vs. 1.2 nmol/mg prot). Activity of the antioxidant enzyme glutathione-peroxidase in erythrocytes was significantly diminished (35.8 vs. 47.4 U/gr Hb).

Conclusion: Our results support the hypothesis that NPD-associated alterations of peroxidable lipids provoke oxidative damage to lipids and proteins. Thus, oxidative stress may be a new pathomechanism of NPD implicated in the phenotypic expression of the disease.

441-P

NIEMANN–PICK A/B DISEASE WITH ACCUMULATION OF SPHINGOMYELIN IN THE AORTIC VALVE AND THE MYOCARDIUM

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The historical classification of sphingomyelinase deficiency by Crocker into types A and B does not describe the complete spectrum of clinical observations. In particular, the mutation p.Q292K of the *SMPD1* gene seems to be associated with an intermediate neuronopathic course, which progresses only slowly and is not lethal in early childhood.

We report the case of a 21-year-old man, who showed first symptoms of the disease within the first year of life. He was diagnosed to have Niemann–Pick disease by enzymatic testing when he was 7 years old. The diagnosis was later confirmed by an elevated activity of chitotriosidase in blood and detection of homozygosity for the p.Q292K mutation of the *SMPD1* gene.

The patient shows severe mental retardation with global cerebral atrophy on MRI. He suffers from a central movement disorder but also has signs of a peripheral neuropathy. He has hepatosplenomegaly with hypersplenism and an impaired lung function caused by interstitial lipid storage. Due to progressive aortic regurgitation (stage III), valve replacement became necessary at age 20. Lysosomal storage material was found both in the myocardium and in the explantated valve. Sclerosis of the coronary arteries was excluded by angiography.

Conclusion: In the intermediate form of Niemann–Pick disease, designated A/B and typical for the p.Q292K mutation of the *SMPD1* gene, the accumulation of lipid storage material may cause cardiomyopathy and progressive aortic valve regurgitation, a complication not previously described in sphingomyelinase deficiency.

442-P

NIEMANN–PICK A DISEASE: CLINICAL SPECTRUM IN A GROUP OF BRAZILIAN PATIENTS

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Objectives: To describe the clinical evolution of Niemann–Pick A disease and to identify possible factors involved in the diagnosis and severity of the disease.
Methods: A clinical retrospective study and review of laboratory analyses, abdominal/brain ultrasounds and chest radiographs were carried out of 9 patients diagnosed with Niemann–Pick type A (five female and four male) in a University Hospital located in southeast of Brazil.
Results: Complete clinical data were obtained from 8 patients; seven were confirmed to have deficiency of sphingomyelinase. Regarding clinical form, 2 were perinatal, 5 severe infantile and 2 were late infantile. Biochemical phenotype was not obtained in two patients whose diagnosis was made by clinical presentation, neuro-ophtalmological findings and presence of numerous foam cells in the bone marrow. Splenomegaly was present in 8 patients with a wide range of age at detection. The first symptom of neurological disease was developmental delay; one patient, however, had as first symptom also seizures which later were characterized as progressive myoclonic epilepsy. Cutaneous manifestations were seen in two patients (one had multiple mongolian spots throughout the body and other had vasculytis in lower limbs). Ocular abnormalities were seen in four patients (one had optic atrophy and three the cherry-red maculae) Other systemic manifestations in the patients included progressive psychomotor deterioration, hypotonicity, muscle weakness, hepatomegaly, microcytic anemia, emaciated appearance, generalized lymph-adenopathy and bronchopneumonia.
Conclusion: The clinical course in Type A Niemann–Pick disease is similar among affected patients and is characterized by a neurodegenerative course with fatal outcome.

443-P

NIEMANN–PICK DISEASE TYPE C: A FURTHER CAUSE OF EXTREMELY ELEVATED ALPHA-FETOPROTEIN LEVELS

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Niemann–Pick type-C (NPC) is a rare autosomal recessive lipid storage disease affecting the viscera and the CNS, caused by a defect in intracellular cholesterol trafficking. Elevated serum alpha-fetoprotein (AFP) levels have been traditionally linked to neoplastic disorders, mainly involving germ cells and hepatocytes. The only metabolic disorder previously reported in association with extreme elevations of AFP levels is tyrosinemia type 1.

A 3-week-old male infant of non-consanguineous Arab parents presented with prolonged jaundice, prominent hepatosplenomegaly and poor weight gain. Evaluation revealed direct bilirubinemia, mildly elevated hepatocellular and cholestatic enzymes and extremely high serum levels of AFP, up to 1 400 000 ng/ml (age-corrected norm <2000). Liver biopsy excluded a neoplastic disorder. Eventually, NPC was diagnosed by means of decreased uptake of tritiated oleic acid and a positive Filipin stain in cultured skin fibroblasts. The diagnosis was confirmed by genetic analysis, which revealed compound heterozygocity of two known mutations, S940L and c2279.81delTCT.

The child's liver disease followed the natural history of hepatic involvement in NPC with spontaneous resolution of cholestasis within the first year of life. Concomitantly, AFP levels returned to near-normal values. He developed progressive neurological deterioration and eventually died at the age of 9 years.

To the best of our knowledge this is the first report in the western literature of elevated AFP levels in NPC. We therefore propose to include NPC in the evaluation of extremely elevated AFP levels. Further, we suggest that in patients presenting with impaired liver function and elevated AFP levels, metabolic disorders should be considered.

444-P

ATYPICAL CLINICAL COURSE IN THE ADULT FORM OF NIEMANN–PICK TYPE C DISEASE

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Background: The main clinical features of adult form of Niemann–Pick type C disease (NPC) are psychiatric disturbancies, cognitive impairment and 'deep brain signs' (cerebellar ataxia, basal ganglia and brainstem dysfunction). Fluctuation of psychiatric symptoms is common. However, a volatile course of both, cognitive impairment and neurologic symptomatology, has not been described yet.
Objective/results: We are reporting a case of 28-year-old female patient with biochemically variant form of disease. After period of fluctuating depression dysexecutive syndrome, mnestic failure, cerebellar ataxia and dysarthria slowly proceeded. Therapeutic attempt with anticholinergic drugs was unsuccessful and confusion with hypobulia and apathy developped. Application of tiapride led to transient coma, rigidity, hypertermia, leucocytosis – symptoms typical for neuroleptic malignant syndrome, a potentionally fatal complication of treatment by neuroleptics, pathogenetically not exactly clarified. No specific acute neurologic disease was confirmed. After recovery of consciousness neurologic status of patient involved severe spastic quadruparesis, dystonia and dysarthria. Confusion and mnestic troubles disappeared completely. 13 months later, the patient is able to walk with minimal help and to comunicate properly. Psychologic evaluation has confirmed no cognitive deterioration in the last 2 years. Surprisingly, the same genotype has been described in another patient with dominating neurologic symptoms.
Conclusion: The clinical course in adult form NPC may fluctuate more widely than it has been presumed. The fact should be taken into account while making a decision on the therapeutic approach by SRT in NPC patients.

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445-P

KRABBE DISEASE: MUTATIONS SPECTRUM IN RUSSIAN PATIENTS

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Krabbe disease is an autosomal recessive sphingolipidosis caused by deficient activity of the lysosomal galactocerebrosidase (GALC). Nearly 70 mutations, including polymorphisms, have been identified in *GALC* gene. The 30-kb deletion accounts for approximately 45% of mutant alleles in the population with European ancestry.

13 Russian patients with Krabbe disease were diagnosed in our department (8 infantile, 6 late infantile clinical form). DNA studies were carried out by automated DNA sequencing of all the 17 exons and the exon-intron boundaries of the *GALC* gene. For detection of common deletion we used standard PCR assay. Only two of previously described mutation were identified (T513M and 30-kb deletion). Common deletion accounts for 27% of all mutation alleles. Most of the mutations found were novel: c1836delA, Y185X, E182K, c1989.1993delTCTT, c246insT, IVS2-2A>G, IVS5+1A>G and I177T. Interestingly, a new mutation, resulting in I>T substitution at amino acid position 177, is found in 23% alleles in Russian patients. High frequency of this mutation may be explained by founder effect.

446-P

α-MANNOSIDOSIS PRESENTING WITH TWO DIFFERENT CLINICAL PHENOTYPES

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Background: We present two non-consanguineous α-mannosidosis patients with different clinical phenotypes: juvenile form manifested with a widespread storage symptoms but without hearing loss, while profound mental retardation, severe hearing loss, psychiatric symptoms and mild storage signs were found in adult variant. Both cases were confirmed by α-mannosidase activity measurement, molecular genetic testing of *MAN2B1* gene is in progress. The first patient was a 6-year-old female of Lithuanian origin. Congenital bilateral hip dislocation was noticed at birth. Frequent respiratory and intestinal infections, permanent nasopharyngeal secretions were noted from very early in infancy. Spinal deformation was noticed at the age of 7 months, lumbar hyperlordosis, bilateral genu valgus and arthritic symptoms in both knees developed later. The other symptoms were psychomotor retardation, coarse facial features, macroglossia, hepatomegaly, macrocephaly, umbilical hernia, mild mitral valve insufficiency, muscular hypotony, severe caries, myopia and strabismus divergens. The second patient was a 25-year-old female of Belorussian/Lithuanian origin. Frequent respiratory infections started from 7 months of age and diminished in frequency at the age of 18 years. At the age of 1.5 years severe hearing loss was noticed. Skeletal symptoms included gibbous, lumbar hyperlordosis and arthritic symptoms in both knees and spine. Other symptoms were profound mental retardation, muscular hypotony, mild facial coarseness, severe caries and umbilical hernia. At the age of 23 the patient developed acute psychiatric symptoms: fobias, delusions, frequent crying and disorganized behaviour, undue attachment to mother. Regress in acquired skills developed afterwards.
Conclusion: This rare disease can present with very different clinical phenotypes.

447-P

A NEW CLINICAL PRESENTATION OF α-N-ACETYLGALACTOSAMINIDASE DEFICIENCY WITH CHILD ONSET REVEALED BY VISCERAL INVOLVEMENT AND ANGIOKERATOMA CORPORIS DIFFUSUM

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Background: Alpha-N-acetylgalactosaminidase (α-GalNac) deficiency is a rare lysosomal storage disorder with atypical features. Type I is an infantile-onset neuroaxonal dystrophy; type II, also known as Kanzaki disease, is an adult-onset disorder characterized by angiokeratoma corporis diffusum and mild intellectual impairment; and type III is an intermediate disorder with mild to moderate neurologic manifestations.
Case Reports: We describe two sibs with α-GalNac deficiency presenting with neonatal hepatosplenomegaly and angiokeratoma corporis diffusum. The boy presented with neonatal hepatomegaly and high liver enzyme levels attributed to auto-immune hepatitis. Liver biopsy was not specific. He was treated for ten years with steroids and his liver tests normalized. He presented at 9 a perception deafness and a vertigo spells. At 19, examination revealed a persistent hepatosplenomegaly, and angiokeratomas. Nerve conduction studies revealed a mild motor and sensitive polyneuropathy. Analysis of urinary excretion revealed an abnormal oligosaccharide profile and accumulation of O-linked glycopeptides. α-GalNac activity was found null in leucocytes.

His sister, presented at four months with hepatosplenomegaly and diffuse telangiectasia. No sign of hepatitis were found. A systematic ophthalmic examination at 15 years revealed a vaso-occlusive retinitis with signs of vasculitis. At 17, clinical examination revealed an hepatosplenomegaly, diffuse angiokeratoma and a sensimotor polyneuropathy.

Both patients were homozygous for the novel mutation c.759+1.759+8del in intron 6 while the parents were heterozygous. The description of these 2 cases broadens the clinical spectrum of α-GalNac deficiency and indicates that Kanzaki disease should be considered in the differential diagnosis of hepatosplenomegaly with angiokeratoma or telangiectasia in infants.

448-P

SEVERE HYPOMYELINATION AS THE LEADING NEURORADIOLOGICAL SIGN IN A PATIENT WITH ENZYMATICALLY PROVEN FUCOSIDOSIS AND TWO NOVEL MUTATIONS IN THE *FUCA1* GENE

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Fucosidosis is a rare autosomal recessive lysosomal storage disease, resulting from a deficiency of alpha-L-fucosidase. The main clinical findings are progressive neurologic deterioration, coarse facial features, growth retardation, recurrent infections, dysostosis multiplex, angiokeratomata, visceromegaly, and seizures.

We report clinical and MRI findings of a 36-month-old girl with this disorder in whom a developmental delay became obvious between 6 and 12 months. Cranial MRI at the age of 16 months revealed severe global hypomyelination, without any basal ganglia involvement or atrophy, or any other accompanying clinical signs typical for this disease. Since the age of 2 years progressive neurologic deterioration occurred. Diagnosis was established by severely decreased activity of alpha-L-fucosidase in plasma and leukocytes and was confirmed by the detection of compound heterozygosity for two novel missense mutations in the *FUCA1* gene (c.1019G>A [p.G340E] and c.1384T>A [p.X462KextX79]).

MRI findings in previously described patients showed alterations in periventricular, lobar, subcortical, and cerebellar white matter including the deep layers, abnormalities within the basal ganglia, in particular globus pallidus and cerebral and cerebellar atrophy. In this patient hypomyelination was the leading neuroradiologic sign.
Conclusion: Diagnostic investigations for lysosomal diseases should be considered early in patients with a severe hypomyelination disorder since early diagnosis may have implications for genetic counselling, and, even if still experimental, for therapeutic approaches.

449-P

CLINICAL AND MOLECULAR FINDINGS OF SIALIDOSIS PATIENTS WITH DIFFERENT PHENOTYPES

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Background: Sialidosis is a lysosomal storage disease caused by the deficiency of alpha-N-acetyl neuraminidase (NEU1), that cleaves terminal sialic acid linkages of glyconjugates. Sialidosis is classified into two main clinical variants: type I, the milder form of the disease, and type II, which can be subdivided into three forms: congenital, infantile and juvenile.
Methods: Here we report the genetic characterization of three sialidosis patients with different phenotype and a clinical review of the previously described patients affected by the most severe type II forms.
Results: Molecular analysis showed two new [c.530A>T (p.D177V), c.1010A>G (p.H337R)] and one known [c. 679G>A (p.G227R)] *NEU1* missense mutations, and the new c.807+1G>A splicing defect, a genetic type of lesion that is extremely rare in this disease. The splicing defect gives rise to at least one aberrant mRNA transcript containing the genetic sequence of intron 4. This insertion results in a premature stop codon UGA starting at position c.807+13. The new missense mutations were identified in patient 1, affected by type I sialidosis. Patients 2 and 3 showed type II phenotypes, infantile/juvenile and congenital forms respectively.
Conclusions: We would like to underline the importance of improving understanding of this rare disorder for a correct diagnosis and genetic counseling and, when available, for enzymatic treatment.

450-P

SCLEROSING CHOLANGITIS IS A HEPATIC MANIFESTATION OF LONG-TERM INFANTILE NEPHROPATHIC CYSTINOSIS

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Cystinosis is a metabolic disease characterized by the accumulation of cystine in different organs and tissues, leading to potentially life-threatening organ dysfunction. Infantile cystinosis for example typically leads to end-stage renal disease, necessitating renal replacement therapy. Liver disease in cystinosis is rare and presents mainly as nodular regenerative hyperplasia leading to portal hypertension. We report here on three patients with infantile cystinosis who developed progressive cholestatic liver disease (increasing alkaline phosphatase, gamma-GT and mild increase in transaminases). Two patients showed changes compatible with sclerosing cholangitis on magnetic resonance imaging of the biliary tree. In all three patients, severe accumulation of cystine was demonstrated on liver biopsy, predominantly localized in Kupffer cells. Two patients also showed morphological signs of sclerosing cholangitis. An extensive literature search yielded no reports of sclerosing cholangitis associated with cystinosis. In all three patients therapy with ursodeoxycholic acid (UDCA) was initiated and led to biochemical improvement (2/3) or stabilisation (1/3).

451-P

MOLECULAR AND CLINICAL CHARACTERIZATION OF MULTIPLE SULFATASE DEFICIENCY CAUSING MUTATIONS IN THE FORMYLGLYCINE-GENERATING ENZYME

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Multiple sulfatase deficiency (MSD) is an inborn autosomal-recessive disorder, which combines clinical and biochemical features of lysosomal storage disorders like metachromatic leukodystrophy, different mucopolysaccharidosis and X-linked ichthyosis. The clinical course ranges from neonatal severe to mild juvenile cases. MSD is caused by mutations in the *SUMF1* gene encoding the formylglycine-generating enzyme (FGE). FGE posttranslationally activates sulfatases by generating formylglycine in their catalytic sites.

We analysed the functional consequences of four FGE missense mutations (A177P, W179S, A279V, R349W) on subcellular localization, residual enzymatic activity, protein stability and resulting sulfatase activities and referred these data to the clinical phenotype of patients carrying the chosen mutations.

All four mutations did not affect localization of FGE in the endoplasmic reticulum of MSD fibroblasts. They decreased its specific enzymatic activity to less than 1% (FGE-A177P and -R349W), 3% (W179S) or 23% (A279V). Protein stability was severely decreased for FGE-A279V and R349W and comparable to wildtype for FGE-A177P and -W179S. The mildest affected patient carries the mutation A279V leading to decreased FGE protein stability, but high residual enzymatic activity and slightly reduced sulfatase activities. In contrast, the most severely affected patient carries the mutation R349W leading to decreased protein stability, very low residual enzymatic activity and significantly reduced sulfatase activities.

This functional study reveal that both residual enzyme activity and protein stability of FGE contribute to the clinical phenotype and provide novel insight into the molecular defect underlying MSD.

452-P

INCIDENCE OF THE 24-bp DUPLICATION IN THE CHITOTRIOSIDASE GENE IN TURKISH POPULATION

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Objective: Chitotriosidase is a human chitinase produced by activated macrophages. Chitotriosidase enzymatic activity is markedly elevated in serum patients suffering from lysosomal storage disorders as well as other diseases in which macrophage activity predominate. Therefore activity pattern of the enzyme may be used as a surrogate marker in order to diagnosing and monitoring the efficacy of therapeutic intervention of these diseases.

This enzyme is encoded by a gene located on chromosome 1q31–32, which is divided into 12 exons. Chitotriosidase deficiency is caused by a duplication of 24 bp in exon10. Homozygosity (0–30%) and heterozygosity (0–58%) prevalences of this mutation are reported at diverse rates in different populations. Consequently, in order to use chitotriosidase activity as a surrogate biomarker, allele frequency determination for that population is regarded as a prerequisite. The aim of this study was to determine the frequency of the 24-bp duplication in the chitotriosidase gene in Turkish population.
Methods: DNA samples were obtained from randomly chosen 900 subjects using a salting out method. Region of interest was amplified by standart PCR followed by electrophoresis analysis.
Results and **Conclusion**: Obtained results displayed a heterozygosity frequency of the duplication of 24 base pairs in exon 10 as 36%, whereas corresponding value for homozygote chitotriosidase deficiency was 8%. High incidence of the 24 bp dublication in Turkish population is demonstrated.

453-P

ENZYME REPLACEMENT THERAPY FOR MUCOPOLYSACCHARIDOSES: OPINIONS OF PATIENTS AND FAMILIES

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Background: We have conducted a study in order to assess the opinions of individuals and parents of individuals with mucopolysaccharidoses (MPS) regarding the use of enzyme replacement therapy (ERT).
Methods: A questionnaire, including a number of hypothetical clinical scenarios about ERT for MPS, was distributed to members of MPS support groups from USA and Australia.
Results: The questionnaire was returned by 249 MPS support group members. Ninety two percent of respondents were in favour of ERT where MPS caused severe physical problems but did not affect intellect. Sixty nine percent were in favour of ERT where the physical limitations were mild and intellect was spared. Only 47% were in favour of ERT where severe physical and intellectual problems were well established, however 77% were in favour of ERT in this situation if begun early in an effort to prolong life and improve quality of life. Ninety three percent were in favour of ERT where there were mild intellectual and physical symptoms, with ERT improving the physical functional capabilities.
Conclusion: The majority of respondents were in favor of ERT for MPS, even where it would not alter the intellectual deterioration. The medical community has a responsibility to advocate for their patients in situations where ERT is appropriate, and recognize the economic burden and 'family function burden' ERT can incur.

454-P

INTRATHECAL ENZYME REPLACEMENT IN MPS1

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A girl with Hurler's disease, diagnosed at the age of two years has been treated with weekly intravenous ERT since the age of 4 years. Although already having central nervous system involvement with psychomotor delay, she was still an active child with good communication skills. Echocardiography showed 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. After two years of ERT treatment she developed episodic hemiplegia with certain headmovements. An MRI of the posterior fossa revealed compression of medulla and cervical cord due to meningeal thickening.

Methods: Using a protocol proposed by P. Dickson (LA, USA) monthly intrathecal enzyme (2 cc AldurazymeR with 4 cc Elliot-B) solution was started at the age of 6 and half years.

Results: No side effects were noted during or after intrathecal injections which are still monthly continued over two years now. CSF protein levels decreased but did not normalize. GAGS in CSF were not found. Clinically the child stabilized and had no further episodes of aggravation with hemiplegia. An MRI of the brain resulted in a mild decrease of meningeal thickening.

Conclusion: Altough the results are not spectacular, intrathecal ERT is safe, and can be useful in early signs of brainstem or cervical cord compression. Early treatment intrathecally in young children with Hurler, might be an option in the time period before and shortly after a bonemarrow transplantation.

455-P

EXPERIENCE IN TREATING SYMPTOMATIC SPINAL CORD COMPRESSION WITH INTRATHECAL ENZYME THERAPY IN THREE BRAZILIAN PATIENTS WITH MPS

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Background: In MPS, deficiency of specific enzymes can cause spinal cord compression due to storage of glycosaminoglycans within the cervical meninges. In 2005, we used intrathecal infusions of recombinant human α-L-iduronidase to treat a MPS I adult patient with spinal cord compression (P1) and recently we conducted the use of IT-ERT in pediatric patients with MPS I (P2) and MPS VI (P3). To our knowledge, these were the first MPS I adult, the first MPS I child and the first MPS VI patient who received IT-ERT.

Methods: The patients underwent a series of 4 monthly courses of IT-ERT (specific enzyme dilluted on Elliotts B solution). Patient P1 performed follow-up evaluations after 12 and 18 months, including 12 MWT, pulmonary function exams, imaging studies and complete neurological examination. P2 and P3 performed the same evaluations as P1 on baseline and on the immediate follow-up, but were not able to perform reliable pulmonary function tests nor 12 MWT.

Results: Neurological symptoms improved on all cases. On patient P1, after an initial improvement, it was noticed on the 12-month evaluation an evidence of neurological worsening; after 18 months this worsening was evident also on pulmonary function tests, especially on pulmonary diffusion.

Conclusions: This procedure seems to be a safe treatment for spinal cord compression in MPS. We speculate that, after and initial set of monthly infusions, a protocol with longer intervals between infusions (2 to 4 times a year) could be enough to maintain the clinical benefits.

456-P

ULTRASTRUCTURAL ANALYSIS OF DERMAL FIBROBLASTS IN PATIENTS WITH MUCOPOLYSACCHARIDOSIS TYPE I (MPS I): EFFECTS OF ERT AND HCT

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Background: In MPS I glycosaminoglycans (GAGs) accumulate in tissues. Delivery of functional enzyme, either by haematopoietic cell transplantation (HCT) or by enzyme replacement therapy (ERT), aims to prevent and reverse this accumulation. We studied clearance of GAGs from fibroblasts by scoring repetitive skin biopsies during treatment with ERT or ERT in combination with HCT.

Methods: Twelve patients were included; four of them received HCT after ERT for a minimum period of three months. Skin biopsies were obtained before and every six months during the first two years of ERT and six months after successful HCT. A scoring system in which 10 randomly chosen fibroblasts were studied (EM) was used in a blinded fashion. Score ranged from 0 to 3 for each fibroblast, based on the number of vacuoles.

Results: Overall, vacuolization score significantly decreased in patients on ERT (Friedman; chi-square = 11, $p = 0.012$). After 6 months of ERT a median decline of 64% (31–90%) was observed. However, response rate varied among patients, since after 1 year of ERT the score was substantially reduced in most patients but remained high in 3 patients. Patients who were successfully transplanted ($n = 3$) showed low scores thereafter.

Conclusions: Enzyme delivery by ERT or by ERT plus HCT resulted in reduction of abnormal fibroblast vacuolization. This correlates well with the clinical observation of improvement in skin suppleness. However, the rate of decline varies among patients. The scoring system might be useful as surrogate marker to determine the efficacy of ERT on connective tissue pathology.

457-P

EXPERIENCE WITH LARONIDASE IN A BONE MARROW-TRANSPLANTED PATIENT WITH SEVERE PULMONARY DISEASE

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Background: Mucopolysaccharidosis type I or Hurler's disease (MPS I-H) is a severe multi-organ lysosomal disease, which untreated is rapidly fatal. Long-term survival has occurred in children with MPS I-H after successful bone marrow transplantation (BMT). Laronidase, a recombinant human alpha-L-iduronidase, safely and effectively alleviates many systemic manifestations of this disease. **Case Report:** We describe a 14-year-old MPS I-Hurler patient that underwent BMT twice, with his heterozygous twin. His clinical course was initially similar to other MPS I-BMT patients, but he developed 5 years ago, a progressive respiratory failure with life-threatening pulmonary hypertension. His pulmonary disease was multifactorial: bone disease responsible for thoracic and spinal deformities; infiltration of upper airways and sleep hypoventilation; interstitial infiltration of lungs with storage material in alveoli. Clinically he was polypneic, fatigable and wheelchair-bound. **Methods** and **Results**: We treated this patient for 18 months so far, with continuous oxygen therapy for pulmonary hypertension, nocturnal non-invasive ventilation and enzyme replacement therapy. The patient's clinical condition improved dramatically. He can now stand and walk alone and climb stairs without significant fatigue or dyspnea. Pulmonary hypertension improved and oxygen therapy during daytime was discontinued. His thoracic CT-scan improved and an upper airways and tracheal endoscopy showed decreased obstruction. Urinary glycosaminoglycans decreased over 50%. **Conclusion**: Enzyme replacement therapy may be an interesting option for treating MPS I BMT patients who develop severe respiratory complications. Further studies should be done to assess the contribution of ERT in other visceral symptoms occurring in these patients.

458-P

STEM CELL TRANSPLANTATION FOR LYSOSOMAL STORAGE DISEASES: THE GHENT EXPERIENCE

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Eight patients followed at our centre for a lysosomal storage disease received a hematopoetic stem cell transplantation (four Hurler, three MLD and one Krabbe patient, age at transplantation 8 months to 20 years). Origin of the stem cells was in two cases unrelated cord blood, in three cases bone marrow from a matched unrelated donor (MUD), in two cases peripheral blood from a MUD and in one case bone marrow from a sibling.

Both patients transplanted with unrelated cord blood had graft failure (one Hurler, and one infantile MLD).

Three other transplanted Hurler patients (two transplanted with bone marrow from a MUD, and one with peripheral blood stem cells from a MUD) had initially normal alfa-L-iduronidase activity, but all evolved to a mixed chimerism with suboptimal alfa-L-iduronidase activity, combined with peripheral disease progression. However, evolution of central nervous pathology is more favorable. Probably remaining alfa-L-iduronidase activity is high enough to protect the brain from further damage and therefore these transplanted patients have a disease progression comparable to Scheie patients. One of these patients is currently under enzyme replacement therapy.

Two juvenile MLD patients and one juvenile Krabbe patient have a favorable evolution, with normalization of the enzyme levels. These patients have in common that they were transplanted at an older age.

In our small series, patients who were transplanted for slower progressing diseases had better engraftment.

459-P

STEM CELL TRANSPLANTATION IN MPS1H. LONG-TERM RESULTS OF A FLUDARABINE BASED REGIMEN

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Background: Hurler syndrome (MPS1H) is a lysosomal storage disease caused by a deficiency of alpha-L-iduronidase activity. Hematopoietic stem cell transplantation (SCT) is regarded the best treatment option, but follow-up investigations of somatic features after SCT are scarce.
Methods: Follow-up data on five Hurler patients transplanted at our institution between 2001 and 2003 using a fludarabine based, radiation free conditioning regimen are presented. After SCT, the children were seen on a regular basis.
Results: All children engrafted and are in ambulatory care. Median age at SCT was 28 months (range 10 to 36 months), median age at last follow-up was 86 months (range 56–101 months). Chimerism analysis showed 95–99% donor chimerism. Graft versus host disease is absent in all patients, the alpha iduronidase activity is normal (n = 3) or low-normal (n = 2). Prior to SCT, all children showed abnormalities in echocardiography that were slightly progressive in all 5 children. Some features however, e.g. cardiac thickening, were regressive. The ophtalmologic follow-up revealed corneal clouding in all five children with fair to good visual functions. Skoliosis was present in 4/5 children before SCT and showed progression all children. Three children underwent surgery for 'carpal tunnel syndrome' and a percutaneous trigger finger release. A varus osteotomy was performed in one patient. In general, pre-existing orthopaedic abnormalities showed aggravation during follow-up in all children.
Conclusions: Despite stable engraftment and lack of SCT associated complications children with Hurler syndrome after SCT deserve special attention and need a multi-disciplinary approach during the long-term follow-up.

460-P

DEVELOPMENTAL OUTCOME IN FIVE CHILDREN WITH M. HURLER AFTER STEM CELL TRANSPLANTATION WITH FLUDARABINE BASED PREPARATIVE REGIMEN

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Introduction: Hurler syndrome (MPS1H) is a lysosomal storage disease caused by the deficiency of alpha-L-iduronidase. The natural course of this disease is neurodegenerative and leads to premature death within the first 10 years of life. Enzyme replacement therapy is effective in controlling extracerebral symptoms only. Hematopoietic stem cell transplantation (SCT) is the only treatment known to prevent psychomotor/neurological deterioration. However, the classical transplantation protocols resulted in a high incidence of graft failure and regimen-related toxicity. Recently, we published a well tolerated, fludarabine based, radiation free conditioning regimen for SCT in Hurler patients.
Methods: We here report the developmental outcome of 5 children (4 females) with MPS1H (mean age at last follow-up 71 months, range 42–87 months) treated according to this strategy. Mean age at SCT was 25 months (range 10–36 months).
Results: All children engrafted and are in ambulatory care. They all show psychomotor development without neurodegeneration. In all patients, a regression of intracranial lesions after SCT could be seen which paralleled the psychomotor improvements. SCT led to a relative reduction of head circumference in all cases. After SCT neither an increase of narrowing at the craniocervical junction nor development of myelopathological lesions could be detected.
Conclusion: Our SCT regimen is a safe and effective strategy for children with MPS1H resulting in an improvement of psychomotor development, relative reduction of head circumference and regression of intracranial lesions – even in children transplanted after the age of 2 years.

461-P

EXPERIENCE WITH IDURSULFASE IN 4 UNDER FIVE MPS II PATIENTS

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Background: Mucopolysaccharidosis II (MPS II or Hunter syndrome) is an X-linked metabolic disorder caused by a deficiency of the lysosomal enzyme iduronate-2-sulfatase (I2S), which catalyzes the catabolism of glycosaminoglycans (GAG). Recently, enzyme replacement therapy (ERT) with recombinant human I2S (idursulfase (Elaprase)) has been approved for the treatment and management of MPS II. Patients and methods: 4 MPS II patients aged 18, 22, 43 and 57 months were started on ERT with idursulfase weekly at 0.5 mg/kg through a Port-A-Cath. They all presented restricted range motion, hepatosplenomegaly and upper airways infiltration. The 3 older patients presented mild psychomotor retardation whereas the youngest patient had a normal intellectual development.
Results: At this stage of treatment (24 weeks) 3 patients presented several mild (rash, fever, ...) or rarely more severe (hypertension, hypotension) adverse effects. However, infusions have been continued in all of them. Significant reduction in liver and spleen volume has been observed in all patients. Joint range motion and performances in the 3-minute stair-climbing test significantly improved in the 2 older patients. Urinary GAG decreased rapidly and significantly but remained above normal range pour age. Overall quality of life scores (CHAQ questionnaire) improved.
Conclusion: These preliminary data show efficacy of idursulfase in patients under five that is comparable to older patients. However urinary GAG reduction was less spectacular and adverse effects quite frequent during the 3 first months of treatment. Further follow-up is needed for assessment of ERT in these young patients including in more severe organ involvement.

462-P

LONG-TERM WEEKLY DOSING OF IDURSULFASE IN THE TREATMENT OF MUCOPOLYSACCHARIDOSIS II (MPS II, HUNTER SYNDROME)

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Background: MPS II is an X-linked lysosomal storage disorder caused by a deficiency in iduronate-2-sulfatase. A recent 1-year, double-blind, placebo-controlled clinical trial of enzyme replacement therapy with idursulfase (Elaprase., Shire HGT, Cambridge, MA, US) showed that both weekly and every other week (EOW) dosing of idursulfase (0.5 mg/kg) significantly improved the primary endpoint (a composite comprising sum of the ranks of changes in percent predicted forced vital capacity (%FVC) and distance walked in 6 min (6MWT) compared to placebo, with the magnitude of the clinical benefit being larger in the weekly compared with the EOW group ($p = 0.13$). **Methods**: This trial has been continued as an open-label extension study designed to evaluate the long-term safety and efficacy of weekly dosing of idursulfase (0.5 mg/kg). **Results**: All patients who completed the double-blind study ($n = 94$) enrolled in the extension study and were treated with idursulfase at 0.5 mg/kg weekly. Changes in %FVC and 6MWT, as well as other assessments, continued to be monitored in the open-label extension study. Safety was assessed continuously during the study by monitoring treatment emergent adverse events and by periodic determination of anti-idursulfase antibodies in blood samples. **Conclusions**: Subsets of the 1-year efficacy and safety results will be presented.

463-P

GENE EXPRESSION-TARGETED ISOFLAVONE THERAPY (GET IT) FOR MUCOPOLYSACCHARIDOSIS TYPE III (SANFILIPPO DISEASE): A PILOT CLINICAL TRIAL

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Background: Enzyme replacement therapy, though effective in treatment of somatic symptoms of some mucopolysaccharidoses (MPS type I, II and VI), leaves neurological problems unmanageable, due to inefficient delivery of enzymes to CNS through the blood–brain barrier (BBB). We found, that genistein (4′,5,7-trihydroxyisoflavone) – that can cross BBB – considerably inhibits synthesis of glycosaminoglycans (GAGs) in cultures of fibroblasts of MPS patients. Therefore a substrate deprivation therapy named 'gene expression-targeted isoflavone therapy' (GET IT) was proposed for mucopolysaccharidoses. Here we describe a pilot clinical trial with patients suffering from Sanfilippo disease (MPS III), as in this MPS type neurological symptoms are main clinical problems. **Methods**: 10 patients suffering from MPS IIIA and 7 MPS IIIB patients were involved in an open-label pilot clinical trial. A genistein-rich soy isoflavone extract was administered orally at the dose corresponding to 5 mg genistein per 1 kg of body weight daily. **Results**: After 12 months of the treatment we have observed (relative to baseline): 1) a decrease in urinary heparan sulfate level, 2) an improvement in hair morphology, 3) an improvement of cognitive functions. **Conclusions:** Results of our pilot clinical trial indicate an improvement in all tested parameters. The enhancement of cognitive functions may be of special importance. Although more detailed studies are necessary to assess efficacy of this treatment more precisely, we may speculate that GET IT might be used not only in MPS III, but also in other MPS types, especially when neurological symptoms occur.

464-P

SIX YEARS EXPERIENCE OF ENZYME REPLACEMENT THERAPY (ERT) WITH RECOMBINANT HUMAN ARYLSULFATASE B (RHASB) IN AN 18 YEAR OLD MALE WITH MPS VI (MAROTEAUX-LAMY)

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Mucopolysaccharidosis (MPS) type VI is a rare lysosomal storage disorder with multiorgan involvement. We report on a male patient who was participating in the phase I/II and III/IV rhASB enzyme replacement study of Biomarin. Our data on flexible endoscopy and MRI imaging on the craniocervical region are presented in context with data on GAG levels, 6-min walk test, and pulmonary function tests, well documented in the study reports.

The patient suffered from chronic upper airway obstruction requiring tracheostomy at the age of 10.7 years. Flexible endoscopy showed thickening of pharyngeal, laryngeal and tracheal mucosa. At the age of 11.9 years the patient entered a phase I/II enzyme replacement trial with weekly rhASB infusions. At study week 240 he showed a 67% reduction of urinary GAGs, improvement (+29%) in the 6-min walking test, improvement in FVC (+53.7%) and FEV1 (+23.4%). Flexible endoscopy, performed as part of the routine patient care, showed a modest reduction of mucosal thickening in the trachea after 5 years on ERT, compared to the pre-treatment investigation. At the age of 16.5 years deep tendon reflexes in the lower limbs became brisk. An MRI of the craniocervical region revealed narrowing of the intervertebral space due to dysostotic changes and incipient compression of the spinal cord.

These findings stress the importance of early initiation of ERT in order to prevent excessive mucosal storage in the upper airway. The infiltration of bones and meninges leading to compression of the spinal cord seems to be less influenced by ERT.

465-O

PHASE 3 EXTENSION 96-WEEK STUDY DATA FOR NAGLAZYME (GALSULFASE) ENZYME REPLACEMENT THERAPY (ERT) IN MPS VI (MAROTEAUX-LAMY SYNDROME) PATIENTS

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Background: MPS VI is a rare, fatal lysosomal storage disease. ERT with recombinant human arylsulfatase B (rhASB) has shown positive results in clinical studies. This study reports the findings of the phase 3 open-label extension study. **Methods**: Efficacy and safety are reported through 96 weeks for the phase 3, 24-weeks' study followed by 72-weeks' open-label extension study. Endpoints included 12-min-walk test (12MWT), 3-min-stair-climb (3MSC), level of urinary glycosaminoglycans (GAGs) and pulmonary function. **Results**: Patients receiving rhASB ($n = 19$) improved by a mean of 183 m ± 26 m (SE) from baseline to week 96 in the 12MWT ($p < 0.0001$). The placebo group ($n = 18$), which was switched to active drug at week 24, improved by a mean of 117 m ± 25 m (SE) from week 24 to week 96 ($p < 0.001$). Similar improvements in the rate of stairs climbed (3MSC) were also observed ($p < 0.001$). Level of urinary GAGs remained reduced after starting rhASB. Forced vital capacity improved in the rhASB-treated group by 0.11 L/min ± 0.05 L/min (mean, SE) from baseline to week 96 ($p = 0.039$), and in the placebo group by 0.07 L/min ± 0.02 L/min (mean, SE) from week 24 to week 96 ($p < 0.001$). Forty-five percent of patients developed a neutralizing antibody response and 55% developed persistent IgE response. **Conclusions**: These data support continued improvement in endurance, pulmonary function, and urinary GAGs over 96 weeks. rhASB antibodies were not associated with increased incidence of IARs or lack of clinical benefit.

466-P

OPHTHALMOLOGIC EVALUATION OF MPS VI PATIENTS FOLLOWING TREATMENT WITH GALSULFASE ENZYME REPLACEMENT THERAPY

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Background: Ophthalmologic abnormalities develop in mild to severe forms of MPS VI, beginning with corneal clouding as one of the first reported symptoms. A novel intravenous enzyme replacement therapy (ERT) provides recombinant arylsulfatase B (galsulfase) to enzyme-deficient patients. This study reports ophthalmologic stability in patients monitored after commencing treatment in clinical trials and during an extension study lasting 144 weeks.
Methods: We describe 12 patients, 7 males and 5 females, aged between 4 and 18 years. Eleven of those patients had severe form of MPS VI and 7 of these were involved in clinical studies for ERT. Patients involved in the study received weekly doses of 1mg/kg recombinant arylsulfatase B (galsulfase) enzyme replacement therapy. Ophthalmologic evaluations were performed at baseline, 24, 48, 96 and 144 weeks. Evaluations included visual acuity, slit-lamp, fundoscopic, intraocular pressure, refractive retinoscopy and subjective evaluations. Four younger MPS VI patients from other institutions, who were not on study drug were evaluated for comparison and complete ophthalmologic evaluations were conducted.
Results: Visual acuity was found to decrease with age; the main cause of decreased visual acuity was optic atrophy. Four of the 7 patients involved in the study had hyperopia. During the 144 weeks of the study, visual acuity and intra-ocular pressure remained stable during ERT.
Conclusions: Results suggest the importance of early diagnosis and early ERT in order to stabilize subsequent vision loss. Further studies will reveal more details about the salutary effects of enzyme replacement therapy on preservation of visual acuity in MPS VI patients.

467-P

SAP-B DEFICIENCY IN CHILDREN WITH LEUKODYSTROPHY: CLINICAL PROFILE AND REPORT OF CORD BLOOD TRANSPLANTATION

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Lysosomal degradation of sphingolipids requires sphingolipid activator proteins (SAP) as cofactors. The SAP precursor, coded by *PSAP* gene, is processed to SAP-A, SAP-B, SAP-C, and SAP-D. The hydrolysis of sulfatides by arylsulfatase-A (ASA) is stimulated by SAP-B. Mutated PSAP resulting in SAP-B deficiency (SAPBD) is known to cause metachromatic leukodystrophy (MLD) variant in which ASA is normal. Of 63 leukodystrophy cases that were evaluated in our centre in three years, 4 (6%) patients were diagnosed to have ASA-deficient MLD. During the same period, 9 (14%) children from 4 unrelated Saudi families were found to have SAPBD. The presentation resembled MLD in seven patients who had normal ASA level. PSAP analysis found that the four families segregate the same homozygous mutation which was a g.722G > C transversion resulting in C241S change. This mutation was previously reported in an Arab patient with SAPBD[1]. Screening the patients' siblings identified two asymptomatic young infants who were homozygous for the mutation. One of them underwent unrelated cord blood transplantation (CBT). She had biochemical response with normalization of urine analysis for sulfatides, however, she died of transplantation complications.

In this work, we report the largest cohort of SAPBD which was found to be the most common known cause of leukodystrophy in the cases we evaluated. We also describe the first CBT in an asymptomatic SAPBD. This work draws the attention to an under-diagnosed cause of neuroregression and reports a potential role for CBT in SAPBD.

[1]Holtschmidt et al. J Biol Chem. 1991;266:7556–60.

468-P

EFFECTIVITY OF ENZYME REPLACEMENT THERAPY IN FABRY DISEASE. STUDIES AT THE CELLULAR LEVEL

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Background: The preliminary studies in Fabry fibroblast cultures showed effective uptake of tested recombinant agalsidases (Fabrazyme, Genzyme and Replagal, TKT) leading to efficient hydrolysis of the critical substrate Gb3Cer directly in lysosomes. We, therefore, used an opportunity to test effectivity of ERT in biopsy samples from the heart and some other tissues of two adult male Fabry patients who received continuous ERT (Fabrazyme) for 29 and 39 months. An autopsy case of Fabry disease in which ERT had been terminated one year before death was included into the study.
Methods: Lipid immuno/histochemistry and electron microscopy for evaluation of the storage process. Immuno/histochemistry for evaluation of the applied enzyme targeting.
Results: In both treated patients endothelial cells were completely cleared from storage. Endothelial storage was resumed one year after ERT withdrawal. Persistent Gb3Cer storage was found in cardiocytes and other cell types even under the continuous ERT regime. In cardiocytes, there was also parallel increase in lipopigment. The applied enzyme was present in detectable quantities and in active state in the storage cells (tested in atrial cardiocytes).
Conclusions: Presence of active applied enzyme in storage cells opens a question if there is, besides other possibilities, an altered connection of the storage lysosomal compartment with the endocytotic pathway. We suggest that the future studies should focus on defining the differences between the storage cell, storage compartment and their wild type counterparts. This may contribute to improvement of effectivity of the ERT.

469-P

A NOVEL APPROACH OF CELL THERAPY FOR METACHROMATIC LEUKODYSTROPHY USING HEMATOPOIETIC STEM CELLS OVER-EXPRESSING HOXB4

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Background: Metachromatic leukodystrophy (MLD) is a lysosomal storage disorder characterized by accumulation of sulfatide in oligodendrocytes followed by widespread demyelination. Although various experimental therapeutic strategies have been proposed, allogenic bone marrow transplantation (BMT) is the only choice for treatment of MLD to delay disease progression at this moment. To improve BMT based therapy, we examined the utility of HOXB4, which is a member of the homeobox family and is thought to play a key role in development of both hematopoietic stem cells (HSC) and oligodendrocytes.
Methods: MLD model mice were treated by BMT using HSC from GFP transgenic mice transduced with retrovirus vector expressing either HOXB4 or control GFP and evaluated by histological examination and the behavior test.
Results: Marked enhancement in HSC expansion and engraftment was observed when transduced with HOXB4 vector. The number of GFP positive donor cells migrated in the brain was also significantly increased by using HOXB4 transduced cells. Immunohistochemical staining showed that a portion of BM derived donor cells differentiated into oligodendrocytes. Alcian blue staining demonstrated that accumulation of sulfatide was inhibited in the brain of treated mice. Improvement of gait disturbance was obsereved by the balanced beam behavior test at 9 months after transplantation.
Conclusions: HSC over-expressing HOXB4 may be useful for treatment of CNS disorders with demyelinations such as MLD, Krabbe, and multiple sclerosis.

470-P

EFFICACY OF WEEKLY INFUSION OF AGALSIDASE-A IN A PATIENT WITH SEVERE GASTROINTESTINAL SYMPTOMS

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Fabry disease is a X-linked storage disorder resulting from a deficiency of alfa-galactosidase A and progressive accumulation of GB3 in different tissues. ERT shows beneficial effects on clinical symptoms. We report on the efficacy of weekly therapy on the severity of GI symptoms in a 20-year-old male patient. ERT was started with recombinant human α-galactosidase A at 0.2 mg/kg body weight i.v. once every two weeks. The patient reported an improvement of the GI symptoms such as diarrhoea and cramps after 4 months of ERT, while acroparesthesia was still present. After 9 months of ERT the patient presented 6 episodes/day of severe abdominal cramps with diarrhoea. Inflammatory parameters, stool culture, oven and parassite, faecal occult blood test, AGA, EMA, tTG, pANCA, cANCA, AMA, AMLO, ANA were normal. After 1 year and 6 months of ERT, abdominal pain and diarrhoea became very severe and for this reason the patient quitted the job. Because of an increase of inflammatory parameters such as VES and PCR a colonoscopy was performed resulting negative. On the basis of these complaints we increased frequency of infusions to once every week for 6 months. We noted a correlation between the severity of GI symptoms and the increase of inflammatory parameters; in fact after 2 months of weekly ERT, abdominal pain and diarrhoea improved and the inflammatory parameters returned normal. Our case suggests a benefit for GI symptoms by weekly infusion of α-galactosidase A.

471-P

ENZYME REPLACEMENT THERAPY IN CHILDREN WITH FABRY DISEASE: PAEDIATRIC PRACTISE DATA FROM FOS – THE FABRY OUTCOME SURVEY

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Background: Fabry disease (FD) is an X-linked lysosomal storage disorder due to α-galactosidase A deficiency. Symptoms start in childhood in both genders. Although ERT is licensed for adults and children, clear guidelines for treatment in children are lacking.
Aim: To analyse current paediatric practise for ERT in FD.
Method: Symptoms and the percentage of patients on ERT were compared in 220 children (118 males, 102 females) and 1109 adults (551 males, 558 females) in FOS. Clinical severity was analysed using a modification of the Mainz Severity Score Index (MSSI).
Results: Of the 220 children, 78 were <10 years of age. Of males, 78% were >10 years of age; 46% of those <10 years of age were on ERT compared with 81% of adult males (>10 years vs adult males, NS; <10 years vs >10 years, $p = 0.01$). A similar difference was noted in females. ERT was given to 47% of girls >10 years of age and to 34% of those <10 years of age, compared with 58% of adult women. MSSI scores were lower (reflecting less severe symptoms) in younger children (males: 6.21 in those <10 years, 8.45 in those >10 years, $p = 0.05$; females: 4.35 in those <10 years, 7.73 in those >10 years, $p < 0.01$).
Conclusion: Although a significant number of children with FD are on ERT, young patients (<10 years) are less likely to be on treatment despite early symptom onset. Differences in symptom severity, cost of ERT and family circumstances may affect the decision of whether to treat children.

472-P

INFLUENCE OF ANTIBODY FORMATION TO ENZYME REPLACEMENT THERAPY FOR FABRY DISEASE

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Background/Objectives: Fabry disease is a lysosomal storage disease which characterized by deficient activity of alfa-galactosidase A (alfa-gal). Enzyme replacement therapy was available now and some patients develop antibody against enzyme. In this study, we analyzed globotriaosylceramide (GL-3) content from seronegative and seropositive patients who received ERT. More over, we studied antibody influence to cellular up take of enzymes *in vitro*. **Methods**: Fourteen hemizygote patients with Fabry disease were divided into seronegative group ($n = 7$) and seropositive group ($n = 7$). The antibody and urinary alfa-gal were assayed by ELISA method and urinary GL-3 contents was measured using MS/MS. The Kaplan-Meier method was used to estimate the cumulative incidence of normalization of urinary GL-3. *In vitro* study, purified enzyme was mixed with various amount of serum from patients and added to cells. 16 h later, enzymatic activities in cells were assayed. **Results** and **Conclusions:** The cumulative incidence curve of seronegative group and seropositive group was tested by log-rank test and there was significant difference between two groups ($p = 0.0007$). The alfa-gal protein concentrations in urine from both groups were determined and concentration of seronegative group (6.36 ± 5.00 ng/mg creatinine) was significantly higher than that of seropositive group (0.11 ± 0.22 ng/mg creatinine ($p = 0.003$, student's *t*-test). *In vitro* study, serum from seropositive patients had significant neutralizing activity and up take to cells was inhibited by antibody. As a conclusion, formation of antibody inhibit the cellular up take of infused alfa-gal and resulted in less reduction of urinary GL-3 during ERT for Fabry patients.

473-O

TREATMENT OF FABRY DISEASE: OUTCOME OF A COMPARATIVE TRIAL WITH AGALSIDASE ALFA OR BETA AT A DOSE OF 0.2 MG/KG

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Objective: Two different enzyme preparations, agalsidase alfa (Replagal™, Shire) and beta (Fabrazyme™, Genzyme), are registered for treatment of Fabry disease. We compared the efficacy of the two agalsidase preparations administered at identical protein dose in a randomized controlled open label trial. **Methods**: Thirty-four Fabry disease patients were treated with either agalsidase alfa or beta at equal dose of 0.2 mg/kg biweekly. Primary endpoint was reduction in left ventricular mass (LVmass) after 12 and 24 months of treatment. Treatment failure (defined as progression of cardiac, renal or cerebral disease), glomerular filtration rate, pain, anti-agalsidase antibodies, and globotriaosylceramide levels in plasma and urine were secondary endpoints. **Results**: After 12 and 24 months of treatment both treatment groups did not show a reduction in LVmass. Also, no differences in glomerular filtration rate, pain and decline in globotriaosylceramide levels were found. Antibodies developed only in males (4/8 in the agalsidase alfa group and 6/8 in the agalsidase beta group). Treatment failure within 24 months of therapy was seen in 8/34 patients: 6 male patients (3 in each treatment group) and 2 female patients (both agalsidase alfa). The occurrence of treatment failures did not differ between the two treatment groups; $\chi 2 = 0.38$ $p = 0.54$.
Conclusion: Our study revealed no difference in reduction of LVmass or other disease parameters after 12 and 24 months of treatment with either agalsidase alfa or beta at a dose of 0.2 mg/kg biweekly. Treatment failure occurred frequently in both groups and seems related to age and severe pre-treatment disease.

474-P

AT1001, A PHARMACOLOGICAL CHAPERONE DRUG CANDIDATE FOR THE TREATMENT OF FABRY DISEASE

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Fabry disease is an X-linked lysosomal storage disorder caused by deficient alpha-galactosidase A (GLA) activity and accumulation of globotriaosylceramide (GL-3). The iminosugar, AT1001 (migalastat hydrochloride), is a pharmacological chaperone designed to selectively bind GLA and increase enzyme stability, trafficking to the lysosome, and cellular activity. We have characterized AT1001 in pre-clinical safety and efficacy studies as well as in safety, pharmacokinetic, and pharmacodynamic studies in healthy human volunteers. AT1001 caused a selective and concentration-dependent increase in GLA levels in a majority of Fabry patient-derived cell lines harboring missense mutations. In rodents, AT1001 is orally bioavailable with a pharmaceutically acceptable half-life and is well-tolerated at doses up to 50 times those required to increase tissue GLA levels. Oral administration of AT1001 (30 mg/kg/day for 28 days) to R301Q GLA Tg/KO mice caused a selective and dose-dependent increase in GLA levels in multiple tissues and reduced GL-3 substrate in skin, heart and kidney. In Phase 1 clinical studies, AT1001 was orally available in healthy male volunteers and was generally well-tolerated at all doses with no serious adverse events. Oral administration of AT1001 (50 or 150 mg twice daily for 7 days) resulted in a dose-dependent increase in GLA levels in white blood cells that persisted for 7 days after drug withdrawal. Enrollment has been completed for Phase 2 clinical trials that are designed to evaluate the safety and tolerability of AT1001 in patients with Fabry disease.

475-P

TEN YEAR FOLLOW-UP OF AN HOMOZYGOUS D409H GAUCHER PATIENT UNDER ENZYME REPLACEMENT THERAPY

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Gaucher's disease (GD) is a clinically heterogeneous sphingolipidosis caused mainly by mutations in the gene encoding lysosomal glucocerebrosidase, resulting in storage of glucosylceramide in cells of the reticuloendothelial system. Type III GD is known as the chronic neuronopathic form. The D409H in homozygosity mutation has been associated with a particular phenotype, including oculomotor apraxia and cardiac valvular calcifications in childhood.

We report a ten-year-old patient, diagnosed of GD at the age of 45 days because of massive hepatosplenomegaly and glucocerebrosidase deficiency. He is homozygous for D409H mutation. Enzyme replacement therapy (ERT) was started at the age of 2 months and has been well tolerated. Clinically he has developed in the past 4 years an oculomotor apraxia. Bone studies (bone MRI and densitometry) and cardiac studies (Ecochardiogram) are normal as well as cranial MRI. Chitotriosidase activity ranged from 240 and 596 nmol/h/ml which are in normal limits for GD patients. Oxidative stress, evaluated in blood samples, was higher than in healthy controls as shown by the higher values of malondialdehyde (0.7 vs. 0.4 nmol/ml) and carbonylated proteins (0.9 vs. 0.5 nmol/mg prot).

To our knowledge this is the youngest type III GD patient, homozygous for mutation D409H, treated with ERT. At the moment there are no signs of cardiac involvement which could be due either to the effect of early ERT treatment or to the heterogeneity of the clinical phenotype.

476-P

PULMONARY INVOLVEMENT IN PATIENT WITH GAUCHER DISEASE TYPE III AFTER 4 YEARS OF ENZYME REPLACEMENT THERAPY

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Background: Symptomatic lung involvement in Gaucher disease (GD) is relatively rare. Pulmonary status in patients with GD is usually monitored by clinical assessment, chest X-ray, pulmonary function tests and high resolution CT (HRCT). **Methods**: In our patient with GD and absent respiratory symptoms we added bronchoalveolar lavage (BAL) and transbronchial biopsy of the lung (TBB) in assessment of pulmonary involvement. **Results**: A 9-year-old girl with GD type III (L444P homozygote) was referred to our hospital for evaluation of infiltrates found on routine yearly CXR and confirmed by HRCT. Regular enzyme replacement therapy (ERT) (Cerezyme.) was instituted during previous 4 years and reversion of severe visceral involvement and hematologic abnormalities occured. She had no respiratory symptoms in this period. Her older sister died from respiratory complications of GD with no prior ERT. Pulmonary work-up in our patient included bronchoscopic BAL and TBB. Cytological analysis of BAL revealed numerous single and clustered plumpy macrophages with striated PAS-positive cytoplasm consistent with the appearance of Gaucher's cells. In the biopsy specimen of lung, Gaucher's cells filling the alveoli and interstitial infiltration of the bronchiolar wall were found. **Conclusion**: BAL and TBB added substantially to diagnostic accuracy and proved to be useful diagnostic tool for evaluation of pulmonary involvement in GD. Presence of L444P mutation in our patient with pulmonary involvement corresponds to previous studies that showed higher risk for lung disease in homozygotes for this type of mutation. Effects of ERT on pulmonary involvement in GD remains unclear in this case.

477-P

POST-MARKETING SURVEILLANCE OF MIGLUSTAT IN TYPE 1 GAUCHER DISEASE (GD1)

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Background: IS3 is a non-interventional post-marketing surveillance programme aimed at enhancing awareness of safety precautions and stimulating appropriate monitoring during miglustat use in patients with GD1.

Methods: From March 2003 to 9 March 2007, information was available on the first 98 GD1 patients (60% female) prescribed miglustat across 11 European countries (45 centres).

Results: Overall exposure to miglustat represented a cumulative period of 147 patient-years, with a median exposure (range) of 17.9 (1.3–107.7) months. Mean patient age (SD) was 44.3 (15.9) years. At baseline, 65 patients (66%) had previously been treated with enzyme replacement therapy, with a median duration (range) of 64.0 (1.0–176.0) months. Baseline neurological assessments were available in 89 patients (91%), amongst whom 23% displayed one or more neurological manifestations (17% tremor, 9% neuropathy, 12% memory problems, 13% cognitive abnormalities). Fifty-three percent of patients had bone manifestations at baseline, the most frequent being osteopenia (41%), bone pain (28%) and avascular necrosis (16%). During follow up, no safety signals were reported in 48 patients (49%). Twenty-three patients (23.5%) discontinued, most frequently due to gastrointestinal disturbances (14 patients); most these cases occurred during the first 6 months of treatment. New tremor was reported in 13 patients (13%), and memory problems occurred in 7 patients (7%). Bone pain was observed in 5 patients, two of whom exhibited skeletal symptoms at baseline.

Conclusions: Long-term miglustat therapy was well tolerated in patients with GD1. Most gastrointestinal side-effects occurred during the first weeks of treatment. No new safety concerns were identified.

478-P

'GIRLS JUST WANNA HAVE FUN'!

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Background/Objectives: To get a group of girls with neuronopathic Gaucher disease (NGD) together for fun activities and to give them a chance to discuss common problems/issues.
Methods: During a clinical trial of substrate reduction therapy for children with NGD 'Aunty Elin day' was conceived. It is supported by the Gaucher Association UK and Wednesday's Child charity. This allowed for a group of six to eight girls, (5 to 21 years old) to meet up regularly with the clinical research nurse and the clinical nurse specialist. Each day consisted of an activity, such as The London Eye, an open-top bus tour or a visit to Blue Peter studios before meeting to discuss important issues to the girls.
Results: This forum allowed for the development of a kids/teen handbook about NGD incorporating the children's own stories. Personal terminology was identified, for example 'medicine box' explaining port-a-cath. 'Key and Lock' group work identified problems as viewed by the girls with the problem written in the 'lock' and solutions in the 'key'. These highlighted problems in school, keeping up with peers and disclosure about disease.
Conclusions: Continuation of this forum will lead to a better understanding of the issues that are important to the girls, allowing for improved care management. Perhaps, more importantly, the girls have fun!

479-P

RESPONSE OF TYPE 1 AND TYPE 2A MUSCLE FIBERS TO ENZYME THERAPY IN CLASSIC-INFANTILE POMPE DISEASE

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Background: Muscle weakness is the main clinical symptom of Pompe disease, a lysosomal storage disorder for which major benefits of enzyme replacement therapy (ERT) have recently been documented. Restoration of skeletal muscle function is a challenging goal[1,2]. It was demonstrated in mice with Pompe disease that type 2 muscle fibers are resistant to therapy[3].
Methods: To investigate the response in humans, we studied muscle biopsies of a severely affected infant before and after 19 months of therapy. Type 1 and 2a fibers were marked with antibodies, and Lamp1 was used as lysosomal membrane marker.
Results: Quantitative measurements showed 2.5–3-fold increase of the cross-area of type 1 and type 2a muscle fibers during therapy and normalization of the Lamp1 signal in ~95% of type 1 and ~75% of type 2a fibers.
Conclusion: The response of type 1 and type 2 muscle fibers in humans affirms the benefits that enzyme therapy can have for patients with Pompe disease.

[1]Kishnani et al. Neurology. 2007;68:99–109.
[2]Van den Hout et al. Pediatrics. 2004;113:448–57.'
[3]Fukuda et al. Ann Neurol. 2006;59:700–8.

480-P

PHARMACOLOGICAL CHAPERONE THERAPY FOR THE TREATMENT OF GAUCHER DISEASE: AT2101 INCREASES β-GLUCOCEREBROSIDASE LEVELS IN CELLS, MICE AND HEALTHY HUMAN VOLUNTEERS

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Gaucher disease is a lysosomal storage disorder caused by a deficiency in beta glucocerebrosidase (GCase). Over 90% of Gaucher patients have at least one allele with the N370S or L444P missense mutation. These GCase variants have impaired exit from the ER and a significant fraction is prematurely eliminated by ER associated degradation (Schmitz et al., 2005; Ron and Horowitz, 2005). The pharmacological chaperone AT2101 binds the GCase active site and forms hydrogen bonds with key residues to increase protein stability (Lieberman et al., 2007). Binding of AT2101 increases N370S GCase export out of the ER and prevents premature degradation; which results in a 2 to 3-fold increase in GCase levels in Gaucher fibroblasts (Steet et al., 2006). Once in the lysosomes, N370S GCase is stable and functional for at least 3 days in the absence of AT2101. AT2101 treatment also increased L444P GCase levels in Gaucher patient-derived fibroblasts and in the liver, spleen, lung and brain of L444P Gaucher mice. In single and repeat-dose Phase 1 clinical trials involving 72 healthy volunteers, AT2101 was well tolerated with no serious adverse events. In the repeat-dose study, a dose-dependent increase in GCase levels (up to 3.5 fold) was observed during the 7 day treatment period, and remained elevated for more than a week upon removal of the drug. Based on these results, Phase 2 clinical trials with AT2101 have begun in Gaucher patients.

481-P

ORAL MAINTENANCE WITH MIGLUSTAT IN ADULT PATIENTS WITH TYPE 1 GAUCHER DISEASE: THE PATIENTS QUALITY-OF-LIFE PERSPECTIVE

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Background: Quality of life (QoL) is impaired in patients with type 1 Gaucher disease (GD1). In a Phase II, randomised, open-label trial, symptom burden and QoL associated with GD1 and treatment with miglustat were assessed in 36 GD1 patients previously stabilised by imiglucerase and then randomised to miglustat (n = 12), imiglucerase (n = 12), or both (n = 12). **Methods**: The QoL questionnaire included the SF-36, a modified Medical Outcomes Study Health Distress scale assessing GD1 patients frustration, distress, and anxiety, and two surveys assessing symptoms and treatment-related issues. Treatment effects were evaluated using analysis of covariance (ANCOVA adjusted for baseline) for numerical QoL scale scores, and the two-sided Fishers exact test for categorical scores. **Results**: Thirty patients completed the questionnaires at baseline, Month 3 and Month 6. There were no dissimilarities in baseline characteristics across groups. At Month 3, no significant differences in QoL score changes were observed. At Month 6, patients receiving miglustat reported improvement (+8.7%) in SF-36 Mental Health compared with a decrease in patients receiving imiglucerase (–8.5%) or combination therapy (–8.1%) (p = 0.057). With respect to treatment, more patients in the miglustat group reported improvement in overall satisfaction (p = 0.04) and in convenience (p = 0.015) by Month 3. At Month 6, 78% of patients receiving miglustat reported improvement in overall satisfaction (p = 0.053) and convenience (p = 0.028) compared with 33% in the imiglucerase group and 30% in the combination group. There were no significant inter-group differences in gastrointestinal symptom scores. **Conclusion**: Maintenance therapy with miglustat improved patients sense of well-being and satisfaction with treatment.

482-P

ENZYME REPLACEMENT THERAPY (ERT) FOR POMPE DISEASE: THE GREEK EXPERIENCE IN CHILDREN

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Background: Pompe disease (PD) is a rare metabolic myopathy due to acid α-glucosidase (GAA) deficiency, resulting in intra-lysosomal accumulation of glycogen, with profound consequences in cardiac, skeletal and respiratory muscles. Infantile and late-onset forms are distinguished, reflecting differences in age of onset and rate of disease progression. ERT with rhGAA is currently available. **Objective/ Methods:** To study the efficacy and safety of ERT with rhGAA for PD. One patient with infantile phenotype (9 months old) and a second with late-onset form (13 years old) received rhGAA for 14 and 10 months, respectively. They have been followed-up by clinical and laboratory assessment.
Results: The first patient, being severely hypotonic and in cardiac failure when initiating ERT, showed an impressive improvement in the first 10 months; she acquired new motor skills and left ventricular muscle index (LVMI) decreased by 60%. She subsequently showed signs of decline and died at 24 months following a respiratory infection. The second patient showed a mild but steady improvement in muscle strength and function (as assessed by standardized testing), following the first 10 months of treatment. His pulmonary function tests, being suboptimal at the start (FVC 76% of predicted), remained stable throughout the course of treatment. Infusion-associated reactions were not observed in either patient.
Conclusions: The availability of ERT has ushered a new era of hope for patients with PD, with clinical benefit being more favourable in late-onset forms. Earlier initiation of treatment in infants is of paramount importance to minimize the consequences of the disease.

483-P

ENZYME REPLACEMENT THERAPY ON JUVENILE ALPHA-GLUCOSIDASE DEFICIENCY – EXPERIENCE OF PEDIATRIC HOSPITAL OF COIMBRA

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Glycogen storage disease type II or Pompe disease is a rare, progressive, neuromuscular, autosomal recessive disorder, caused by lysosomal storage of glycogen due to alpha-glucosidase deficiency. **Aim**: To analyze the safety and efficacy of two years of alglucosidase in juvenile Pompe pediatric patients followed at our hospital. **Methods**: Prospective study of 4 female patients submitted to Alglucosidase. The dose used was 20 mg/kg, bimonthly, 4 h i.v. infusion, with pre medication. They all have important muscular atrophy, muscular strength deficiency with absent reflexes, nocturnal non invasive ventilation and slight pulmonary hypertension. None have echocardiographic signs of cardiomyopathy. Subjective improvement, muscular functional testes (Walter and Gardner-Medwin scores, functional scores of arms and legs), muscular strength test (muscular manual test), weight, ventilatory pressure, pletismography and side effects were evaluated at 3rd, 6th, 12th and 24th months. Clinical follow-up was also analysed. **Results**: Patients begin Alglucosidase at 5 to 17 years old and have an ERT follow-up time of 6 to 28 months. One child died from severe respiratory infection 2 months after the beginning of therapy. The main clinical results were: subjective improvement shortly after the first infusions; improvement of muscular function and muscular strength; maintenance of weight, ventilatory pressure and respiratory testes. None showed any sign of deterioration. **Conclusions**: Results have shown that Alglucosidase in paediatric juvenile Pompe patients is safe and effective in slowly improving neuromuscular signs and symptoms, especially concerning quality of life and muscular function. We believed that this therapy will change the natural course of Pompe disease.

484-P

2-YEAR FOLLOW-UP OF ENZYME THERAPY IN 5 CHILDREN WITH POMPE DISEASE

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Background: Pompe disease is a rare neuromuscular disorder caused by deficiency of the lysosomal enzyme acid alpha-glucosidase. Patients with the classic-infantile form die within the first year of life, older children and adults show a more protected course of disease and eventually become wheelchair bound and ventilator dependent. Enzyme therapy with recombinant human alpha-glucosidase has recently been approved by EMEA and FDA based on results in infants. Here we present two year follow-up of 5 older children treated with enzyme therapy. **Methods**: 5 children diagnosed between 1.1 and 11.6 years of age were included in a single-center open label study. Age at start of treatment ranged from 5.9–15.2 years. All patients were ambulant. Three patients had decreased pulmonary function (FVC below 80%) both in upright and supine position. One required non-invasive ventilation at night. Patients were treated with intravenous infusions of recombinant human alpha-glucosidase (Myozyme™, dose 20 mg/kg every two weeks). Safety and efficacy was monitored on a regular basis. **Results**: No infusion associated reactions were observed. Three patients showed significant improvement of muscle strength as measured by MRC-score and Hand Held Dynamometry. Two of the three patients with decreased FVC at baseline showed significant improvements in predicted FVC. **Conclusion**: Treatment with recombinant human alpha-glucosidase in 5 older children with Pompe disease was tolerated well over two years and showed significant improvements in all patients in either predicted FVC or muscle strength.

485-O

RESPONSE TO ENZYME REPLACEMENT THERAPY IN 18 JUVENILE AND ADULT PATIENTS WITH ADVANCED POMPE DISEASE

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Background: Pompe disease is a rare autosomal recessive disease due to a deficiency of lysosomal acid-α-glucosidase. In older patients it causes progressive proximal muscle weakness, respiratory insufficiency, substantially decreased quality of life and early death.
Methods: We reviewed physician-reported outcomes for 18 advanced patients treated with enzyme replacement therapy (ERT) at 20 mg/kg/day intravenously bi-weekly. Fıfteen were treated through a compassionate usage program and 3 in an open-label extension study.
Results: At baseline, all patients were wheelchair bound. Seventeen required respiratory assistance: 9 invasive, 7 non-invasive, 1 combined invasive and non-invasive ventilation. Mean age at ERT initiation was 30.8 ± 14.3 years. Treatment duration ranged from 8 to 75.6 months. Ten of 17 patients demonstrated improvements in respiratory function, including a 50% reduction in ventilation for one patient. Motor function improved in 13 of 18 patients and stabilized in the remaining 5 patients; no declines in muscle strength or tone were noted. Most patients (15/16) reported positive improvements in their quality of life since commencing ERT. Treatment was well-tolerated, with only one report of a transient infusion-associated reaction (chills) during the first infusions.
Conclusions: Enzyme replacement therapy for juvenile and adult patients with advanced Pompe disease is associated with gains in both respiratory and motor function. Intervention earlier in the disease course was associated with greater improvement in clinical parameters. Overall, patients were satisfied with their treatment, and reported positive improvements in their quality of life regardless of the magnitude of clinical gains or baseline disease involvement.

486-P

POMPE DISEASE: CLINICAL TRIAL WITH ENZYME REPLACEMENT THERAPY. CASE PRESENTATION

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Background: Pompe disease is an autosomal recessive disorder caused by deficiency of the enzyme acid alpha glucosidase (GAA) which results in intralysosomal accumulation of glycogen in multiple organs with prominent involvement of heart and skeletal muscles.

The clinical presentation is heterogeneous, largely due to the residual enzyme activity associated with different mutations in the *GAA* gene, thus it encompasses a range of phenotypes, all of which include varying degrees of myopathy but differ with respect to age of onset, extent of organ involvement and rate of progression to death. The most severe form is the classical infantile onset disease, described by Pompe with hypertrophic cardiomyopathy, hypotonia, hepatomegaly and death due to cardiorespiratory failure.

Methods:A female patient presenting with repeated heart failure and huge cardiomegaly at the age of 3 months, diagnosed as having infantile Pompe disease at the age of 7 months, has been receiving enzyme replacement therapy with myozyme at a rate of 20 mg/kg every 2 weeks for a period of 6 months.

Results: Enzyme replacement therapy with acid alpha glucosidase administered to the patient resulted in improvement of both the cardiac and skeletal muscles functions.

Conclusion: Enzyme replacement therapy with acid alpha glucosidase offers hope for patients suffering from this lethal disease.

487-O

LONGER SURVIVAL BY ENZYME REPLACEMENT THERAPY UNMASKS THE UNDER-RECOGNITION OF OTONEUROLOGICAL INVOLVEMENT IN INFANTILE POMPE DISEASE

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Introduction: The recent introduction of enzyme replacement therapy (ERT) for Pompe disease altered the disease course, increased survival, and improved cardiomyopathy and achievements of motor skills. However, in few patients, ERT unmasked previously unrecognized medical issues including sensory neural hearing loss (SNHL) and CNS involvement. **Objective**: To describe the results of otoneurological evaluation in a cohort of nine patients, age 3 months–8 years treated with ERT at one center in Israel. **Methods**: Hearing thresholds were determined for air and bone conduction. Auditory brain-stem evoked potentials (ABEPs) recorded peak latencies of waves I, III and V, and the central conduction time in terms of interpeak latency interval. Brain neuroimaging included CT or MRI. **Results**: All nine patients demonstrated various degrees of conductive hearing loss which in 6/9 improved following conservative treatment. However 3/9 had SNHL indicating cochlear pathology, and increased interpeak latency differences denoting abnormal CNS auditory pathways. These three patients had nonsense mutations, 2/3 had brain white-matter changes on neuroimmaging, and after 6–8 months on ERT, they experienced infusion-associated reactions. Most of ERT treated patients develop antibodies, but only few have inhibitory antibodies as did 2/3 patients. All nine patients experienced major clinical benefits including improvement of cardiomyopathy and achievements of motor skills. However, following 10–18 months, the two patients with nonsense mutations experienced neurological deterioration and died of cardiorespiratory failure. **Conclusions**: Further studies are necessary to clarify the impact of genetic and inhibitory antibodies on clinical efficacy of ERT in a subgroup of patients with nonsense mutations who might have CNS involvement.

488-P

FAVOURABLE RESPONSE TO RECOMBINANT HUMAN ACID ALPHA-GLUCOSIDASE AND HIGH-PROTEIN LOW-CARBOHYDRATE DIET IN PATIENTS WITH POMPE DISEASE

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The prognosis of Pompe disease has changed with the availability of recombinant human acid alpha-glucosidase. We report here the favourable response to high-protein low-carbohydrate diet in a cohort of 5 patients with Pompe disease (2 atypical infantile, 2 juvenile and one adult). Excellent outcome without side effects was achieved after a year of enzyme therapy in 2 with atypical infantile Pompe disease. Initially, all were managed conservatively with an individually designed, closely monitored high protein (3 g/kg/day) but low carbohydrate (40–50% of calories) diet and all showed favourable response. One infantile onset patient in frank cardiac failure improved. Her left ventricular ejection fraction (LVEF) improved from 25% to 44%. Anti-failure therapy could be weaned off but left ventricular mass (LVM) did not change. She still could not walk at 2. After 3 months of enzyme replacement and normal diet she started walking. Her cardiac hypertrophy regressed; LVM reduced from 70 g to 58 g and LVEF improved to 55.2% after 10 months of enzyme replacement. Her elder sister could carry out normal activities in school after enzyme therapy. The adult who required invasive ventilation for 6 months could be weaned off and discharged home after a month of dietary treatment. She remained well on the diet and only required occasional nasal oxygen therapy but still wheel chair bound without enzyme for past one year. High-protein low-carbohydrate diet may buy time for patients with Pompe disease while waiting for enzyme therapy in countries with limited resources. Enzyme therapy eventually provides hope for all of them.

489-P

BONE MARROW TRANSPLANTATION FOR NIEMANN–PICK C2 DISEASE

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Background: Niemann–Pick type C disease (NPC) is an autosomal recessive lipid storage disorder with a variable clinical phenotype and age of onset. NPC is most commonly characterized by hepatosplenomegaly and a severe progressive neurological dysfunction. Two different genetic complementation groups, *NPC1* and *NPC2* have been established. Even though unsuccessful bone marrow transplantation (BMT) has been reported in NPC1 patients and in the mouse model, it has never been proposed in NPC2 patients.

Case report: We report here the first trial of BMT in a 7-month-old female patient affected with NPC2. She presented early symptoms since 2 months of age, including feeding difficulties and failure to thrive. She developed later a severe lung interstitial disease with hepatosplenomegaly, that lead to the diagnosis of NPC disease by filipin staining in fibroblasts and molecular analysis which revealed a homozygous IVS1+2 T > C mutation on the *NPC2* gene. At the moment of the diagnosis her respiratory condition deteriorated. BMT was performed with her geno-identical brother.

Results: The patient has been engrafted after a usual conditioning regimen. Within 2 months after BMT a clinical improvement in her pulmonary function and splenomegaly was noted and the lung X-rays remained stable. She developed, 3 months after BMT a severe adenovirus pulmonary infection that lead to a respiratory failure and death despite anti-viral therapy, mechanical ventilation and aggressive life support.

Conclusion: BMT performed in this patient transiently improved the visceral signs of the disease. These data, should encourage early BMT in NPC2 patients before life-threatening symptoms appear.

490-P

TREATMENT OF NIEMANN–PICK DISEASE TYPE C IN TWO CHILDREN WITH MIGLUSTAT: INITIAL IMPROVEMENT COULD BE MAINTAINED OVER 1 YEAR

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Background: Niemann–Pick disease type C (NP-C) is a lipid storage disorder characterized by the accumulation of unesterified cholesterol and glycolipids in the lysosomals of cells in the CNS and visceral organs. Clinical symptoms include progressive neurological deterioration and visceral organomegaly. Miglustat, a small iminosugar molecule approved for the treatment of Gaucher disease, reversibly inhibits glucosylceramide synthase, which catalyses the first committed step in glycosphingolipid synthesis. The physico-chemical properties of miglustat allow it to cross the blood-brain barrier and suggest possible benefits in lysosomal storage diseases affecting the CNS.
Methods: We present findings in two children with NP-C aged 14 (Case 1) and 9 years (Case 2), treated with miglustat for 1 year.
Results: Before treatment, Case 1 presented with severe difficulties in swallowing and walking, and Case 2 with problems mostly affecting communication and social interaction. Over the first 6 months, there were improvements in swallowing according to function evaluation and Videofluoroscopic study in Case 1, improvements in motor function according to ambulation index measurements in Case 1, and improvements in cognitive function by Mini Mental-State Examination (MMSE) assessments in Case 2. These functions stayed stable over the latter 6 months of the study period. Liver/spleen volume and plasma chitotriosidase activities were not changed significantly over the whole study period.
Conclusion: This study suggests that miglustat can provide therapeutic benefits in CNS symptoms and allows stabilization of systemic disease in childhood-onset NP-C.

491-P

INFILTRATION OF NIEMANN–PICK CELLS IN BONE MARROW CAN BE DETECTED BY QUANTATIVE CHEMICAL SHIFT IMAGING (QCSI)

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Background: Nieman-Pick disease type A and B (or NPD type IA and IS) is a lysosomal storage disease caused by acid sphingomyelinase deficiency. Type B/IS is the chronic non-neuronopathic form and is characterized by slowly progressive hepatosplenomegaly and deterioration of pulmonary function. The relationship between bone marrow infiltration and cytopenia or growth retardation is unknown. We hypothesized that QCSI could be useful tool to study Niemann–Pick cell infiltration in bone marrow.
Methods: Two adult male patients aged 36 and 21 years with classical non-neuronopathic NPD were studied. Both had hepatomegaly (2198 cc and 3357, respectively) and splenomegaly (1421 cc and 1349 cc, respectively). Non-invasive bone marrow infiltration assessment by means of fat fraction measurement was performed with Dixon Quantitative Chemical Shift Imaging, according to Maas et al. (Am J Roentgenol., 2002). Fat fraction was compared to those measured in healthy volunteers and Gaucher disease patients.
Results: Patient A had a fat fraction of 0.16, which remained stable after 12 months. Patient B had a fat fraction of 0.23. Fat fraction was severely decreased compared to 16 normal controls (0.27–0.55 mean 0.37). Fat fraction was comparable to those seen in 30 patients with Gaucher disease (0.08–0.4, mean 0.20).
Conclusion: In patients with non neuronopathic Nieman-Pick disease (type B or IS), bone marrow fat fraction is decreased, as it is in Gaucher disease. QCSI may be a useful tool to study natural history of bone marrow involvement in NPD and serve as a surrogate clinical endpoint in future clinical trials.

492-P

BONE MARROW TRANSPLANTATION IN NEWBORN TWITCHER MICE; BIOCHEMICAL AND PATHOLOGICAL FINDINGS

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Globoid cell leukodystrophy (GLD, Krabbe disease), one of lysosomal storage diseases (LSDs), is rapidly progressive, invariably fatal disease of infants and caused by genetic deficiency of the lysosomal enzyme galactocerebrosidase (GALC). For GLD, various treatment has been enforced same as the other LSDs but bone marrow transplantation (BMT) is the only available effective treatment for the moment. In this study we enforced BMT to a Twitcher, the murine model of GLD, this time for the newborn baby period and evaluated it biochemically and pathologically.

The BMT was carried out on twitcher on the birth day. At 4 weeks after BMT, The enzyme activity of a treatment group increased in comparison with a non-treatment group in some organs, especially hematopoietic organs. The demyelination of treatment group was improved pathologically in comparison with non-treatment group. We suggest that BMT in the newborn may be more effective treatment than initiated later in life.

493-O

PYRIMETHAMINE AS A NOVEL POTENTIAL PHARMACOLOGICAL CHAPERONE FOR LATE-ONSET FORMS OF GM2 GANGLIOSIDOSIS

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Background: Late-onset GM2-gangliosidosis (GM2) is composed of two related, autosomal recessive, neurodegenerative diseases, both resulting from deficiency of lysosomal, heterodimeric beta-hexosaminidase A (Hex A, alpha, beta). Pharmacological chaperones (PC) are small molecules that can stabilize the conformation of a mutant protein, allowing it to pass the quality control system of the ER.
Hypothesis: To date all successful PCs have also been competitive inhibitors. We believe that PC for GM2 can be identified by screening competive inhibitors of Hex A.
Methods and Results: Screening for Hex A inhibitors in a library of 1040 FDA-approved compounds identified pyrimethamine (PYR) as the most potent inhibitor. Cell lines from 10 late-onset Tay-Sachs (11 alpha-mutations, 2 novel), and 7 Sandhoff (9 beta-mutations, 4 novel) disease patients, were cultured with PYR at concentrations corresponding to therapeutic doses. Cells carrying the most common late-onset mutation, alphaG269S, showed significant increases in residual Hex A activity, as did all 7 of the beta-mutants tested. Cells responding to PC-treatment included those carrying mutants resulting in reduced Hex heat stability and partial splice junction mutations of the inherently less stable alpha-subunit. PYR, which binds to the active site in domain II, was able to function as PC even to domain I beta-mutants.
Conclusions: We concluded that PYR functions as a mutation-specific PC, variably enhancing residual lysosomal Hex A levels in late-onset GM2 patient cells.

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494-P

CLINICAL AND URINE FINDINGS 7 YEARS AFTER BONE MARROW TRANSPLANT FOR ALPHA-MANNOSIDOSIS

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Aim: To assess the effect of matched unrelated bone marrow transplantation at age 6 on clinical outcomes and urinary oligosaccharides in a patient with α-mannosidosis. **Methods**: Hearing was assessed by brain stem evoked potentials and pure tone audiometry, intellectual functioning by standard tests including WISC4, Neale, Westwood and Adaptive Behaviour Assessment Scale. Records of outpatient consultations were reviewed before and after transplantation. Matched unrelated bone marrow transplant was undertaken at age 6 after conditioning with busulphan, cyclophosphamide, fludarabine and ATG. Urine was derivatised with phenyl-methyl-pyrazalone according to the method of Ramsay et al.[1] and prepared for mass spectrometry using C18/amino columns [baseline n = 300]. The oligosaccharides H2-HNAc (man-man-glcNAc), H3-HNAc, H4-HNAc, H5-HNAc, H6-HNAc were measured using the multiple reaction monitoring mode by electrospray ionisation-tandem mass spectrometry. **Results**: There was a significant improvement in hearing, most obvious at 2 KHz, to the extent that hearing aids were no longer required. Performance IQ at age 4.9 years was 63, at 5.4 years it was 69. At age 11, overall IQ was 41–49. She enjoyed school where she was in a Special Unit. Flat feet and scoliosis were less obvious. Macrocephaly did not develop. Urinary H2-HNAc was reduced from 30-fold elevated to 5-fold elevated 11 months following BMT and continued to decrease post transplant. **Conclusion**: Bone marrow transplant improved hearing and stabilised skeletal features but there appeared to be slow intellectual decline. Urinary oligosaccharide analysis demonstrated a biochemical response to BMT.

[1]Ramsay et al. Anal Biochem. 2005;345:30–46.

495-P

CORD BLOOD TRANSPLANTATION FOR A BOY WITH WOLMAN DISEASE. VOD WITH FATAL OUTCOME DESPITE PROMPT ENGRAFTMENT

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Wolman disease is a rare autosomal recessive metabolic disorder. Deficiency of lysosomal acid lipase results in accumulation of triglycerides and cholesteryl esters most importantly in liver, spleen, intestinal mucosa, adrenal glands and lymphocytes. The disease is fatal during first year of life. Only one patient with long-term survival after successful stem cell transplantation has been reported in the literature and no more than 13 patients are registered at CIBMTR – less than 1% of all transplants for metabolic disorders during 1980–2005.

Case Report: First child of non-consanguineous parents. At the time of diagnosis at five months of age, he showed typical features of Wolman disease: diarrhea, vomiting, abdominal distension, liver and spleen enlargement and adrenal glands with characteristic calcifications. Acid lipase activity in leukocytes and fibroblasts was low. He was transplanted at 6.5 months of age after conditioning with BU16/CY200 plus Thymoglobulin 10 mg/kg. He was given an unrelated 5/6 antigen HLA-matched cord blood. 4.6×106 CD34+ cells/kg were infused. Engraftment was prompt and at day +9 ANC was $>0.5 \times 109$/L. Already at day +15 he fulfilled criteria for VOD with a hepatorenal syndrome. His VOD evolved into multiorgan failure and the boy died at day +36

Acid lipase in leukocytes increased to normal levels already day +18 and at day +28 after SCT the child was a full chimera

Conclusion: Wolman disease might be cured by SCT. However, due to severe and early organ involvement complication rate is high and for successful outcome the transplant must be done within the first months of life.

496-O

NON-INHIBITORY ANTIBODIES IMPEDE LYSOSOMAL STORAGE REDUCTION IN ENZYME REPLACEMENT THERAPY OF A LYSOSOMAL STORAGE DISEASE

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Enzyme replacement therapy (ERT) is a treatment option for several lysosomal disorders including the sphingolipid storage disease metachromatic leukodystrophy (MLD). MLD is caused by a deficiency of arylsulfatase A (ASA) resulting in intralysosomal deposition of sulfatide, a severe neurological phenotype and early death. Treatment of an *ASA* knockout mouse model of MLD by intravenous injection of recombinant human ASA (rhASA) reduced sulfatide storage and improved nervous system pathology and function (Matzner et al., Hum Mol Genet. 2005;14:1139–52). Repeatedly treated mice develop, however, anti-rhASA antibodies which impede sulfatide clearance. Reduced sulfatide clearance is not caused by antibody-mediated inhibition of ASA activity, but due to a blockade of mannose 6-phosphate receptor-dependent enzyme uptake, retargeting of rhASA to macrophages, intracellular rhASA misrouting and diminished enzyme stability. To abrogate immune-mediated side effects we constructed a novel ASA knockout strain which constitutively expresses an inactive hASA mutant from a stably integrated transgene. The expression of the mutant *hASA* on the *ASA* knockout background confers absolute immunotolerance to substituted wildtype rhASA, does not induce an overt new disease phenotype and restores sulfatide clearance upon long-term treatment. Our data indicate that effects of non-inhibitory antibodies must be more intensively considered in evaluating the therapeutic efficacy of ERT in lysosomal storage disorders in general and in patients without cross-reacting material specifically.

497-O

WITHDRAWN

498-P

DIAGNOSING INBORN ERRORS OF LIPID METABOLISM USING ^{1}H-NMR SPECTROSCOPY

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Background: Many severe diseases are caused by defects in lipid metabolism. As a result, patients often accumulate unusual lipids in their blood and tissues and proper identification of these lipids is essential for correct diagnosis. In this study, we investigated the potential use of proton nuclear magnetic resonance (1H-NMR) spectroscopy to simultaneously identify and quantify (un)usual lipids present in the blood of patients suffering from different inborn errors of lipid metabolism. **Methods**: Blood plasma or serum lipids were extracted in 2:1 (v/v) chloroform-methanol. After addition of the non-volatile chemical shift and concentration reference compound octamethylcyclotetrasiloxane, 1H-NMR measurements were performed on a 500 MHz spectrometer. Assignments were based on literature, computer simulations and reference spectra of relevant authentic standards. **Results**: Spectra of normal plasma samples allowed the identification of nine lipid species. For cholesterol and triglyceride levels, a good correlation was found between conventional methods and 1H-NMR. Furthermore, four inborn errors of lipid metabolism were investigated (three in sterol metabolism and one in fatty acid metabolism). NMR analysis led to a correct diagnosis for all four diseases, while the concentration of the diagnostic metabolite could be determined for three. **Conclusions**: We showed that 1H-NMR spectroscopy of blood plasma or serum lipid extracts can be used to accurately identify lipids. Identification was also possible for unusual lipids in the blood of patients suffering from an inborn error of lipid metabolism. Additionally, simultaneous lipid quantification was possible for the first time. The technique is therefore applicable in clinical diagnosis and follow up.

499-P

EFFECTS OF CHOLESTEROL AND SIMVASTATIN TREATMENT IN PATIENTS WITH SMITH-LEMLI-OPITZ SYNDROME (SLOS)

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Background: Smith-Lemli-Opitz syndrome (SLOS) is caused by deficiency of 7-dehydrocholesterol reductase resulting in an accumulation of 7- and 8-dehydrocholesterol (7+8-DHC) and, in most patients, a deficiency of cholesterol. Current therapy consists of dietary cholesterol supplementation, which raises plasma cholesterol levels but clinical effects have only been reported in few patients. HMG-CoA reductase inhibitors were shown to reduce 7+8-DHC levels and increase cholesterol concentrations in two small trials with divergent clinical outcome.

Methods: We evaluated the effects of cholesterol only and of cholesterol plus the HMG-CoA reductase inhibitor simvastatin on plasma sterols in a retrolective study in 41 and on anthropometrical measures in 24 SLOS patients.

Results and **Conclusions:** Cholesterol as well as additional simvastatin decreased the plasma (7+8-DHC)/cholesterol ratio. However, the mechanism leading to the decreasing ratio was different. Whereas it was due to an increasing cholesterol concentration in the cholesterol only cohort, a decreasing 7+8-DHC concentration was demonstrated in the cohort receiving additional simvastatin.

We could not confirm a positive effect of simvastatin treatment on anthropometric measures or behaviour, as previously reported.

500-P

3β-HYDROXY-Δ5-C27-STEROID DEHYDROGENASE (3β-HSD) DEFICIENCY WITH FRIEDREICH-LIKE ATAXIA

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The 3β-HSD deficiency impairs bile acids synthesis. Patients present at birth or childhood with cholestatic jaundice and steatorrhea. There are few data about affected cases from adolescence to adulthood, but death can happen by liver injury. Therefore, early identification and bile acid therapy could probably avoid neurological deterioration and liver transplantation.

Case Report: A 20-year-old male presenting since newborn diarrhoea and failure to thrive, and during infancy malabsorption, steatorrhea and severe coagulopathy was studied. Later, he developed Friedreich-like ataxia, liver fibrosis, biliary lithiasis and nephrocalcinosis. Plasma cholesterol level, triglycerides and ApoB were decreased, 7-dehydrocholesterol was slightly increased. CoQ10 levels were reduced in plasma but normal in muscle tissue. Urinary bile acids were analyzed by electrospray-tandem mass spectrometry in the precursor ion scan negative mode (glycoconjugates: m/z 74, sulfated: m/z 80).

Results showed almost absence of primary bile acids (di- and trihydroxycholanoic glycine or taurine conjugated acids) with high excretion of di- and trihydroxycholenoic acids, glycine and/or sulfate conjugated (m/z 462, 469, 485, 526, 542). These findings were consistent with a 3β-HSD defect. This was confirmed by molecular analysis of the *HSD3B7* gene showing that patient was homozygous for a substitution mutation in exon 4 that changes glutamic acid to lysine (E167K). The mutation has not been previously reported, but this glutamic acid residue is conserved between human and animal species. Patient improved clinically after chenodeoxycholic acid therapy.

We highlight the neurological phenotype in a patient with 3β-HSD defect, usually associated with hepatic and gastrointestinal symptoms.

501-P

MONITORING CHOLESTEROL PRECURSORS IN SIBLINGS WITH CEREBROTENDINOUS XANTHOMATOSIS: A FOLLOW UP

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Cerebrotendinous xanthomatosis (CTX) is an inborn error of bile acid synthesis in which hepatic conversion of cholesterol to cholic and chenodeoxycholic acids is impaired. Patients with CTX have abnormal bile alcohols in urine, normal/increased plasma cholesterol concentrations and increased concentrations of plasma cholestanol. Little is known about complete cholesterol profiles in CTX.

Therefore, we studied cholesterol- and phytosterol profiles in 2 siblings with CTX during follow up. While cholesterol concentrations were low in both patients (2.9 mmol/L and 2.1 mmol/L, respectively), plasma cholestanol was 6-fold higher compared to control values. In addition, both sibs had a more than 100-fold increase in 7-dehydrocholesterol (7DHC) and 8-dehydrocholesterol (8DHC). Lathosterol, lanosterol and sitosterol were increased in both patients while concentrations of desmosterol and campesterol were normal. In addition, plasma lathosterol/cholesterol ratio's (marker for cholesterol synthesis) were significantly elevated. After two weeks of treatment with chenodeoxycholate (15 mg/kg/day, in three times daily), both patients showed a marked decrease in cholestanol, 7DHC, 8DHC, lathosterol, lanosterol and sitosterol. In addition, lathosterol/cholesterol ratio normalized indicating that overall cholesterol synthesis was sufficiently suppressed. Bile alcohols excretion normalized after 14 weeks of treatment. The levels of plasma metabolites cholestanol, 7DHC, 8DHC and sitosterol declined further upon treatment (measured at 14 and 48 weeks). However, 8DHC was still increased in both patients at t = 48 weeks (13-fold and 27-fold, respectively).

In **conclusion**, the present study shows that bile alcohols were completely normalized after 14 weeks treatment with chenodeoxycholate, while some cholesterol intermediates were still increased after 48 weeks of treatment.

502-P

CLINICAL AND MOLECULAR GENETIC ANALYSIS OF A CHINESE PATIENT WITH SITOSTEROLEMIA

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Sitosterolemia is a rare inherited plant sterol storage disease. It is characterized by tendon and tuberous xanthomas from early childhood and by a strong propensity toward premature coronary atherosclerosis. Mutations in the ATP-binding cassette (ABC) proteins *ABCG5* or *ABCG8* are now known to cause this disease. To date, only a few mutational studies of sitosterolemia have been reported and interestingly, most of the Caucasian patients were caused by the *ABCG8* mutations and most of the Asian patients were caused by *ABCG5* mutations. Recently, we encountered a 1 year 6 months old girl who has marked xanthomas over the bilateral ankles and elbows and severe hypercholesterolemia. Because her parents had normal serum cholesterol levels and she had a good response to bile-acid resins (cholestryamine) therapy, sitosterolemia was highly suspected. The mutational analysis of *ABCG8* and *ABCG5* genes were performed. The result showed that the proband had a compound heterozygous mutations in exon 9 (c.1306G>A; p.R389H) and in exon 10 (c.1476C>T; p.R446X). The p.R446X mutation is a novel mutation leading to a premature stop codon that generates a truncated protein. Such truncated protein remains the largest portion of ABCG5 compared with other premature forms that have been reported. The missense p.R389H mutation has only been found in Japanese patients. In this study, we describe the clinical, biochemical and molecular genetic features of an index patient with sitosterolemia in Taiwan. These findings could extend the range of clinical phenotypes of sitosterolemia in Asian population.

503-P

FIRST 2 YEARS EXPERIENCE WITH EZETIMIB THERAPY OF PRIMARY HYPERCHOLESTEROLEMIA IN CHILDREN AND ADOLESCENTS

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Background: Ezetimib – a selective inhibitor of intestinal absorbtion of cholesterol belongs to new hypolipidemic drugs recently started to be used for treatment of hypercholesterolemia.
Aims: To present first 2 years experience with Ezetimib monotherapy (10 mg/day) in children and adolescents with primary hypercholesterolemia.
Subjects and methods: 21 patients (age: 7 to 17 years, average 13.5) treated 1 year, 8 of them 2 years. Lipid analysis (total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, apolipoprotein A 1, apolipoprotein B) was performed after 1, 6, 12, 18 and 24 months of treatment. The somatic and sexual development was observed, side effects were evaluated clinically and by laboratory methods.
Results: Significant decrease ($p<0.01$) of average pre-treatment lipid values was observed after one month therapy in total cholesterol (from 7.9 mmol/l to 6.3 = 19.2%), LDL-cholesterol (from 5.7 mmol/l to 4.3 = 24.8%) and apolipoprotein B (from 1.6 g/L to 1.2 = 23%). The biggest decrease was in 18 month (cholesterol 27.5%, LDL-cholesterol 31.9%, apolipoprotein B 36.2%). The decrease persisted during all 24 months of treatment, differences between months values were not significant. Differences between pre-treatment and post-treatment levels of other lipids were not significant. No side effects were observed till now.
Conclusion: Ezetimib appears to be the effective and safe drug in the long-term treatment of primary hypercholesterolemia in childhood.

504-P

EFFECTS OF DOCOSAHEXAENOIC AND EICOSAPENTAENOIC ACIDS ON BLOOD LIPID PROFILE IN HYPERCHOLESTEROLEMIC CHILDREN

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Background and **Aims**: Dietary n-3 fatty acids (FA) can reduce cardiovascular risk in dyslipidemic adults. We have investigated the effects of eicosapentaenoic (EPA) and docosahexaenoic (DHA) FA on blood lipid profile in dyslipidemic children.
Methods: In a double blind placebo-controlled trial, 36 children 3–13 years of age, 19 males and 17 females, referring to our clinic for primary hypercholesterolemia, were put on Step-I diet and randomised to receive 500 mg/day of purified DHA, or EPA+DHA mixture (41% and 45%, respectively), or wheat germ oil as placebo. They underwent at baseline and after a 4-month treatment: anthropometrics, blood lipid profile [total cholesterol (TC), HDL-C, TG levels (enzymatic method), LDL-C (Friedewald formula)], dietary habits (food frequency questionnaire). Statistics: non-parametric tests and ANOVA as appropriate. Descriptive data: mean values.
Results: 31 children completed the study (11, DHA; 10, DHA+EPA; 10, germ oil). At baseline, groups were not different as far as age, anthropometrics and blood lipid profile (trend for difference in HDL levels, $p = 0.06$). After 4 months the DHA group showed increase of HDL-C (+39.8%, $p = 0.003$) while the placebo showed a decrease of LDL-C (–11.7%, $p = 0.007$). Both DHA and placebo groups showed a decrease of fat intake (–14.7% and –13.0%, $p = 0.004$ and 0.009, respectively) compared with no changes in the EPA+DHA group.
Conclusions: DHA supplementation combined with step-I diet in dyslipidemic children resulted in raised HDL-C. The EPA+DHA group, who was less compliant with STEP-I diet than the other two groups, did not show significant changes of the lipid profile.

505-P

A FIVE YEARS EXPIRIENCE OF LDL-APHERESIS IN PEDIATRIC PATIENTS WITH HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLAEMIA

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Background/Objectives: Low density lipoprotein (LDL) apheresis provides a safe and effective means of treating adult patients with homozygous familial hypercholesterolaemia (FH).The aim of the presented study is to investigate the efficacy LDL-apheresis in children with homozygous familial hypercholesterolaemia.
Methods: Data were collected from 10 patients with FH (11.4 ± 5.8 years old). Two techniques of LDL-apheresis treatment were used: (1) Cascade filtration (Evaflux 5A) (in combination with centrifugal cell separator), (2) Direct absorption (Lipcollect 200 with ADAsorb system and DIDECO cell separator). The most of the sessions were carried out at biweekly or monthly intervals in combination with medical and diet treatment.
Results: The mean patient follow-up was 21.9 ± 21.4 months. The number of sessions averaged 31.8 ± 29.38 per patient.Total number of session was 318. The mean LDL-C pre treatment value was 375.5 ± 127.5 mg/dl, and post-treatment value was 147.5 ± 73.9 mg/dl. Acute reductions ranged from 43 to 73% for LDL-C ($62.77\pm10.27\%$). The chronic reduction ranged from 18 to 52% ($36.36\pm11.74\%$). **Conclusions**: (1) LDL apheresis, combined with lipid-lowering drugs, provides a safe and effective means of improving the prognosis of patients with homozygous FH, especially if started before the age of seven. (2) Because of economic constraints restricted the use of LDL apheresis to the treatment of potentially fatal FH, the most important is to start apheresis instead of choice of the apheretic technique. (3) The frequency of sessions and continuity of treatment are keys of success in management of pediatric patients with FH.

506-A

LONG-TERM EFFECT OF LOW-DENSITY LIPOPROTEIN APHERESIS: EXPERIENCE IN FOUR CHILDREN WITH FAMILIAL HOMOZYGOUS HYPERCHOLESTEROLEMIA

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Heterozygous familial hypercholesterolemia effect one in every 500 persons and is the most common cause of markedly elevated cholesterol levels in children. In children with heterozygous familial hypercholesterolemia, the short-term risk of clinical events is low; therefore, managements starts with stratification of risk, followed by dietary modification, and in high-risk cases, pharmacologic treatment initiated after puberty. But children with homozygous familial hypercholesterolemia require expert management with LDL apheresis, high doses of effective statins and cardiologic follow-up. LDL apheresis has been widely accepted as an effective treatment for hypercholesterolemic patients who are resistant to drug and conventional therapy such as in case of familial hypercholesterolemia. There are various techniques for performing LDL apheresis including heparin – induced extracorperal LDL precipitation, specific immunoadsorption, double membran filtration, dextran sulphate adsorption (liposorber) and direct adsorption of lipoproteins. The purpose of the present study was to clarify the efficacy and safety of LDL apheresis in children with familial hypercholesterolemia. Four girls aged between 12 and 16.2 years old with familial hypercholesterolemia who were highly resistant to dietary regimens and to drug therapy were treated with double membran filtration and direct adsorption of lipoproteins once every 2 weeks. Duration of treatment was beetwen 8 and 25 months. One patient who received direct adsorption of lipoprotein (DALI) had severe anaphylactic reaction. Apheresis was effective the remaining 3 patients. In our patients the acute mean LDL cholesterol reduction was $51.2 \pm 4.2\%$. We conclude that DALI and double membran filtration were effective in children with familial hypercholesterolemia.

507-P

A NOVEL NEUTROPENIA SYNDROME WITH TRANSIENT BILIARY DUCTOPENIA, FACIAL DYSMORPHY AND PROGRESSIVE SENSORIMOTOR POLYNEUROPATHY

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This boy was born after a pregnancy of 43 weeks. He developed physiological hyperbilirubinemia up to 15.5 mg/dl. At the age of 9 weeks he was admitted because of persistent jaundice due to cholestasis. Hepatosplenomegaly was present. Laboratory findings included an increase of serum total and conjugated bilirubine, alkaline phosphatases, transaminases and bile salts. PT and APTT were severely disturbed. Serum triglycerides, cholesterol, apo A1, apo B, vitamins A and E were normal. Liver biopsy showed hypoplasia of the intrahepatic bileducts. During the first year of life, clinical and biochemical abnormalities normalized. Liver biopsy at 12 months was normal on light microscopy. In the next years we noticed mild mental retardation and dysmorphic features including high set ears, slight retrognathia and thick lips together with intermittent endotropia and two large cafe-au-lait spots on his back. He developed neutropenia, and mild elevation of unconjugated bilirubine reappeared at the age of 12 years. Blood acylcarnitine abnormalities were mild and aspecific. Electron microscopy of hepatocytes at 13 years showed a cystically dilated rough endoplasmic reticulum, a prominent Golgi apparatus with large vesicles filled with VLDL droplets and pleiomorphic mitochondria. He developed hyporeflexia of the lower extremities with high arched feet and hammer toes. EMG and nerve conduction velocities showed axonal and demyelinating sensorimotor polyneuropathy. Brain MRI was normal.

Conclusion: This seems to be a new neutropenia syndrome, different from the known neutropenia syndromes such as Barth syndrome, Cohen syndrome, GSD-Ib and Shwachman-Diamond syndrome. We hypothesize an underlying defect in lipid metabolism.

508-P

IDENTIFICATION OF A NEW CASE OF CREATINE TRANSPORT DEFECT BY DETERMINING URINE CREATINE AND GUANIDINOACETIC ACID IN CHILDREN WITH SUSPECTED METABOLIC DISORDER

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Background: Defects in the biosynthesis and cerebral transport of creatine (CR) may be underdiagnosed in our paediatric population due to the unspecific clinical symptoms.
Objective: In order to detect these defects in a cohort of patients with suspected metabolic disorder, a prospective screening measuring CR and guanidinoacetic acid (GAA) levels in urine was undertaken. Biochemical and molecular studies were also performed.
Methods: Electrospray tandem mass spectrometry (MS/MS) has been used to quantify both metabolites. Persistent increased creatine/creatinine (CR/CRN) ratio (>1.5) or abnormal GAA levels (less than 11 or higher than 300 mmol/mol creatinine) were further investigated. A method using [14C] creatine uptake at physiologic concentrations was developed in fibroblasts. Molecular analysis of creatine metabolism related genes (*SLC6A8* and *GAMT-1*) was implemented.
Results: We selected a four year-old boy with persistent high CR/CRN ratio (>2), presenting slight psychomotor and speech retardation with normal karyotype, EEG and brain MRI. An impaired deficiency in the specific high-affinity creatine uptake was confirmed in patient's fibroblasts. The mutational analysis of the *SLC6A8* gene led to identify the new sequence variation c.1210G>C in exon 8, which predictable effect would be the missense probably disease-causing mutation (p. A404P), affecting to a conserved transmembrane domain.
Conclusions: The identification of a new CR transport patient with a mild neurological picture by measuring urinary CR levels highlights the importance of screening for these disorders.

509-P

CREATINE TRANSPORTER DEFICIENCY: PREVALENCE AMONG PATIENTS WITH MENTAL RETARDATION AND PITFALLS IN METABOLITE SCREENING

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Creatine transporter (CT) defect is the most frequent cause of creatine deficiency syndromes. The hallmark for CT defect is an increase in creatine/creatinine (Cr/Crn) ratio in urine, that might be influenced by several external factors. We aimed to determine those factors, as well as the prevalence of CDS in mentally retarded patients.

We have searched for CDS in 1600 cases with mental retardation, autism and/or epilepsy, of which 2.1% showed an increased Cr/Crn ratio in urine, suggesting a CT deficiency. However, a definite diagnosis was only established in 4 patients (0.25%), while in 21 cases the Cr/Crn ratio normalised in the second or third urine sample, and/or Cr uptake in fibroblasts and H-MRS studies showed normal results. To assess the dietary influence in Cr/Crn ratio, we analyzed 65 first-morning urine samples from thirteen healthy volunteers eating different kind of foods.
Results showed only an increased ratio after eating oily fish and beef. Therefore, in case of an increased ratio, care should be taken with meals rich in Cr and in case of an increased urinary Cr/Crn ratio, the results should be confirmed in a second sample of urine under controlled diet, followed by brain H-MRS, Cr uptake studies and/or molecular analysis of the *SLC6A8* gene.

510-P

CREATINE PRECURSORS STIMULATE CR SYNTHESIS IN CT1 DEFICIENT LYMPHOBLASTS

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Background: Creatine (Cr) transporter (CT1) defect is the most frequent disorder of Cr metabolism. Like the defects of Cr synthesis (due to the failure of AGAT or GAMT enzymes) it results in brain Cr depletion, that, however, cannot be restored by exogenous Cr supply. According to the existence of Cr synthetic enzymes inside different tissues, an alternative approach might consist in the stimulation of the endogenous synthesis of Cr by increasing the availability of its precursors.
Objectives: We performed an *in vitro* study aimed at verifying: (a) the use of lymphoblasts as a tool to study the CT1 defect and (b) the effects of the Cr precursors, L-glycine (Gly) and L-arginine (Arg), on Cr synthesis in these cell lines.
Methods: Control and CT1 deficient cells were incubated: (a) for 24 h with Cr concentrations ranging from 25 to 500 μM in either the absence or the presence of 500 microM of 3-guanidinopropionic acid (GPA), and (b) for 8, 24, 30 h in media containing rising concentrations of Arg and Arg+Gly 2.5 to 20 mM.
Results: (a) CrT activity was detectable in lymphoblasts; (b) substrate enrichment with Arg and Arg+Gly (5 to 15 mM) increased the Cr synthesis in affected as well as in control lymphoblasts.
Conclusions: These in vitro results demonstrate that Cr uptake mediated by CT1 is present in lymphoblasts, and support the hypothesis that Cr synthesis can be stimulated by increasing the availability of its precursors. The viability of this approach *in vivo* remains to be explored.

511-O

EXPERIMENTAL MODEL TO PROVE THE PATHOGENIC NATURE OF *GAMT* MISSENSE MUTATIONS

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Guanidinoacetate methyltransferase (GAMT) deficiency is an inherited neurometabolic disorder of creatine biosynthesis. The *GAMT* gene is 4.46 kb in size and it contains six exons, which encode a protein of 237 aminoacids. Currently, there are 7 missense mutations/variations described throughout the gene, The diagnosis of GAMT deficiency can be ascertained by cerebral creatine deficiency, increased levels of guanidinoacetate, impaired GAMT activity in cultured cells and pathogenic mutations in the *GAMT* gene.

In case of novel missense mutations it is essential to prove the pathogenic nature of the mutation. Hereto, we set up an experimental model in which this can be tested. Transfection studies were performed where both, the *GAMT* open reading frame (ORF) containing one of the mutations (c.59G>C; p.Trp20Ser) as well as the wild-type *GAMT* ORF, were transfected into primary GAMT-deficient fibroblasts (c.59G>C, p.Trp20Ser) or HeLa cells. The expression of the GAMT-EGFP fusion protein was analyzed by Western blot confirming its presence. Subsquently, GAMT activity was performed using stable isotope labeled substrates. In contrast to wildtype transfectants, GAMT-deficient fibroblasts/HeLa cells transfected with *GAMT* ORF containing the c.59G>C; p.Trp20Ser did not result in increased GAMT activity, proving the pathogenic nature of the mutation. These results provide an experimental set up that can be used to ascertain if the missense mutations are pathogenic, which is of pivotal importance in case of first-trimester prenatal diagnosis. Currently, both reported and novel missense mutations are being tested.

512-O

THE DISSOCIATED EXPRESSION OF *AGAT*, *GAMT* AND *CT1* IN THE CENTRAL NERVOUS SYSTEM SUGGESTS THE TRANSPORT OF GUANIDINOACETATE BETWEEN BRAIN CELLS FOR CREATINE SYNTHESIS TO OCCUR

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The lack of creatine transporter (CT1) in astrocytes makes the import of creatine from blood inefficient in CNS, which relies more on endogenous creatine synthesis through AGAT and GAMT expression. This seems contradictory with CT1 deficiency, which, despite AGAT and GAMT expression, leads to creatine lack in CNS. To elucidate this, our aim was to finely dissect the cell-to-cell (co-)expression of AGAT, GAMT and CT1 in CNS.

AGAT, GAMT and CT1 (co-)expression was analyzed by combining in situ hybridization and immunohistochemistry. The proportions of cells expressing AGAT, GAMT, CT1, AGAT+GAMT, AGAT+CT1, GAMT+CT1, AGAT+GAMT+CT1, or none, were calculated in various regions of the rat brain (cortex, caudate putamen, hippocampus, hypothalamus, inferior colliculus, pons, cerebellum).

In most structures, cells co-expressing AGAT+GAMT, equipped to self-synthesize creatine, were <20%. Cells co-expressing GAMT+CT1 were also <20%. In whole CNS, 30–50% of cells did not express AGAT nor GAMT, and only 2–15% express CT1 alone. In cortex and caudate putamen, very few cells seemed able of their own creatine synthesis, in agreement with the creatine lack observed by MRS in CT1-deficient patients.

Our work suggests that to allow CNS synthesis of creatine, guanidinoacetate must be transported from AGAT- to GAMT-expressing cells possibly through CT1, thus explaining why CT1-deficient patients lack creatine in CNS. Moreover, high proportion of cells with no expression of AGAT, GAMT and CT1, and low proportion of cells expressing CT1 alone, suggest that brain cells express AGAT, GAMT and CT1 on demand to timely adapt their creatine needs.

513-P

SUCCESSFUL TREATMENT OF A GUANIDINOACETATE METHYLTRANSFERASE DEFICIENT PATIENT: FINDINGS WITH RELEVANCE TO TREATMENT STRATEGY AND PATHOPHYSIOLOGY

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Objective: To describe the clinical course of a patient with guanidinoacetate methyltransferase deficiency (GAMTD) before and after the start of treatment, which resulted in a very favorable outcome, and to discuss the findings in this patient in relation to treatment strategy and monitoring of treatment in this inborn error of Creatine (Cr) biosynthesis.
Methods: Developmental testing (5×), proton magnetic resonance spectroscopy of the brain (3×), measurement of GAA and Cr concentration in plasma and urine, GAMT enzyme assay, mutation analysis of the *GAMT* gene. Treatment, starting at 3 years and 8 months consisted of Cr supplementation 375 mg/kg/day in the first 14 months with addition of ornithine (Orn) supplementation up to 800 mg/kg/day thereafter.
Results: After the start of Cr treatment developmental abilities improved gradually from quotient scores of 50 to 68, while there was partial normalization of plasma, urine and cerebral Cr and GAA. After addition of high dose Orn IQ further improved to 78, plasma and urine GAA and Cr further improved, but brain GAA and Cr remained comparable
Conclusion: The favorable outcome may be due to the early start of treatment. At this moment, the combination of Cr and Orn supplementation might be a good start for initial treatment of GAMTD. Further strategies to further normalize metabolites in plasma and the cerebral compartment should be developed.

514-A

DON'T FORGET MENKES' DISEASE IN THE DIFFERENTIAL DIAGNOSIS OF NON-ACCIDENTAL INJURY PRESENTING WITH SUBDURAL FLUID COLLECTION AND FRACTURES

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Introduction: Menkes disease is a rare X-linked disorder resulting from a defect in copper metabolism. Tortuosity of the cervical and intracranial vessels leads to sudural hematoma and effusion. Clinical features include seizure, developmental delay, and hair changes. Fracture with callus formation is not uncommon in Menkes disease. Diagnosis is often difficult to establish early in childhood. Some patients may benefit from early treatment with parenteral copper-histidine.
Aims: To report a case of Menkes disease who had subdural effusion and radiological evidence of previous fracture mimicking non-accidental injury.
Case report: A male infant born to consanguineous parents. Pregnancy and delivery were uneventful. He developed intractable seizures at 6 weeks of age. Clinical examination revealed macrocephaly, gray iris, absent eyebrows, divergent squint, sparse, thin, hypo pigmented, and kinky hair. MRI brain showed massive bilateral subdural fluid collection. Skeletal survey revealed perosteal reaction and callus formation with evidence of old fracture of the left and right humerus, which raised the question of non-accidental injury. Family and social history disputed child abuse. Serum copper and cerloplasmin were persistently low.

The clinical features, biochemical and radiological findings were consistent with Menkes disease. Subdural drains were inserted, however fluid re-accumulated.
Conclusion: Menkes disease may be difficult to differentiate from non-accidental injury especially when subdural effusion and fractures are present. High index of suspicion is needed to out rule Menkes disease.

515-A

DIAGNOSIS IN WILSON DISEASE

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Wilson disease (WD), or hepatolenticular degeneration, is an autosomal recessive disorder of copper metabolism caused by *ATP7B* gene mutation. The clinical manifestation of copper accumulation produced by enzyme deficiency usually present after birth, but occurred before 5 years old. Patients with WD most often present with either progressive liver degeneration liver degeneration or neurological symptoms, or both. Three major clinical patterns of live disease in WD are hepatic cirrhosis, chronic active hepatitis and fulminant hepatic failure. Neurological manifestations are bradykinesia, rigidity, tremor and dyskinesa. Copper-transporting P-type *ATP7B* gene has been identified as a detective gene in WD and WD locus consisting of 21 exons. Seven missnes mutation including (R778L, C656X, G943D, V1140A, V1106I, V1216M) and 1384del 17 bp. PCR-SSCP, PCR-RFLP and direct sequencing were used for genetic diagnosis. R778L is the most common mutation of *ATP7B* gene in Wilson disease. There is a correlation between R778L and hepatic manifestations in WD patient. Genetic diagnosis for Wilson is available in Iran.

516-P

ACUTE PSYCHIATRIC SIGNS IN WILSON DISEASE CAUSED BY ZINC ACETATE OVERDOSAGE

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Wilson disease (WD) is an autosomal recessive inborn error of copper metabolism that leads to a toxic copper accumulation with hepatic, neurologic, psychiatric and haemolytic disorders.

We report a boy with neurological WD: this presentation is rare in childhood. He was born from non consanguineous parents, walking alone at 18 months. At 11 years he had an acute haemolytic crisis. Since he was 14 years he showed dysarthria, stammering, attentive disorders, akinetic rigidity, gait disturbancy, extrapyramidal parkinsonism, intentional tremors. At 15 years WD was diagnosed: urinary copper 576 mcg/24 h (20–50), serum ceruloplasmin <1.81 mg/dl (22–61), normal transaminases; Kayser-Fleischer ring was present. Brain MRI showed involvement of basal ganglia, midbrain, temporal areas and brainstem; decreased NAA and Cho in MRS. Molecular analysis *ATP7B* gene showed N1332K/T997M mutations.

He started chelating therapy with trientine for 8 weeks and with zinc acetate (ZA, Galzin) 25 mg $\times$ 3/day (59 kg) with clinical improvement. According to pharmacinetic indications at 16 years (61 kg) we increased the ZA to 50 mg $\times$ 3/day. After 3 months he showed state of anxiety, insomnia and severe behavioural abnormalities. Brain MRI showed new multiple hyperintense lesions in frontal subcortical regions and urinary copper was very low (36 mcg/24 h). The decrease of dosage of ZA leads to clinical and neuroimaging improvement. We conclude that the overdosage of ZA can cause neurological involvement explained by copper deficiency with impaired activity of copper enzymes (i.e. COX).

517-P

ANALYSIS OF THE *ATP7B* GENE IN WD PATIENTS FROM BELARUS

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Introduction: Wilson disease (WD) is an autosomal recessive disorder of copper metabolism. The disease is conditioned by the damage in the *ATP7B* gene. More than 200 mutations of the *ATP7B* have been identified and spectrum of mutations appeared to be population specific.
Objectives: To investigate the mutations of *ATP7B* gene and genotype-phenotype correlations in a cohort of Byelorussian WD patients.
Methods: DNA samples from 37 WD patients from 32 unrelated families, their relatives and sibs (51 is the total number) were investigated for *ATP7B* gene mutations. Nested PCR-amplification method and restriction endonuclease digestion of the PCR product was used to detect the H1069G mutation. A direct sequencing of 8 and 15 exons was performed.
Results and **Conclusions**: 66% of all mutant alleles were identified. The frequency of H1069G in Byelorussian WD patients was 55%. In two family cases of WD the 2299insC was detected as the second mutant allele. Two patients were heterozygous for I1102T and one individual was heterozygous for 3400delC. Substitution C→A in codon 745 was detected in two specimens. The C→A transition is supposed to be a novel mutation, since it produces amino acid substitution in the third transmembrane domen of Cu-ATPase. The H1069G mutation dominated in patients with liver disease, 14 of them revealed to be homozygous and 15 – compound heterozygous for H1069G. 4 patients died from acute fulminant hepatitis were homozygous for the H1069G. Our study provides possibility of early presymptomatic diagnosis, carrier detection and prenatal DNA diagnosis in Byelorussian WD patients.

518-P

MICROARRAY TECHNOLOGY – A NEW APPROACH FOR DETECTION OF MUTATIONS IN ATP7B GENE IN PATIENTS WITH WILSON DISEASE

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Background: Wilson disease is an autosomal recessive inherited disorder of copper metabolism which is caused by the mutations in *ATP7B* gene. Molecular diagnostics of Wilson disease utilizes mainly restriction enzyme digestion, MLPA or a direct sequencing of *ATP7B* gene. Nowadays, the significance of microarray technology in molecular biology is indisputable for its advantages such as reduction of time and material of analyses.
Methods: We have analysed total of 100 patients with Wilson disease from Czech Republic by APEX (arrayed primer extension) technology. APEX consists in incorporation of fluorescently labeled ddNTPs to the 3′ end of sense/antisense probes spotted on chip which hybridize with complementary fragments of analyzed DNA sample. According to which ddNTP is linked to each DNA probe, it is possible to detect sequence variants in hetero- or homozygote form.
Results: In our laboratory, we have developed the Wilson chip for detection of 87 mutations and 17 polymorphisms in *ATP7B* gene, which represent the most common reported sequence variants across population groups. Up to date, we have detected 39 mutations and 17 polymorphisms correctly in comparison with direct sequencing in all tested DNA samples. All mutations were observed either from both (sense/antisense) directions or just from one strand. We have obtained the similar results for polymorphisms.
Conclusion: The establishment of the Wilson chip could speed up and facilitate molecular diagnostics of Wilson disease in future.

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519-O

MOLYBDENUM COFACTOR DEFIENCY: DRUG PRODUCTION AND THERAPY

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Molybdenum cofactor (Moco) deficiency is a rare (orphan) autosomal-recessive metabolic disease and results in progressive neurological damage leading to early childhood death. The main cause of neurotoxic symptoms is the cytotoxicity of sulfite, which accumulates in these patients due to the loss of hepatic sulfite oxidase activity, one out of three molybdenum-dependent enzymes in humans. The majority of patients with Moco deficiency belong to group A that have a defect in the first step of the biosynthesis of Moco, the conversion of GTP to the intermediate cyclic pyranopterin monophosphate (cPMP). This defect is caused by mutations in the *MOCS1* gene, which was simulated in knockout mice. *MOCS1*-deficient mice show a maximum lifespan of 12 days and display all biochemical characteristics observed in human patients. We have developed a procedure for biotechnological fermentation and subsequent purification of cPMP, the missing metabolite in *MOCS1*-deficient mice and human patients. Subsequently, purified cPMP was used to cure the lethal symptoms in *MOCS1*-deficient mice. If started shortly after birth, this treatment neutralizes the disease phenotype completely and results in healthy and fertile animals. The described treatment provides the basis for the first effective means against human Moco deficiency. Currently, large amounts of cPMP are generated to ensure sufficient quantities for first clinical trials. Not only intrahepatic but also oral application of the drug has been proven. Due to the low number of patients and high number of possible gene products that could cause Moco-deficiency a more carefully screening for this disease is needed.

520-P

LONG TERM FOLLOW-UP OF A PATIENT WITH PRIMARY HYPOMAGNESAEMIA AND SECONDARY HYPOCALCAEMIA DUE TO A *TRPM6* MUTATION

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Hypomagnesaemia with secondary hypocalcaemia (HSH), also known as primary infantile hypomagnesaemia (PIH), is a rare condition of autosomal recessive inheritance in which lack of this cationic bioelement is due to defective absorption through intestinal epithelium, and with accompanying renal wasting of Mg^{2+}. It is characterized by extremely low levels of serum magnesium associated with symptomatic hypocalcaemia. It usually presents during the newborn period or first years of life, with seizures and delay of physical and psychomotor development, as well as an abnormal bone metabolism, mainly due to secondary hypocalcaemia. We herein report a case with a new *TRPM6* mutation which had not been previously described, and long term follow-up during 10 years.
Case report: A 16-day-old infant girl born to consanguineous parents presented with generalized seizures and hypocalcaemia, refractory to conventional antiepileptic drugs and intravenous calcium. After demonstration of hypomagnesaemia, treatment with magnesium sulphate was started, and seizures ceased without any clinical recurrence during a ten years follow-up. A slight renal magnesium wasting was present despite hypomagnesaemia. Up to now, she has presented normal growth and bone mineral density. Molecular study revealed a homozygous truncating mutation in the *TRMP6* gene that confirmed the clinical diagnosis.
Conclusion: Clinical suspicion is essential for an early diagnosis and to ensure a long term treatment with oral magnesium supplements in order to avoid abnormalities of neurological and physical development. Molecular study of the different types of hereditary hypomagnesaemia may be critical for an accurate diagnosis and to further improve our knowledge of magnesium homeostasis.

521-P

TRMA SYNDROME (THIAMINE-RESPONSIVE MEGALOBLASTIC ANEMIA): A CASE REPORT WITH EARLY DIAGNOSIS

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Thiamine-responsive megaloblastic anemia syndrome (TRMA) is characterized by diabetes mellitus, megaloblastic anemia and sensorineural hearing loss. Mutations in the *SLC19A2* gene, encoding a high-affinity thiamine transporter protein, THTR-1 are responsible for the clinical features associated with syndrome in which treatment with thiamine correct the megaloblastic anemia and diabetes mellitus. We report a female patient with TRMA syndrome which was diagnosed early before development of diabetes mellitus and deafness. A 20-month-old patient presented with vomiting, pallor and petechia when she was at one months of age. Physical examination revealed tachypnea, tachycardia with cardiac failure and hepatomegaly. Laboratory analyses showed bicytopenia with a hemoglobin 6.2 gr/dl, hematocrit 19.7%, mean corpuscular volume 110 ft/lt, white blood cell count 5200/mm^3, platelets 21 000/mm^3. The presence of megaloblastic anemia and death of a sibling guided us to suspicion of high output cardiac failure due to thiamine deficiency. 100 mg intravenous thiamine was given to patient. After administration of thiamine, the levels of Hb and platelets began to elevate. The cardiomegaly and hepatomegaly started to resolve on the fourth day of treatment. Three weeks after thiamine therapy, anemia ameliorated completely. The genetic evaluation showed that the proband was homozygous for a mutation, 242insA in the nucleic acid sequence of exon 2. TRMA is a rare genetic disease which can result with death in a short period if not diagnosed. The possibility of this disease is of great concern in cases which refer with megaloblastic anemia especially in infancy and those with a family history.

522-P

THIAMINE-RESPONSIVE MEGALOBLASTIC ANEMIA (TRMA) ASSOCIATED WITH ATP SYNTHASE DEFICIENCY PRESENTING WITH PANCYTOPENIA AND STROKE IN INFANCY

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Background: TRMA, previously known as Rogers syndrome, is an autosomal recessive disorder characterized by diabetes, anemia and deafness. TRMA results from mutations of the human thiamine transporter-1 protein (THTR1), causing a defective function of thiamine dependent enzymes which are important for intermediate metabolism. **Case Report:** The patient, the first child of healthy consanguineous parents, presented with pancytopenia in the newborn age. Bone marrow examination revealed increased erythropoesis with megaloblastic erythroblasts and ringed sideroblasts. Transfusions of erythrocytes and platelets were necessary. At the age of three months an ischemic infarction of the medial cerebral artery caused first convulsions. Thrombophilia was excluded. Sensorineural deafness and diabetes mellitus manifested in late infancy. Based on a decreased ATP synthase activity in a muscle biopsy, a mitochondrial disorder was assumed. Treatment with vitamins, including thiamine, ameliorated anemia, thrombocytopenia and diabetes, prevented further metabolic strokes but did not prevent macular degeneration and deafness. The diagnosis of TRMA was established after recurrence of anemia and diabetes at the age of 6 years, due to pour compliance and therapy interruption. Homozygocity for the previously described *THTR1* mutation p.G383fsX384 was found. **Conclusions**: The clinical manifestations of TRMA syndrome may resemble respiratory chain disorders probably due to a secondary impairment of energy production. The importance of thiamine for the activity of complex I has been previously reported. Here we report a unique presentation of TRMA with neonatal pancytopenia and stroke already in infancy and we provide evidence that thiamine deficiency affects the function of ATP synthase.

523-P

CEREBROSPINAL FLUID PYRIDOXAL 5′-PHOSPHATE VALUES: REFERENCE VALUES AND RELATION WITH NEUROTRANSMITTERS IN A PAEDIATRIC POPULATION

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Introduction: Pyridoxal 5′-phosphate (PLP) deficiencies have been described to produce a secondary biogenic amines (BA) decrement due to a reduce activity of the enzyme aromatic L-aminoacid decarboxilase. PLP measurement may be useful as biochemical marker for pyridoxine and pyridoxal-phosphate-dependent seizures. Our aim was to establish reference values for cerebrospinal fluid (CSF) PLP in a paediatric population and to correlate them with BA metabolites (5-HIAA and HVA).

Material and methods: For reference values, CSF samples from 141 paediatric controls (age range: 1 day–18 years, average: 3.8 years) were analysed. Cerebrospinal fluid PLP and BA concentrations were analysed by HPLC with fluorescence and electrochemical detection. Cerebrospinal fluid PLP concentration in a patient with a proven mutation in *PNPO* gene was determined.

Results: A negative correlation between CSF PLP values and age of controls was observed ($r = -0.401$; $p < 0.0001$). Reference values were stratified into 3 age groups (Group A (1 day–11 months) PLP = 23–99.06 nmol/L; Group B (1–2 years) PLP = 8.8–59 nmol/L; Group C (3–18 years) PLP = 11.1–39 nmol/L). No correlation was observed in the different age groups between CSF PLP values and BA metabolites. PLP values in a patient with *PNPO* mutation were clearly decreased (PLP = 3.4 nmol/L. Age of the patient 3 days).

Conclusions: According to our data, reference values for CSF PLP should be stratified according to age and no association was observed between PLP values and BA metabolites in CSF. In our first case with PNPO deficiency, CSF PLP values were clearly below the reference values.

524-P

AGE RELATED REFERENCE INTERVALS FOR CSF PYRIDOXAL PHOSPHATE. POTENTIAL UTILISATION FOR THE DIAGNOSIS OF PNPO DEFICIENCY

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Pyridox(am)ine 5′-phosphate oxidase (PNPO) deficiency is an inborn error of metabolism affecting pyridoxal phosphate (PLP) availability. PNPO deficiency is a cause of early onset epileptic encephalopathy and intervention with PLP may be curative (J Inher Metab Dis. 2007;30:96–9). Consequently, identification is important. Whilst CSF analysis can reveal a profile that resembles aromatic amino acid decarboxylase deficiency, this does not appear to be a consistent finding. Similarly, elevations of CSF threonine and glycine may also be indicative. In view of the pivotal role PNPO plays in PLP generation, it seems logical that PLP status should be evaluated in patients with suspected PNPO deficiency. However, generation of appropriate reference intervals are essential before this approach can be tested. Here, we have validated an HPLC method for PLP determination in CSF. To date, we have analysed 23 samples from children aged between 1 month and 14 years. Significantly higher levels of PLP were recorded in samples obtained from 12 children aged 1 month to 2 years when compared to those from 11 individuals aged from 2 to 14 years (58 ± 17 vs 31 ± 5 nmol/L, $p < 0.0001$). A plot of PLP concentration against age also revealed a clear decline with age over the first 2 years. Inclusion, on this plot, of the PLP status of 3 children previously reported by us to have PNPO deficiency (Hum Mol Genet. 2005;14:1077–86) clearly demonstrates low PLP concentrations.

525-P

A NEW FATAL CASE OF PYRIDOX(AM)INE 5′-PHOSPHATE OXIDASE (PNPO) DEFICIENCY

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Introduction: Pyridox(am)ine 5′-phosphate oxidase (PNPO) is involved in the synthesis of pyridoxal 5′-phospate (PLP), which is a cofactor of several enzymes. PNPO deficiency is characterised by neonatal epileptic encephalopathy with severe seizures, unresponsive to anticonvulsant drugs or pyridoxine, but with a dramatic response to PLP. **Results**: We present a patient with epileptic encephalopathy born prematurely at 33 weeks of gestation. Repetitive rhythmic foetal movements had been detected during pregnancy. After birth the boy developed a faltering cry, severe seizures, myoclonus, hepatomegaly, lip-smacking automatism, microcephaly and signs of poor postnatal adaptation. Convulsions were unresponsive to pyridoxine, folinic acid, and biotin. EEG showed characteristic burst-suppression pattern. Biochemical studies showed an increase of vanyl-lactate in urine: 11 mmol/mol creatinine (C.V. undetectable) and of glycine in plasma: 1.116 mM (C.V.189–291), compatible with a deficiency of several PLP-dependent enzymes in this patient. The suspicion of PNPO deficiency was confirmed in CSF by neurotransmitter studies. Treatment with 50 mg PLP/kg/day was started at 23 days of life. However, despite the disappearance of the burst-suppression the clinical condition worsened and the patient died at 48 days of age. Molecular studies revealed a novel homozygous nonsense-mutation p.A174X (c.520C > T) in the *PNPO* gene that causes a premature stop codon and is expected to completely remove enzyme function. **Conclusions**: Diagnostic and therapeutic trial of PLP should not be delayed until the complete biochemical evidence is obtained. It is noteworthy that simple non-invasive tests, such as organic acids and amino acids, may support the clinical suspicion of PNPO deficiency, allowing early treatment.

526-O

THE PHENOTYPIC SPECTRUM OF PYRIDOXINE-DEPENDENT EPILEPSY (PDE) DUE TO ANTIQUITIN DEFICIENCY

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Background: Pyridoxine dependent epilepsy (PDE; OMIM 266100) was historically a clinical diagnosis based on seizure response to pyridoxine administration. Recently, infants with classical PDE, (seizures within the first 28 days) have been shown to carry mutations in the *ALDH7A1* gene encoding antiquitin. Deficiency of this enzyme leads to accumulation of L-α-amino adipic semialdehyde in body fluids including urine. **Methods**: Clinical and biochemical data was collected on patients we have proven to have PDE by demonstrating accumulation of urinary L-α-amino adipic semialdehyde and by mutation analysis. **Results**: Patients with atypical PDE may also have antiquitin mutations. Seizures in some patients are partially responsive to conventional antiepileptic drugs. CNS involvement may encompass malformations and dysfunction in addition to seizures. Systemic effects of PDE are diverse. These may be more slowly responsive to pyridoxine and may obscure the diagnosis particularly as some are themselves epileptogenic e.g. hypocalcaemia, hypomagnesaemia. CSF aminoacid and amine metabolite profiles can be similar to those seen in pyridoxamine phosphate oxidase deficiency. **Conclusions**: The phenotypic spectrum of pyridoxine-dependent epilepsy is wide. We advocate the use of biochemical and DNA investigation for PDE in a wide range of infants and children with epilepsy.

527-P

DYSMORPOLOGY IN PATIENTS WITH PYRIDOXINE-DEPENDENT SEIZURES AND MUTATIONS OF THE ANTIQUITIN (*ALDH7A1*) GENE

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Pyridoxine-dependent seizures are a rare recessively inherited condition due to a defect of alpha-amino adipic semialdehyde (AASA) dehydrogenase (antiquitin) in the cerebral lysine degradation pathway. Clinical diagnosis depends on a successful therapeutic trial with pyridoxine and further proof of pyridoxine after a withdrawal with recurrence of seizures. Atypical forms with late-onset during childhood and episodes of status epilepticus are present. Pipecolic acid and AASA elevations in the plasma, urine and/or CSF have been described as a biochemical marker in this disorder.

We would like to present clinical, biochemical and molecular characteristics of seven patients (2 girls, 5 boys) between the ages of 2 months and 6 years, with pyridoxine dependent seizures. Clinical findings are seizures beginning in the neonatal period followed by afebrile seizures in the infancy. All patients respond to pyridoxine treatment. Three of these patients had elevated pipecolic acid and AASA in the urine and showed mutations of the antiquitin (*ALDH7A1*) gene. Patients had megalencephaly, prominent forehead, flattened nasal bridge, delay in speech and walking, and ataxic gait. In one of the patients there is additional cortical neuronal migration disorder and in the other there is a history of siblings lost with a suspected diagnosis of neonatal adrenoleukodystrophy.

To our knowledge, dysmorphic features were not reported in patients with antiquitin gene mutations before. There may further be genetic heterogeneity, since four of the patients in this cohort do not have dysmorphic features and increased levels of pipecolic acid and AASA.

528-P

GENOTYPING OF PATIENTS WITH PYRIDOXIN-DEPENDENT EPILEPSY (PDE) BY RT-PCR IN CDNA OF LEUKOCYTES IS DISTURBED BY AN ANTIQUITIN PSEUDOGENE

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Background and **Methods**: Patients with pyridoxine dependent epilepsy (PDE, MIM# 266100) present with pyridoxine-responsive seizures and elevated concentrations of pipecolic acid as well as alpha-aminoadipic semialdeyde in urine, plasma and cerebrospinal fluid. It is caused by a deficiency of alpha-aminoadipinic semialdehyd-dehydrogenase (antiquitin; *ALDH7A1*, MIM#107323;). At the gene level a total of 18 pathogenic mutations in 31 patients have recently been described by Mills P et al. Nature Med. 2006;12:307–9) and Plecko B et al. (Hum Mutat. 2007;28:19–26). Among these, we have recently detected four heterozygous patients with a new common transversion of G>T at a highly conserved donor splice site in intron 17 (c.1482-1G>T; 12% of alleles) and a novel A>G transition affecting the acceptor splice site in intron 7 (c.612 2A>G). Further characterization of these alleles by RT-PCR was hampered by the replication of an *ALDH7A1* pseudogene in leukocyte cDNA.

Results: We systematically analyzed preparations of cDNA from leukocytes of normal individuals for specificity of RT-PCR products and found that only the first, amino-terminal fragment of the six replicons covering the entire *ALDH7A1* cDNA contained the *ALDH7A1*-specific sequence gene, while all others were replicated from the pseudogene sequences. In contrast, analogous replicons from fibroblast were all entirely free of pseudogene sequences if processed at an elevated annealing temperature of 64°C.

Conclusion: These findings will be of importance for the future characterization of disease causing mutations of the antiquitin gene.

529-O

DUTCH COHORT WITH α-AMINOADIPIC SEMIALDEHYDE DEHYDROGENASE DEFICIENCY (PYRIDOXINE DEPENDENT EPILEPSY): A FOUNDER EFFECT AND AN INTRIGUING 'SILENT' MUTATION IN ANTIQUITIN (*ALDH7A1*)

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Background: Recently, α-aminoadipic semialdehyde (α-AASA) dehydrogenase deficiency was shown to cause pyridoxine-dependent epilepsy in a considerable number of patients. α-AASA dehydrogenase deficiency is an autosomal recessive disorder characterized by a neonatal onset epileptic encephalopathy in which seizures are resistant to anti-epileptic drugs but respond immediately to the administration of pyridoxine (MIM266100). Increased plasma and urinary levels of α-AASA are associated with pathogenic mutations in the α AASA dehydrogenase (*ALDH7A1*/antiquitin) gene. **Methods**: The *ALDH7A1* gene was sequenced in a panel of 10 Dutch patients (7 families) with biochemically proven α-AASA dehydrogenase deficiency. The primers were designed to amplify the open reading frame (ORF) and splice sites of the *ALDH7A1* mRNA only. **Results**: In 7 Dutch index patients from apparently unrelated families 9 alleles carried the c.1195G>C; p.Glu399Gln mutation suggesting a founder effect. In two Dutch families, a novel 'silent' mutation (c.750G>A) was found, a type of DNA variation that is often considered to be non-pathogenic. Interestingly, this variant proved to be the pathogenic mutation, since it results in erroneous splicing. **Conclusion**: It may be desirable – at least in the Netherlands, but likely in a broader area – first to analyze the DNAs for the presence of mutations in selected exons of *ALDH7A1*, before sequencing the complete ORF. The present study further emphasizes that elevated urinary α-AASA is associated with pathogenic mutations in *ALDH7A1* and thus that α-AASA should be used as a non-invasive pathognomonic marker in diagnostic laboratories.

530-P

IMERSLUND-GRÄSBECK SYNDROME VERSUS NUTRITIONAL COBALAMIN DEFICIENCY IN CHILDREN

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Background: Imerslund-Gräsbeck syndrome (IGS) is a childhood disorder characterised by serum vitamin B12 (cobalamin, Cbl) deficiency resulting in megaloblastic anemia, homocystinuria, methylmalonic aciduria, proteinuria and neurological symptoms. IGS is caused by autosomal recessive mutations in cubilin (*CUBN*) or amnionless (*AMN*). Owing to the metabolic profile and the rapid response to Cbl treatment, IGS can be mistaken for nutritional Cbl deficiency (nCbld). We present the clinical, biochemical and genetic data of two patients with IGS in comparison with those of four patients with nCbld.
Results: Neurological symptoms and macrocytosis were present in all patients, except for IGS case 2 with microcytosis owing to thalassaemia minor. Low serum Cbl (<74 pmol/L in IGS vs. 88–180 pmol/L in nCbld), methylmalonic aciduria (1041 mmol/L vs. 120–1601 mmol/L), and elevated plasma as well as urine homocysteine (72 μmol/L vs. 30–159 μmol/L and 490 μmol/g creatinin vs. 100–1890 μmol/g, respectively) were always present. Proteinuria was present and the Schilling test was indicative for IGS. Genetic studies revealed that IGS case 1 is compound heterozygous for two *CUBN* mutations (nonsense L479X and a large deletion) and IGS case 2 carries a homozygous mutation in the *AMN* gene (c.208-2A>G, skipping exon 4). Patients with nCbld were breast-fed infants of Cbl deficient mothers (one vegan and three with antibody positive-intrinsic factor deficiency).
Conclusion: Differential diagnosis of Cbl malabsorption and malnutrition can be difficult based on clinical and biochemical data alone. Genetic analysis of the *CUBN* and *AMN* genes offers a definitive diagnosis in paediatric cases of suspected IGS.

531-P

MATERNAL AND UMBILICAL COBALAMIN AND METHYLMALONIC ACID CONCENTRATIONS AND THEIR RELATION TO BIRTH WEIGHT

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Background: Cobalamin is essential for the conversion of methylmalonyl-CoA to succinyl-CoA and for the conversion of homocysteine to methionine. Cobalamin deficiency leads to an accumulation of methylmalonic acid (MMA) and total homocysteine (tHcy). Adequate intracellular cobalamin concentrations are vital for cellular function in general and for the central nervous system in particular. **Aim**: to study whether a relation between maternal and umbilical cobalamin and/or MMA concentrations was present and to investigate the possible relation between maternal MMA and cobalamin and birth weight of the child. **Methods**: Maternal blood was drawn between 30–34 weeks of gestational age. Umbilical cord blood was drawn immediately after delivery of the child. Birth weights were collected from delivery charts. **Results**: From 386 mothers and their 406 newborn babies blood was drawn. The geometrical mean of maternal MMA was 0.17 (0.16–0.18) μmol/L compared to 0.31 (0.30–0.33) μmol/L in umbilical cord blood ($p<0.0001$). Geometrical mean of plasma cobalamin was 174 pmol/L (120–252) for the mothers and 232 pmol/L (134–402) in umbilical cord blood ($p<0.0001$)). Significant correlations between maternal and umbilical MMA values and maternal and umbilical cobalamin concentrations were found ($r = 0.566$, $p<0.0001$ and $r = 0.546$, $p<0.0001$ respectively). Both variables did not show a significant relation with birth weight. In our study population, neither maternal cobalamin nor maternal MMA concentrations seem to be related to birth weight. Strong correlations between maternal and umbilical cobalamin and MMA values exist, suggesting the importance of maternal vitamin B12 status for the child.

532-O

IDENTIFICATION OF THE GENE RESPONSIBLE FOR THE cbl D DEFECT OF VITAMIN B12 METABOLISM: *MMADHC*, ONE GENE AND THREE PHENOTYPES

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Background: Vitamin B12 (cobalamin) is increasingly linked to common diseases such as vascular disease, dementia, Alzheimer's disease and cognitive dysfunction. Cobalamin is converted to two coenzymes, adenosylcobalamin in mitochondria and methylcobalamin in the cytoplasm. These are essential for methylmalonic acid and homocysteine homeostasis respectively. Several steps in intracellular metabolism have been defined as complementation groups cbl A/B/C/D/E/F/G/. The cbl D defect of cobalamin metabolism (OMIM 277410) causes combined or isolated methylmalonic aciduria (MMA) and homocystinuria (HC). Multisystemic clinical abnormalities including developmental, hematological, neurological and metabolic abnormalities are found in affected individuals. **Methods**: Microcell-mediated chromosome transfer and refined genetic mapping were used to locate the exact cbl D locus. Sequencing and functional analyses were performed to determine how mutations of the cbl D gene (*MMADHC*) cause the three variant forms of the cblD defect. **Results**: cbl D-MMA patients have truncations in the N-terminal region with reinitiation of translation preserving methylcobalamin synthesis; cblD-HC patients have missense mutations in the C-terminal region; cblD-MMA/HC patients have truncating mutations downstream of an alternative start codon. Transfection of wild-type MMADHC into patient cells corrected the cellular phenotype. The MMADHC protein shares sequence homology with an ABC transporter found in bacteria and contains a putative motif for cobalamin binding. **Conclusions**: We have identified the gene responsible for the cbl D defect. It is a unique example of one gene with three biochemical phenotypes involving pathways in different cellular compartments. Our findings suggest the existence of a novel mechanism for the channelling of cobalamin to cytosolic and mitochondrial targets in mammals.

533-P

EXPRESSION OF WILD-TYPE AND MUTANT cbl D-cDNA IN FIBROBLASTS OF THE THREE TYPES OF THE cbl D-DEFECT OF VITAMIN B12 METABOLISM

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Background: Vitamin B12 (cobalamin, cbl) in mammals is converted intracellularly to two coenzymes, adenosylcobalamin (AdoCbl; mitochondrial) and methylcobalamin (MeCbl; cytosolic). The cbl D defect is caused by mutations of the *MMADHC* gene and uniquely presents with 3 biochemical phenotypes: (1) cblD-MMA/HC (MMA = methylmalonic aciduria; HC = homocystinuria) with combined deficiency of AdoCbl and MeCbl synthesis, associated with C-terminal truncating mutations, (2) cblD-HC with isolated deficiency of MeCbl synthesis, associated with C-terminal missense mutations, and (3) cblD-MMA with isolated deficiency of AdoCbl synthesis, associated with N-terminal truncations and reinitiation of translation preserving MeCbl synthesis.
Methods: Transient or stable transfection of mutant and/or wt cblD-cDNA into immortalized cblD fibroblasts and determination of cellular function by measurement of AdoCbl and MeCbl synthesis.
Results: MeCbl synthesis was normalized in cblD-MMA/HC and cblD-HC cells by wt-cDNA and a cblD-MMA deletion transcript, but not by cblD-HC missense mutations. In contrast, there was no or little correction of AdoCbl synthesis with wt-cDNA after transient transfection using two vectors (pTracer, pcDNA3) or after stable transfection. However AdoCbl synthesis was corrected by wt-cDNA in the pcDNA3.2/V5 vector in cblD-MMA/HC cells but not in cblD-MMA cells. Also a cblD-MMA truncating mutation transcript abolished correction of AdoCbl synthesis when co-transfected with wt-cDNA in cblD-MMA/HC cells.
Conclusions: We show that transfection with wild type *MMADHC* corrects the cbl D defect and demonstrate functional significance of mutant alleles. The lack of consistent correction of the mitochondrial defect reflects the complexity of interaction of the cblD-protein with cytosolic and mitochondrial targets in this unique disorder.

534-P

THE COBALAMIN F DEFECT: A RARE DISEASE OF THE LYSOSOME

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In the cobalamin F (cblF) defect, transcobalamin bound cobalamin is internalized into the lysosome, but transport of free cobalamin into the cytoplasm is blocked. So far, only five patients have been reported.

Our male patient is the first child of unrelated parents. At thirteen days of age, he presented with feeding difficulties and glossitis. Propionylcarnitine (7.0 μmol/L, controls <5 μmol/L) and homocysteine in plasma (27.9 mmol/L) were increased, and urinary methylmalonic acid was elevated (2310 mmol/mol creatinine). Cobalamin was not detectable in the patient's plasma, whereas maternal plasma cobalamin levels were normal. The Schilling test revealed a markedly reduced excretion of the applied 57Co-labelled cyanocobalamin in the urine ($<1\%$, controls >8–10%). Intrinsic factor did not increase the excretion of the tracer. Serum transcobalamin levels were normal. Fibroblast studies revealed a normal uptake of cobalamin but deficient production of methyl- and adenosylcobalamin. CblC and cblD mutant cell lines, but not cblF cell lines complemented the patient's defect, indicating the cblF defect. During treatment with subcutaneous hydroxycobalamin (2.5 mg per week, gradually tapered to 2.5 mg twice a month), laboratory values gradually normalized. Now, at the age of two years, the patient's psychomotor developmental status is normal, but he presents with short stature (10 cm below the 3rd percentile).

In the cblF defect, cobalamin is trapped in the lysosome. This might affect the transport of cobalamin across the placenta and the terminal ileum. Studies to identify the molecular basis of the cblF defect are under way.

535-P

Call for Collaborators

GENOME WIDE LINKAGE FOR COBALAMIN F DISEASE

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In the cobalamin F (cblF) defect, intracellular cobalamin is not released from the lysosome. The defect is most likely due to a putative lysosomal transport protein. Affected patients present with symptoms of cobalamin deficiency. The cblF defect can be identified by complementation analyses in cultured fibroblasts. In the literature, only few cases of this rare disease have been previously reported.

We have collected DNA material from cblF patients in an effort to build up a resource for linkage analysis to identify the underlying disease causing gene. Genome wide linkage analysis will be performed applying 250K Affymetrix Chips in the affected patients and their families.

We are looking for collaborators, who would like to share their patient material for this study.

536-P

URINE SCREENING STRATEGIES FOR 2-AMINOADIPIC SEMIALDEHYDE DEHYDROGENASE DEFICIENCY

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Background: Deficiency of 2-aminoadipic semialdehyde dehydrogenase (antiquitin) has recently been described as a cause of pyridoxine dependent epilepsy (PDE). The main diagnostic metabolites are pipecolate, 2-aminoadipic semialdehyde (AASA) and its equilibrium product, piperideine-6-carboxylate (P6C).
Methods: Two novel urine screening tests have been developed. The first uses gas chromatography-mass spectrometry (GC-MS) of ethoxime/propyl chloroformate derivatives to simultaneously measure AASA and pipecolate. The second uses electrospray tandem mass spectrometry of butyl derivatives to detect P6C and related products. We have investigated metabolite excretion in controls and patients with varying degrees of clinical suspicion of having PDE.
Results: The GC-MS method had sufficient sensitivity to detect AASA in controls. Ten patients with increased AASA were detected, all being clinically classified as 'probable' or 'definite' PDE. Pathogenic mutations have been identified in three out of three of these patients investigated to date. Pipecolate was normal in nine patients who were being treated with pyridoxine and increased in one patient who was sampled before pyridoxine treatment commenced. All ten also had increased P6C products as determined by the tandem mass spectrometry method.
Conclusions: These GC-MS and electrospray tandem mass spectrometry methods can be used in the diagnosis of PDE. The GC-MS method is suitable for small-scale batch processing of samples with a high index of suspicion. The tandem mass spectrometry method is suitable for the rapid screening of larger numbers of samples and can be carried out simultaneously with testing for a wide range of other inborn errors of metabolism.

537-P

SERUM URIC ACID LEVELS AND SEQUENCE VARIANTS OF METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) GENE

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Background. Recent data suggest that an elevated serum uric acid (UA) levels may play a significant role in the development of cardiovascular, renal disorders, and of metabolic syndrome. We have analysed biochemical, anthropometric, and genetic determinants of physiological UA levels including the *MTHFR* sequence variants. **Methods**. The linear generalized ANOVA regression approach was used to identify the structure of statistically significant latent and covariate influence factors with fixed effects, where logarithm of UA figured as dependent variable. **Results**. Factor analysis and regression model identified four statistically significant clusters determining UA levels: anthropometric (sex, age, BMI, W/H), renal (cystatine, creatinine), metabolic syndrome (triglycerides, HDL-cholesterol, blood glucose, blood pressure, GMT) and homocysteine metabolism (cysteine, homocysteine, folic acid, B12, B6, MTHFR c.1298A>C). We have found association between c.1298A>C *MTHFR* sequence variants and reduced serum UA levels in women. The mean UA levels for the A/A (135 cases), A/C (127 cases) and C/C genotype (36 cases) were 235 ± 4.6, 243 ± 5.7 and 214 ± 8.9 μmol/l ($p = 0.039$), respectively. We did not corroborate the statistically significant correlation between the c.677T>C MTHFR and increased serum UA. **Conclusions**. Four most significant components of factor analysis interpret 52% of variance UA levels. Our results did not confirm the role of common mutation c.677T>C in *MTHFR* gene as a risk factor for hyperuricemia. In contrast the c.1298A>C *MTHFR* sequence variant was associated with reduced UA level. Further studies are needed to assess the role of the *MTHFR* polymorphisms and serum uric acid levels.

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538-P

SEVERE NEUROLOGICAL SYMPTOMS CONCEAL INHERITABLE DISORDER CAUSED BY PARTIAL DEFICIENCY OF HYPOXANTHINE-GUANINE PHOSPHORIBOSYLTRANSFERASE AS LESCH NYHAN SYNDROME VARIANT – THE FIRST CASE DETECTED IN LITHUANIA

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Lesch-Nyhan syndrome is a rare, X-linked recessive inheritable disorder caused by a deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase (HPRT). *HPRT* gene has been mapped to Xq26-q27 and more than 200 mutations responsible for this disease have been characterized. Depending on the amount of residual enzyme activity the spectrum of the disease is wide: from isolated hyperuricemia and gout, to hyperuricemia with profound neurobehavioral dysfunction. Diagnosis could be made according to clinical symptoms, biochemical blood and urine test results, enzyme activity and molecular genetic testing.

We present a one year and eight months old boy with severe neurological symptoms and mild dysmorphism of phenotype: macrocephaly, thin upper lip and cryptorchism. The patient is the first child of healthy non consanguineous parents with uncomplicated genealogy of family. His development was normal till first five months. Later, muscle hypotonia with psychomotoric development delay and indifference to pain were observed. Clinical follow-up showed symptoms of frontal lobes atrophy and internal hydrocephaly (CT), kidney ultrasound results without pathology.

Laboratory investigations revealed increased serum uric acid, 0.5 mmol/l (ref 0.13–0.23 mmol/L) and urinary uric acid, 6.3 mmol/mmol crea (ref <2.1 mmol/mmol crea). Moreover, increased amounts of urinary hypoxanthine, xanthine and inosine (195; 109; 10 μmol/mmol creat. were found, respectively. Subsequently severely decreased HPRT activity – 0.10 μmol/mmol Hb/hr (ref 0.92–4.37) was detected in lysed erythrocytes. This led to diagnosis of HPRT deficiency. Allopurinol therapy is started from the first week after confirmation of the diagnosis. Mutation analysis will be performed in the nearest future.

539-P

DYSREGULATION OF DOPAMINERGIC MARKERS IN PERIPHERAL CELLS FROM LESCH-NYHAN DISEASE PATIENTS

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Background: Lesch-Nyhan disease (LND) is an X-linked recessive disorder caused by mutations in the gene encoding the purine salvage enzyme, hypoxanthine-guanine phosphoribosyltransferase (*HPRT*). LND patients have an over-production of uric acid and characteristic neurological problems including mental retardation, motor disability and self injurious behaviour. Recent studies show that LND is associated with loss of striatal dopamine. Several authors suggest that platelets could be a good synaptic model and that they express many dopaminergic markers. Also, previous studies on other diseases such as Parkinson's disease use peripheral blood lymphocytes to show differential expression of dopaminergic markers.
Objectives/Methods: The present study evaluates the expression of a large number of dopaminergic markers by quantitative PCR in peripheral blood lymphocytes in four LND patients compared to four healthy volunteers. We also studied several genes involved in the purine metabolism.
Results: As expected, *HPRT* expression was significantly decreased in lymphocytes from LND patients. Interestingly, the expression of other purine metabolism genes such as *APRT*, *ADSL*, *PRPS1* and *PRPS2* were also significantly decreased. Several genes involved in the dopaminergic system were significantly up-regulated in LND lymphocytes compared to controls: *DAT*, *Nurr1*. A significant down regulation was observed for *COMT*, *parkin* and *POLG*. *GCH1* and *MAOB* expression were variable depending on the LND patient.
Conclusion: These results suggest that peripheral blood cells could be a good model for studying dopaminergic modifications in LND and could help elucidate the mechanisms that lead to their neurological problems and self injurious behaviour.

540-P

DIAGNOSTIC APPROACH TO ADENOSINE DEAMINASE DEFICIENCY

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Backround: Adenosine deaminase (ADA) deficiency is a rare, autosomal recessive disorder of purine metabolism. One striking feature is that although the enzyme is missing in every cell in the body, the immune system is most severely affected. Enzyme deficiency results in accumulation of 24-deoxyadenosine and its phoshorilation to deoxyadenosine triphosphate (dATP). Elevation of cellular dATP is believed to account for the severe combined immunodeficiency (SCID) seen in humans.
Methods and **Results**: As this disorder remains often undiagnosed we have set up a diagnostic procedures, which include estimation of: (1) 24-deoxyadenosine in urine, (eventually dATP in erythrocytes); (2) activity of ADA in erythrocytes. Urinary purine nucleosides and enzyme activity were measured by method adapted to HPLC. Confirmation could be done with analysis of ADA gene. Using these tests we have detected one patient of Czech origin from the 40 urine samples received in our department with suspicion of SCID during last two years. 4-month-old boy with diarrhoea, and respiratory insufficiency had concentration of deoxyadenosine in urine 236 mmol/mol/ Cr. Activity of ADA in erythrocytes was 3,0 nmol/h/mg Hb, (healthy controls: 8.0–89.0 nmol/h/mg Hb). Enzyme activities were found several times within normal limits when assays were performed after blood transfusion.
Conclusion: Suspicion of SCID is clear indication for detailed purine metabolic investigation, which is essential for diagnosis of ADA deficiency. In samples after blood transfusion, where characteristic urinary deoxyadeosine disappers, estimation of the red cell dATP is the only clue for early diagnosis.

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541-P

CLINICAL, ENZYMATIC AND MOLECULAR CHARACTERISTICS OF POLISH PATIENTS WITH ADENYLOSUCCINATE LYASE DEFICIENCY

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Background: Adenylosuccinate lyase deficiency (ADSL) is an autosomal recessive disorder of purine metabolism. The TLC screening for SAICAr detection was introduced in our Institute in 1984. In 1999 we identified first patient based on the clinical picture of surprising, autistic behaviour. The screening of newborns and infants with epileptic encephalopathy (2000 samples since 1999) revealed six more cases. The aim is to describe characteristics of these patients.
Results: Clinical findings indicate a severe neurological involvement with a high incidence of seizures, that were present in six of seven cases. In five children, seizures were initially observed in the first month and in one case in 4th year of life. Delayed motor milestones were evident in all patients ranging from moderate to severe. One patient died at 2.5 months. Currently, the oldest patient is 8.5 years old. Most of the patients display severe psychomotor retardation, epilepsy and impressive autistic features including absent or poor eye contact and stereotypies. Enzymatic measurements of the ADSL activity in expressed proteins ranged from 16 to 142% and were similar for both S-AMP and SAICAr in all patients. Four apparently unrelated patients carried a R426H mutation (two homozygous and two compound heterozygous). Other mutations (including four novel T242I, S23R, D215H and I351T) were identified only in single alleles. No correlation was uncovered between studied parameters.
Conclusion: Our experience confirms that all children with neonatal seizures, severe infantile epileptic encephalopathy and developmental delay should be screened for ADSL deficiency as it may be underdiagnosed in our population.

542-P

SUBSTRATE SPECIFICITY OF HUMAN INOSINE TRIPHOSPHATE PYROPHOSPHOHYDROLASE, CONSEQUENCES FOR PHARMACOGENETIC SIGNIFICANCE

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Background: Deficiency of inosine triphosphate pyrophosphohydrolase (ITPase) is an inborn error of metabolism with a supposedly benign phenotype. Polymorphisms in *ITPA* are thought to be associated with adverse drug reactions in patients treated with mercaptopurines. This is, however, a focal point of discussion. Any pharmacogenetic significance of ITPase is dependent on its substrate specificity towards the mercaptopurine metabolites that theoretically accumulate. The substrate specificity of human erythrocyte ITPase towards its natural substrate ITP and the mercaptopurine nucleotides 6-thio-ITP and 6-methylthio-ITP was determined.
Methods: Substrate specificity was determined in a erythrocyte lysate, which was incubated for 30 min in the presence of Mg^{2+} and the appropriate nucleoside triphosphates, i.e. ITP, 6-thio-ITP or 6-methylthio-ITP. The reaction was terminated by acid precipitation and nucleotides were analysed using ion-pair HPLC method with a UV-Vis detector.
Results: The pyrophosphohydrolysis of ITP, 6-thio-ITP and 6-methylthio-ITP followed Michaelis-Menten kinetics, having the following characteristics: ITP K_M = 107 μM, V_{max}=1068 μmol/mg protein/h, 6-thio-ITP KM = 283 5M, V_{max}= 512 μmol/mg protein/h, 6-methylthio-ITP K_M = 924 μM, V_{max}= 335 μmol/mg protein/h.
Conclusions: Both ITP and 6-thio-ITP were efficiently hydrolysed, however 6-methylthio-ITP was a poor substrate. We propose that in vivo human ITPase catalyses the pyrophosphohydrolysis of 6-thio-ITP, but not of 6-methylthio-ITP. Our findings provide further evidence that ITPase deficiency has pharmacogenetic consequences.

543-P

β-UREIDOPROPIONASE DEFICIENCY PRESENTING WITH CONGENITAL ANOMALIES OF THE UROGENITAL AND COLORECTAL SYSTEMS

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Background: β-ureidopropionase deficiency (McKusick 606673) is an autosomal recessive condition caused by mutations in the UPB1 gene. β-ureidopropionase (E.C.3.5.1.6) catalyzes the third step of the pyrimidine catabolic pathway and biochemical diagnosis of the condition is made by measuring the accumulation of the enzyme's substrates, N-carbamyl-β-alanine and N-carbamyl-β aminoisobutyric acid, in urine, plasma and cerebrospinal fluid. To date, five patients have been reported including one putative case detected through newborn screening. Clinical presentation includes neurological and developmental problems whereas the child detected through newborn screening was asymptomatic.
Results: Here, we report another case of β-ureidopropionase deficiency who presented with congenital anomalies of the urogenital and colorectal systems and with normal neurodevelopmental milestones. Analysis of a urine sample, because of the suspicion of renal stones on ultrasound, showed strongly elevated levels of the N-carbamyl-β-amino acids using tandem mass spectrometry, suggestive of a β-ureidopropionase deficiency. Subsequent analysis of UPB1 identified a novel mutation 209G>C (R70P) in exon 2 and a previously reported splice receptor mutation IVS1-2A>G. Expression studies of the R70P mutant enzyme showed undetectable residual activity.
Conclusions: Our case demonstrates that urine screening by electrospray tandem mass spectrometry is a powerful tool for detecting patients with a β-ureidopropionase deficiency. However, the clinical significance remains uncertain in our patient with congenital structural anomalies and normal neurodevelopmental milestones.

544-P

DIHYDROPYRIMIDINE DEHYDROGENASE DEFICIENCY DUE TO A DE NOVO INTERSTITIAL DELETION del(1)(p13.3p21.3)

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Background: Dihydropyrimidine dehydrogenase (DPD) deficiency is an autosomal recessive disease characterized by thymine-uraciluria and is often accompanied by a neurological disorder. To date, no patients have been described with a DPD deficiency caused by compound heterozygosity for an inherited mutation and a de novo deletion of *DPYD*. **Methods**: The concentrations of the pyrimidine bases were determined using HPLC-MS/MS. The activity of DPD was determined using radiolabeled thymine and the genomic sequences of *DPYD* were sequenced. **Results**: The patient presented with severe irritability, respiratory distress and dysmorphia. His development was severely retarded and he showed epilepsy. Analysis of a urine sample showed strongly elevated levels of uracil and thymine and absent DPD activity in PBM cells. Analysis of *DPYD* showed that the patient was apparent homozygous for the 295–298delTCAT mutation. However, carriership for this mutation could only be confirmed in the mother and not in the father. Subsequent analysis using MLPA showed LOH of the *DPYD* gene in the patient. FISH- and array-CGH analysis demonstrated a de novo deletion of approximately 14 Mb of chromosome band 1p13.3–1p21.3 including *DPYD*. **Discussion:** The patient proved to be compound heterozygous for the 295–298delTCAT mutation in *DPYD* and a de novo deletion of chromosome band 1p13.3–1p21.3, including *DPYD*. Only a few patients have been described with an interstitial deletion of chromosome 1p and they all presented with a severe clinical phenotype. The clinical presentation of our patient is most likely caused by the chromosomal anomaly and aggravated by the presence of a DPD deficiency.

545-P

DETERMINATION OF THYMIDINE PHOSPHORYLASE ACTIVITY IN WHITE CELLS AND PLATELETS

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The autosomal recessive condition of mitochondrial neurogastrointestinal encephalopathy (MNGIE) is caused by a deficiency of thymidine phosphorylase (TP). Thymidine in urine is used as a marker for TP deficiency, and deoxyuridine has also been shown to accumulate. Thymidine phosphorylase activities have been reported in white cells. We report a simple HPLC method for TP assay and show that white cells and platelets have similar enzyme activities.

Briefly, lysates of white cells and platelets were incubated with 100 mM phosphate buffer and 10 mM thymidine for 1 h. The assay was stopped by the addition of trichloroacetic acid and the supernatant injected onto an ion-pair HPLC system with UV detection at 254 nm for quantitation of the product thymine.

The **results** showed that levels of TP were comparable between white cells and platelets.

Activities expressed as nmol/h/mg protein

	White cells	Platelets
Ave *n* =10	710	716
SD	216	187
Range	(336–1341)	(377–1320)

Four patients diagnosed by metabolite screening were confirmed as TP deficient by enzyme assay and mutation analysis

In **conclusion**, platelets ought to be considered as an alternative to white cells for enzyme assay. Platelets, unlike white cells, are present in large amounts in blood, and can be easily obtained by a single spin or even by leaving the blood to settle. This greatly facilitates the preparation of samples for enzyme assay.

546-P

CEREBRO-SPINAL FLUID NEUROTRANSMITTERS STUDY IN CHILDREN WITH EARLY-ONSET SEVERE EPILEPTIC SYNDROMES

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We performed, along a prospective study, CSF neurotransmitters analysis in 37 children with severe, often drug-resistant epilepsy. Onset of seizures occurred before age 1 year in 25 patients, in 8 of whom during the neonatal period. Partial migrating seizures were seen in the neonates, followed by infantile spasms (3cases) or myoclonic seizures (1 case). A severe encephalopathy was constantly seen during the evolution, with drug-resistant epilepsy (5/8). These epilepsies were classified as cryptogenic (7), or symptomatic, with cerebral ischemic lesions (1). In the infantile forms (29 patients), we observed West syndrome (4), Doose syndrome (4), Lennox-Gastaud syndrome (4), infantile spasms with partial seizures (4), and partial epilepsy, often complex with multifocal foci (12). 21 of these epilepsies were of the cryptogenic type, 8 were symptomatic (cortical dysplasia 3, ischemic lesions 3, mesial sclerosis 1, tuberous sclerosis (1). During evolution, severe encephalopathy or mental deficiency were constantly observed; 15/29 patients were drug-resistant. CSF neurotransmitters were studied in the months following the seizure onset: 5-HIAA, HVA, 3-O-methylDopa, 5-HTP, BH_4, 5-MTHF, aminoacids, and also CSF lactates and urine organic acids were investigated. In cryptogenic epilepsies, cerebral MRI was either normal, or showed a non-specific generalized atrophy.

All the analysis gave normal results, showing that a dysfunction of the main neurotransmitters pathways can be excluded in most cases of early-onset severe epileptic encephalopathy;

547-P

ANALYSIS OF CEREBROSPINAL FLUID (CSF) FOR THE DIAGNOSIS OF BIOGENIC AMINES METABOLISM DISORDERS; POLISH EXPERIENCE

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Objectives: Disorders of biogenic amines neurotransmission belong to inherited neurological defects diagnosed only by CSF analysis. Our aim was to introduce a measurement of biogenic amines in CSF for differential diagnosis in the patients presenting with rigidity, axial hypotonia, movement disorders as dystonia or parkinsonism, spasticity, oculogyric crises, temperature instability, developmental delay. **Methods**: Lumbar puncture and CSF samples collection and storage was applied according to standardized protocol. Biogenic amines metabolites (HVA, 5-HIAA and 3-OMD) were analysed by HPLC with electrochemical detection in CSF from 46 patients, aged 2 months–23 years. **Results**: In six cases (13%) abnormal results were revealed. In 4 patients strongly decreased concentrations of HVA and 5-HIAA were detected. In further 2 patients (siblings) – a diminished concentration of HVA and a low-normal concentration of 5-HIAA was found. Further studies (pterins CSF levels, phenylalanine loading test) led to biochemical diagnosis of neurotransmission disorders in four patients, who presented with progressive extrapyramidal symptoms under 5 years of age. They were: PTPS deficiency in one patient, GTPCH deficiency in one patient, and DRD (Segawa) in two patients. All these patients responded positively to a low dose L-dopa treatment. The remaining two patients with abnormal CSF biogenic amines concentration presented with progressive dystonia later, since the age of 13–14 years, and did not respond to L-dopa treatment. **Conclusion**: Our preliminary results confirm usefulness of the method, newly introduced in our lab, for the detection of neurotransmission disorders among patients with the special neurological symptoms of unknown etiology.

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548-P

AN ATYPICAL PRESENTATION OF CEREBRAL FOLATE DEFICIENCY

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Cerebral folate deficiency (CFD) typically manifest from early infancy, starting with marked unrest, irritability, and sleep disturbances followed by psychomotor retardation, cerebellar ataxia, spastic paraplegia, dyskinesia, epilepsy, acquired microcephaly, progressive visual and hearing impairment. Autoantibodies to folate receptors are present in majority of patients. Only one patient was reported to have progressive spasticity and speech difficulties resembling a spastic paraplegia phenotype. We report a patient with an atypical presentation of CFD. This boy is the first child of a non-consanguineous Chinese couple. His developmental milestones were normal till 2 years old. He began to fall frequently. He was noted to have progressive cognitive decline, drooling and speech difficulty. Physical examination revealed prominent spasticity and dystonia over both lower limbs with dysarthria. The upper limbs were not involved. Magnetic resonance imaging of the brain was normal. Extensive neurometabolic investigations were unrevealing. Cerebrospinal fluid (CSF) for analyses was screened. 5-methyltetrahydrofolate (5MTHF) was very low 38.2 nmol/L (normal 63–111). There was no systemic folate deficiency. He was then started on oral folinic acid and the dosage was adjusted according to the CSF 5-MTHF level. After replacement with folinic acid, his cognitive decline stopped with no further deterioration in motor function. 1 year after treatment, this boy had dramatic developmental progress. The motor disorder became static. Serum for autoantibodies to folate receptors assay is pending. Screening for CSF 5-MTHF is recommended for any patient with undiagnosed neurodegenerative disease as CFD can be a potentially treatable condition.

549-P

NEW MUTATION IN INTRON 5 OF GTP CYCLOHYDROLASE I GENE CAUSES DOPA-RESPONSIVE DYSTONIA (SEGAWA SYNDROME) IN A BRAZILIAN FAMILY

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Dopa-responsive dystonia (DRD), also known as Segawa syndrome or hereditary progressive dystonia with diurnal fluctuation (HPD), is clinically characterized by occurrence of simultaneous or late parkinsonism and by an excellent response to treatment with low doses of L-Dopa. Diagnostics of DRD is essentially clinical, based on the clinical history and response to the treatment with low doses of L-Dopa. However, due to the low penetrance of the disease asymptomatic carriers may exist. In these cases, mutational analysis of the *GCH1* gene is one alternative to diagnose DRD. This gene is constituted by 6 exons, and the complete transcript codes for GTP cyclohydrolase I, an enzyme that regulates BH_4 synthesis. In this work we investigated a large DRD-carrier family in an attempt to identify the disease-causing mutation. The proband, a young woman diagnosed at 13 years of age, is a daughter of a healthy non-consanguineous couple with history on the maternal family of several cases of tip-toeing, disturbance of gait, parkinsonism, rigidity and cramps in the lower limbs. Using the single strand conformational polymorphism (SSCP) and DNA sequencing techniques to analyze DNA extracted from blood samples, we identified a new mutation in the *GCH1* gene, IVS5+5insA that would preclude the formation of the active enzyme due to the formation of truncated peptides.

To our knowledge, this work represents the first case of identification of a mutation causing DRD in Brazil.

550-P

AUTOSOMAL RECESSIVE GTP CYCLOHYDROLASE I DEFICIENCY WITHOUT HYPERPHENYLALANINEMIA: A UNIQUE PRESENTATION IN TWO SIBLINGS

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GTP cyclohydrolase I (GTPCH I) deficiency is a disorder of tetrahydrobiopterin (BH_4) synthesis, causing decreased production of dopamine and serotonin. Autosomal recessive GTPCH I deficiency presents with hyperphenylalaninemia, whereas plasma phenylalanine (Phe) is normal in autosomal dominant forms. We report a unique case: autosomal recessive GTPCH I deficiency presenting without hyperphenylalaninemia, in two siblings. **Case Report:** A Pakistani male presented at birth with tremor, dystonia, marked irritability, excessive drooling, oculogyric episodes, vomiting, failure to thrive and microcephaly. At 10 months, CSF neopterin and BH_4 were low, HVA marginally low and 5HIAA normal. Plasma Phe was normal at newborn screening, and on several occasions later. Oral Phe loading test yielded markedly elevated plasma Phe and clearly abnormal Phe/Tyr ratio. Fibroblasts showed decreased GTPCH activity. GCHI gene sequencing revealed homozygosity for a novel mutation, V206A (both parents heterozygous and asymptomatic). Now three years old, he is treated with BH_4, L-Dopa/Carbidopa and 5-hydroxytryptophan, showing dramatically improved tone and motor development. His younger brother was diagnosed neonatally by mutational analysis. At that time, CSF HVA, 5HIAA and BH_4 were normal, CSF neopterin slightly decreased, and plasma Phe normal. Treatment (as above) was started promptly. Twelve months later, motor and cognitive development remain age-appropriate. **Conclusions**: Autosomal recessive GTPCH I deficiency can present without hyperphenylalaninemia. CSF neurotransmitter profiles can be extremely subtle. Testing for GTPCH I deficiency (using CSF pterin analysis and/or oral Phe loading) should be considered for patients with unexplained neurological symptoms associated with extrapyramidal movement disorder; even with normal plasma Phe and/or unremarkable CSF neurotransmitters.

551-P

A CASE OF SEPIAPTERIN REDUCTASE DEFICIENCY DETECTED IN THE SCREENING FOR BIOGENIC AMINE NEUROTRANSMITTER DISEASES

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Sepiapterin reductase deficiency is an autosomal recessive defect in the biosynthesis pathway of BH_4 presenting without hyperphenylalaninemia that leads to a severe biogenic amine neurotransmiter deficiency. We present a new case with this disorder identified during the screening for neurotransmitter diseases in CSF that we routinely perform to all CSF samples from patients with neurological affectation.

Case report: This patient is a 23-months-old boy presenting with psychomotor retardation and hypotonia, ataxia and extrapyramidal signs. Analysis of biogenic amine neurotransmitters by HPLC with electrochemical detection in CSF (nmol/L) revealed very low levels of 5-HIAA (9; N > 125), HVA (111, N > 274) and MHPG (10, N > 30); normal value of 3OMD (20, N < 40). Analysis of pterins in CSF by HPLC with fluorescence detection showed mildly elevated biopterin (52, N < 40) and normal neopterin (18, N < 30) levels. Further investigation of pterins revealed a clear elevation of sepiapterin (16, N < 0.5) in the CSF. Amino acids and organic acids concentrations in body fluids were normal. These biochemical findings were fully consistent with sepiapterin reductase deficiency. Confirmation of the disease by molecular analysis revealed a homozygous nonsense mutation c. 751 A > T (p.K251X) in exon 3 of the *SPR* gene. Treatment with L-DOPA and carbidopa has been introduced.

CSF is the only informative sample for the detection of this disease. This case illustrates the importance of CSF investigations in patients with neurological disorder when other metabolic diseases have been excluded.

552-P

FIRST REPORT OF DOPAMINE RESPONSIVE DYSTONIA AS THE PRESENTATION OF DIHYDROPTERIDINE REDUCTASE DEFICIENCY

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Background: Dihydropteridine reductase deficiency (DHPR) typically presents with myoclonic epilepsy, microcephaly, intellectual impairment, and hyperphenylalaninemia. We report the case of a young woman of superior intelligence in whom dopamine responsive dystonia (DRD) was the manifesting feature of DHPR deficiency.

Case Report: Our patient is a woman of age 28 years, and a happily married university graduate. Severe talipes was noted at birth. Diurnal weakness and dystonia progressed from age 10 years, and by 14 she had became wheelchair-bound. A successful trial of L-dopa was conducted at age 18: over the space of 1 week she regained upper limb function, standing, and walking. Current treatment: L-dopa, 5-OH-tyrptophan, baclofen, and folinic acid.

Results: Phenylalanine and tyrosine levels are normal. During a phenylalanine load, the P/T ratio rose to 6 at 1.5 h, and was 2.4 at 5 h. CSF neurotransmitters, HVA 0.1 (RR 0.22–0.66 μmol/L) and 5-HIAA 0.04 (RR 0.13–0.21 μmol/L), were both reduced. GTPCH activity in cultured skin fibroblasts was normal. The predominant form of urine biopterin was fully oxidised rather than the reduced form, prompting consideration of DHPR. Blood spot DHPR assay results were indicative of a 'heterozygous range' (0.68 to 0.96), while her parents displayed homozygous normal levels (father 1.84 and mother 1.80). Mutation analysis of the causative gene, quinoid dihydropteridine reductase (*QDPR*), is pending.

Discussion: This case represents the first presentation of DRD due to DHPR deficiency. We postulate that the milder and atypical phenotype may reflect a residual DHPR capacity, perhaps secondary to impaired dimerisation.

553-P

DOPAMINE AGONISTS IN TETRAHYDROBIOPTERIN DEFICIENCY

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Background: Substitutive therapy with L-dopa represents the most critical aspect in the treatment of tetrahydrobiopterin (BH_4) deficiency, because of pharmacological disadvantages and adverse effects, and despite different adjustments, such as drug fractionation and/or administration of peripheral decarboxylase (PD), monoamino oxidase (MAO), and catechol-O-methyl transferase (COMT) inhibitors. Though L-dopa therapy is the physiological cure of dopaminergic dysfunction, new long-acting dopamine agonists may provide continuous, rather than pulsatile, neuroreceptor stimulation.

Methods: Pramipexole, a recent nonergoline dopamine agonist provided of specific activity at both D2 and D3 post-synaptic receptors, was orally administered at the daily dosage of 0.0041–0.033 mg/kg to 5 patients affected by 6-pyruvoyl-tetrahydrobiopterin synthase (PTPS) deficiency. The patients, 4 males and 1 female, were all on treatment with L-Dopa, and PD, MAO, and COMT inhibitors, with satisfactory results. Cognitive and motor performances were evaluated by the UDPRS questionnaire. Blood prolactin profile and plasma and urine catecholamine were analyzed before and during the treatment.

Results: Pramipexole administration allowed to reduce by 20–40% the daily dosage of L-dopa and the number of daily L-dopa administrations from 3-4 to 2. Concurrently, the prolactin profile was stabilized and catecholamine excretion was reduced. Improvements in the UDPRS scale were obtained in all patients, with a significant reduction of on-off, wearing-off and end-of-dose phenomena.

Conclusions: Dopamine agonists may well result in facilitated clinical response to L-dopa therapy in BH_4 deficiency.

554-P

TETRAHYDROBIOPTERIN (BH_4) IN THE TREATMENT OF ADD/ADHD

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Background: Attention deficit disorders with or without hyperactivity, ADD/ADHD are caused by inadequate levels of neurotransmitters, primarily dopamine. BH_4 is a co-factor for dopamine synthesis, and therefore we tested BH_4 administration on subjects with ADD/ADHD.
Methods: Seven individuals with ADD/ADHD, 5 males and 2 females were studied using Conner Continuous Performance test (CCPT) before, during and after administration of BH_4. Five individuals, 4 males and 1 female had been on stimulant therapy which was suspended 4 weeks prior to BH_4 trial. The other 2 had no therapy prior to the study. All patients received 100 mg of BH_4 daily in a single dose, while one patient was given 350 mg daily. Prior to treatment, CCPT was administered, then after 1 week, 2, 3 and 4 weeks while patient remained on BH_4. CCPT was followed after discontinuation of treatment.
Results: After one week, 4 of the 5 male patients had significant improvement on CCPT. Two of the female patients showed moderate improvement. CCPT returned to baseline 1–2 weeks after BH_4 was discontinued. The patient who received 350 mg daily continued to show benefit for 4 weeks before returning to baseline.
Conclusion: Treatment of ADD/ADHD with BH_4 shows improvement using a single dose in 6 out of the 7 patients in the trial. Dosage response had not been studied and was varied only in one patient. The preliminary data in this study are encouraging and further work is needed to establish the optimal level of BH_4 and the length of time to achieve positive response.

555-P

AROMATIC L-AMINO ACID DECARBOXYLASE ENZYME ACTIVITY IN DEFICIENT PATIENTS AND HETEROZYGOTES

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Background: Aromatic L-amino acid decarboxylase (AADC) deficiency is a severe, autosomal recessive neurometabolic disorder. Low cerebrospinal fluid (CSF) concentrations of homovanillic acid (HVA) and 5-hydroxy indole acetic acid (5-HIAA) are suggestive, but not specific, for this disorder. Confirmation of the diagnosis AADC deficiency is required by enzyme activity measurement or genetic analysis.
Methods: We describe assays for plasma AADC enzyme activity using both of its substrates, 5-hydroxytryptophan (5-HTP) and 3,4-dihydroxyphenylalanine (L-dopa). We measured AADC activity in controls, AADC deficient patients and heterozygotes.
Results: AADC enzyme activity in control plasma on average is a factor 8–12 higher with L-dopa as substrate than with 5-HTP. Both substrates of AADC compete for the same active site of the enzyme resulting in equally decreased residual enzyme activities in AADC deficient patients. In AADC deficient patients, the enzyme activity towards both substrates, L-dopa and 5-HTP, are equally decreased, as are the CSF concentrations of HVA, 5-HIAA and MHPG, whereas heterozygotes have intermediate AADC activity levels
Conclusions: The presently described assays for AADC activity measurement allow an efficient and reproducible way to diagnose AADC deficiency. For diagnostic purposes, AADC enzyme activity is better measured with L-dopa than with 5-HTP as substrate.

556-O

TYROSINE HYDROXYLASE DEFICIENCY: THE SPECTRUM OF ITS CLINICAL, BIOCHEMICAL, AND MUTATIONAL CHARACTERISTICS IN A SERIES OF 36 PATIENTS

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Introduction: Tyrosine hydroxylase (TH) catalyzes the conversion of L-tyrosine to L-dopa. TH deficiency (THD) is an autosomal recessive condition due to mutations in the TH gene. Originally, THD was reported as a L-dopa responsive extrapyramidal movement disorder. THD is diagnosed by neurotransmitter analysis in cerebrospinal fluid (CSF), and mutation analysis of the *TH* gene. THD has been reported in less than 25 patients worldwide. In this study we aim to provide the first large review on the clinical, biochemical, and mutational characteristics of THD.
Methods: In our laboratory we have a longstanding tradition on CSF neurotransmitter analysis and *TH* gene mutation analysis. An extensive questionnaire was send to the physicians from all THD patients ($n = 36$) diagnosed.
Results: It seemed appropriate to lump the phenotypical varieties at presentation into three major groups: (1) infantile or childhood onset progressive 'isolated extrapyrimidal movement disorder', (2) more complex phenotype with earlier onset, originally described as 'neonatal encephalopathy', and (3) 'intermediate phenotype'. Cerebral imaging studies were generally normal. CSF homovanillic acid concentrations were always decreased, and lowest values were found in group (1). Mutation analysis revealed many different mutations. Depending on the phenotype, the respons to l-dopa varied from excellent to none.

557-P

MUTATION SPECTRUM IN NON-KETOTIC HYPERLGYCINEMIA

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Patients with non-ketotic hyperglycinemia (NKH) have mental retardation and seizures. NKH is characterized by deficient glycine cleavage enzyme activity. This system is composed of 4 peptides, P, T, H, and L.
Methods: We characterized mutations in 73 patients with proven NKH by direct sequencing of all exons and intron-exon borders of the *AMT*, *GLDC*, and *GCSH* genes. Parental studies identified the phase. To identify deletions in *GLDC*, we analyzed 6 highly polymorphic markers in the core family, an MPLA and new real time PCR method.
Results: Mutations were found in *AMT* in 23% of patients, and in *GLDC* in 70% of patients. In 5 patients with deficient enzyme activity, no mutations were found. In *AMT*, 97% of the alleles were identified, 71% missense mutations, with the common mutation R320H accounting for 21% of alleles. A founder mutation I106T in the Dutch population was associated with a mild phenotype. In *GLDC*, 91% of the 102 alleles were identified. There were 54 alleles with missense mutations, 11 alleles with splice-site mutations, and 3 small deletions. There was proof of a large deletion in at least 11 alleles of varying length and position. Recurring mutations were R515S, A389V, R424X and IVS9(-1)G > A. New Zealand patients had a common mutation. Mutations with residual activity were associated with a milder phenotype.

This testing allows confirmation of diagnosis, and has been used successfully in prenatal diagnosis. It further aids in identifying patients affected by a milder disease in which treatment may be more effective.

558-P

DNA ANALYSIS FOR NON-KETOTIC HYPERGLYCINAEMIA IN THE UK POPULATION

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Background: We are a centre offering metabolite, enzymological and DNA testing for non-ketotic hyperglycinaemia (NKH) to the UK. We present the data of DNA analysis in all our proven cases of NKH.
Methods: A diagnosis of NKH was confirmed in 30 patients by measurement of liver glycine cleavage system and/or DNA analysis. DNA analysis involved mutation screening for the more commonly reported mutations (R515S, A389V in the *GLDC* gene and R320H, IVS7-1 G>A and R296H in the *AMT* gene) and MLPA analysis for deletions within the *GLDC* gene and an exon 1 specific deletion assay. Further DNA analysis was carried out in selected patients and microsatellite markers developed for gene tracking.
Results: Mutations in the *GLDC* gene were found in 21 of 30 patients. One patient was found to have a mutation in the T protein gene (*AMT*) and no mutations were found in 8 patients. In Caucasian patients the R515S mutation accounted for 21% of alleles with evidence of a founder effect. Deletions spanning one or more exons of the *GLDC* gene were present on 19% of all alleles. Eight other point mutations were identified, all being private. Microsatellite markers proved effective in gene tracking in all cases where performed.
Conclusions: In the UK, screening for the R515S and deletions in the *GLDC* gene detects approximately 50% of alleles in Caucasians and 20% in other ethnic groups. In conjunction with microsatellite markers for gene tracking, DNA analysis can be used for a majority of antenatals for NKH.

559-P

EVIDENCE OF INCREASED PROTEIN SULFOXIDATION IN CSF OF PATIENTS WITH NON-KETOTIC HYPERGLYCINAEMIA WITH PRIMARY PULMONARY HYPERTENSION (NKH WITH PHN) FOUND BY 2-D FLUORESCENCE DIFFERENCE GEL ELECTROPHORESIS ANALYSIS (DIGE)

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NKH associated with PHN is an atypical variant of NKH with severe neurological and pulmonary implications leading to exitus before 1 year in the studied cases. Small increment of CSF glycine, absent GCS and protein P activities without significant molecular findings in protein P, T and H genes, brought us to consider another methodological approach to search for ethiopathological implications.

CSF of 4 NKH/PHN patients and 4 controls (3 non-NKH and one classical NKH) was studied by DIGE, a powerful methodology that permits the reliable study of a small number of samples, followed by MS analysis.

Patients showed a different proteomic pattern from controls while these differences were not observed between classical NKH and non-NKH controls. Marked increment in the more acidic isoforms of several series of protein spots was found. An increase in the sulfoxidation of methionine residues in cystatin C and vitamin-D binding protein was found by MALDI TOF-TOF MS that, which in addition to an increased serum malondialdehyde, provide evidence of oxidative stress. Alterations related with oxidative stress has been reported in other neurodegenerative diseases. Particularly, a decreased in CSF asialotransferrin, found in vanishing white matter disease (a spongyform leukodystrophy with glycine increase) was not observed in our patients, suggesting a different neuropathological process.

As increase in sulfoxidation does not sufficiently explain the shift observed in the protein spots pattern, other oxidative processes must be involved. Whether these findings are important as biomarkers for diagnosis and/or as pathophysiological factors remain to be verified in more patients.

560-P

THE DIAGNOSTIC VALUE OF EXERCISE TESTING IN PATIENTS WITH EXERCISE INTOLERANCE

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Background: Exercise intolerance (EI) is a frequent cause of medical attention, but is only rarely caused by a (metabolic) myopathy. The diagnosis EI is hampered by the fact that classical symptoms are not always found in patients with a (metabolic) myopathy and are there of difficult to distinguish from patients with chronic fatigue.

Goal developing an exercise induced test for distinguishing patients with (metabolic) myopathies from patients with chronic fatigue.
Methods: Maximal exercise test (to determine the maximal oxygen uptake and workload) and a prolonged submaximal exercise test (90 minutes at 30% of peak workload) were performed in 39 children. Thirteen patients (9 ♂, 4 ♀, age 5–15 years) had a (partly later) confirmed diagnosis (GSD1a, GSD3, GSD7, MCAD (2×), SCAD, MADD (2×), ketothiolase deficiency, mitochondrial myopathy, Becker dystrophinopathy (2×) and hypokalemic episodic paralysis). Twenty-six children (19 ♂, 7 ♀, age 5–17 years) were based upon chronic fatigue referred for EI, but had normal exercise tolerance during testing. During exercise respiratory gas-exchange and heart rate were monitored, blood was drawn at set time points for biochemical analysis (CK, NH3, lactate, acylcarnitine, FFA, glucose). Urine was collected during and up to 3 h after the test for biochemical analysis (organic acids, oligosaccharides and myoglobin).
Results: During exercise, several significant differences between patients with a known disorder and the clinical control group were found.
Conclusion: Exercise testing is a useful screening tool in the diagnostic approach of patients with EI and can be used to differentiate patients with EI and chronic fatigue.

561-P

ACUTE CHRONIC FATIGUE AS AN INDICATOR OF INBORN ERRORS OF METABOLISM IN ADULTS

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We have mounting evidence that acute chronic fatigue and hepatomegaly can be indicators of inborn errors of metabolism in adults. Our metabolic analysis of 20 such patients resulted in the diagnosis of deficiencies in 3-methylcrotonyl-CoA carboxylase (MCC), ornitine transcarbamylase, short and medium chain acyl CoA dehydrogenase, mitochondrial deficiencies, mild hyperphenylalaninemia, vitamin B12 responsive and non-responsive methylmalonic aciduria and X-linked adrenoleukodystrophy.

An adult patient presented with extreme chronic fatigue and muscle weakness was diagnosed with a non-classical, very late onset MCC deficiency. A molecular follow-up revealed no known disease associated mutations in either his MCC alpha or beta subunit, but a heterozygous single nucleotide polymorphism, 1391 A→C (H456P), in his alpha subunit. His MCC enzyme activity is around 70% relative to the controls. This and other data suggest that chronic long-term exposure to accumulating metabolites and energy draining detoxification reactions resulting from mild, untreated IEMs can result in chronic fatigue.

Our data indicate the importance of analyzing the long chain fatty acid and organic acid metabolite profile of chronic fatigued adults. Young women presenting with Parkinson's-like symptoms and their children should also be screened for PKU to prevent mental retardation. For patients with vitamin B12 defects, the impact of vegetarian diets should also be considered. The physical and emotional condition of these chronic fatigued patients generally improved significantly with the identification of their defect and when their diets were supplemented with glycine and/or carnitine to improve detoxification and mitochondrial ATP production and thus energy levels.

562-P

POSSIBLE ASSOCIATION BETWEEN ADULT TYPE PRIMARY HYPOLACTASIA (ATPH) AND TWO SNP MARKERS A COLOMBIAN SUBPOPULATION

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Adult type primary hypolactasia (ATPH) is an autosomic recessive condition in which the activity of lactase phlorizin hydrolase (LPH) decreases after weaning. Two SNPs markers localized upstream of the LPH gene have been found to be associated with LPH persistence in Caucasian populations and are considered potential diagnostic tools for ATPH.

Objective: To identify and give an initial description of the allele frequencies of C→T-13910 and G→A-22018 markers in a Colombian subpopulation.

Methods: DNA from 86 individuals was subjected to PCR and restriction analysis. Genotypes of the two markers were determined and the frequency of ATPH was calculated in this sample. Hardy-Weinberg equilibrium (HWE) was calculated for both alleles.

Results: The expected alleles of the two markers were found in the population studied. According to the genotype occurrence, the frequency of hypolactasia predicted was between 60% and 70%. This is consistent with the frequency of lactose non-digesters found previously in the Colombian population and with estimations made for a population originated from a mixture of Amerindians and Caucasians. Neither SNP was found to be in HWE. C-13910 and G-22018 alleles are in highly significant linkage disequilibrium. This pattern of allelic association, already reported in other populations, agrees with the hypothesis that selective forces are acting upon the ATPH haplotypes.

Conclusion: Although it is advisable to continue the study using a larger population, our results suggest that the SNPs can be used as a screening method for ATPH in the Colombian population.

563-P

THE GC-MS URINARY STEROID METABOLOME CORRELATED BETTER WITH THE REAL ENZYMATIC ACTIVITY STATUS OF A PATIENT THOUGHT TO HAVE AN ISOLATED 17,20-LYASE DEFICIENCY THAN MOLECULAR BIOLOGY

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We studied an adolescent male with clinical and biochemical features of isolated 17,20-lyase deficiency, including micropenis, hypospadias, and gynecomastia, who is homozygous for *CYP17* mutation E305G. The *CYP17* gene encodes a unique enzyme that possess two enzymatic reactions, 17α-hydroxylase and 17,20-lyase activities. We have previously published (JBC 2003, 278:48563), the finding, in this patient, that the E305G mutation caused isolated 17,20 lyase deficiency. Expression studies in transfected HEK-293 cells and in yeast microsomes, expressing the mutant E305G, suggested intact 17-hydroxylase activity. This was at odds with subnormal ACTH stimulated cortisol in the patient, which suggested that the 17-hydroxylase activity was also affected in this mutation. Two years later we developed a gas chromatography-mass spectrometry (GC-MS) profiling of urinary steroid metabolites, which allowed us to study *in vivo* the global enzymatic activities and metabolic pathways of steroid hormones. Assessment of enzymatic activity was based on precursor/product ratios. Elevated metabolic ratios of corticosterone metabolites/C19-steroids (B-M/An+Et: 22.71; Control: 0.32) reflected impaired global activity of the enzymatic system 17-hydroxylase/17,20-lyase. 17,20-lyase activity was markedly impaired, especially P5T/(A5-3β,17β): 362.5; Control: 2.47. 17-hydroxylase activity was also impaired as indicated by the significantly elevated ratio corticosterone metabolites/cortisol metabolites (7.63; Control: 0.17).

Conclusions: In this patient, the GC-MS steroid metabolome expressed the real steroids enzymatic activity status more accurately than molecular biology, and lead to the conclusion that this mutation does not solely affect 17,20-lyase, as we previously reported, but also impairs 17-hydroxylase activity.

564-P

PHENOTYPIC SPECTRUM OF PATIENTS WITH INFANTILE NEUROAXONAL DYSTROPHY (INAD) CAUSED BY MUTATIONS IN THE *PLA2G6* GENE

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Background: Neurodegeneration associated with brain iron accumulation comprises a heterogeneous group of disorders where disruption of different cellular mechanisms results in accumulation of iron in the basal ganglia. This group includes patients with recently discovered mutations in the PLA2G6 gene encoding a calcium-independent phospholipase A2 enzyme which catalyses the hydrolysis of glycerophospholipids. This enzyme appears to be critical in maintaining cell membrane integrity and *PLA2G6* mutations may lead to a relative abundance of phosphoditylcholine in the membrane resulting in neurodegeneration. **Patients and Method:** Retrospective analysis of the medical records and brain imaging from a cohort of 14 patients with *PLA2G6* mutations from 3 major UK paediatric centres. **Results:** Median age of symptom presentation was 15 months. One third of the cohort presented following intercurrent illness. All children had progressive cognitive and motor skill regression, with evidence of axial hypotonia, four limb spasticity and bulbar dysfunction. Over time, all patients developed cerebellar signs, dystonia, strabismus and, in the majority of cases, optic atrophy. The brain imaging of all patients showed cerebellar atrophy and gliosis. Increased iron deposition was identified in the globus pallidus, substantial nigra and subthalamic nuclei. Optic pathway atrophy was present in the majority of the cohort. **Conclusions:** We describe a cohort of patients with Infantile Neuroaxonal Dystrophy due to mutations in *PLA2G6* gene. Careful assessment of the natural history and radiological features of this disorder will allow the proposal of clinical criteria, which in conjunction with molecular gene analysis will provide early definitive diagnosis.

565-P

UNIPARENTAL DISOMY IN CONGENITAL METHEMOGLOBINEMIA: A RARE CAUSE OF CYANOSIS IN CHILDREN: A CASE REPORT

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Congenital methemoglobinemia is a vary rare disorder characterized by lifetime cyanosis, mostly caused by either an inherited mutant hemoglobin (Hemoglobin M disease) or deficiency of cytochrome b5 reductase. Cytochrome b5 reductase deficiency leads to two different types of recessive congenital methemoglobinemia. In type I, cyanosis is the only major symptom and cytochrome b5 reductase deficiency is restricted only to the red blood cells. In type II, cyanosis is associated with severe mental retardation and neurological impairment. Recently, we experienced an 11-year-old boy with cytochrome b5 reductase deficiency type I. The mutational analysis of *CYB5R3* gene revealed that the boy is homozygous (L72P mutant). Surprisingly, only his mother carries this L72P mutant, but his father does not have any identified mutant. Nine microsatellite markers of chromosome 22 were selected to analyze the origins of the patient's chromosome 22. The result showed that both of the chromosome 22 of the patient came from one of the maternal allele. This finding gives the first case of cytochrome b5 reductase deficiency type I resulting from uniparental disomy and also discloses an alternate mechanism whereby this enzymatic disorder can derive from a single parent.

566-P

CRISPONI SYNDROME AND COLD-INDUCED SWEATING SYNDROME TYPE 1: TWO FACES OF THE SAME COIN

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Crisponi syndrome (CS) is a severe autosomal recessive condition, characterized by abnormal, paroxysmal muscular contractions resembling neonatal tetanus, large face, broad nose, anteverted nares, camptodactyly, hyperthermia and sudden death in most cases. Homozygosity mapping in five Sardinian and three Turkish families with CS using high-density SNP arrays revealed a critical region on chromosome 19p12–13.1. The most prominent candidate gene was *CRLF1*, recently found to be involved in the pathogenesis of cold-induced sweating syndrome type 1 (CISS1). CISS1 belongs to a group of conditions with overlapping phenotypes, also including cold induced sweating syndrome type 2 (CISS2) and Stüve-Wiedemann syndrome (SWS). All these syndromes are caused by mutations of genes of the ciliary neurotrophic factor (CNTF) receptor pathway, which is known to be involved in neuron differentiation and survival. We identified four different *CRLF1* mutations in eight different Crisponi families, including a missense mutation, a single nucleotide insertion, a nonsense and an insertion/deletion (indel) mutation, all segregating with the disease trait in the families. Comparison of the mutation spectra of CS and CISS1 suggests that neither the type nor location of the *CRLF1* mutations point to a phenotype/genotype correlation that would account for the most severe phenotype in CS. Our findings provide evidence that CS is allelic to CISS1.

567-O

SYNDROME OF LIVER CIRRHOSIS, DYSTONIA AND HYPERMANGANESAEMIA – A NEW METABOLIC DISORDER?

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We report a new constellation of clinical features consisting of hypermanganesaemia, liver cirrhosis and extrapyramidal motor disorder in a 15 year-old Arabic girl born to consanguineous parents. Plasma manganese (Mn^{2+}) levels were increased above 3000 nmol/L (normal range <320 nmol/L). Liver biopsy revealed a micronodular cirrhosis of the liver and an MRI of the brain showed signal abnormalities of the basal ganglia consistent with heavy metal deposition in these regions. An older brother showing the same clinical phenotype died at a young age, thereby suggesting that the disease follows an autosomal recessive trait.

This newly described disorder is probably caused by a defect of Mn^{2+} metabolism resulting in the accumulation of Mn^{2+} in the liver and the basal ganglia similar to copper accumulation in Wilson's disease. In order to assess the genetic basis of this syndrome we investigated two candidate genes: *ATP2C2* and *ATP2A3* encoding the two Mn^{2+} transporting calcium ATPases SPCA2 and SERCA3, respectively. Genotyping the patient and the family for microsatellite markers surrounding *ATP2C2* and *ATP2A3* excluded these genes in this case. The patient was found to be heterozygous for both gene loci.

Even though the pathophysiology of this disorder is not fully understood yet, we were able to develop a successful treatment regime. Chelation therapy with calcium edetate combined with iron supplementation lowered plasma Mn^{2+} levels significantly and improved the girl's dystonia.

568-P

PERSISTENT METABOLIC ACIDOSIS IN ARC SYNDROME: A CASE REPORT

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ARC syndrome is a rare disorder inherited with autosomal recessive pattern. The characteristic clinical features are arthrogryposis, renal Fanconi syndrome and cholestasis.

Case Report: A three-months-old male patient was referred to our department because of jaundice, failure to thrive, pes equinovarus, antenatal femur fracture, persistent metabolic acidosis, hepatomegaly, glucosuria and proteinuria. He was born after a noncomplicated pregnancy at 39 weeks from consanguinous parents. His physical examination revealed failure to thrive, hypotonia, ichtiosis, slopping forehead, hepatomegaly, unilateral inguinal hernia, multipl contractures and an old, dislocated fracture in left femur. Direct hyperbilirubinemia, metabolic acidosis, renal tubular dysfunction, steatore, large platelets and hypothyroidism were detected. Trasminases, PT, aPTT and electrolytes were normal. The ultrasound revealed hyperechogenic megalic kidneys and a liver with rough hyperechogenic parenchyme. The clinical and laboratory findings were found consistent with ARC syndrome. Intravenous and oral bicarbonate, pancreatic enzyme extracts with vitamins A, D, E, K and thyroxine therapy were initiated. As iatrogenic hypernatremia developed oral rehydration solutions was commenced as an alternative treatment for metabolic acidosis. The patient died at four months age because of lung hemorrhage.

While arthrogryposis, renal tubular acidosis and cholestasis are the main features of ARC syndrome; failure to thrive, ichthyosis, nephrogenic diabetes insipitus, congenital heart disease, low set ears, high arched palate, frontal bossing, cryptorchidism, hypothyroidism and large platelets in blood films have also been described which were also found in our patient. All reported cases had died of unexpected bleeding, persistent metabolic acidosis, sepsis and diarrhea before the age of 8 months, like our patient.

569-P

A CASE OF ISOLATED NEONATAL HYPERINSULINISM DUE TO AN ATYPICAL BECKWITH-WIEDEMANN SYNDROME

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Background: The Beckwith-Wiedemann syndrome (BWS) is an overgrowth syndrome with macrosomia, visceromegaly, macroglossia and abdominal wall defects. Many of the affected patients have episodes of hypoglycemia during the first days of life. During childhood, a frightening complication of BWS is the development of specific tumors. BWS is caused by different types of anomalies at the 11p15.5 imprinted locus.

Case report: A female patient was born at 36 weeks by cesarian section because of preeclampsia. She was eumorphic, her birth occipito-frontal circumference was 33 cm (P50-P75), weight 3140 g (P90), length 46.5 cm (P50). From day 3, repetitive episodes of severe hypoglycemia occurred, leading to increase the glucose intake up to 12 mg/kg/min for stabilizing glycemia. At day 15, normoglycemia was obtained by continuous enteral alimentation and diazoxide. As a diffuse form of hyperinsulinism was evidenced, a subtotal pancreatectomy was performed at 2 months of age which was followed by normoglycemia even with normal discontinuous alimentation. There was no organomegaly. At 6 years of age, neurological assessment was normal except for cognitive functions with an IQ in the lower normal range and attention deficit. At 8.5 years, FISH analysis was performed on lymphocytes using the RP11-534I22 probe, containing the *IgF2* gene, and the *CDKN1C* probe. Using these probes a duplication in the 11p15.5 region was shown in 50% of patient's lymphocytes. At 10 years of age, follow up did not reveal any tumoral pathology.

Conclusions: Neonatal hyperinsulinism could be the only manifestation of BWS.

570-P

ASSOCIATION OF GENETIC POLYMORPHISMS IN *UGT1A1* GENE WITH GILBERT SYNDROME IN BELORUSSIAN PATIENTS

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Introduction: Gilbert syndrome is a benign inherited unconjugated hyperbilirubinemia without structural liver disease or overt hemolysis, usually characterized by episodes of mild intermittent jaundice. It is the most common inherited variant of bilirubin metabolism disorders, occurring in 2–12% of population. The most prevalent genetic polymorphism encountered in whites with Gilbert syndrome is an additional TA insertion in the TATAA box of the *UGT1A1* gene promoter – TA(7).
Objective: To elucidate the genetic background of Gilbert syndrome in Byelorussian patients with unconjugated hyperbilirubinemia without hemolysis by the analysis of the A(TA)nTAA promoter region of the UGT1A1 gene.
Methods: Genomic DNA was isolated from peripheral blood leucocytes collected from 252 patients with suspected Gilbert syndrome. Polymerase chain reaction amplification was used to examine sequence variation of the promoter upstream of the UGT1*1 exon. The population prevalence of UGT1A1*28 allele was determined in DNA samples from volunteers.
Results and **Conclusions**: Different genotypes were identified due to the presence of (TA)5, (TA)6 and (TA)7 repeats. 77% of patients with unconjugated hyperbilirubinemia without hemolysis appeared to be homozygous for additional TA repeats, that is authentically higher than the homozygote frequency in population (18%, χ^2=76.7, p = 0.005). The gene frequency of this mutation in population is extraordinarily high, 0.39 in the present study, similar to the frequency found in series of studies of Caucasians published previously. Our results show that among Byelorussians the most common mutation associated with Gilbert's syndrome is insertion of an extra TA into the promoter region of the *UGT1A1* gene.

571-P
WITHDRAWN

572-P

PAMIDRONATE IN OSTEOGENESIS IMPERFECTA: EVALUATION AFTER 5 YEAR OF TREATMENT

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Introduction: Osteogenesis imperfecta (OI) is characterized by multiples bones fractures.
Objective: To evaluate the time of treatment with pamidronate in a patient with OI.
Materials and Methods: Retrospective study of a child with OI type 3, starting treatment with pamidronate at 5 months of age until 5 years, using Plokin protocol. Annual evaluation of number of fractures and growth, urine calcium and phosphorus, bone mineral density (BMD and T score between L1 to L4).
Results: Two bones fractures. Stature below percentile 3 in the first year of treatment and percentile 5 at the age of two. Before treatment with pamidronate, the calcium value was 2.70 mg/d (100–300), phosphorus: 0.02 g/d (0.4–1.3), BMD: 0.000 g/cm^2 and T-score: 9.5. At two years – calcium: 48.50, phosphorus: 0.40, BMD: 0.468, T-score: 5.3. After five years – calcium: 46.40, phosphorus: 0.51, BMD: 0.486 and T-score: 5.1.
Conclusions: (1) Treatment with pamidronate reduced numbers of fractures, increased quality of life and inverted the evolution to very short stature of patient with OI. (2) Two years of treatment is enough because after that time there is no significant recovery of calcium / phosphorus metabolism and mineral bone density compared to the rest of treatment until five years.

573-O

A NOVEL FORM OF CEREBELLAR ATAXIA WITH INCREASED FREE SIALIC ACID IN CEREBROSPINAL FLUID

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Background: Three patients of two unrelated families, 2 sisters and one sporadic male, presented in their early twenties with cerebellar ataxia, abnormal eye movements, axonal neuropathy and neurobehavioral decline. Other symptoms included (i) myoclonic dystonia and epilepsy in only one of the 2 affected sisters and (ii) progressive deafness, pigmentary retinopathy and ptosis in the isolated case. Brain MRI showed cerebellar atrophy, and abnormal bilateral hyperintensities of the basal ganglia, the cerebellum and the pons in 2 unrelated patients. Extensive metabolic work-up, including various lysosomal assays, was negative. **Methods**: In order to identify new metabolic markers, we used 1H-NMR spectroscopy to study cerebrospinal fluid (CSF) and urine. **Results**: One- and two-dimensional correlation spectroscopy (COSY) 1H-NMR analyses revealed elevated free sialic acid in the CSF of all 3 patients (35, 36 and 40 micromol/L respectively). Normal free sialic acid in CSF in 71 adult controls and patients with various neurodegenerative conditions was 7.4 ± 3.7 micromol/L. In contrast, urine sialic acid excretion was normal. Fibroblasts' analyses in one patient showed normal free sialic acid content. Common disorders associated with increased free sialic acid levels in CSF (pyogenic meningitis, brain tumors) were ruled out. No mutation was found in the *SLC17A5* gene, thus excluding Salla disease. **Conclusion**: We describe 3 patients presenting with a novel form of cerebellar ataxia characterized by an isolated CSF elevation of free sialic acid, for which the primary defect remains to be determined.

574-A

METABOLIC CARDIOMYOPATHIES IN A PEDIATRIC POPULATION

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Background: Inherited errors of metabolism (IEM) are associated with a wide variety of cardiac manifestations, including hypertrophic and dilated cardiomyopathy (CMP). The cardiac manifestations may be late or may be the first presentation of the metabolic disease. Assess the clinical evolution of patients with cardiomyopathy secondary to IEM.
Methods: The authors analysed the clinical records of all patients with IEM and selected those with clinical and echocardiographic criterions of CMP in a paediatric hospital. The following variables were studied: sex, age of presentation of the metabolic and cardiac disease, metabolic diagnosis, type of cardiomyopathy, evolution, treatment and time of follow up.
Results: This study included 13 children (7 males and 6 females). The age of presentation of CMP ranged between 9 days and 15 years. The CMP was hypertrophy in 7 and dilated in 6. There was progression from hipertrophic to dilated type of CMP in one case and in another case the hypertrophy has diminished after therapy with carnitine. In the majority of children (54%), the IEM associated was a mitochondrial disorder. Six children died, 5 from heart failure and one from sudden death.
Conclusions: Hypertrophic CMP was present in all storage disorders; dilated CMP was found in mitochondrial disorders and fatty acid oxidation and was associated with elevated mortality. The authors emphasize the need of metabolic screening in all CMP because an early diagnosis and treatment can have a favourable impact on the outcome. Specific treatment can lead to recovery of the CMP in some cases.

575-P

DIAGNOSTIC OUTPUT OF MUSCLE BIOPSIES FROM 1990 TO 2006

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Introduction: The performance of a muscle biopsy is an important step in the diagnosis of muscular and mitochondrial diseases, sometimes also performed in other disorders. In this retrospective analysis the muscle biopsies performed over a period of 15 years were evaluated to determine the diagnostic output and evaluate indications for this invasive procedure.
Patients: From 1990 to 2006, 145 patients (88 male, 57 female) underwent a muscle biopsy, mainly due to symptoms like hypotonia, metabolic acidosis, seizures, developmental delay. Age range was 1–7 years for 126/145 patients; 62 were biopsied during the first year of life. We grouped the patients in six categories (i.e. spinal muscular atrophy, muscular dystrophy, mitochondrial disorders, developmental delay, epilepsy, and others).
Results: In 53 out of the 145 biopsies the results were diagnostically relevant; 18 by light microscopy and 35 by biochemical investigation. In total, 85 patients were assigned a definite diagnosis; 33 a probable/possible diagnosis, in 27 cases the diagnosis remained unclear.
Conclusion: The diagnostic output was 36.5%. The main factor for a conclusive result is the indication for the muscle biopsy, which is still mainly supported by a good clinical history and evaluation of the patient. Recently, the analytical procedures have developed and improved substantially, so we think that some of the earlier patients would get a definite diagnosis nowadays.

576-P

ROUTINE NUTRITIONAL EVALUATION IDENTIFIES DISORDERED PEROXISOMAL FUNCTION IN A NEONATE WITH ISOVALERIC ACIDEMIA DETECTED THROUGH NEWBORN SCREENING

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Background: Isovaleryl-CoA dehydrogenase deficiency (IVDD; OMIM 243500) is one of several heritable organic acidurias detectable via expanded newborn screening with tandem mass spectrometry. We report a 13 month-old asymptomatic male with enzyme confirmed IVDD who, upon routine nutritional follow-up, presented evidence of peroxisomal dysfunction.
Methods/Results: Expanded newborn screen (2 days of life) revealed an isolated elevation of C5-carnitine (2.95 μmol/L; normal <0.09). Plasma acylcarnitine and urine organic acid profiles were consistent with IVDD, further supported by fatty acid oxidation probe analysis in fibroblasts (C5-carnitine 0.24 μmol/g protein; normal <0.04) and absent fibroblast isovaleryl-CoA dehydrogenase activity. Plasma fatty acid (FA) analysis, for assessment of nutritional status during protein restriction/L-carnitine supplementation, revealed elevated C26:0 (5.0 μmol/L; normal <1.3). Very long chain fatty acid (VLCFA) analysis corroborated this finding: C26:0, 3.8 μmol/L; C24:0/C22:0, 1.79 (normal <1.39) and C26:0/C22:0, 0.09 (normal <0.02). VLCFA analysis in a second biochemical genetics laboratory was also supportive of peroxisome dysfunction: C26:0 (1.25 μmol/L; normal <0.19); C24:0 (30.9 μmol/L; normal <20.2); C24:0/C22:0, 1.84 (normal <0.9); and C26:0/C22:0, 0.04 (normal <0.01).
Conclusions: Confirmatory testing for X-linked adrenoleukodystrophy (XALD) is in progress. The fortuitous identification of XALD in the currently asymptomatic patient has significant treatment and counseling ramifications for this family. Coinheritance of X-linked and autosomal recessive disorders (IVDD, chromosome15q14) is likely coincidental but previously unreported.

577-P

IMPROVEMENT OF BONE DENSITY AFTER THE USE OF BIPHOSPHONATES IN A PATIENT WITH HYPOPHOSPHATASIA

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At the age of two years the patient was referred to our out-patient clinic because of dental problems. He also suffered from pain in the legs, tiredness upon walking for which he had seen the physiotherapist. The history did not show bone fractures. Family history: dental problems, rickets and osteoporosis. Physical examination: Normal weight and height for age. Abnormal dentition: incisors were absent. He had an enlarged liver. Radial broadening of the wrists. No other signs of rickets were seen.

Metabolic investigation into bone diseases revealed a low level of serum alkaline phosphatase and an increased level of phosphoethanolamine in urine. The diagnosis of childhood type hypophosphatasia was made.

The child was treated with calcium suppletion and after informed consent also with biphosphonates (alendroninic acid) to prevent decreased bone density and fractures. His total body bone density increased from a Z-score of –4.6 at 3 years 8 months to –0.7 SD at the age of seven. At the time of the decreased bone mineral density the boy suffered a lower arm fracture, which took a long time too heal. Thereafter no fractures occurred. Patients with hypophosphatasia could benefit from treated with biphosphonates.

(A.M.Boot, januari 1996, Lunar DPXL).

578-P

CONTRIBUTION OF CEREBRAL PROTON MAGNETIC RESONANCE SPECTROSCOPY IN THE DIAGNOSTIC EVALUATION OF DEVELOPMENTAL DELAY

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Background: Proton magnetic resonance spectroscopy (1HMRS) of the brain is a relatively new technique yielding semi quantitative information on levels of a few brain metabolites, which might be of value in the process of determining the etiology of developmental delay.
Objective: To assess the contribution of 1HRMS to the diagnostic evaluation of children with developmental delay.
Methods: In 96 patients admitted to our multidisciplinary outpatient clinic neuroimaging including 1HMRS was performed because of neurological abnormalities in addition to the developmental delay or an IQ below 50. Multivoxel 1HMRS was performed in a plane superior to the lateral ventricles, enabling to study gray and white matter separately. Main metabolites assessed were N-acetylaspartate (NAA), choline (Cho), creatine (Cr), glutamine/glutamate (Glx) and lactate.
Results: 1HMRS contributed to the diagnosis in 3 patients (1× guanidinoacetate methyltransferase deficiency, 2× mitochondrial respiratory chain disorder). In 8 patients clear abnormalities (elevated Cho, decreased NAA, increased Glx) were observed but did not contribute to the diagnostic evaluation of DD. One patient, with markedly elevated Cho and decreased NAA later was diagnosed with a thyroid hormone transporter defect.
Conclusion: 1HMRS contributes to the diagnostic evaluation of DD in a few cases. At present, the clinical significance of the more often observed changes in metabolite pattern is unclear. Still, when performing magnetic resonance in patients with DD, addition of 1HRMS is clearly justified.

579-P

VARIABILITY OF PHENOTYPE IN TWO PATIENTS WITH DIFFERENT MUTATIONS IN MONOCARBOXYLATE TRANSPORTER 8

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Background: Monocarboxylate transporter 8 (MCT8) plays an important role in neuronal uptake of triiodothyronine (T3). MCT8 deficiency is an X-linked disorder with a clinical phenotype of severe mental retardation, infantile hypotonia gradually changing into bilateral spasticity, and sometimes dyskinesias of the hands. Serum T3 levels are elevated.
Objective: To demonstrate the genetic and clinical variability of this disorder.
Methods: Patient 1 is a 4-year-old boy who presented at the age of 8 months with severe developmental delay, axial hypotonia, and progressive spasticity. He is microcephalic, severely retarded and shows athetoid movements of both hands. Patient 2 is a 2-year-old boy who presented at 9 months with delayed motor development. He is macrocephalic with bilateral spasticity. His mental performance is only mildly behind. Both have white matter abnormalities on cerebral MRI and elevated free T3 of 8.3 and 9.1 pmol/l, respectively (N 2.6–5.7 pmol/l). *MCT8* mutation analysis was performed and T3 uptake *in vitro* was analyzed in patient's fibroblasts and transfected cells.
Results: In patient 1 a Gly564Arg substitution was identified causing a complete inactivation of MCT8 *in vitro*. In patient 2 a 3-nucleotide deletion (1497–1499) was found, resulting in a loss of phenylalanine (delPhe501) causing a partial defect in MCT8 function *in vitro*.
Conclusion: Patient 1 shows the classical clinical MCT8 deficiency phenotype as a result of complete loss of MCT8 function. In contrast, patient 2 has a small deletion in *MCT8* with less functional consequences and milder clinical symptoms. This substantiates a genotype-phenotype correlation in MCT8 deficiency.

580-P

NUTRITIONAL STATUS OF 23 PATIENTS SUBMITTED TO LOW PROTEIN DIET

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Patients submitted to restrictive diets are at risk for several nutritional deficiencies that could have an important impact on growth and body composition if not prevented.
Aim: To assess nutritional status of patients followed at our outpatient clinic, submitted to low protein diet.
Methods: Prospective study of 23 patients (mean age 5 years 9 months; 15 girls) under a low protein diet with no signs of infection or disease decompensation. The pathologies and respective number of analysed patients were: fifteen aminoacidopathies (eight PKU), five organic acidurias and three urea cycle disorders. Anthropometric measurements (height/weight, body mass index, triceps and subscapular skinfold and arm circumference), bioimpedance analysis (body fat and body lean) and biochemical parameters (haemoglobin, ferritin, albumin, pre-albumin, branched-chain plasma amino acids, retinol binding protein, urea, creatinine) were evaluated.
Results: The great majority of the patients showed anthropometric and biochemical evaluations in the normal range for age and sex. About the anthropometric evaluations the exceptions were: 2/8 boys with low weight/height; 6/23 and 1/23 with subscapular skinfold and arm circumference out of the normal range, respectively. About the biochemical evaluations the exceptions were: 2/23 had low haemoglobin and albumin levels, 5/23 had low plasma urea level, 6/23 had high ferritin level and 3/23 had branched chain plasma amino acids out of range. In what concerns to bioimpedance analysis 14/23 had high body fat values.
Conclusions: Patients submitted to low protein diets can have a good nutritional status if minimum natural protein, vitamin and mineral requirements are achieved.

581-P

EVIDENCE BASED NATIONAL DIETARY GUIDELINE OF THE KETOGENIC DIET (THE DUTCH EXPERIENCE)

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That fasting can be a cure for seizures is known for centuries. Since the early 20th century the medical use of the ketogenic diet (KD) emerged as a strategy to mimic the biochemical effects of fasting. KD is now an accepted therapy in drug-resistant epilepsy and the following metabolic disorders: glut-1 deficiency syndrome (GLUT-1), pyruvate dehydrogenase complex deficiency (PDHC) and mitochondrial disease (complex 1 deficiency). In the Netherlands, every year, 45–50 patients with intractable epilepsy or metabolic diseases start with KD.

Since June 2004 a working party of clinical (paediatric)neurologists, (metabolic) paediatricians,(paediatric) dieticians and pharmacists (known as the Dutch Multidisciplinary Collaboration Ketogenic Diet) has joint forces with the aim to achieve a medical protocol for KD that will be used nationwide in the Netherlands. An evidence-based dietary guideline is part of this protocol. The national database for collecting data about patients on KD diet was realized in 2005. All conditions now meet for scientific research in the future.

The dietary guideline includes both Classical KD and KD with MCT. Consensus has been achieved about dietetic issues as calculation energy, macro- and micronutrients need, initiation-, monitoring, and termination of KD. Nutritional advice for special conditions are given like intercurrent illness, growth delay etc.

The authors want to share their experiences with the audience and debate about the pitfalls during the process of writing, implementation and use of this national dietary guideline of the KD.

582-P

RENAL TUBULAR ACIDOSIS WITH FANCONI SYNDROME CAUSED BY KETOGENIC DIET: REPORT OF TWO CASES

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We report two children with symptomatic renal tubular acidosis with Fanconi syndrome after being put on ketogenic diet (KD). KD is a valuable therapeutic option for patients with intractable epilepsy. Potential known adverse effects are: metabolic acidosis due to excessive amount of organic acids, nephrolithiasis, and osteoporosis.
Case report 1: The girl suffered from adenylsuccinate lyase deficiency presented with intractable seizures from the neonatal period. Before introducing of KD at age of 3 years. there were no blood gas disturbances. During evaluation at the age of 5 years. hyperchloremic metabolic acidosis (Cl 108 mmol/L, pH 7.21; HCO3 14.9 mmol/L; SBE –14.9 mmol/L; AG 19.1 mmol/L), hypouricemia with increased FEUA (0.9 mg/dl; 69.9%), hypophosphatemia with low Tmp/GFR (0.72 mmol/L; 0.25 mmol/L respectively) and glucosurie were noted. 1.5 month after discontinuation of KD all above parameters normalized.
Case 2: The second patient was a boy with epileptic encephalopathy of unknown origin from the 5th week of life. After introducing of KD at the age of 2 years. The patient developed hyperchloremic metabolic acidosis (Cl 112 mmol/L; pH 7.15; HCO3 10.8 mmol/L; SBE –18.2 mmol/L) with hyperphosphaturia (Tmp/GFR 0.1 mmol/L) and glucosuria. All above symptoms disappeared after discontinuation of the diet.
Conclusion: Although metabolic acidosis occurred in the majority of children treated with KD acidosis in described cases was caused by massive proximal dysfunction resulted in Fanconi syndrome. The nature of this phenomenon remains unclear. Patients treated with KD should be monitored towards renal tubular dysfunction.

583-P

HOME ENTERAL TUBE FEEDING IN IMD CHILDREN: A REVIEW OF CARER KNOWLEDGE AND TECHNIQUE

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Careful home management of enteral tube feeding (HETF) is vital in children with inherited metabolic disorders (IMD). Skills and practice of carers in administering feeds should periodically be reviewed by health professionals in the home where feeding takes place.
Aim: To assess carers of HETF children with IMD for knowledge and technique with regard to: hygiene; feed preparation and storage; checking tube position; care of tube; changing tube; and use of feeding pumps and equipment.
Methods: 40 patients (median age 5.1 years: range 0.3–13.6 years) with IMD requiring pump tube feeding were recruited. 12 had GSD; 11 organic acidaemias; 8 fatty acid oxidation disorders; 4 urea cycle disorders and 5 other conditions. 50% were fed by gastrostomy and 50% nasogastric tube. A questionnaire and practical assessment of feeding process was completed with carers by a dietitian and paediatric nurse in the child's home.
Results: The main issues identified were; poor hygiene practices (78% unclean work surfaces; 25% no hand washing); nightly carer sleep disturbance (78%) mainly due to children lying on tubes (70%); children sleeping in parent's room as a safety precaution (58%); inaccurate ingredient measuring (43%); irregular checking of tube position (40%); inadequate tube flushing (50%); poor knowledge of how to clear tube blockages (80%); incorrect priming of pump sets (50%); incorrect position for night feeding (63%); untrained secondary carers (43%); and poor knowledge of pump alarms, battery life and charging time.
Conclusions: Carers of children with IMD on HETF would benefit from regular updates on knowledge and techniques of HETF.

584-P

EFFECTS OF THE REFSUM DISEASE CAUSATIVE AGENT PHYTANIC ACID ON MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION

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Introduction: In Refsum Disease (RD) the fatty acid alpha-oxidation pathway is deficient due to mutations in the gene encoding phytanoyl-CoA-hydroxylase in the majority of RD patients. As a result, the alpha-oxidation substrate phytanic acid accumulates in RD patients, which is thought to be the main cause of the pathology. However, until now it is not clear in what way phytanic acid exerts its toxic effects. Recent studies have shown that phytanic acid has multiple detrimental effects on mitochondrial functioning.
Methods: In order to generate more insight into the toxic effects of phytanic acid on mitochondria we examined the effects of unesterified phytanic acid on mitochondria using: (1) ATP synthesis measurements in digitonin permeabilized fibroblasts, and (2) mitochondrial membrane potential measurements in intact fibroblasts with confocal microscopy.
Results: (1) The results of our study show that phytanic acid inhibited ATP synthesis in mitochondria of digitonin permeabilized cells with substrates entering at the level of complex I (malate), or complex II (succinate). This inhibition was specific for phytanic acid when compared to other analogues. (2) Membrane potential studies in intact cells showed that phytanic acid decreased the membrane potential dose dependently with the branched-chain fatty acids again being the most effective.
Conclusions: Our *in vitro* data was in good agreement by with data from intact cells. From this we conclude that phytanic acid primarily acts as a rather specific uncoupler of mitochondrial oxidative phosphorylation.

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