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Contents

Workshop 2: Mitochondrial Disorders

Workshop 3: Screening for Inborn Errors of Metabolism 1

Workshop 4: Screening for Inborn Errors of Metabolism 2

Workshop 5: Lysosomal Storage Disorders 1

Workshop 6: Lysosomal Storage Disorders 2

Workshop 7: Inborn Errors of Metabolism In Asia

Workshop 8: Citrin Deficiency

Workshop 9: Enzyme Replacement Therapy

Workshop 10: Gene and Cell Therapy

Workshop 11: Long Term Management of Inborn Errors of Metabolism

Workshop 12: Management of Urea Cycle Disorders in Asia

Workshop 13: Peroxisomal Disorders and Fatty Acid Oxidation Defects

Workshop 14: Stem Cell Biology

Workshop 15: Neuroscience in Inborn Errors of Metabolism

Workshop 16: Dietetic Management

Oral Presentations

Oral 1: Organic Acid Disorders 1

Oral 2: Screening for Lysosomal Disorders

Oral 3: Lysosomal Disorders 1

Oral 4: Mitochondrial Disorders 1

Oral 5: Mitochondrial Disorders 2

Oral 6: Enzyme Replacement Therapy

Oral 7: Fabry Disease 1

Oral 8: Urea Cycle Disorders 1

Oral 9: Neurometabolic Disorders 1

Oral 10: Endocrinological and Metabolic Disorders

Oral 11: Hyperphenylalaninaemias and Biopterin Defects 1

Oral 12: Organic Acid Disorders 2

Oral 13: Organic Acid Disorders 3

Oral 14: Screening for Inborn Errors of Metabolism

Oral 15: Fatty Acid Oxidation Defects

Oral 16: Urea Cycle Disorders 2

Oral 17: Other Amino Acid Disorders

Oral 18: Neurometabolic Disorders 2

Oral 19: Glycogen Storage Diseases

Oral 20: Carbohydrate Disorders

Oral 21: General Aspects of IEM

Oral 22: Enzyme Replacement Therapy

Oral 23: Enzyme Replacement Therapy 3

Oral 24: Neueromuscular Disorders

Oral 25: Hyperphenylalaninaemias and Biopterin Defects 2

Oral 26: Hyperphenylalaninaemias and Biopterin Defects 3

Oral 27: Hyperphenylalaninaemias and Biopterin Defects 4

Oral 28: Hyperphenylalaninaemias and Biopterin Defects 5

Oral 29: Fabry Disease 2

Oral 30: Lysosomal Disorders 2

Oral 31: Mitochondrial Disorders 3

Oral 32: Lysosomal Disorders 3

Oral 33: Mucolipidoses

Oral 34: Peroxisomal Disorders

Oral 35: Vitamin Disorders

Oral 36: Porphyria and Metal Disorders

Poster Presentations

Poster 1: Screening for Inborn Errors of Metabolism

Poster 2: Hyperphenylalaninaemias and Biopterin Defects

Poster 3: Organic Acid Disorders

Poster 4: Other Amino Acid Disorders

Poster 5: Fatty Acid Oxidation Defects

Poster 6: Mitochondrial Disorders

Poster 7: Carbohydrate Disorders

Poster 8: Urea Cycle Disorders

Poster 9: Purines and Pyrimidine Disorders

Poster 10: Lipidoses/Fabry Disease

Poster 11: Peroxisomal Disorders

Poster 12: Metal and Vitamin Disorders

P-13: Congenital Anomalies

Poster 14: Endocrinological Disorders

Poster 15: Neurometabolic Disorders

Poster 16: Lipoprotein and Lipid Disorders

Poster 17: Mucopolysaccharidoses and Mucolipidoses

Poster 18: Other Disorders

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.

All authors are invited to provide a colour picture that may be used for the front cover of the issue in which the article appears.

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Garrod's Memorial Lecture

LEGACIES OF GARROD'S BRILLIANCE: ONE HUNDRED YEARS AND COUNTING

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In 1906, precisely one hundred years ago, Archibald E. Garrod was modestly and methodically making his observations about albinism, alcaptonuria, cystinuria, and pentosuria. He referred to these conditions as 'inborn errors of metabolism' a term still current (as the title of our Congress signifies). It is impossible to overstate the prescience the brilliance of his work. Years before the word 'gene' was coined, Garrod knew that these disorders behaved as Mendelian recessives. Decades before the realization that genes encode enzymes, Garrod proposed that these inborn errors altered catabolic pathways controlled by the activity of 'special enzymes set apart for each particular purpose.' A half century before the discovery of DNA's double helix, Garrod laid the ground work for the analysis and confirmation of its principles and predictions in humans. These are but the early scientific legacies of Garrod's work. Today we recognize hundreds of inborn errors, affecting virtually every compartment and every step in every pathway of anabolism and catabolism. Thanks to progress in protein chemistry and gene cloning, we understand much about the mutations responsible for these conditions and can account for some of the genotypic and phenotypic heterogeneity that mark them. Thanks to the genome effort, many of the responsible genes have been mapped and sequenced. The significance of these conceptual and molecular truths is far reaching, but Garrod's legacies extend well beyond them to the clinical management of affected patients. Our impressive capabilities to detect these disorders in neonates, in children, and in adults have been constructed directly from application of Garrod's concepts of metabolism normal and abnormal, blocked and unblocked. So, too, have our approaches to treatment and prevention. Looking only narrowly at the disorders Garrod studied, one can discern the many ways we intervene on behalf of patients and families: medically and surgically; with diets and drugs; through avoidance and administration. As the 21st century unfolds, Garrod's legacies will evolve, but not lessen: extending the general approach honed for rare conditions (in which single gene mutations are necessary and sufficient to cause disease) to common conditions (in which mutations predispose to, but are not sufficient to produce disease); convincing our societies that these so-called orphan diseases merit attention from the biopharmaceutical and medical device industries; assuring that advanced diagnostic technology respects the privacy rights of patients and families. Finally, each participant at this Congress is part of Garrod's legacy the human part working to maximize our understanding of, and minimize the consequences of, the inborn errors of metabolism. To paraphrase President John F. Kennedy's words on coming to Berlin when it was being blockaded, each of us can proudly and rightly say, 'I am a Garrodian.'

President Lecture

NOVEL TREATMENT FOR NEUROGENETIC DISORDERS

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Many inherited metabolic disorders (IMD) exhibit serious neurological clinical manifestations, in which there is a few therapy to treat these disorders in the past. But, recently, these disorders could be treated by several possible procedures. These therapeutic approaches include (1) pharmacological therapy including small molecules such as chaperones therapy or substrate deprivation therapy, (2) organ transplantation/ bone marrow transplantation (BMT), (3) enzyme replacement therapy (ERT), (4) cell therapy and gene therapy or combined therapies. Among various IMD, we focused on the studies for the treatment of lysosomal storage diseases (LSD). In order to treat neurological LSD, more than 100 cases with LSD have been treated by BMT in Japan. Among these disorders, MPS-I (Hurler) and II (Hunter) plus aderenoleukodystrophy are most common disorders treated by BMT. High dose of ERT may effect for the improvement of CNS in Gaucher disease. More than 100 cases with Gaucher disease have been treated by ERT and about 40% of these cases are neurological form. The outcome for neurological effects by ERT exhibited minor, but the ERT has an efficacy to prolong their life span in neurological Gaucher disease. The experimental therapeutic trials have performed in MPS VII (Sly) and Krabbe mice using mesenchymal stem cell and neural stem cell therapy, and gene therapy. The cell therapy and gene therapy using adenovirus, retrovirus vectors successfully treated the neurological abnormalities of murine model of Krabbe disease and Sly disease. A small molecule therapy using chemical chaperones and substrate deprivation may have a potential importance for the treatment of neurogenetic disorders, since these chemical compounds can across blood brain barrier. These results suggest that several modes can be applied for the treatment of genetic neurological disorders.

Plenary 1

INBORN ERRORS OF METABOLISM: PAST, PRESENT AND FUTURE

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The field of inborn errors of metabolism (IEMs) has come a long way since Sir Archibald Garrod coined this terminology in the early 20th century. Garrod first used the phrase IEM in his Croonian lectures to the Royal College of Physicians of London in 1908, following his concept of 'chemical individuality' that he first published in the Lancet in 1902. Garrod's ideas were truly prescient, and, despite how far we have come, we not only use his terminology, but also pursue his notion about individuality to this very day. Through the latter half of the 20th century we saw a dramatic increase in the number of recognized IEMs and the understanding of their etiology. This growth in the number of disorders was fueled by constantly improving diagnostic technologies, including the amino acid analyzer, gas chromatography-mass spectrometry and tandem mass spectrometry (MS/MS). Advances in enzymatic analyses permitted the understanding of the enzyme activity deficiencies that resulted in the metabolite alteration in these disorders. The growth of molecular genetic diagnostic capability subsequently led to a fundamental knowledge of the mutations responsible for these IEMs. The Human Genome Project brought us rapidly increasing sequence information over the 1990s into the early 2000s, along with increased ease and decreased expense of sequencing. The concept of genetic determinism was inherent in the Human Genome Project. Genetic determinism posits that our future is determined by the sequences of our genes and many of us believed that we would be able to predict the phenotypes of our patients from their genotypes. As we gained more knowledge about the genotypes of our patients, we found that in general they did not predict the phenotype. Scriver and Waters first argued that 'monogenic traits are not simple,' using phenylketonuria as the example. Subsequently, beginning in 2000, Dipple and McCabe generalized the concept 'simple' Mendelian disorders are in fact complex traits involving modifying genes and systems dynamics. Systems biology and the mathematical reduction of biological networks began to be applied to our understanding of IEMs. Genomic medicine brings together our knowledge of genetics, abilities in population-based screening and opportunities in large population clinical trials. The promise of genomic medicine will be clinical care that is predictive, preventive and personalized. These concepts have been part of biochemical genetics since Garrod, and we have the opportunity to demonstrate the value of genomic medicine for our patients with IEMs as we lead the way in understanding and managing common complex diseases.

Plenary 2

MEDICAL GENOMICS? A WALK IN THE GARDEN OF EDEN

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'The beginning is the most important part of the work' Plato, The Republic. Book II (377 BC).

Research on inborn errors of metabolism, established in part, the framework for the fundamental discipline of genetics, and brought the ? laboratory bench? to the art of medicine. Approximately 40 years ago the application of tissue culture technology allowed one, for the first time, to utilize *in vitro* techniques to diagnosis rare disorders of metabolism. Scientific and technological advances now allow contemplation of gene therapy and the prospect of stem cell therapy. Molecular genomics, bioinformatics, the development of new models systems have revolutionized our understanding of birth defects, complex diseases and provided the basis for new approaches to therapeutics. Functional imaging techniques coupled with molecular genomics have provided the basis not only for the dissection of cognition and behavior, but make possible avenues to explore the interaction of environment and its influence on disease.

However, during this half century of scientific revolution our capacity to conceptualize the context in which these advances, and their limitations, may be applied has provided dilemmas for society. Ethical and societal issues raise the specter of concerns from eugenics to invasion of privacy, and additionally provoke questions of genetic discrimination in employment and healthcare. Societal forums identify the need for community norms, and involvement in the management of the scientific knowledge generated as a result of these advances. Scientific investigators and biomedical researchers/physicians must strive to educate and provide the basis for an informed citizenry.

Plenary 3

MOLECULAR MECHANISM OF DEVELOPMENT AND DIFFERENTIATION OF THE BRAIN: TOWARDS THE UNDERSTANDING OF THE PATHOLOGICAL CONDITION

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Brain has a beautiful architecture and plays an important role in learning and mortor coordination. Since I devoted to the work on the cerebellum by analysing the cerebellar mutant mice with abnormal behavior comparing with wild type mice, we are able to correlate behavior, morphology and molecules. We have found various molecules which are essential for the cerebellar development or neural plasticity such as Zic, reelin/CR-50 antigen, and IP_3 receptor. (1) We found a novel gene, *zic* (zinc finger protein enriched in the cerebellum). Odd-paired was found to be a *Drosophila* homologue of zic that regulates wingless and engrailed. Zic has five isoforms and they were found to be involved in brain development (Zic 1, J Neurosci. 18, 284–93, 1998) (Zic 2, PNAS 97,1618–923, 2000) (Cell 114, 545–57, 2003) or right-left axis formation (Zic 3, Development 127, 4787–95, 2000). (2) Reeler is a mutant characterized by neuronal malpositioning and lack of lobule formation. We succeeded in raising the antibody (CR-50) against *reeler* gene product by immunizing wild embryo brain in *reeler* mouse (Neuron 14, 899–912, 1985). CR-50 reacts with reelin (J Neurosci. 17, 23–31, 1997). We further discovered *yotari* mutant mouse which has abnormality in mouse *disabled-1* (*mDab-1*) gene exhibiting reeler-like phenotype (Nature 389, 730–3, 1997). mDab-1 is a dowstream target of reelin. Reelin/mDab-1 signaling are found to be essential in neuronal positioning. (3) We discovered IP_3 receptor as a P400 protein (enriched in the Purkinje neuron but is greatly decreased in *pcd* and *staggerer* mice) (Nature 342,32-38,1989). IP_3 receptor is important in fertilization (Science 257, 251–5, 1992), dorso-ventral axis formation (Science 278, 1940–3, 1997) (Nature 417, 295–9, 2002), neurite extension (Science 282, 1705–8, 1998) and neuronal plasticity (Nature 408, 584–8, 2000). We discovered that IP_3 receptor plays a role in redox regulation (Cell 120, 85–98, 2005) and is essential for excrine secretion (Science 309, 2232–4, 2005). The data obtained from these analysis give us a clue to understand the pathological condition of our health.

Plenary 4

CLINICAL APPROACH TO TREATABLE INBORN ERRORS OF METABOLISM IN EMERGENCY

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In view of the major improvements in treatment, it has become increasingly important that in order for physicians in the front line not to miss a treatable disorder, that they should be able to initiate a simple method of clinical screening, particularly in the emergency room, not only for children but also for adults. The clinical diagnosis of IEM relies upon a small number of important principles:

- In the appropriate clinical context consider an IEM in parallel with other more common conditions.
- Be aware that symptoms that persist and remain unexplained after the initial treatment and the usual investigations have been performed for more common disorders may be due to an IEM.
- Do not confuse a symptom or a syndrome with aetiology – the underlying cause may be an IEM yet to be defined.
- Remember that an IEM can present at any age, from fetal life to old age.
- Initially consider IEM which are amenable to treatment.
- First provide care for the patient (emergency treatment) and then the family (genetic advice).

We propose a new diagnostic approach to treatable IEM: first list the life-saving treatments (such as vitamins, cleansing drugs, extra-corporeal removal procedures, special diets, etc.), and then check if the presenting symptoms of the patient are compatible with one IEM treatable by these life-saving procedures.

Plenary 5

'BIOBANK JAPAN INITIATIVE' AND WHOLE-GENOME SNP ASSOCIATION STUDY

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The 'Biobank Japan' project started in June 2003 by the support of the Japanese government. The aims of this project are (1) discovery of genes susceptible to diseases, or those related to effectiveness or adverse reactions of various drugs, (2) identification of molecular targets for evidence-based development of drugs or diagnostic tools, (3) identification of the important genetic information that can be applied for establishment of 'personalized medicine', and (4) studies on gene-environment interaction for prevention of diseases. To achieve these goals, we planed to collect of DNAs, sera and clinical information from 300 000 patients who mainly have common diseases by March 2007. As the research resource bank, we constructed 'Biobank Japan' that consisted of facilities for storing DNAs (the maximum capacity of 1 000 000 tubes; fully-automated sample handling system) and sera (the maximum capacity of 3 000 000 tubes in liquid nitrogen; semi-automated handling system). We also established clinical information database as a part of 'Biobank Japan' activity. For protection of individual privacy, we introduced (1) two-step anonymalization of individual identification code by two-dimension code system and (2) avoidance of placing individual identification information with genotyping information together. By the end of February in 2006, we have obtained a written informed consent from more than 135 000 patients (a total number of disease cases is more than 194 000 because of multiple diseases in one patient) from 66 hospitals participating in this project. We have been performing the genome-wide association studies using 250 000 SNPs (covering most of our haplotype blocks) to identify genes of medical importance using these samples. Accumulative data should provide us the basis to identify genes associated with common diseases and to establish the personalized treatment.

Plenary 6

GLYCOGEN STORAGE DISEASE TYPE I AND TYPE II: TREATMENT UPDATES

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Glycogen storage disease type I is caused by a deficiency in glucose-6-phosphatase-α (GSD-Ia) or by a defect in glucose 6-phosphate transport protein (GSD-Ib). Patients affected by GSD-I are unable to maintain glucose homeostasis and present with hypoglycemia, growth retardation, hepatomegaly, nephromegaly, hyperlipidemia, hyperuricemia, and lactic academia. Early diagnosis and early initiation of dietary therapy, primarily using uncooked cornstarch to maintain normoglycemia, have improved the outcome of the disease. However, some long-term complications remain. A modified cornstarch is being tested for its ability to maintain longer duration of normoglycemia than the current unmodified starch. Gene therapy has been tested in mouse model as well dog model of GSD-Ia; an AAV vector containing the G6Pase promoter driving G6Pase expression pseudotyped as AAV8 appears promising in long-term survival and reversal of growth retardation and biochemical abnormalities.

Pompe disease, also known as glycogen storage disease type II, is a debilitating and often fatal genetic muscle disorder for which no approved treatment currently exists. In its most severe form, most infants with the disease die from cardiac-respiratory failure by age one year. We have been engaged in the development of recombinant human acid (α-glucosidase (rhGAA) – the enzyme that Pompe patients lack – for enzyme replacement therapy since 1991. We have produced in the laboratory via the genetic engineering of Chinese hamster ovary cells, a cell line overproducing the rhGAA. Pre-clinical study showed that rhGAA helped relieve symptoms and reduced glycogen storage in animal models of Pompe disease. The first human clinical study with rhGAA was started in 1999, followed by several trials including a pivotal study with Myozyme (rhGAA manufactured by Genzyme) in patients with infantile onset Pompe disease that completed in 2004. Available data from various trials showed that rhGAA is well tolerated and is capable of improving cardiac and skeletal muscle functions in these patients although skeletal muscle improvements are more variable among patients. The longest survival is in an infant who was started on ERT at age 2 months, he is currently age 7 years and doing well clinically and developmentally. Challenges in the development of this novel therapy and potential causes for differential patients and tissue responses will be discussed.

Plenary 7

RECENT ADVANCES IN ENZYME REPLACEMENT THERAPIES FOR LYSOSOMAL DISEASES

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In 1964, Christian de Duve first suggested that lysosomal storage diseases (LSDs) could be treated by replacing the defective enzyme with its normal counterpart. Early experiments in tissue culture, animal model systems, and pilot clinical studies demonstrated 'proof of concept' for this therapeutic strategy. In the 1970s, the discovery of the mannose-6-phosphate receptor-mediated pathway for the intracellular delivery of glycoproteins to the lysosome provided the rationale for the treatment of lysosomal disorders by enzyme replacement therapy (ERT). However, it was not until the early 1990's that ERT became a reality with the demonstration of its safety and effectiveness in patients with Type 1 Gaucher disease.

Currently, ERT for Type I Gaucher disease, Fabry disease, and mucopolysaccharidosis (MPS) I and VI is approved in Europe, the United States, and many other countries. Clinical trials for Pompe disease and MPS II have been completed and submitted for approval, and trials for Niemann-Pick B disease are about to begin. Experience with over 4000 Gaucher, 1000 Fabry, XXX MPS I and XXX MPS IV patients have demonstrated the safety and efficacy of ERT in these diseases, and taught general principles for effective therapy in these diseases. The biodistribution of these recombinant enzymes, which are administered intravenously, is determined by the receptor-mediated uptake and delivery via the mannose 6-phosphate (mannose for Gaucher disease) receptors for targeting cellular lysosomes. However, little, if any, enzyme crosses the blood/brain barrier, so enzyme replacement does not alter heterodegenerative symptoms. Moreover, enzyme delivery to cells and tissues is a function of the presence and density of the receptors on different all types. Thus, the biodistribution of the enzyme and the substrate clearance from cells depends on the dose of enzyme, its mannose 6-phosphate content, and the density of the receptors on a given cell type. Certain cells are easily reached while others are not and require larger doses. Maintenance of substrate clearance requires continuous treatment since ceasing replacement results in rapid reaccumulation. Finally, early initiation of treatment is essential to avoid the irreversible manifestations of the disease.

Plenary 8

ADVANCES IN THE TREATMENT OF AMINO ACID AND ORGANIC ACID METABOLISM

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In this review three areas will be discussed briefly.

(1) Cofactor responsive disorders: *Tetrahydrobiopterin and phenylketonuria*. It is now clear that the protein tolerance of a significant number of patients with phenylketonuria will improve if given large doses of tetrahydrobiopterin, although the response may not always be completely clear. This may simplify the treatment but it is expensive and it can be difficult to decide who should receive this therapy. The mechanism is important and may have implications for the treatment of other disorders. *Pyridoxine dependent seizures.* Although not strictly a disorder of amino acid metabolism, pyridoxine is a cofactor in many reactions of intermediary metabolism. The elucidation of the metabolic basis of pyridoxine dependent seizures has identified a new mechanism to explain cofactor responsive disorders. The defect is in D(1)-piperideine-6-carboxylate-a-aminoadipic semialdehyde dehydrogenase and as a result of which pyridoxine-5'-phosphate is inactivated. A similar mechanism is likely to be responsible for the fits in hyperprolinaemia type II.

(2) Mechanisms of neurological damage: The neurological outcome of many inborn errors of metabolism remains disappointing but the mechanisms that are responsible for this poorly understood. Many of the inborn errors of amino acids and organic acid metabolism are steps on catabolic pathways of essential amino acids and as a result there is accumulation of potentially toxic metabolites. Traditionally the aim of treatment has been to give just sufficient protein to meet the essential requirements for growth and development but with minimal residue that would normally be catabolised in the pathway. However this traditional 'protein turnover model' has shortcomings. For example patients with glutaric aciduria type 1 who are 'low' excretors and yet have the same neurological complications as those with classical disease. This and other lines of information have focused on the properties of the blood brain barrier. This has important implications for the management of these disorders.

(3) The role of liver transplantation and isolated cell transfusions: The prognosis for severe organic acidaemias has improved gradually but remains disappointing. It is quite natural therefore that the possibility of organ transplantation to replace missing enzyme has been given careful consideration. However the role of liver and isolated cell transplantation is still not completely clear although many patients have done very well, there is still a significant mortality and morbidity. The management of end stage renal failure in patients with methylmalonic acidaemia, kidney or liver and kidney transplant, remains uncertain.

There remains the need for carefully documented and controlled studies to answer questions about the management of these disorders. As they are rare, international collaboration between centres is going to be essential if evidence based advances are to be made.

Plenary 9

A HEREDITARY DISEASE CAUSED BY CHROMATIN REMODELER DEFECTS: THE ROLE OF WILLIAMS SYNDROME TRANSCRIPTION FACTOR (WSTF) IN CHROMATIN REMODELING

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We have purified a novel ATP-depending chromatin remodeling complex (WINAC) directly interacting with vitamin D receptor (VDR), and the purified complex composed of the SWI/SNF-type complex components and a Williams syndrome transcription factor (WSTF) (Kitagawa et al., Cell, 113, 905, 2003). Deletion of the locus of *WSTF* and some other genes at chromosome 7q11. 23, causes diverse abnormalities in Williams syndrome patients as an autosomal dominant trait. However, the physiological impact of WSTF is still largely unknown. Ligand-induced transactivation of VDR was potentiated by WINAC through chromatin remodeling and histone modifications (Kitagawa et al., Cell, 113, 905, 2003). We then further studied the function of WINAC on the ligand-induced transrepression function of VDR for the newly identified a negative VDRE in the 25-hydroxyvitamin D3 1alpha-hydroxylase gene promoter (Murayama et al., EMBO J, 23, 1598, 2004). For the ligand-induced transrepression of VDR, VDR did not bind to the nVDRE, but physically associated with an activator (VDIR) bound upon the nVDRE together with a HDAC complex. The interaction of the WSTF bromodomain with acetylated histones and consequent assembly of WSTF, VDR and DNA-bound VDIR is required to initiate transrepression (Fujiki et al., EMBO J., 24, 3881, 2005). Finally to address the physiological role of WSTF, we generated *WSTF*-deficient mice. The *WSTF*-deficient mice were born with Mendelian frequency, but died within a few days. Histological analysis of embryos revealed severe cardio-vascular defects seen in Williams syndrome patients in all $WSTF^{-/-}$ and $\sim$10% of $WSTF^{+/-}$ E9.5 embryos and neonates (P0). In conclusion, our study provides the first genetic evidence that Williams syndrome represents a novel class of disorders hereditary chromatin remodeling disease.

Plenary 10

MITOCHONDRIAL DISEASES: AN UPDATE

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After a brief overview of the multiple functions of mitochondria and of the approximately 1300 proteins that compose them, I will consider recent epidemiological data (mitochondrial diseases are not all that rare!), and a 'neurological classification' of the mitochondrial diseases. I will then focus on defects of the mitochondrial respiratory chain, which comprise most of the disorders that we conventionally call 'mitochondrial encephalomyopathies'.

These disorders are especially interesting from a genetic pint of view because they may be due to defects of mitochondrial DNA (mtDNA) or defects of nuclear DNA (nDNA). As mtDNA is the 'slave' of nDNA, an interesting subgroup of Mendelian mitochondrial diseases is due to errors in the 'dialogue' between the two genomes.

Recent progress in mtDNA-related diseases includes: (i) new pathogenic mutations in protein-coding genes, especially those encoding subunits of complex I (ND genes); (ii) the pathogenic nature of homoplasmic mutations, whose expression is regulated by environmental and genetic factors; (iii) increasing interest in the functional and pathophysiological role of haplotypes. In contrast, there has been relatively little progress in our understanding of pathogenic mechanisms.

Advances in mendelian mitochondrial diseases include: (i) new mutations in genes for complex I subunits; (ii) identification of new mutant ancillary proteins associated with deficiencies of complexes I, III, IV, and V; (iii) better molecular understanding of disorders due to faulty intergenomic communication, which are associated with multiple mtDNA deletions, mtDNA depletion, or defects of mtDNA translation; (iv) the pathogenic role of alterations of the inner mitochondrial membrane phospholipid components, especially cardiolipin; (v) the emerging importance of defects in mitochondrial motility, fission, or fusion.

Therapy is woefully inadequate, although ingenious therapeutic strategies are being developed, at least in the laboratory. But this is another story deserving a separate lecture.

Plenary 11

MULTIPLE SULFATASE DEFICIENCY

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Multiple sulfatase deficiency (MSD) is a rare inborn neurometabolic disorder with a significant reduction in all sulfatase activities combining the clinical features of single sulfatase deficiency disorders including metachromatic leukodystrophy, different mucopolysaccharidoses, X-linked ichthyosis and chondrodysplasia punctata. MSD results from a failure to posttranslationally convert a cysteine residue which is located in the catalytic centre of all sulfatases into a Cα-formylglycine residue. The latter is essential for the cleavage of sulfate ester bonds and is generated in the lumen of the endoplasmic reticulum by the formylglycine generating enzyme (FGE) encoded by the *SUMF1* gene. FGE is a N-glycosylated, homodimeric Ca^{2+}-binding protein which contains in addition to three disulfide bonds a redox-active disulfide bond. The latter is located at the bottom of a solvent accessible cleft forming the active site. FGE catalyzes the conversion of cysteine to formylglycine by a novel oxigenase mechanism utilizing molecular oxygen and forming a covalently linked FGE-sulfatase intermediate. FGE escaping ER-retention is N-terminally trimmed by a furin type protease and secreted. This N-terminally truncated FGE is catalytically active but unable to activate sulfatases in the ER suggesting a role of the N-terminus for the interaction of FGE with nascent sulfatases. Mutations in the *SUMF1* gene cause MSD. Seven nonsense and 18 missense mutations have been identified. Only the latter have been observed in patients in homozygous form. Expression of a selected number of mutant alleles revealed that some of the missense mutations are associated with up to 20% residual activity.

Plenary 12

PATHOGENESIS AND PATHOPHYSIOLOGY OF CITRIN DEFICIENCY

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Citrin is a mitochondrial aspartate-glutamate carrier predominantly expressed in the liver, which plays a role in various metabolic pathways, including aerobic glycolysis, gluconeogenesis, urea cycle, and protein and nucleotide synthesis. We found that human citrin deficiency causes adult-onset type II citrullinemia (CTLN2) and neonatal cholestatic hepatitis (NICCD). CTLN2 patients suffer from hyperammonemia accompanied with disorientation, aberrant behavior, convulsion, and death. Hyperlipidemia, pancreatitis and hepatoma are often seen complications of CTLN2. NICCD patients suffer from jaundice, hypoglycemia, galactosemia, and multiple aminoacidemias including citrulline, Thr, Met, Tyr, and Arg. We propose that metabolic disturbances in citrin deficiency mainly result from aberrant management of liver cytosolic NADH. This is because citrin is a member of malate-aspartate shuttle which plays a role in aerobic glycolysis through transport of NADH reducing equivalent from cytosol to mitochondria. In the absence of citrin, aspartate can not be supplied from mitochondria, and is hardly formed through cytosolic aspartate aminotransferase because oxaloacetate is scarcely formed from malate due to a high $NADH/NAD^+$ ratio in the cytosol. This results in hyperammonemia, because lack of aspartate stops argininosuccinate synthetase reaction. A large amount of carbohydrate intake or administration results in deterioration due to cytosolic NADH accumulation: individuals with citrin deficiency take much less carbohydrate. Newly established citrin deficiency model mice show hyperammonemia which is exaggerated by administration of sucrose. Thus, several evidences reveal toxicity of carbohydrate in citrin deficiency. Therapies for hyperammonemia in general may not be adopted for citrin deficiency.

Plenary 13

SOMATIC STEM CELLS FOR ENDODERMAL ORGANS

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The pancreatic β8-cell is one of the most important targets in regenerative medicine. Many approaches regarding inducing insulin-prodicing cells by use of various types of pancreas-derived stem/progenitor cells such as pancreatic islets-derived stem cell and pancreatic duct epithelial cells have been tried. However, differentiation studies of extra-pancreatic tissue stem/progenitor cellsare limited. Among these, studies of hepatic oval cells are representative example. In liver injury model of 2-acethyaminofluorene administration combined with partial hepatectomy, oval cells with small duct-like appearance and with atypical ductular proliferation appeared form portal triad in hepatic lobules. These hepatic oval cells express not only hepatic markers such as α-fetoprotein, albumin and cytokeratin19, but also Thy-1 antigen known surface markers of the hematopoietic stem cell. Hepatic oval cells obtained by sorting Thy-1 strong positive fractions differentiate into pancreatic endocrine hormone-producing cells. Correspondingly, stem/progenitor cells prepared from the pancreas capable of differentiating into hepatic cells have been demonstrated. For example, cytokeratin19-positive pancreatic epithelial progenitor cells that transplanted into rat liver differentiate into hepatocytes. These reports give implication of a commonality between stem/progenitor cells of the different tissues.

The salivary gland consists of cells originating from the ectoderm and the endoderm. We previously reported isolation and characterization of progenitor cells that have ability to differentiate into both hepatocyte- and pancreatic-endocrine phenotypic cells *in vitro* from injured salivary gland. There are many experimental injuries caused by chemicals, heat shock and physiological methods. The progenitor cells from the rat, mouse and swine salivary gland share cellular characters such as expression of CD49f (integrin α6), c-Kit or Thy-1 antigen, intracellular laminin-immunoreactivity and abilities to differentiate into albumin-producing or insulin-producing cells *in vitro*. Human salivary glands-derived progenitor cells also share cellular character that we previously reported, and have ability to differentiate into insulin-producing cells *in vitro*.

These somatic stem cells from adult tissues will provide useful tool for treatment fo inborn errors of metabolism which involve endodermal organs.

Plenary 14

PHENYLKETONURIA: DIETARY AND THERAPEUTIC CHALLENGES

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Since the late 1960s, the main goal of the dietary treatment of PKU has been the prevention of mental impairment, based on an early diagnosis by means of neonatal screening and a low-Phe, low-protein diet supplemented with Phe-free amino acids. Long-term follow up studies have shown that normal growth in children and optimal health outcome in adults need further efforts to improve the composition of protein substitutes and the quality of the dietary intervention while ameliorating the patients' compliance. In planning the nitrogenous supply, Phenylalanine needs, and the balance with tyrosine and competing amino acids should be considered. Also the release of amino acids within the gut could impact the amino acid metabolism. The dietary manipulation of non-protein components has also progressed. Human milk and breastfeeding, together with the opportunity of long-chain polyunsaturated fatty acids supplementations, may be relevant for PKU infants due to their neuroprotective effects. Identification of BH_4 responsive patients, biochemical and genetic markers of responsiveness and the therapeutic dose of BH_4, are new diagnostic and therapeutic challenges. Present studies suggest that some HPA patients can benefit from BH_4 instead of dietary treatment, but further functional (not just biochemical) investigations are necessary. The early recognition of neurodegenerative processes are the next step in the treatment of the 'older' treated, PKU patients, even if still on diet. Nevertheless, the dietary treatment of PKU women in childbearing age still needs to be optimised as far as optimal requirements not just in pregnancy but through all the childbearing age.

Plenary 15

CURE OF ESTABLISHED NEUROLOGIC DISEASE AND EVIDENCE OF IN VIVO CROSS CORRECTION IN METACHROMATIC LEUKODYSTROPHY AFTER HSC GENE THERAPY

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Metachromatic leukodystrophy (MLD) is a demyelinating lysosomal storage disorder with an urgent medical need. We previously showed that transplantation of lentiviral vector-transduced hematopoietic stem cells (HSC) in pre-symptomatic MLD mice prevented disease manifestations. By reversing the neurological deficits and neuronal damage of affected mice, we now provide direct evidence of correction of an established neurological disease by HSC gene therapy. We show that the unique efficacy of gene therapy is dependent on over-expression of arylsulfatase-A (ARSA) in microglia cells derived from the transplanted gene-corrected HSC. We demonstrate the occurrence of widespread enzyme distribution from microglia, and of robust cross correction of neurons and other CNS cell types *in vivo*. By establishing a sustained hepatic source of ARSA in chimeric mice harboring ARSA over-expressing hepatocytes from transgenic donors, we failed to deliver the enzyme to the CNS, thus highlighting the role of microglia as exclusive source of bioavailable enzyme to the brain. These results provide a strong rationale for implementing HSC gene therapy in MLD patients.

Plenary 16

APPROACHES FOR THE TREATMENT OF LYSOSOMAL STORAGE DISEASES

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In recent years, remarkable advances have been made towards the treatment of lysosomal storage disorders (LSDs). Enzyme replacement therapy (ERT) is currently available for several disorders, and is under development for others. Small molecule, gene and stem cell-based approaches are also being developed in animal model systems, and in some cases are being evaluated in human patients. However, despite these remarkable advances, significant limitations remain. Our research focuses on three LSDs: acid sphingomyelinase (ASM)-deficient Niemann-Pick disease (Types A and B NPD), Farber Disease (acid ceramidase deficiency), and MPS VI (*N*-acetylgalactosamine-4-sulfatase deficiency). Several years ago, a complete knock-out mouse model of ASM-deficient NPD was developed (ASMKO mice). In these animals, as in human patients, the major target sites of pathology are the liver, spleen, lung and brain. ERT in the ASMKO mice was particularly effective in the liver and spleen, and to a lesser degree in the lung. As expected, it was ineffective in the brain. This led us to undertake efforts to gain a better understanding of the pathobiology of this disorder, particularly in these organs, and to develop new therapeutic approaches. For example, to achieve better enzyme delivery to the lung, we have used nanocarriers coated with recombinant ASM and an antibody fragment against Intercellular adhesion molecule I (ICAM1). After IV injection, these nanocarriers have excellent biodistribution to the lung ($>20\%$ of injected dose as compared to $<5\%$ for free enzyme), are delivered to lysosomes, and can degrade accumulated sphingomyelin. Gene therapy and stem cell-based approaches also have been studied in these animals, and several small molecules have been evaluated as well. Among these, zinc supplementation and chaperone therapy using a novel sphingomyelin analogue have yielded some success *in vitro*. In addition, we have used microarray analyses to identify genes that are abnormally expressed in the lungs and brains of ASMKO mice. This has led to the identification of several new biomarkers for this disorder, some of which can be used to monitor disease efficacy. For example, several inflammatory cytokines were found to be elevated in the serum of ASMKO mice, and the levels were reduced to normal following ERT or gene therapy. Serum growth hormone levels were also elevated in these mice, but were only reduced following brain-directed gene therapy. Overall, these studies have led to the development of several new therapeutic approaches for this disorder, and have provided new insights into the pathogenic mechanisms.

Plenary 17

MUCOPOLYSACCHARIDOSES: DIAGNOSIS, PATHOGENESIS AND TREATMENT

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Over the past ten years there has been a revolution in the options available for the early diagnosis, predictive testing and therapy for a group of eleven lysosomal storage disorders known as the mucopolysaccharidoses (MPS). Following isolation and characterisation of MPS genes, mutations were used to identify MPS patients and heterozygotes, investigate the relationship between genotype and phenotype – and to offer a prognosis. Further, this molecular genetics revolution enabled both enzyme and gene replacement therapies to be researched and trialed. As a result clinical trials to demonstrate the efficacy and safety of intravenously delivered enzyme replacement have been completed or are in trial. Enzyme replacement for MPS I and VI patients have FDA approval, with phase III trial completed for MPS II patients. A major concern – and therefore research activity – is how to deliver replacement enzyme to the brain of those patients destined to develop brain pathology. Gene therapies for most MPS types are actively being researched to evaluate the efficacy and safety of using gene vectors in a number of MPS animal models. Other therapies: stem cell implantation, chaperone, substrate depletion and stop codon read through are also under evaluation in MPS animal models. Early diagnosis and intervention have been shown to maximize benefits from therapies applied to MPS patients. New technologies to enable newborn screening for most MPS types have been developed. Mass spectroscopy based methods has enabled the use of sulphated oligosaccharides as biomarkers to diagnose, predict clinical severity and to monitor therapies in MPS patients.

Plenary 18

RECENT ADVANCES IN NEWBORN SCREENING

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Perhaps the general expansion of availability of newborn screening is the most exciting advance for many – in the Asia-Pacific region at least ten countries now have near-universal coverage. But by far the most revolutionary recent development has been 'expanded newborn screening' by tandem mass spectrometry (MSMS), allowing simultaneous testing for many rare disorders of amino acid, organic acid, and fatty acid metabolism. Affected babies are often now first detected by newborn screening, rather than by the metabolic service. This is resulting in better organised follow-up testing and management. Many problems remain: evaluating benefits remains difficult because the disorders are rare; sensitivity varies, and decisions about tolerance for false positives vary with the disorder concerned; newborn screening detects more cases than are detected clinically, and some identified babies may need no treatment; some disorders as a whole may be largely benign. Nevertheless, the new technology is very promising for the future. The next candidate disorders for routine screening are almost certainly the lysosomal storage disorders.

The ultimate criteria for newborn screening have not yet changed: a clear benefit from early diagnosis, and the benefit balanced against costs and harms. Adding new disorders will depend on new ways to diagnose disorders, new treatments or preventive measures, and new attitudes to what is desirable. With the burgeoning of affordable DNA testing there will be pressure to screen for genetic risk and for carrier status, and this will pose substantial ethical and practical problems, but used with care may open the way for exciting new possibilities.

Plenary 19

NONKETOTIC HYPERGLYCINEMIA (GLYCINE ENCEPHALOPATHY)

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Nonketotic hyperglycinemia (NKH), also called as glycine encephalopathy, is an inborn error of glycine metabolism characterized by accumulation of glycine in body fluids, brain malformations, and various neurological symptoms. More than 40 years has passed since first description of this disorder in 1965. In 1969 the enzymatic lesion for NKH was identified to be the mitochondrial glycine cleavage system (GCS), which was followed by identification and characterization of the three genes encoding the GCS components, GLDC, AMT and GCSH during 1991–2000. This lecture will focus on recent advance in study of NKH.

Phenotypic variations: Natural history of NKH has been clarified by the extensive study of Hoover-Fong et al. (2004). There were several reports describing cases with atypical NKH. In few of the cases, however, the diagnosis was confirmed by enzymatic or genetic analysis. Recently, detailed clinical pictures of atypical cases with identified GLDC mutations have been reported, extending the phenotypic spectrum of NKH (Dinopoulos et al., 2005; Flusser et al., 2005; Korman et al., 2004; Kure et al., 2004).

Diagnosis: Clinical diagnosis of NKH should be confirmed by enzymatic or genetic testing. Confirmation of diagnosis is, however, currently problematic, requiring either invasive liver biopsy for measurement of GCS activity or exhaustive mutational screening of three GCS genes. We have developed a novel diagnosis method using the ^{13}C-glcyine breath test, by which we can evaluate the *in vivo* GCS activity without technical expertise or invasive biopsy (Kure et al,. 2006). The ^{13}C-glycine breath test would be useful for confirmation of clinical diagnosis of NKH.

Genetic background: A comprehensive mutation screen of three GCS genes has revealed complex nature of NKH genetics (Kure et al., 2006). The *GLDC* mutations account for $\sim$70% of NKH alleles, and are heterogeneous. Mutation detection rate by exon-sequencing analysis of the GLDC gene was far lower than we had expected. We have developed a detection system for genomic deletion within GLDC by multiplex ligation-dependent probe amplification (MLPA) method, and found that *GLDC* deletions are prevalent in NKH. We propose the MLPA screening for *GLDC* deletions as a first line of NKH genetic testing (Kanno et al., 2006).

Neuropathogenesis: To elucidate the neuropathogenesis we have established NKH model mice by knocking out of the GCS genes. The knockout mice resemble NKH patients in phenotypes: Accumulation of glycine was obviously observed. They had brain malformations, and died within a few days of life. *In situ* hybridization study revealed that the GCS abundantly expressed not only in astroglial cells, but also in neural stem cells (Ichinohe et al., 2004). In degradation of glycine the GCS generates methylene-tetrahydrofolate, which is essential for DNA synthesis. Defect of GCS may impair proliferation of neural stem cells, and lead to brain malformations.

Plenary 20

MITOCHONDRIAL FATTY ACID OXIDATION DISORDERS: PHENOTYPES, ENZYMOLOGY, AND PATHOPHYSIOLOGY

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Fatty acid oxidation (FAO) in mitochondria is an essential physiological process as exemplified by the existence of a growing number of mitochondrial FAO disorders in humans. Hypoketotic hypoglycaemia is a general characteristic of all FAO disorders and is independent of the type of defect. Other features are dependent on the type of the enzymatic defect and include cardiac failure in all long-chain defects and retinopathy/peripheral neuropathy in LCHAD/MTP deficiency. The introduction of tandem-MS technology has revolutionized the identification of FAO deficient patients and has allowed neonatal screening programs for these disorders. Unfortunately, the number of false positives picked-up upon neonatal screening is quite high. Our finding that virtually all mitochondrial FAO enzymes are properly expressed in lymphocytes, now allows definitive and unequivocal identification of FAO deficient patients, with clear distinction between true and false positives, subsequently followed by molecular analysis to pinpoint the molecular defect. Now that FAO disorders are picked-up very early in life there is a growing need for improved therapies, which requires a detailed knowledge about the pathophysiological mechanisms. Some new developments will be discussed.

Plenary 21

MOLECULAR AND CLINICAL ASPECTS OF PEROXISOMAL DISEASES

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Peroxisomes are single-membrane lined organelles present in all eukaryotic cells and catalyzing a range of essential metabolic functions. Inborn errors of peroxisomal metabolism, an expanding group of genetic disorders in humans, have been divided into two groups with disorders of peroxisome biogenesis (PBD) and single peroxisomal enzyme deficiencies. PBD include Zellweger syndrome (ZS), neonatal adrenoleukodystrophy (NALD), infantile Refsum disease (IRD) and rhizomelic chondrodysplasia punctata (RCDP), all caused by a defect in *PEX* genes which encode peroxins, proteins necessary for peroxisome biogenesis and the import of peroxisomal matrix and membrane proteins. The peroxisomal matrix contains over 50 enzymes mainly related to lipid metabolism, of which eleven single peroxisomal enzyme deficiencies have been identified. Furthermore, a novel phenotype similar to ZS caused by a contiguous deletion spanning the 5′ ends of X-linked adrenoleukodystrophy gene and DXS1357E in Xq28 (CADDS) has been reported, therefore, we classify the peroxisomal diseases into three groups including contiguous gene syndrome.

We have been studying peroxisomal diseases for more than 20 years, as the only diagnostic center in Japan, and doing molecular analysis on PBD and their related disorders. In 2004, we identified a new PBD group K with PEX14 as the defective gene, which means there are 13 genotypes in PBD, and all of the responsible genes have been identified since we reported the first responsible gene for PBD, PEX2, in 1992. We also found interesting phenomenon, temperature sensitivity of peroxisome biogenesis, which is the import of peroxisomal matrix proteins and biochemical defects in the cells from patients with milder phenotypes of PBD are restored at the lower temperature, 30 degree. This phenomenon may be useful for investigating peroxisome biogenesis and for developing effective therapy for PBD patients.

We have developed the screening system of peroxisomal diseases, using GC/MS analysis of very long chain fatty acids, phytanic acids and plasmalogen, and identified many Japanese patients, using biochemical and molecular analysis: 28 patients with ZS, 3 with NALD, 3 with RCDP, 3 with acyl-CoA oxidase deficiency, 7 with D-bifunctional protein deficiency, 1 with CADDS and over 100 patients and carriers with ALD. Japanese PBD patients were genetically subdivided into complementation group A, B, C, E, F, K, R and 2, and mutation analysis of PEX 1, 2, 5, 6, 7, 10, 14 and 26 has been done.

Here we present the molecular and clinical aspects of peroxisomal diseases, and those screening and diagnostic system in Japan.

WS-1-1

FREQUENCY OF *PAH* MUTATIONS IN BH_4-RESPONSIVE PKU

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Patients with BH_4-responsive PKU respond to BH_4 by lowering their blood Phe levels 8–24 h after oral administration (20 mg/kg). Based on the BH_4 loading test, an frequency of BH_4-responsiveness was calculated to be 60–70% in mild HPA/PKU, ~30% in moderate PKU, and ~5% in classic PKU (overall ~40%). Most of BH_4-responsive patients are compound heterozygotes, carrying at least one mutant allele with residual phenylalaine hydroxylase (PAH) activity, 29 are homozygotes for two active alleles, but none was homozygote or compound heterozygote for two null mutations. In order to estimate the population of BH_4-responsive PKU patients on basis of the genotype, we established a database of *PAH* mutations BIODEF (www.bh4.org).

So far 264 patients with BH_4-responsive HPA/PKU and 519 alleles with 112 different mutations are tabulated in the database. 340 alleles (66%) are located in the catalytic domain, 74 (14%) in the regulatory domain, 53 (10%) in the tetramerization domain, and 52 (10%) are intornic. 316 alleles (61%) exert residual PAH activity (~43% compared with the wt enzyme), 29 alleles (5%) are potentially active, 87 alleles (17%) are inactive, and 87 alleles (17%) are not yet defined. The most common BH_4-responsive mutations (occurring in >10 alleles) are A403V (8.5%), R261Q (6.9%), Y414C (6.7%), V245A (4.7%), A300S (4.7%), R241C (4.0%), I65T (3.6%), E390G (3.6%), V388M (2.4%), and L48S (2.2%).

WS-1-2

A PHASE 3 STUDY OF THE EFFICACY OF SAPROPTERIN IN REDUCING PHE LEVELS IN SUBJECTS WITH PHENYLKETONURIA

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Sapropterin dihydrochloride, a synthetic form of 6R-BH_4 (tetrahydrobiopterin), is under development for treatment of Phenylketonuria (PKU). This Phase 3, multicenter, randomized, double-blind, placebo controlled trial evaluated safety and efficacy of sapropterin in reducing blood phenylalanine (Phe) in PKU subjects after 6 weeks treatment. Subjects, previously screened for BH_4 responsiveness, received sapropterin 10 mg/kg or placebo orally once daily for 6 weeks. Of 89 subjects enrolled, 87 completed treatment (1 withdrew prior to dosing, 1 was discontinued due to poor compliance). 59% were male, 41% female. Age ranged from 8 to 49 years (mean 20±9.7). At baseline, mean (±SE) blood Phe was 843 (±47) μM in the sapropterin group and 888 (±47) μM in the placebo group. At week 6, the sapropterin group mean blood Phe decreased by 236 (±40) μM (–29%) compared with a 3 (±35) μM (+3%) increase in the placebo group ($p<0.0001$). At baseline, the proportions of subjects with blood Phe levels fl600 uM were17% and 19% for the sapropterin and placebo groups, respectively. At week 6, the percentage increased to 54% in the sapropterin group compared with 23% in the placebo group ($p = 0.004$). The type and incidence of adverse events was similar in the sapropterin and placebo groups. The most frequent adverse events occurred in the Infections and Infestations and Gastrointestinal system organ classes. Sapropterin was well tolerated and effective in reducing Phe levels in BH_4-Sencitive PKU patients, and importantly, increasing the proportion of patients able to achieve Phe levels within recommended guidelines.

WS-1-3

DOUBLE BLIND PLACEBO CONTROL TRIAL IN PKU WITH NeoPhe

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Studies in our center with large neutral amino acids (LNAA), resulted in decrease in blood phenylalanine(phe). The initial formula, PreKUnil, was deficient in lysine. New formula of LNAA, NeoPhe, with lysine was made. The decrease in blood phe concentration using NeoPhe was documented in mice and patients with PKU. One week, double blind placebo controlled trial was conducted with NeoPhe. Sixteen patients were enrolled from six different centers in the US, Italy, Ukraine and Russia. The average baseline of phenyalanine in these patients was 1120 μmol/L. Patients were given 0.5 g/kg of NeoPhe or placebo tablets, in 3 divided doses, taken with meals. Baseline blood phe was determined prior to the trial, then every other day when on treatment. There was significant blood phe drop in patients on NeoPhe, which averaged 27% from baseline levels. During the placebo trial, blood phe did not vary significantly, and in some cases, blood phe went slightly higher. This double blind study indicates that LNAA can compete with phe on the transporter in the GI tract. The number of NeoPhe tablets to be taken should be adjusted to the needs and the blood phe concentrations of each patient. Longer term study of NeoPhe and placebo needs to be conducted in order to establish the efficacy and tolerance of NeoPhe in long term treatment of PKU. These results are encouraging as more LNAA can cross the blood brain barrier since blood phenylalanine is lowered, which should result in improved neurotransmitter concentrations of PKU patients.

WS-1-4

LONG-TERM TREATMENT OF TETRAHYDROBIOPTERIN (BH_4)-RESPONSIVE MILD PKU IN JAPAN

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Background: In 2000 a novel therapeutic strategy for phenylketonuria (PKU) was initiated in Japan. Twelve patients with mild PKU responsive to tetrahydrobiopterin (BH_4) were identified by neonatal PKU screening between 1995 and 2001 and a clinical trial of BH_4 treatment for mild PKU was performed. **Object**: To evaluate the long-term treatment of BH_4 we performed an extension study and followed up these patients. **Methods**: In the extension study for BH_4 treatment among 12 patients with BH_4-responsive mild PKU, 5 patients were treated with BH_4 and their plasma phenylalanine levels were measured between 2001 and 2005. **Results**: All 5 patients were controlled well under normal diet with BH_4 alone. Their mean values of plasma phenylalanine were around 4 mg/dl. However, in some patients when the dosage of BH_4 was decreased to less than 15 mg/kg per day, their plasma phenylalanine levels increased and sometimes over control range so that these patients needed 18 mg/kg per day to maintain control range. All of them were developed normally and no apparent side effects were found during these 5 years. **Conclusions**: We continued to treat with BH_4 and followed up 5 patients with BH_4-responsive mild PKU more than 5 years. All of them were controlled well in plasma phenylalanine levels under normal diet and showed no side effect. BH_4 treatment of responsive patients can eliminate or reduce the need for phenylalanine-restriction.

WS-1-5

LONG-TERM TREATMENT OF PATIENTS WITH MILD AND CLASSICAL PHENYLKETONURIA BY TETRAHYDROBIOPTERIN: PROBLEMS DURING CATABOLIC CONDITIONS

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Tetrahydrobiopterin (BH_4), the natural cofactor of phenylalanine hydroxylase (EC 1.14.16.1), can reduce blood phenylalanine (Phe) in BH_4 sensitive patients with hyperphenylalaninemia (McKuisick 261600). We report on the long-term treatment of eight patients with mild and classical phenylketonuria (blood Phe levels maximum blood Phe levels between 771 and 1500 μmol/L) using BH_4 at a dosage of 812 mg/kg BW per day. In all patients reduction of blood Phe was $>30\%$ after BH_4 loading test. Three patients were treated from birth by BH_4 only, five after initial low Phe dietary treatment. Seven of them continue to be on BH_4 treatment only, one has a relaxed low protein diet. No side effects could be observed (longest observation time 6 years), somatic and psychomotor development were normal. The main problem of BH_4 treatment is finding an optimal dosage at different ages and under special conditions like infectious diseases. One patient is demonstrated where high phe 'peaks' occurred during catabolic conditions. Further studies are necessary to find optimal dosages in individual patients and strategies to prevent phe elevations in blood under catabolic conditions.

WS-1-6

THE DIAGNOSIS, TREATMENT AND LONG-TERM FOLLOWING UP OF 223 PATIENTS WITH HYPERPHENYLALANINEMIA (HPA) DETECTED BY NEONATAL SCREENING PROGRAMS

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Objective: To evaluate the growth and mental development for patients with various types of hyperphenylalaninemia (HPA) detected by neonatal screening and early treated. **Method**: Two hundred and twenty-three patients with HPA detected by neonatal screening programs were performed by BH_4 (20 mg/kg) loading test, urinary pterin and dihydropteridine reductase (DHPR) activity determination at the age of 41 ± 27 days after birth. The phenylalanine (Phe) levels, growth and mental development were evaluated in all treated patients. **Results**: (1) one hundred and ninty-three of 223 patients were diagnosed as having various phenylalanine hydroxylase deficiency and 30 patients as having 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency. (2) Totally 135 patients were treated with low or free phenylalanine formula or BH_4 combined with DOPA and 5-hydroxytryptophan according to etiology. These patients were followed up until 4.5 y (0.25–22 y) years old. All of them have a normal growth development and nutrition. (3) One hundred and seven of 135 (79.3%) had normal mental development and the percent of good control for Phe concentration was $65.3 \pm 27.8\%$, other 28 patients had mental retardation and the percent of good control for Phe was $33.7 \pm 30.7\%$. (4) Nine kinds of PTPS gene mutations were found in 9 cases with PTPS deficiency and the R241C is common mutation in patients with BH_4 responsive HPA. **Conclusion**: The BH_4 loading test combined with urinary pterin and DHPR analysis are rapid methods of differential diagnosis for patients with HPA. About 80% patients who were early diagnosed, treated and had a good control for Phe level, had a normal mental development.

WS-1-7

WILD-TYPE PAH ACTIVITY IS ENHANCED BY BH_4 SUPPLEMENTATION *IN VIVO*: IMPLICATION FOR MECHANISM OF BH_4 RESPONSIVENESS

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We previously proposed a novel disease entity, tetrahydrobipoterin (BH_4)-responsive phenylalanine hydroxylase (PAH) deficiency, in which administration of BH_4 can reduce elevated levels of serum phenylalanine (Phe) (Kure et al., J Pediatr, 1999;135:375). Recent studies indicate that 30–50% of patients with PAH deficiency are expected to respond to BH_4 therapy. Although growing attention has been paid to a new treatment with BH_4, the mechanism of the BH_4 responsiveness remains largely unidentified. We devised a breath test using ^{13}C-Phe on mice, and examined BH_4 responsiveness of wild-type PAH enzyme *in vivo* (Kure et al., Mol Genet Metabol, 2004;83:150). Reliability of the test was verified by using PAH-deficient mice. C57BL/6 mice with BH_4 administration generated significantly higher level of $^{13}CO_2$ than those without BH_4. This augmentation was constantly observed only with preloading of Phe prior to breath sampling, but not without preloading of Phe. These observations suggest that normal PAH enzyme is not fully active *in vivo* under the physiological concentration of BH_4 and its activity is rapidly enhanced by supplementary BH_4 under high concentration of Phe. Recently, Pey and Martinez studied kinetics of PAH by an isothermal titration calorimetry method (Mol Genet Metabol, 2005;86:S43). They beautifully showed that the apparent affinity for BH_4 decreases at increasing Phe concentration, and that the affinity for the substrate also depends on the cofactor concentration. Our *in vivo* study, together with the *in vitro* study by Pey and Martinez, suggests complex regulation of PAH activity by Phe and BH_4.

WS-1-8

TETRAHYDROBIOPTERIN AND MATERNAL PKU

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CJ was born in 1973 and was diagnosed by newborn as classical PKU. Her phenylalanine hydroxylase PAH mutation was R408W/F39L. AT 29 years of age, CJ and her husband decided to have their own baby. Two years prior to this time, she was ingesting 20 mg of BH_4 twice a day on a normal diet. Phenylalanine (Phe) levels ranged around 900 μmol/L. After conception, her BH_4 therapy continued, but she ingested a Phe restricted product in addition. At the beginning of her second trimester, her BH_4 was increased to 40 mg twice a day. The remarkable thing about her pregnancy was the complete absence of any nausea or vomiting. A male baby was born in 2004, weighing 3440 g, measuring 52 cm in length with a head circumference of 36 cm. An echo examination of his heart was normal. At one years of age, the baby appears to be perfectly normal. The authors currently have one more woman with PKU, whose pregnancy is being treated with both diet and BH_4. She is due to deliver in October, 2006. The Food and Drug Administration has approved the use of BH_4 in these two patients. An Informed Consent was also required for its use. **Discussion**: Imamura et al. in 1993 showed that when BH_4 was administered to a pregnant guinea pig, it stimulated PAH production in the fetal liver. He suggested that BH_4 administration should be part of the therapy for maternal PKU. **Conclusion**: Two women with PKU are presented. The first was successfully treated with both Phe restriction, as well as oral BH_4 during her pregnancy, with a normal outcome. The second is currently in her second trimester with a normal course so far.

WS-2-1

IDENTIFICATION OF A CANINE MODEL OF PYRUVATE DEHYDROGENASE PHOSPHATASE 1 DEFICIENCY

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We have discovered that a null mutation in the pyruvate dehydrogenase phosphatase 1 gene (*PDP1*) is the cause of lactic acidemia and a severe exercise intolerance syndrome in Clumber and Sussex spaniels. The native activity of the pyruvate dehydrogenase complex (PDHc) in the fibroblasts of affected dogs is approximately 10 and 25% of control fibroblasts respectively. The addition of dichloroacetate, which increases the activity of PDHc in control fibroblasts, does not appear to stimulate PDHc activity in affected dog fibroblasts. However, the addition of 20 μg of recombinant human PDP1 restores affected PDHc activity to approximately 50% of native control. We demonstrate here that a c.754C>T mutation in *PDP1* in both Clumber and Sussex spaniels segregates with the clinical features of the disease, and the inheritance follows a classic Mendelian autosomal recessive pattern. In addition, one hundred Clumber spaniel DNA samples were randomly selected, blind, and tested for the presence of the c.754C>T mutation using a restriction enzyme test. Twenty dogs (20%) were shown to be carriers (*P/p*) and one (1%) was homozygous mutant (*p/p*). The allele frequencies can be determined as 0.89 for *P* and 0.11 for *p*. Real-time quantitative RT-PCR, used to determine the effects of the canine c.754C>T mutation on *PDP1* mRNA transcript stability, showed expression of *PDP1* relative to reference transcripts, within the range of the control. This canine model should provide valuable information about PDP1 deficiency and the role of *PDP1* and *PDP2* in metabolism.

WS-2-2

POLG MUTATIONS IN ALPERS SYNDROME CAUSE PROGRESSIVE LOSS OF MITOCHONDRIAL DNA

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Alpers syndrome, an autosomal recessive hepatocerebral syndrome of early onset, has been associated with mitochondrial DNA (mtDNA) depletion and *POLG* mutations. We identified pathogenic *POLG* mutations in 20 children with Alpers syndrome, from 17 apparently unrelated families. Most missense mutations were located in the linker region of the POLG protein and three common *POLG* mutations (A467T, W748S and G848S) accounted for ~70% of the mutant alleles. Haplotype studies suggest that A467T and W748S mutations are of ancient European origin and that patients with the G848S mutation have a common ancestor. G848S is associated with earlier onset of disease. Liver biopsies were available from 16 patients and each showed marked mtDNA depletion (80% to 96% estimated by qPCR). Liver respiratory chain enzyme profiles were diagnostic of a defect affecting complexes with mtDNA-encoded subunits in all patients studied. One patient did show normal liver respiratory chain enzymes and normal liver histology in a biopsy obtained shortly after they developed seizures at 5 years of age. However, liver obtained at 6.5 years of age showed a typical enzyme defect, abnormal mitochondrial morphology and a decline in the mtDNA:nDNA ratio. Respiratory chain enzymes were often normal in skeletal muscle biopsies, but two patients also developed a progressive enzyme defect in skeletal muscle. These data are consistent with progressive loss of mtDNA in liver, to a lesser degree in muscle, and presumably in brain. The sudden onset of seizures in this condition may correspond to mtDNA depletion reaching a critical threshold level in brain.

WS-2-3

ENZYME REPLACEMENT THERAPY FOR MITOCHONDRIAL DISORDERS – THE LIPOAMIDE DEHYDROGENASE DEFICIENCY AS A MODEL

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Modern medicine offers no cure for patients suffering from mitochondrial disorders such as lipoamide dehydrogenase (LAD) deficiency. LAD is the E3 subunit shared by all α-ketoacid dehydrogenase complexes in the mitochondrial matrix, which are crucial for the metabolism of sugars and amino acids. Clinical presentation of LAD deficiency is variable, ranging from severe neurological disease in infancy to recurrent episodes of liver failure or myoglobinuria later in life. Conventional treatment is mostly palliative with the aim of postponing or circumventing the massive damage caused by the free radicals, the accumulation of toxic metabolites and mainly the low rate of energy production. We propose a novel concept for the treatment of mitochondrial disorders such as LAD deficiency using enzyme replacement therapy. Our approach entails fusion of the LAD enzyme with the HIV-transactivator of transcription (TAT) peptide, capable of rapidly crossing biological membranes in the form of a fusion protein. TAT will deliver LAD into cells and their mitochondria, thus replacing the mutated endogenous enzyme. We present here for the first time successful enzyme replacement in cells taken from patients with LAD deficiency. TAT-LAD is delivered into these cells and their mitochondria rapidly and efficiently, restoring LAD activity to normal values. Most importantly, TAT-LAD is naturally incorporated into α-ketoacid dehydrogenase complexes such as the pyruvate dehydrogenase complex while augmenting its activity. We believe that this novel approach for enzyme replacement therapy could be applied to additional mitochondrial and other metabolic disorders and will profoundly revolutionize the management of these types of disorders.

WS-2-4

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

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Objective: 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 20 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**: No abnormality was detected except for Complex I. Abnormal assembled pattern for Complex I was identified in 11 out of 20 cell lines. Among those, three groups were categorized by amount and size of assembled Complex I. Grossly deficient of fully assembled 900 kDa species (<30%) was in 2 cell lines, moderately decreased of fully assembled 900 kDa species (30–70%) was in 7 cell lines and smaller sub-supercomplexes were in 2 cell lines. **Conclusion**: BN-PAGE is a useful guide to prompt and correct diagnosis, and future molecular analysis for categorizing Complex I deficiency.

WS-2-5

HEPATOCEREBRAL MITOCHONDRIAL DNA DEPLETION CAUSED BY DEOXYGUANOSINE KINASE (*DGUOK*) MUTATIONS

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Autosomal recessive mutations in deoxyguanosine kinase (*DGUOK*) were repeatedly described in the hepatocerebral form of mitochondrial DNA (mtDNA) depletion (MDS). We report 6 cases of DGUOK-deficiency and summarize the literature to describe the clinical spectrum of *DGUOK*-related MDS. We identified pathogenic mutations in the *DGUOK* gene in 7 patients with the hepatocerebral form of mtDNA depletion. We describe the clinical, neuroradiological, histological and genetic features in these children. Liver involvement seems to be the most prominent feature (7/7), leading to liver cirrhosis and causing early-onset liver failure. In contrast, the encephalopathy may be relatively moderate or even absent. We identified five novel mutations (one of them in two patients) and two previously described mutations. Three different mutations affected the initiation methionine, suggesting a mutational hot spot. One of our patients underwent liver transplantation and pathology revealed, in addition to diffuse hepatopathy, a hepatocellular carcinoma, implying a possible link between mtDNA depletion and tumorigenesis. **Conclusions:** We studied 12 cases with infantile hepatoencephalopathies and MDS depletion, and found pathogenic *DGUOK* mutations in 7, suggesting that this gene defect is a frequent but not exclusive cause of the hepatic form of mitochondrial DNA depletion syndrome.

WS-2-6

THE MOLECULAR GENETIC BASIS OF RESPIRATORY CHAIN COMPLEX I DEFICIENCY: CLINICAL PRESENTATIONS AND mtDNA MUTATIONS

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Defects of the mitochondrial respiratory chain are associated with a diverse and ever-growing spectrum of clinical phenotypes, and may be caused by mutations in either the nuclear or mitochondrial genome (mtDNA). Isolated complex I deficiency is the most commonly reported enzyme defect in mitochondrial disorders, particularly in children where family history is often consistent with sporadic or autosomal recessive inheritance, implicating a nuclear genetic cause. In contrast, pathogenic mtDNA mutations have historically been perceived as rare causes of paediatric complex I deficiency.

We reviewed the clinical and genetic findings in a large cohort of 87 paediatric patients with isolated complex I deficiency, of whom 41 had extensive mtDNA analysis performed. Pathogenic mtDNA mutations were found in 25/87 (29%), 18 in *MTND* subunit genes and 7 in *MTTL1*. Autosomal recessive defects could be inferred in 31/87 (36%) patients based on cell hybrid studies, parental consanguinity, mtDNA sequencing or mutation analysis (nuclear gene mutations were identified in 8 patients). Leigh/Leigh-like disease or lethal infantile mitochondrial disease was diagnosed in 52% of mtDNA and 71% of nuclear patients. Median age of onset was higher in mtDNA patients (10.5 m c.f. 4 m) but ranged from 0 m to >60 m in both groups. The prevalence of mtDNA mutations is significantly higher than previously thought in these patients. The mtDNA patients differ slightly as a group in clinical phenotype, age of onset, family history and tissue specificity. However these differences are too subtle for the clinician to predict the likely mode of inheritance in most individual patients.

WS-2-7

CLINICAL AND GENETIC HETEROGENEITY OF DIHYDROLIPOAMIDE DEHYDROGENASE DEFICIENCY

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Dihydrolipoamide dehydrogenase (DLD, E3) deficiency is rare in populations other than Ashkenazi Jews, where patients typically have prominent hepatic involvement and a common G229C mutation. The few other reported patients had a more variable presentation and different mutations. We describe five new patients with DLD deficiency from four families. Two presented with severe lactic acidosis in the newborn period, the remainder presented later, most commonly with developmental delay and hypotonia. One child died aged 8, the two siblings are currently in their teens and have normal mental development, although one has episodic dystonia. Two patients had recurrent hypoglycaemia. Blood and CSF lactate were variably elevated and were normal in one patient. Levels of branched chain amino acids and their metabolites were highly variable and did not suggest the diagnosis. Fibroblast pyruvate dehydrogenase activity ranged from 5–20% of normal. All patients have missense mutations in the *DLD* gene; three patients from two families, are homozygous, the other two are compound heterozygotes. Four of these *DLD* mutations are novel. In groups other than Ashkenazi Jews, DLD deficiency may be under recognised because of this clinical and genetic heterogeneity.

WS-2-8

A NOVEL MITOCHONDRIAL DNA tRNA GENE MUTATION IN A FAMILY WITH MITOCHONDRIAL ENCEPHALOPATHY

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Pathogenic mutations in mitochondrial DNA (mtDNA) tRNA genes are widely associated with a spectrum of different clinical presentations. The majority of these tRNA mutations affect tRNA structure by disrupting Watson-Crick base pairing, impairing cellular function by a number of mechanisms including effects on tRNA stability, aminoacylation, maturation or post-transcriptional modification. We have studied two brothers who presented with a similar picture of stroke-like episodes, headache, bilateral optic atrophy, encephalopathy and occipito-temporal infarcts on brain MRI. Muscle histochemistry demonstrated a significant number (60%) of cytochrome *c* oxidase (COX)-deficient fibres. Sequencing of the entire mitochondrial genome revealed a novel mutation (12206C>T) in the *MTTH* gene encoding $tRNA^{His}$ which was present at high levels of heteroplasmy in both muscle and urinary sediments, but absent in blood. Single muscle fibre analysis demonstrated highest levels of the 12206C>T mutation in COX-deficient fibers, confirming both segregation of the mutation with respiratory chain dysfunction and pathogenicity. Of particular interest, this specific mutation alters the $tRNA^{His}$ discriminator base at position 73 of the tRNA molecule, which is important for the correct recognition of the $tRNA^{His}$ by its aminoacyl-tRNA synthetase and hence crucial to the maintenance of translational stability. Whilst investigations into the underlying molecular mechanism are continuing, we believe that this is the first known example of a pathogenic mtDNA tRNA mutation affecting the discriminator base.

WS-3-1

THE BENEFIT OF TESTING SECOND NEWBORN SCREENING SPECIMENS BY TANDEM MASS SPECTROMETRY

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The Northwest Regional Newborn Screening Program implemented expanded newborn screening using full scan tandem mass spectrometry in October, 2002. As of December 31, 2005, the laboratory has tested 388 398 first newborn screening specimens using tandem MS. Collected either by mandate or by recommendation following early hospital discharge, 300 054 second screening specimens (77.3%) were also tested using tandem MS. 147 confirmed cases representing 19 different inborn errors of metabolism were detected by tandem MS during this period yielding an overall disease incidence of 1:2642 live births. To date, out of 147 total cases, 40 cases (27%) representing 13 metabolic disorders have been detected on second screen specimens only. The diagnoses detected on second screens have included homocystinuria, MAT deficiency, hyperphenylalaninemia, tyrosinemia type 2, 3-methylbutyrl-CoA dehydrogenase deficiency, 3-MCC deficiency, arginase deficiency, argininosuccinic aciduria, carnitine transporter deficiency, VLCAD deficiency, MCAD, and a CPT1 variant in Alaska Natives. Excluding the 22 CPT1 cases in Alaska, 12% of cases detectable by tandem MS were identified only on a second screen. This outcome is similar to that seen in our program for congenital hypothyroidism (10–12% of cases detected on second screen) and congenital adrenal hyperplasia (10%). Routine collection and testing of second newborn screening specimens should be seriously considered by all screening programs.

WS-3-2

IMPACT OF EXPANDED NEWBORN SCREENING ON DIAGNOSTIC AND CLINICAL BIOCHEMICAL GENETICS SERVICES IN NEW SOUTH WALES

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Expanded newborn screening by tandem mass spectrometry began in New South Wales in 1998. All laboratory, and almost all clinical follow up has been performed in a single centre, enabling accurate determination of the impact of the change.

From 1st April 1998 to 28th February 2006 730 736 babies were screened for selected amino acids and acylcarnitines. Excluding disorders of phenylalanine metabolism, 393 patient samples were referred for further biochemical genetics testing comprising 114 plasma amino acid quantitations, 348 urine metabolic screens and 176 plasma acylcarnitines. The additional samples represent 0.8% of the total samples received by the biochemical genetics laboratory. Eight patients with positive screens were investigated before the result was available because of clinical presentation or family history. Of the 401 patients investigated, 125 (31%) had positive follow up results, 99 were diagnosed with an IEM, 12 had proven or suspected maternal defects (16 babies), and 10 cobalamin deficiency.

Almost all patients attended a single genetic metabolic clinic. Data on outpatient visits from the most recent three years show 35% involved phenylketonuria, and 14% other disorders diagnosed by expanded newborn screening. Of the latter, 60% were 'extra' visits, made by patients who may not have had symptoms until later, or in some instances, not at all.

Thus we estimate, while the numerical increase in workload was trivial for the laboratory it was about 8% for clinical services. However, the real increase was probably smaller, as the 'extra' patients were not ill, and often not on taxing treatment regimens.

WS-3-3

OUTCOME STUDY OF TANDEM MASS SPECTROMETRY ON CLINICAL SELECTIVE SCREENING IN CHINESE PATIENTS

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Tandem mass spectrometry has demonstrated the broad spectrum of disease covered, specificity and high sampling throughput. We report here the outcome study of 2110 cases suspected with inborn error of metabolism collected in China from 2003 to Dec. 2005. The amino acids and acycarnitine profiles were analyzed by API 2000. The blood was collected on filter paper, derivatized with butanolic-HCI with stable isotope labeled internal standards. Positive cases for the test were referred to metabolic specialist who made a final diagnosis with other clinical and laboratories data. From 2110 cases that we were analyzed, 228 cases (10.8%) were made a final diagnose. Among these cases, 156 cases were diagnosed as amino acid diseases (127 hyperphenyl-alaninemia, 10 OTC, 5 tyrosinemia type I, 5 MSUD, 3 citrullinemia type I, 3 citrullinemia type II, 2 homocystinuria, 1 arginasemia); 63 cases were diagnosed as organic academia (32 MMA, 10 PA, 4 IVA, 3 GA-I type I, 5 3-MCC, 2 3-HMC, 3 biotinidase deficiency, 1 multiple CoA carboxylase deficiency, 3 β-keto thiolase deficiency; 9 cases were diagnosed as fatty acid disorders (4 MCAD, 1 CPT type II, 1 SCAD, 1 VLCAD, 2 MACD). Our results have demonstrated that the tandem mass spectrometry) were efficiency and important tool in diagnosis of amino acids diseases, organic acidemia and fatty acid oxidation disorders by measurement of one small punch of blood spot.

WS-3-4

SCREENING OF HIGH-RISK NEONATES AND CHILDREN USING GC/MS IN INDIA – THE FIRST STEP TOWARDS NEWBORN SCREENING

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More than 28 million annual births in India pose a huge burden on nation while offering genetic healthcare services to community. Genetic epidemiology data is not yet available for Indian population. There is a lack of high-throughput laboratories and skilled professionals in genetics. Newborn screening, well accepted health policy in some countries is lacking here due to other national priorities. The present study reports, for the first time in India, the screening of inborn errors of metabolism (IEM) in high-risk children using gas chromatography/mass spectrometry (GC/MS). Of 2351 high-risk screened, 684 were neonates with clinical symptoms like seizures, vomiting, poor feeding, metabolic acidosis, lethargy, and mental and/or motor delay. The 28% had metabolic abnormality. Amino and organic acidopathies accounted for 31%, followed by 4–6% of sugar metabolism disorders. Most common IEM were MMA, MSUD, PA, UCD, OTC, glutaric aciduria, galactosemia, FDPD, Fanconi syndrome and tyrosinemia. Interestingly, 8 cases of Canavan disease were diagnosed. Religious, racial and ethnic diversity with cultural and traditional misconcepts are important in our population while genetic counselling. The simultaneous chemical diagnosis of about 100 IEM by GC/MS is cost effective in the long run considering prevention and rehabilitation cost of the disabled child, and can be used for mass screening program. It was evident that which metabolic disorders are more frequent in our population, and a need of public health programs of 'Newborn Screening' to prevent the burden of mental retardation.

WS-3-5

GAS CHROMATOGRAPHY-MASS SPECTROMETRY OFFERING PATIENT ASSISTANCE FOR THE DIAGNOSIS AND TREATMENT OF INHERITED METABOLIC DISEASES: A PILOT STUDY OF 4 YEARS IN CHINA

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Objective: This study is aimed to screen for inherited metabolic diseases (IMD), by analysis of urine components in children suspected to have IMD, providing laboratory assistance for the diagnosis and therapy. **Methods**: A procedure known as urease-pretreatment gas chromatography-mass spectrometry (UP-GC-MS) was involved in the screening, to analyze organic acids, amino acids, sugars, polyols, purines and pyrimidines simultaneously. Some positive results were further studied with confirmative tests, and the patients were treated after the establishment of diagnosis,and the therapeutic effects were followed up. **Results**: Urine samples of 489 patients from 23 provinces, municipalities and autonomous regions in China were analyzed, and 21 kinds and 49 cases of IMD were found (positive rate 8.26%), including 8 cases of methylmalonic aciduria, 5 phenyl ketonuria, 4 multiple carboxylase deficiency, 4 glyceroluria, 4 von Gierke's disease, 3 Leigh syndrome, 2 propionic acidemia, 2 ornithine transcarbamylase deficiency, 2 citrin deficiency, 2 fructose-1,6-diphosphatase deficiency, 2 Fanconi's syndrome, 2 fructosuria, 1 glutaric acidemia type I, 1 maple syrup urine disease, 1 hyperglycinemia, 1 beta-aminoisobutyric aciduria, 1 tyrosinemia type I, 1 Canavan's disease, 1 galactosemia, 1 succinyl semialdehyde dehydrogenase deficiency, and 1 primary hyperlactic acidemia. Some diagnosis have been confirmed by gene mutation alaysis and enzymatic activity analysis, together with blood biochemical and MS-MS analysis, or ultrastructural observation by electronic microscopy, and satisfactory therapeutic effects have been achieved in some patients, such as those with multiple carboxylase deficiency,citrin deficiency or galactosemia. **Conclusion**: As a patent tool in the screening for IMD, UP-GC-MS, in association with other confirmative tests, could provide effective diagnostic and therapeutic assistance for IMD patients.

WS-3-6

HOW RELIABLE ARE METABOLITE MEASUREMENTS IN IEM? EXPERIENCE FROM ERNDIM QUALITY CONTROL SCHEMES

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Agreed thresholds of metabolite levels for treatment, inclusion in multicentre studies, mobility of patients between countries and agreed critical cutoff values in newborn screening by tandem MS all demand satisfactory quality assurance, including external quality control. National schemes for quality control of IMD analytes are unrealistic and have been organised on a Europe-wide basis by ERNDIM since 1994. Today ERNDIM offers nine different external quality assurance schemes, (operated according-to guidelines summarised by Sciacovelli et al: CCA 2001;309: 183) as follows with participant numbers in brackets: quantitative amino acids (177); special assays in urine (121); special assays in serum (158); quantitative organic acids urine (63); purines and pyrimidines in urine (45); cystine in white blood cells (27); proficiency testing of organic acids in urine (137); diagnostic proficiency testing (93); acylcarnitines in blood spots (67).

Results of the schemes indicate widely differing levels of performance both between the different types of metabolites and within each scheme. For example the coefficient of variation between laboratories for amino acids ranges from 6.4% for valine to 253% for hydroxyproline compared with a mean value for all analytes of 98.2% in the purines and pyrimidines scheme.

Presently ERNDIM activities are being co-ordinated with those of other genetic disciplines within the EuroGentest project (www.eurogentest.org/). EQA will play an increasing role in promoting the much needed improvements of quality of analyte measurement in IEM and will become essential for the accreditation of laboratories.

WS-3-7

A TIME STUDY OF EXPANDED NEWBORN SCREEN (NBS) REFERRALS

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Objective: Emergency referrals from the New York State NBS program are received by our metabolic nurse practitioners, who are the first contact for families of infants referred for an abnormal NBS. We evaluated the phone counseling time used during the initial processing of NBS referrals. **Method**: Telephone records from a random sample of 13 infants referred to us over a six month period were analyzed. **Results**: The mean number of phone calls per NBS referral was 2.4 (range 1–4), divided between the family and pediatrician. Discussions focused primarily on the infant's current health status, newborn screening processes, description of the metabolic disorder, sick management, and scheduling the clinic appointment. The mean phone time per referral was 45 min (range 5–105 min). We identified a number of barriers to care including language difficulties, inadequate transportation and lack of phone service. **Conclusions**: The first interactions between the treatment center and family in a NBS referral require expert communication skills. This initial contact provides the groundwork for a positive clinical experience for families. Substantial case management is needed to care for these families initially and throughout the NBS process. Clinics are not typically reimbursed by insurers for consultation phone time and follow-up. This represents a critical unfunded mandate requiring consideration in development of expanded NBS programs.

WS-3-9

METABOLOME-BASED CHEMICAL DIAGNOSIS OF IEM

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Metabolite analysis is comprehensive for mutant genes. Enzyme dysfunction caused either by abnormal structure of enzyme, abnormally reduced quantity of normal enzyme/apoenzyme or by the lack of coenzyme is also involved. Enzyme dysfunction either by abnormal structural gene, abnormal regulatory gene, abnormal subcellular localization, abnormal post transcription or abnormal post-modification is included. Mutation on a gene either known or unknown or common or uncommon is involved. We performed metabolome-based chemical diagnosis of IEM for high-risk patients using simplified urease-pretreatment, stable isotope dilution, and capillary gas chromatography-mass spectrometry (GC/MS). The simultaneous screening or chemical diagnosis of over 130 disorders, including hyperammonemias, lactic acidemias, IEMs of amino acids, pyrimidines, purines, and carbohydrates, primary hyperoxalurias, neuroblastoma, and acquired deficiencies of folate, biotin and vitamin B_{12}. In high-risk patients, the incidence was 1 per 12 (8.3%) for newborns and 1 per 41 (2.4%) for patients of all ages. Moreover, methylcitrate, orotate and glycerol-3-phosphate, extremely polar acids, can be analyzed more quantitatively or sensitively than by conventional organic solvent extraction followed by GC/MS. It was suggested that our practical yet specific procedure for chemical diagnosis can be applied to early diagnosis, prenatal diagnosis, tailored medicine, and also secondary and acquired metabolic disorders. Our technique is promising not only for clinical diagnosis but also for newborn mass screening.

WS-4-1

NEONATAL SCREENING FOR VERY LONG-CHAIN ACYL-CoA DEHYDROGENASE DEFICIENCY: EVALUATION OF NEONATES WITH ELEVATED C14:1-CARNITINE LEVELS

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Neonatal screening programs for very long-chain acyl-CoA dehydrogenase deficiency (VLCADD) have been implemented in various countries. C14:1-carnitine serves as disease-specific marker and elevated C14:1-carnitine on day 3 of life strongly suggests VLCADD. We characterized 14 neonates with elevated C14:1-carnitine on neonatal screening by enzyme and molecular analysis. Palmitoyl-CoA dehydrogenase activity was measured in lymphocytes. Sequencing of all 20 exons of the VLCAD gene was performed from genomic DNA. Palmitoyl-CoA dehydrogenase activity revealed significantly decreased residual activities consistent with VLCADD in 10 neonates. In two individuals, residual activities of 48% and 44%, respectively, suggested heterozygosity. Two neonates presented with a normal palmitoyl-CoA dehydrogenase activity. Two disease-causing mutations were detected in eight out of ten neonates with VLCADD; in two patients with VLCADD, only one mutation was identified. Out of two individuals with residual activities consistent with heterozygosity one was heterozygous for a VLCAD mutation. The other child as well as the three individuals with normal palmitoyl-CoA dehydrogenase activity had a normal genotype. Overall, in four out of 14 neonates identified on neonatal screening with elevated C14:1-carnitine, VLCADD was excluded. A C14:1-carnitine level of >1 μmol/l strongly suggests VLCADD, whereas concentrations <1 μmol/l do not allow a clear discrimination between patients, carriers and healthy individuals. Normalization of C14:1-carnitine on days 5–7 of life occurred in one patient with VLCADD and in two healthy individuals and, therefore, does not exclude VLCADD. Further enzymatic and molecular work up is essential to correctly identify VLCADD.

WS-4-2

A NOVEL TIME- AND COST-EFFECTIVE METHOD FOR SENSITIVE MUTATION SCANNING AND FREQUENCY STUDIES IN THE *ACADVL* GENE

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Very-long-chain acyl-CoA dehydrogenase deficiency (VLCADD) is an autosomal recessive inborn error of fatty acid oxidation. Three mutations (c.779C>T, c.830.832del and c.848T>C) are frequent in clinically affected patients with VLCADD and may account for up to approximately 30% of mutant *ACADVL* alleles in newborns identified by tandem MS based newborn screening. Because newborn screening by acylcarnitine profiling for fatty acid oxidation defects, including VLCADD, is becoming standard in many countries world-wide, there is a need for more time- and cost-effective gene analysis methods for confirmation of screening positive samples. Moreover, knowledge on the carrier frequent-cies of the most common *ACADVL* mutations in the general population may enable evaluation of the diagnostic specificity of newborn screening for this defect. High-resolution melting of PCR amplicons amplified in the presence of the DNA binding dye LCGreen Plus™ has recently been introduced as a homogeneous, 'closed-tube' method for genotyping using the LightScanner™ instrument from Idaho Technology. Here we validate the LightScanner™ as a mutation scanning instrument by blinded genotyping of 160 different sequence variants that we have identified in patients with VLCADD. We also demonstrate that this method is a very time- and cost-effective method for population carrier testing allowing simultaneous genotyping of three mutations (c.779C>T, c.830.832del and c.848T>C) in 2030 Danish blood spots using only one set of primers and LCGreen Plus™.

WS-4-3

CONSENSUS CASE DEFINITIONS FOR MCADD AMONG INFANTS WITH PRESUMPTIVE POSITIVE NEWBORN SCREENING RESULTS

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Background: There is uncertainty about the criteria required to confirm the clinical relevance of deficiency of medium chain acyl CoA dehydrogenase (MCADD) in infants with positive newborn screening results. Some children with *ACADM* gene mutations and high residual enzyme activity may remain asymptomatic even during metabolic stress. We used a standard diagnostic algorithm to review independently data from a prospective multicentre study, in which infants were investigated using predefined protocols to determine MCADD status. **Methods**: Infants were screened at 5–8 days with electrospray MSMS of underivatised blood spots to quantitate octanoylcarnitine (C8). An independent expert panel reviewed blood acyl carnitine, urine organic acid and mutation analysis results in newborns with elevated C8 values ($\geqslant$0.5 micromol/l) and assigned them to one of four categories: MCADD of definite phenotype (c.985A>G homozygotes, or presence of two mutations, both either disease associated or predicting truncated protein); MCADD of uncertain phenotype (genotype of uncertain pathogenicity); carriers; none of these. **Results**: Of 93 infants reviewed, 55 (59%) were classified as MCADD of definite phenotype (45 homozygous c.985A>G), 25 (27%) MCADD of uncertain phenotype, nine (10%) carriers, and four, none of these. 108 of 160 (68%) alleles were c.985A>G. **Conclusion**: The phenotype in 69% (55/80) of MCADD cases identified through newborn screening is consistent with an increased risk of clinical decompensation. A consensus case definition is needed to allow data from newborn screening programmes to be compared.
Funder: Department of Health, UK

WS-4-4

NEWBORN SCREENING FOR MEDIUM CHAIN ACYL CoA DEHYDROGENASE DEFICIENCY (MCADD): FINDINGS FROM A MULTICENTRE PROSPECTIVE UK COLLABORATIVE STUDY

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Background: We report MCADD prevalence and test performance from a prospective multicentre UK study, using predefined screening and diagnostic protocols. This study differs from others worldwide, which report data from single centres on babies screened around day 2 using butylated samples. **Methods**: Newborns aged 5–8 days were screened using MSMS of underivatised blood spots to quantitate octanoylcarnitine (C8). An independent expert panel reviewed blood acyl carnitines, urine organic acids and mutation analysis results in newborns with elevated C8 values ($\geqslant$0.5 micromol/l) and assigned infants to MCADD of definite phenotype, MCADD of uncertain phenotype, carriers, or none of these. **Results**: 98 of 745 000 infants screened positive (1.3 per 10 000; 95% CI: 1.1,1.6). Of 93 reviewed to date, 55 (59%) are MCADD of definite phenotype (45 homozygous c.985A>G), 25 (27%) MCADD of uncertain phenotype, nine (10%) carriers, and four none of these, with 108 c.985A>G alleles (68%) among the 80 definite and uncertain cases. The positive predictive value is 59% (55/93) for definite MCADD, 86% (80/93) including uncertain phenotypes. **Conclusion**: Although a spectrum of MCADD phenotypes are diagnosed following newborn screening, in this UK population the phenotype in 69% (55/80) is consistent with an increased risk of clinical decompensation.
Funder: Department of Health

WS-4-5

FOUR TIMES THE EXPECTED NUMBER OF NEWBORNS IN DENMARK HAVE *MCAD* DEFICIENCY – THIS CAN BE EXPLAINED BY A DIFFERENT MUTATION DISTRIBUTION THAN THAT PREVIOUSLY OBSERVED IN CLINICALLY PRESENTING PATIENTS AND A REDUCED PENETRANCE OF THE DISEASE

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Medium-chain acyl-CoA dehydrogenase deficiency (MCADD) is the most common defect of fatty acid oxidation. Many countries have introduced newborn screening for MCADD, since it can be rapidly indicated by identification of acylcarnitines in blood spots by tandem mass-spectrometry. In Denmark we have screened 213 500 newborns for MCADD as part of a pilotstudy, and identified 24 with a positive profile. The diagnosis of MCADD was established in all 24 newborns by mutation analysis. This gives an estimated incidence of MCADD in Denmark of 1/8900, which is much higher (3.5–4X) than expected (1/32 000) from the carrier frequency of the prevalent c.985A>G mutation or from the observed incidence of clinically presenting patients (1/37 000) in the 10 year period preceding the pilotstudy. Much of this discrepancy can be explained by a reduced penetrance of c.985A>G, with only 25–50% of c.985A>G homozygotes presenting with recognized disease.

The mutation spectrum in the newborns was different from that observed in clinically presenting patients. A much higher proportion (54% vs 28%) of newborns had other genotypes than c.985A>G homozygous. The deleterious nature of all identified mutations was confirmed by functional testing (Analysis of patient cDNA or recombinant protein or minigenes). Although a few of the mutations were 'mild' and may represent a minor risk for disease most of them caused a severe defect.

We conclude that MCADD is more frequent than expected, has a reduced penetrance, but since other factors play an important role in triggering disease all identified newborns should be considered at risk for disease presentation.

WS-4-6

A MILD AMISH VARIANT OF PROPIONIC ACIDEMIA IS DETECTABLE BY NEWBORN SCREENING WITH TANDEM MASS SPECTROMETRY

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Propionic acidemia (PA) is a disorder of propionate metabolism caused by propionyl CoA carboxylase (PCC) deficiency. Acylcarnitine profiles in PA are characterized by an isolated elevation of propionyl carnitine (C3). The phenotype of PA ranges from fatal neonatal ketoacidosis and hyperammonemic coma to a complete absence of symptoms. We describe an Amish kindred of 9 children in which 3 siblings were diagnosed with PA following a positive newborn screening result in the youngest child revealing a C3 of 11.3 μmol/L (abnormal >5.6), a C3/C2 ratio of 0.69, and a C3/C16 ratio of 5.62. Follow-up plasma acylcarnitine analysis confirmed the screening result (C3: 14.7 μmol/L; ref. range: <1.78) and urine organic acid analysis suggested a mild form of PA by the elevated excretion of 2-methylcitric acid with undetectable levels of 3-hydroxy propionic acid and propionylglycine. The proband's follow-up results were similar to another asymptomatic Amish PA infant, homozygous for the N536D mutation in PCCB, also identified through newborn screening. Work-up of the proband's siblings underscored a mild phenotypic presentation because 2 sisters at 10 and 13 years of age had C3 levels of 17.6 μmol/L and 16.0 μmol/L, respectively, while their clinical histories were significant for infantile febrile seizures. Cognitive delay and seizures are ongoing issues for the 10-year-old while the 13-year-old is asymptomatic. Molecular studies of the alpha and beta genes of PCC are pending. This sibship emphasizes the presence of a mild PA phenotype segregating in specific populations within the United States that is detectable through newborn screening with MS/MS.

WS-4-7

COMPREHENSIVE GENE ANALYSIS IN A ROUTINE CLINICAL SERVICE LABORATORY ENABLED BY HIGH RESOLUTION MELT PROFILING AND FREEZE-DRIED ASSAY PANELS

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Analyzing common mutations will characterize patients with some metabolic deficiencies (i.e. CF, MCAD). In many metabolic diseases, common mutations are not observed, necessitating full gene analysis. Analyzing coding regions and adjacent sequence critical to mRNA processing is complex and requires specialized knowledge of the gene. Laboratories with research interests in a particular gene have traditionally provided this type of full gene analysis. Enabling comprehensive gene analysis to be rapidly performed in a routine service laboratory would speed assessment of candidate patients. High-resolution DNA melt profiling rapidly determines the presence of sequence variation. PCR reaction components (buffer, dNTPs, primers, LCGreen dye, taq polymerase, $MgCl_2$) may be preserved in a freeze-dried format and retain function for 6–12 months. Using the ornithine transcarbamylase (OTC) gene as a model, PCR products were developed to assess the coding regions and adjacent sequence critical to mRNA processing. These reagents were freeze-dried in a 96-well plate. The plate format includes no-template PCR controls, positive controls that generate a wild type profile, and sites to evaluate the patient specimen. Also included are assays to specifically query common polymorphisms. Using samples from characterized OTC-deficient patients, freeze-dried assay panels are assessed for ability to support robust amplification and identify sequence variants. The OTC panel has thus far demonstrated stability out to 8 months. Freeze-dried reagents are resuspended using either water (positive and negative controls) or diluted DNA (10–20 ng) of the sample being evaluated. Melt profiling using freeze-dried assay panels expedites the evaluation of candidate patients by reducing the turn around time for comprehensive molecular genetic analysis.

WS-5-1

GENZ-112638, AN ORAL CERAMIDE ANALOG FOR SUBSTRATE INHIBITION THERAPY OF GAUCHER DISEASE

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Glucosylceramide synthase inhibitors, including imino-sugar-based analogs and ceramide-based analogs, may be useful in the treatment of Gaucher disease. We have evaluated GENZ-112638, a novel ceramide analog, through a series of *in vitro* and *in vivo* assessments and Phase I clinical trials. *In vitro* characterization of GENZ-112638 has shown significantly increased potency and specificity in comparison to imino-sugar analogs, with up to 3500-fold greater potency (IC_{50}) for inhibition of GL-1 synthesis, and specific inhibition of glucosylceramide synthase. Using a Gaucher mouse model, we have demonstrated GENZ-112638 efficacy at well-tolerated oral doses in young mice prior to substrate accumulation and in older mice with established disease. In young mice treated with GENZ-112638 for 10 weeks, the liver and spleen were devoid of Gaucher cells and the lung had reduced storage cells; all three tissues had significantly decreased levels of GL-1. In older mice with established disease, treatment with GENZ-112638 resulted in a reduction of Gaucher cells in liver, lung and spleen and substantially reduced tissue levels of GL-1. In a Phase Ib study, the target efficacious exposure level (at and above *in vitro* IC_{50}) was achieved at doses well-tolerated by normal volunteers; plasma GL-1 reductions were observed after 10 days of dosing. These data suggest that GENZ-112638 may be a promising oral ceramide analog candidate for the treatment of Gaucher disease. A Phase II study in type 1 Gaucher patients is underway to investigate the safety and efficacy of GENZ-112638.

WS-5-2

GAUCHER PATIENTS HOMOZYGOUS FOR THE L444P MUTATION: PITFALLS OF TREATMENT

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We describe two sisters of Turkish origin, with consanguineous parents, affected by Gaucher disease type 3A or chronic neuronopathic type of this lysosomal storage disease. Both patients were homozygous for the L444P mutation. The older sister was diagnosed at the age of five years with sever hepatosplenomegaly, haematological disturbances and oculomotor dyspraxis. The younger sister was diagnosed shortly after birth. Both patients receive enzyme substitution therapy (ERT): Cerezyme 240 U/kg body weight/4 weeks;in the younger girl treatment was started in the presymptomatic stage of the disease. Abnormalities found during ERT included: in the older patient the appearance of polyclonal IgG and IgM elevation and in the younger patient the occurrence of oculomotor disturbances (brain stem involvement) and a pulmonary reticulonodular infiltration (Rx-thorax; CT-scan of the lungs). An open lung biopsy showed invasion of the lungs by Gaucher cells responsible for the abnormalities seen on imaging. **Conclusions**: (1) Consecutive detailed neurological evaluation is warranted in every patient who carries the L444P mutation; (2) Involvement of the lungs (fibrosis; pulmonary hypertension) is rare in Gaucher disease; relapse of the disease in the lungs under ERT as seen in our patient is extremely rare; (3) The case of our younger patient proves that ERT solely cannot prevent further disease progression in Gaucher patients with severe disease as is the case in type 3 patients.

WS-5-3

ENHANCED DIFFERENTIATION OF OSTEOCLASTS FROM MONONUCLEAR PRECURSORS IN PATIENTS WITH GAUCHER DISEASE

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Gaucher disease is an autosomal recessive disorder caused by deficiency in β-glucocerebrosidase resulting in storage of glucosylceramide in reticuloendothelial cells. This results in multiorgan pathology including cytopenias, hepatosplenomegaly and bone disease. Bone disease may remain problematic despite treatment with recombinant enzyme. Whilst features of bone pathology such as osteopenia and osteonecrosis have been described their underlying pathophysiology is not well understood and the role of monocyte-derived osteoclasts is unknown. **Aim**: To isolate, culture and characterise osteoclasts from patients with Gaucher Disease and to compare kinetics of *in vitro* osteoclastogenesis and bone resorptive activity with cells derived from normal subjects. **Method**: PBMC were isolated from Gaucher patients and normal controls, cultured with M-CSF and RANK-L to induce osteoclastic differentiation and analysed for evidence of osteoclast markers and activity. **Results**: Multinucleate giant cells expressing markers of osteoclast differentiation occurred earlier and in greater numbers in cultures derived from Gaucher patients than normal controls (171 ± 53 v 48 ± 13 cells per coverslip, $p = 0.04$). In addition the functional capacity of osteoclasts for bone resorption was enhanced in Gaucher patients (Pit size $286u^2$ v $176u^2$ $p = 0.01$). Increases in both osteoclast number and activity correlated with clinical markers of severity of bone disease in individual patients. **Conclusion**: Patients with Gaucher related bone disease exhibit enhanced capacity for osteoclast differentiation. Elucidating the underlying mechanisms will suggest rational therapies for the most disabling aspect of this condition.

WS-5-4

PHENOTYPIC HETEROGENEITY OF N370S HOMOZYGOTES WITH TYPE 1 GAUCHER DISEASE: AN ANALYSIS OF 798 PATIENTS FROM THE INTERNATIONAL COLLABORATIVE GAUCHER GROUP GAUCHER REGISTRY

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Gaucher disease (GD) is the most common lysosomal storage disorder. Type 1 GD has no primary CNS manifestations. Homozygosity for the N370S mutation generally confers a mild phenotype with presentation in adulthood. To determine whether a subgroup of N370S homozygotes with a more severe phenotype exists, we examined the clinical characteristics at or near the time of diagnosis of the 798 N370S homozygotes in the ICGG Gaucher Registry; data for all parameters were not available for all patients. 32% (251/788) were diagnosed before age 20. Many already had significant bone disease at diagnosis: 26% (51/198) showed evidence of infarctions, lytic lesions, or avascular necrosis; 49% (34/70) had decreased lumbar spine bone density. 18% (59/327) were anemic; 9% (29/327) were thrombocytopenic ($< 60\,000/mm^3$). 41% (78/190) had hepatomegaly > 1.25 $2.5\times$ normal; 3% (5/190) had hepatomegaly $> 2.5\times$ normal. 73% (142/193) had splenomegaly $> 5\times$ normal. We conclude that some N370S homozygotes present with a moderate-to-severe phenotype. The observed proportions of more severely affected N370S homozygotes may be overestimates as patients with an asymptomatic phenotype may not be brought to medical attention nor be enrolled in the Registry. Nevertheless, these data suggest that N370S homozygosity is not invariably a benign late-onset disorder, but may present with a more severe phenotype and may present before adulthood. The phenotypic heterogeneity of N370S homozygosity should be addressed in genetic counseling and clinical decision-making.

WS-5-5

HEMATOPOIETIC CELL-SPECIFIC KNOCKOUT OF THE MURINE GLUOCEREBROSIDASE LOCUS: A GAUCHER DISEASE MODEL

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Total loss of function of the lysosomal enzyme glucocerebrosidase (GBA) leads to a perinatal lethal phenotype in the mouse, similar to that seen for severe type II Gaucher patients inheriting two null alleles. As it is clear that some residual activity is required for viability in the mouse as well as humans, a conditional approach was used to selectively target GBA function. Using an engineered murine GBA locus with flanking loxP sites and a Cre mouse strain expressing the recombinase in endothelial and hematopoietic cells (Tie2Cre) we have produced a mouse with decreased GBA activity in the liver, spleen, bone marrow and peripheral white blood cells. These mice store glucocerebroside in the liver and spleen and have significant pathology by 26 weeks of age with obvious rafts of lipid-engorged macrophages (Gaucher cells) in the spleen. Interestingly, liver pathology is somewhat attenuated in comparison and no bone marrow involvement can be seen at 26 weeks. This work represents the first conditional murine model of Gaucher disease. Although significant visceral pathology is produced by targeting hematopoietic cells alone, other Cre-expression approaches can be used to address the various symptoms seen across the Gaucher spectrum, including neurological manifestations. The flexibility of this conditional approach to vary both the timing and distribution of Cre expression will allow for extensive phenotypic modulation and the production of a complete set of models for investigating the pathophysiology of Gaucher disease and testing novel therapeutic approaches.

WS-5-6

STRUCTURAL BASIS OF FABRY DISEASE AND CORRECTIVE EFFECT OF YEAST RECOMBINANT HUMAN ALPHA-GALACTOSIDASE ON FABRY MICE

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To clarify structural basis of Fabry disease, we built structural models of mutant alpha-galactosidases resulting from 161 missense mutations, and evaluated the influence of each replacement on the structure by calculating the numbers of atoms affected. Structural changes caused by classic Fabry mutations are generally large or are located in functionally important regions. In 82% of the cases, three atoms or more in the main chain are affected. On the other hand, structural changes caused by variant Fabry ones are small and located apart from the active site. In 85% of the cases, less than three atoms in the main chain are influenced. Structural investigation is useful for elucidating the molecular pathology of Fabry disease. Furthermore, we produced a recombinant human alpha-galactosidase in a mutant yeast cell line. The products were incorporated into cultured fibroblasts from a Fabry disease patient via mannose-6-phosphate receptors. Repeated intravascular administration of the enzyme to Fabry mice led to successful degradation of the accumulated ceramide trihexoside in the liver, kidneys, heart and spleen but not in the dorsal root ganglia. The recombinant enzyme produced in yeast cells is promising as an enzyme source for enzyme replacement therapy, as the culture of yeast cells is easy and economical and it does not require fetal calf serum.

WS-5-7

HETEROGENIC BIOCHEMICAL, METABOLIC AND GENETIC FEATURES OF FABRY DISEASE

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Fabry disease is an X-linked metabolic disease which is caused by deficiency of the lysosomal alpha-galactosidase A (AGA). We describe here a total of 101 patients (77 females and 24 males) with variable clinical manifestations, in whom a secure diagnosis was made by the enzyme and molecular analysis. The AGA activity in leukocytes among female patients is well correlated with the severity of clinical symptoms. As an example, in a 55 year-old female patient with multiple symptoms the AGA activity was found to be as low as her relatively healthy 26 year-old son (0.05 and 0.02 nmol/min/mg protein; normal range: 0.4–1.0). The variability in clinical manifestation of heterozygous females emphasizes the necessity of early diagnosis of carriers for Fabry disease. The mutations of the AGA gene are spread out through the whole gene and mostly private or rare without prevalent aberrations. We have also found 22 novel mutations and three polymorphisms, –10C>T, IVS4-16A>G and IVS6-22C>T with the allele frequency of 5–10%. The plasma and urinary concentration of GB3 was determined using by HPLC-MS/MS technique in 45 patients during enzyme replacement therapy. GL-3 was first separated by a C18 column and quantified in multiple reaction monitoring mode by using C17 GB3 as an internal standard. In urine the GL-3 excretion normalized within 2–10 months after therapy. On the other hand the plasma level reaches to the normal range somewhat slower. The measurement of GL-3 and the molecular testing may be necessary for securing the diagnosis of female patients due to extreme heterogeneous clinical conditions particularly in cases with inconclusive enzyme levels.

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WS-5-8

HETEROZYGOUS FABRY FEMALES SUFFER FROM SIGNIFICANT MULTISYSTEMIC DISEASE AND IMPAIRMENT IN QUALITY OF LIFE

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Fabry disease is an X-linked disorder of glycosphingolipid degradation caused by a deficiency in the lysosomal enzyme α-galactosidase A, resulting in systemic deposition of globotriaosylceramide. Heterozygous women with α-galactosidase mutations were, until recently, thought to be asymptomatic. Data from 45 females with Fabry disease we have evaluated were compiled and analyzed for manifestations of the disease. Nearly 75% were referred due to an affected male relative; 76% reported acroparasthesia as their first symptom. A mean of 15.7 years elapsed from the onset of first symptoms to diagnosis. 24.3% experienced cerebrovascular compromise with an average age of first event of 52 years. 62% reported symptoms of depression or treatment with antidepressants, while 39% reported generalized anxiety. 75% had electrocardiographic abnormalities, 58% had valvular dysfunction, and 6% had ventricular hypertrophy on echocardiography. 40% reported significant abdominal cramping and postprandial diarrhea. 58% had reduced creatinine clearance; 13% had end-stage renal disease. Almost 60% complained of fatigue and 82% of exercise intolerance due to cardiopulmonary involvement. 47% had evidence of small airway disease on spirometry. At maximal exercise, mean oxygen uptake was 78% of predicted; 89% of the women tested showed abnormal decrease in diastolic blood pressure. Our series indicates that the asymptomatic female Fabry heterozygote is the exception rather than the rule; in fact, heterozygotes suffer from significant multisystemic disease and reduction in their quality of life.

WS-6-1

A RAPID AND SIMPLE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY (LC-MS/MS) METHOD FOR URINARY GL-3 ANALYSIS IN FABRY DISEASE

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Fabry disease is an X-linked inborn error of glycosphingolipid catabolism resulting in fatty deposits in lysosome of various tissues and body fluids. A predominant accumulation of one biomarker, globotriaosylceramide (GL-3 or CTH), has been detected in the urine of Fabry patients. We present a simple, rapid and efficient method for the analysis of GL-3 in urine samples collected on filter paper. One ml of urine sample from Fabry disease patients and controls are deposited on a 5 cm disc of Whatman 903 filter paper and left to dry for at least 4 h at room temperature. Elution of urine samples is performed by shaking the filter papers with 4 ml of methanol in glass vials for 1 h. The eluates are poured in glass tubes and homogenized for 30 s. Fifty µL is injected into a Waters Micromass Quattro micro tandem quadrupole system equipped with an Alliance 2795XE LC. A two-step gradient of ammonium acetate (2 mM) + 0.1% formic acid in water (A) and methanol (B) mobile phases was used. MS/MS analyses were carried out by positive electrospray ionization. QuanLynx software was used to measure the total urinary GL-3 content and specific isoforms from C16 to C24:OH (internal standard: C17). The coefficient of regression for all isoforms was high with a mean of 0.993. We validated the technique (linearity, limit of detection, quantitation, recovery) and the stability at different temperature conditions was established for a period of 8 weeks. Urine sample results were expressed according to creatinine by multiplex assay.

WS-6-2

SCREENING FOR NEURONAL CEROID LIPOFUSCINOSES USING FILTER PAPER BLOOD SPOTS

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Neuronal ceroid lipofuscinoses (NCL) constitute a heterogeneous group of neurodegenerative disorders characterized clinically by the combination of dementia, epilepsy, and retinopathy. At least nine different genetic forms of NCL are known. Among those, the lysosomal enzyme deficiencies result from mutations in the genes for CLN1 and CLN2. In the remaining forms, deficient membrane proteins or unknown defects occur. The clincial symptoms may already confer a suspicion for NCL. However the ultimate diagnosis can be difficult and may require to send specimens to distant specialized laboratories. We have therefore developed biochemical assays that allow the use of dried blood spots for the detection of the deficient enzymes palmitoylprotein thioesterase 1 (PPT 1) or tripeptidyl peptidase 1 (TPP 1) and the direct molecular genetic analysis of the most frequently encountered mutation in CLN3, a 1.03 kb deletion, from the same specimen. These biochemical markers are stable for at least 1 year at room temperature. Our experience over the last three years confirmed the reliablity of the assays (total ca. 600 samples, of these 37 tested positive for an NCL). No false negative results were noted. Therefore, dried blood spots provide a reliable and convenient tool for the initial diagnostic work-up of clinically suspicious patients.

WS-6-3

NEONATAL SCREENING FOR POMPE DISEASE – THE NOVEL DRIED BLOOD SPOT ASSAY IN COMPARISON TO LYMPHOCYTE ENZYME ACTIVITIES

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Pompe Disease is an autosomal recessive disorder which results in deficiency of acid alpha-glucosidase. As enzyme replacement therapy has been shown to be effective, neonatal screening for this disorder may significantly ameliorate the outcome. Enzymatic diagnosis is hampered by the presence of the neutrophil enzyme maltase-glucoamylase (MGA) which shows a significant overlap in activity within the acidic pH range. Recently, Chamoles et al. described a novel method for the selective inhibition of MGA in dried blood spots (DBS). To allow an evaluation of its diagnostic potential we compared results from the lymphocyte assay with the DBS assay. Neglible activity was found in lymphocytes of all patients. Recently, acarbose, a potent mechanism-based alpha-glucosidase inhibitor, was used to inhibit non-specific enzyme activity. All of our Pompe patients ($n = 10$) showed significantly increased ratios of pH 7.0/pH 3.8 (with inhibitor >1000; normal range 200–1000) while at the same time the activity at pH 3.8 using acarbose was reduced (patients mean: 0.02 nmol/spot; normal range >0.09 nmol/spot). In summary, we were able to demonstrate that the novel DBS assay compares well in its diagnostic value with the lymphocyte assay and therefore, may be evaluated in newborn screening.

WS-6-4

NEONATAL SCREENING FOR POMPE DISEASE: RESULT FROM THE TAIWAN SCREENING PROGRAM

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Pompe disease is caused by the deficiency of acid-alpha-glucosidase (GAA). Recently, recombinant human GAA has been used to treat infantile-onset Pompe disease (IOPD) with good success, resulting in prolonged survival, reversal of cardiomyopathy, and growth and motor gains, although not all patients achieve ambulation. Best motor outcomes are reached when rhGAA treatment is initiated early. The timing of treatment initiation may be critical for patient outcomes. Newborn screening will permit early diagnosis and treatment, thus, a neonatal screening program for Pompe disease started in Oct. 2005. Blood spots were obtained from babies around 3 days of age. Blood spot GAA activities were measured using 4-MU-glucoside as the substrate, and acarbose as an inhibitor of maltase-glucoamylase (MGA). The assay employed a first screen for GAA activity, and for those with a decreased activity, a retest assay including the ratio between GAA and neutral maltase, and percent inhibition by acarbose. The recall rate was below one percent. After screening 39 422 newborns, two cases of GAA deficiency were confirmed. One case is currently 4 months of age with muscle weakness being suspected. Another case was clinically normal at the age of one month. During this period, one case of IOPD was diagnosed clinically at the age of 4 months. She was not screened in our program, but her newborn bloodspot GAA activity was in the deficiency range when assayed retrospectively. Currently the screening program continues, and the result suggests that neonatal screening for Pompe disease is feasible and would be helpful in its early diagnosis.

WS-6-5

IMINO SUGARS DEOXYNOJIRIMYCIN AND N-BUTYL DEOXYNOJIRIMYCIN ENHANCE ALPHA-GLUCOSIDASE ACTIVITY IN FIBROBLASTS FROM PATIENTS WITH INTERMEDIATE AND LATE ONSET POMPE DISEASE

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Pompe disease (PD) is a metabolic myopathy due to the deficiency of the lysosomal acid alpha glucosidase (GAA) and characterized by generalized tissue glycogen storage. The phenotype of PD is highly variable and ranges from severe classic infantile forms to intermediate, childhood-, juvenile- and adult-onset forms. We studied the effects of two imino sugars (IS), deoxinojirimycin (DJ) and N-butyl deoxynojirimycin (BDJ) on GAA activity in fibroblasts from 2 patients with the classic infantile PD, 2 with an intermediate form and 1 with a childhood-onset form. The cells were cultured for variable periods (3–15 days) in the presence of IS concentrations ranging from 10 to 80 mM. Treatment with both IS resulted in enhanced GAA residual activity (1.7 to 8.2-fold) in cells from patients with intermediate and childhood-onset PD. No increase was observed in fibroblasts from patients with the classic infantile form. Enzyme enhancement peaked at 9 days after addition of IS and was already detectable at low concentrations (10 mM). IS treated fibroblasts from intermediate and childhood-onset Pompe patients showed detectable amounts of the active GAA molecular forms (76–70 kDa) by Western blot analysis and decreased intracellular glycogen concentrations after 20 days. Our results indicate that DJ and BDJ may be effective as pharmacological chaperones in some forms of PD, and may also provide the rationale foralternative therapeutic approaches to PD, such as enzyme enhancement.

WS-6-6

EFFICACY ESTIMATION OF BONE MARROW TRANSPLANTATION ON THE BRAIN IN MUCOPOLYSACCHARIDOSIS TYPE II: A COMPARATIVE STUDY WITH NATURAL HISTOTRIES

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Bone marrow transplantation (BMT) is one of the therapies for mucopolysaccharidosis type II (MPS II). However, its efficacy on the brain is a point of controversy. It is difficult to estimate the efficacy because of the variation of brain involvement. MPS II is divided into mild form without mental retardation and severe form with mental retardation. We further divided the mild form into two groups (A and B) and the severe form into three groups (C to E). **Group A**: they showed normal intelligence and brain MRI. **Group B**: they showed borderline intelligence in the adult age or some abnormalities in brain MRI. **Group C**: they showed normal speech development with one-word or two-word sentences, and mental retardation was found around age three. **Group D**: they spoke one-word sentences but did not show further development, and speech retardation was found before age two. **Group E**: they showed developmental delay around one year of age, and hardly get one-word sentences. The severities of the 7 patients who received BMT were classified by their initial speech development. Out of these 7 patients, 3, 2, and 2 patients were classified to group B, C, and D, respectively. Although the patients of group C or D without BMT usually show convulsion and complete loss of speech before age 10, two of group C and two of group D with BMT kept their speech abilities after BMT and showed no convulsion.

WS-6-7

ECONOMICAL DIAGNOSIS OF NEURONAL CEROID LIPOFUSCINOSES

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The neuronal ceroid lipofuscinoses (NCL) are characterized by the combination of dementia, epilepsy, and retinopathy and by the intracellular storage of a material defined by its ultrastructural morphology. Presently nine different genetic forms of NCL are known, designated CLN1 through CLN9. Lysosomal enzyme deficiencies occur in CLN1 and CLN2, deficient membrane proteins in other forms. Some NCL types manifest at a characteristic age. Suspecting an NCL is at hand by the typical combination of symptoms and the progressive nature of the condition, whereas arriving at a diagnosis may be difficult and require facilities not readily available. Our diagnostic strategy starts with the use of dried blood spots for the detection of deficient palmitoylprotein thioesterase (CLN1), deficient tripeptidyl peptidase (CLN2), and the presence of the most frequently encountered mutation (in ~80%) in CLN3. These tests will identify the large majority of NCL cases. When results are negative, we encourage consultation between the local physician and our center. Depending on the clinical picture, molecular genetic analysis of genes for rare NCL types is initiated. Patients in whom known NCL mutations have been excluded and where ultrastructural studies demonstrate storage material are investigated scientifically. The combination of expert advice (*http://ncl-net.com*) and analysis of dried blood provides an efficient approach to diagnose NCL disorders.

WS-6-8

LYSOSOMAL SIALIDASE Neu4: A NOVEL ROLE IN GLYCOLIPID CATABOLISM

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Mammalian sialidases, described so far, have been classified according to their subcellular localization as lysosomal (Neu1), cytosolic (Neu2) and plasma membrane associated (Neu3). These 3 classes of sialidases are different in their substrate specificity, Neu1 being active mostly against oligosaccharides and glycopeptides, whereas Neu2 and Neu3 are also capable of hydrolysis of sialylated gangliosides. Recently we identified a novel lysosomal sialidase, Neu4 and showed that it is equally active against all classes of sialylated glycoconjugates. The sialylated gangliosides (GD1a, GM2, GM3) were hydrolyzed by Neu4 in the presence of saposins and GM2-activator protein that are required for degradation of glycolipids by other lysosomal glycosidases. To further investigate whether Neu4 is involved in the cell in ganglioside catabolism, we transiently expressed it in hexosaminidase A-deficient neuroglia cells from the patient affected with Tay-Sachs disease. Electron micrographs of the control cells showed lysosomes containing membranous profiles and vesicular structures, characteristics of lysosomal storage disease, whereas the lysosomes in the Neu4-transfected cells had homogenous electron dense-content, indicating the correction of storage due to the reduction of accumulated GM2 ganglioside. Using Neu4 siRNAs we have generated stable loss-of-function phenotype in HeLa cells. Transfected cells showed large heterogeneous lysosomes containing lamellar structures and vesicular profiles of different sizes indicating that down-regulation of Neu4 causes lysosomal storage. Taken together, our results strongly suggest the potential involvement of Neu4 in the lysosomal catabolism of glycolipids.

WS-7-1

INBORN ERRORS OF METABOLISM IN KOREA

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This presentation will deal with current status of IEM in Korea, from the view points of screening, diagnosis and management of IEM. The field of inborn errors of metabolism (IEM) used to be barren until the introduction of newborn screening program in 1985 as a pilot study. Newborn screening program has been extended from regional basis to nation-wide program since 1997. Nowadays, every single Korean newborn can be screened for 6 IEMs by government sponsored nation-wide program free of charge. However, about half of newborns are enrolled in nation-wide program. The other half are being screened for 18–24 IEMs by MS/MS at parents' own costs. Tandem mass spectrometry was introduced in 2001. The facilities for biochemical and molecular diagnosis have been available from early 1990 in commercial laboratory as well as academic institutions. Basic epidemiological and clinical data have been accumulating in many IEMs. Special formulas for diet therapy have been manufactured by a Korean dairy company. The organ transplantation using liver or hematopoietic stem cells is also very active when appropriately indicated. Enzyme replacement therapies for Gaucher, Fabry, Hurler, and Pompe diseases are also underway with a financial support from both a special government grant and nation wide insurance. Also, a small number of physicians, scientist and dietitian initiated the study group on IEM in 1994, which was established as the Korean Society of Inherited Metabolic Disorders (KSIMD) in 2000. The KSIMD has about 100 active members, holding annual academic meeting and publishing periodicals for scientific communications.

WS-7-2

INBORN ERRORS OF METABOLISM IN INDONESIA

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Inborn errors of metabolism (IEM) services in Indonesia were begun on July 2000, after the returned of first Indonesian's pediatrician who formally trained and got qualification as pediatric metabolicist in the Netherlands. It was integrated with Pediatric Nutrition service because failure to thrive or malnutrition is listed as a component in a number of metabolic disorders, and nutrition play a vital role in the multidisciplinary management of metabolic disorders. In the beginning there were two main problems, the unawareness of physicians to symptoms and signs of IEM and the unavailability of diagnostic tools. The first problem is solved by educate the physicians to think metabolic. Since 2001, IEM is integrated to the syllabus of pediatric specialities, 2002 to the clinical syllabus for medical student, and 2006 to the Cell and Genetics Module for the first grade medical students. Since we do not yet have the facilities to do metabolic screens, the diagnosis is entirely depending on clinical symptoms and the oriented laboratory investigations that available in Indonesia. If necessary and possible financially, more specific investigations were conducted in the referral laboratories such as in Japan, The Netherlands, and Australia. Until now, we could diagnosed clinically and proved enzymatically GSD type IX, MPS types III, IV and VII, Niemann-Pick, MLD, MMA, and mitochondrial diseases. Further, it is estimated 1000 IEM will be born in Indonesia every year. For that reason, the Pediatric Nutrition and Metabolic Diseases Working Group of Indonesian Society of Pediatrician has been performed introducing workshop on IEM and participated by 21 pediatricians representing 11 Faculties of Medicine all over Indonesia. Presently, nine pediatricians had been further trained IEM for 3–6 months in Jakarta and The Netherlands. Finally, the network was built for referring the suspected patients by e-mail/fax/phone to Jakarta as a National Referral Hospital, wherever necessary facilitated further investigations.

WS-7-3

CHALLENGES IN ORGANIZATION OF SERVICES FOR INBORN ERRORS OF METABOLISM IN A DEVELOPING COUNTRY

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Inborn errors of metabolism (IEM) have tremendous public health impact on morbidity, mortality and medical cost of a country. After achieving good general health care, it is imperative to establish a coordinated nationwide service for IEM as many of them are treatable and preventable. For any developing country, the lack of trained personnel, equipment and financial support pose great challenges. In Malaysia, a nationwide coordinated IEM service was established in 1999 under Ministry of Health. A national referral center for IEM was established in Kuala Lumpur. Basic IEM tests such as plasma amino acid, urine organic acid, acylcarnitine, urine GAG were made available. Quality assurance activity for the laboratory services was achieved through ERNDIM. International collaboration and help was sought for rarer diagnostic testing. Continuous training of clinical and laboratory personnel was carried out. Regional centers were set up and networking with the national center was established. For the past 6 years, 724 cases of IEM (324 cases of mitochondrial diseases, 95 cases of organic aciduria, 81 cases of amino acid disorders, 59 cases of urea cycle defects, 50 cases of carbohydrate disorders, 33 cases of fatty acid acid oxidation defects, 32 cases of lysosomal disorders, 22 cases of pediatric neurotransmitter defects and 44 others) were diagnosed and therapy offered. About a third of them survived with good outcome but a third had significant handicap. Support group was established to achieve better care. Further challenges include the establishment of a newborn screening program and to obtain financial resources for expensive enzyme therapies.

WS-7-4

INBORN ERRORS OF METABOLISM IN CHINA

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China has a big population of 1.3 billions, of whom 0.26 billions are children under 14 years old. As a developing country, we are facing a great challenge to launch a nationwide study on Inborn Errors of Metabolism (IEM) in China. As the beginning of the research on IEM, the study on glucose-6-phosphate dehydrogenase deficiency was conducted in south China from the early 1950s. Up to now, more than 200 labs took part in the work to provide biochemical genetic service for the screening, diagnosis, treatment and prenatal diagnosis of IEM. Neonatal screening for phenylketonuria and congenital hypothyroidism has been established in most provincial cities, covering 20% of the live births. In the past 10 years, high risk screening for organic acidurias, urea cycle defects and fatty acid beta-oxidation defects using gas chromatography – mass spectrometry and tandem mass spectrometry has been applied in some cities. The study on rarer disorders (e.g. mitochondrial disorders, lysosomal storage disorders, peroxismal disorders and glycogen storage disorders) has been performed in some university labs. The study on IEM has now been in very fast progress in China ever since. More and more patients with varied disorders of IEM were detected. A collaborative network has been built to cater for exchange of expertise in diagnosis and management of the patients. Although the network system is at an early stage, it has contributed much to provide information, training of healthcare professionals and sharing experience.

WS-7-5

INBORN ERRORS OF METABOLISM IN ASIA – AN INDIAN PERSPECTIVE

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IEM are increasingly being recognized in India because of improved ICU care, better diagnostic facilities, higher frequency of consanguinity, and a large population. Moreover the realization among pediatricians that majority of IEM have treatment, has led to increased interest in IEM. It is estimated that almost 10 000 infants with amino-acid disorders, and 9600 infants other metabolic disorders are born every year. Therefore the Government has launched a national program of neonatal screening. Initially congenital hypothyroidism, congenital adrenal hyperplasia and G-6-PD deficiency will be screened. In neonatal practice organic acidurias are frequent, methylmalonic acidemia being the commonest, followed by propionic acidemia, and MSUD. Urea cycle disorders are the next most frequent, citrullinemia being the commonest followed by arginino-succinic aciduria and OTC deficiency. Galactosemia is often observed, and we are determining the causative molecular mutations. Glycogen storage disease is also frequent, type 1 and 3 being the commonest. A number of cases of Pompie's disease have been identified, and hopefully enzyme replacement therapy will help them to survive in future. Tyrosinemia is often observed, and some patients have been treated with NTBC. Wilson disease is common, and mutation analyses are being carried out to help in managment. The common mutations in tyrosinase negative albinism have been determined to help in prenatal diagnosis, for which there is demand. The lysosomal disorders that are common are mucopolysaccharidoses (MPS 1, 2, 4 and 6), Gaucher disease, Niemann Pick disease, metachromatic leukodystrophy and Tay Sach disease. There is great demand for prenatal diagnosis as the socio-economic burden of having an affected infant with any IEM is high.

WS-7-6

INHERITED METABOLIC DISORDERS IN THAILAND AND THE ASIA-PACIFIC

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The study of inherited metabolic disorders (IMD) in Thailand started in 1987. Majority were clinically diagnosed since there were only a handful of clinicians/scientists with expertise in IMD. There is lack of governmental interest/support due to high prevalence of infectious diseases and congenital infections. A multicenter survey in 1994 and 2001 revealed numerous cases of IMD from all over the country. Reports and publications on IMD in Thai medical journals in past 18 years has undoubtedly raised awareness among Thai pediatricians/scientists. The collaboration with Japanese experts since 1998 with support from JICA has introduced Thailand to the GC/MS technology; in conjunction with funding from Siriraj Hospital has led to the establishment of the 'Genetic Metabolic Center', the only one of its kind in Thailand in 2001. Biochemical and molecular diagnoses of IMD has just begun. National neonatal screening program has been implemented into public health infrastructure since 1996. At present, approximately 80% of all newborns are being screened for congenital hypothyroidism (CH) and phenylketonuria (PKU). However, many problems still exist due to poor organization and lack of systematic and holistic approach. Treatment of IMDs in Thailand and other developing countries in the Asia-Pacific are quite difficult. IMD in developed countries in the Asia-Pacific was well established since 1960s (Australia and Japan). As for other developing countries e.g. Indonesia. Philippines and Thailand, the study of IMD is still in its infancy. Data on common IMDs, diagnosis and treatment in different countries in the Asia-Pacific will be presented.

WS-8-1

SLC25A13 MUTATIONS IN CITRIN DEFICIENCY AND THE FREQUENCY

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Citrin deficiency results in adult-onset type II citrullinemia (CTLN2) and neonatal intrahepatic cholestasis (NICCD). So far, we have identified twenty-eight SLC25A13 mutations and diagnosed the patients not only in Japan (152 CTLN2 and 182 NICCD) but also in other countries. We have detected 3 CTLN2 Chinese, 22 NICCD Chinese, 4 NICCD Vietnamese and one NICCD Korean with the same mutations as Japanese. In Israel, USA, UK and Czech, we have detected 11 NICCD patients with mutations different from those found in Japanese, indicating a wide distribution of citrin deficiency. On the other hand, the DNA diagnoses of 12 known SLC25A13 mutations revealed that the carrier frequency was high in control individuals from East Asia. We noticed some regional specificity in mutation type in East Asia and regional difference in mutation frequency in China. Mutations 851del4 and 1638ins23 were found in all Asian countries tested, and 851del4 was especially frequent. From haplotyping analysis of microsatellite marker D7S1812, it is reasonable to consider that 851del4 associated with 290-haplotype occurred in the south China, and IVS11+1G>A found in Japan and Korea associated with 281-haplotype occurred in north Mongolia or southeast Siberia. We found a remarkable difference in carrier rates in China (including Taiwan) between north (1/940) and south (61/2933 = 1/48) of the Yangtze River. We detected many carriers in Chinese (64/4169 = 1/65), Japanese (21/1372 = 1/65) and Korean populations (22/2455 = 1/112), suggesting that near 100 000 East Asians are homozygotes with two mutated SLC25A13 alleles. It is now important to find out patients with CTLN2 and NICCD, to treat them, and to prevent onset of severe CTLN2.

WS-8-2

CLINICAL PICTURES OF 75 PATIENTS WITH NEONATAL INTRAHEPATIC CHOLESTASIS CAUSED BY CITRIN DEFICIENCY (NICCD)

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To clarify the clinical features of NICCD, we sent a detailed questionnaire to pediatricians in charge of patients with NICCD and collected data from 75 patients. We retrospectively reviewed symptoms, management and long term outcomes of these patients. Thirty patients were referred to hospitals because of positive results for newborn screening (hypergalactosemia, hypermethioninemia, and hyperphenylalaninemia). The rest of 45 patients were negative for newborn screening. The chief complaints of 37 patients were similar; i.e. jaundice and/or discolored stools, and they were referred to hospitals as suspected cases of neonatal hepatitis or biliary atresia. Eight patients were revealed to exhibit a failure to thrive. Most of patients visited hospitals before 4 months of age. Laboratory data showed elevated serum bile acid levels, hypoproteinemia, low levels of vitamin K-dependent coagulation factors, and hypergalactosemia. Hypoglycemia was detected in 16 patients. Serum amino acid analyses showed a significant elevation of citrulline and methionine. Most of the patients were given a lactose-free and/or medium chain triglycerides-enriched formula and lipid-soluble vitamins. The symptoms resolved in all but two patients by 12 months of age. Two patients were reported to have suffered from progressive liver failure and have undergone transplantation therapy before 1 year old. Another patient was reported to have developed citrullinemia type 2 (CTLN2) at the age of 16 years. We must realize that patients with NICCD are not always benign. It is now important and urgent to find the genetic or environmental factors which lead to the deterioration to CTLN2.

WS-8-3

SLC25A13 MUTATION ANALYSIS IN CHINESE HEPATITIS SYNDROME INFANTS

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Objects: SLC25A13 mutations cause not only adult-onset type II citrullinemia (CTLN2) but also early onset of citrin deficiency (NICCD) which resembles galactosemia in clinical manifestation. In order to know if Chinese hepatitis syndrome infants are citrin deficiency, SLC25A13 gene mutations were analyzed in those sick infants. **Subjects**: 27 sick infants were involved: all Chinese aged 2 months to 3 years, 8 females and 19 males. Among them 16 infants were diagnosed as 'galactosemia', 2 'tyrosinemia' and 9 infant hepatitis syndrome. All diagnoses were made depend on clinical manifestation, plasma amino analysis, urine GC-MS analysis and therapy effect. **Methods**: All infant's DNA samples collected from peripheral blood or dried peripheral blood spots on filter paper were analyzed at Kagoshima University for DNA diagnosis of known SLC25A13 mutations identified in Japanese patients with citrin deficiency. **Results**: 11 of 'galactosemia' and 1 of infant hepatitis syndrome infants had SLC25A13 mutations. None of 'tyrosinemia' infant had mutation on SLC25A13 gene. Among those mutations 5 were homozygote with I, 1 was compound heterozygote with I and X, 1 was compound heterozygote with I and XIX, 1 was compound heterozygote with X and XIX, 1 was compound heterozygote with I and III, 1 had XIX in one allele and 2 had I in each one allele. **Conclusion**: Most of our 'galactosemia' might be caused by citrin deficiency rather than really 'galactosemia'. We need to do more work, such as Western blotting and enzyme analysis to differentiate those two similar diseases in near future.

WS-8-4

NEONATAL SCREENING OF CITRIN DEFICIENCY: COMPARISON WITH CLINICALLY DIAGNOSED CASES IN TAIWAN

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Deficiency of citrin causes both adult-onset type II citrullinemia (CTLN2) and neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD). Previous reports suggest a high carrier frequency of the citrin gene SLC25A13 mutations in Japan and in East Asia including Taiwan; however, the clinical spectrum of citrin deficiency is still far from clear in this area. Neonatal tandem mass (MS/MS) screening monitors the blood spot citrulline level for the diagnosis of either type I citrillinemia or citrin deficiency. All cases were confirmed by both biochemical and mutational analyses. From Jan 2001 to Jun 2005, two cases of citrin deficiency were detected by the screen program. During the same period, two cases of CTLN2 were followed, and another six cases of NICCD were confirmed by retrospectively. Patient with CTLN2 presented intermittent hyperammonemia since their third decades. One patient had chronic renal failure and the other had hepatocellular carcinoma and liver cirrhosis. Neither of them survived to their fifth decades. The six cases detected retrospectively had transient intrahepatic cholestasis and hepatic steatosis. One case detected by screening presented during his first year of life with prominent jaundice, hypoproteinemia, aminoacidemia, fatty liver, hypoglycemia, disturbed coagulation, and high α-fetoprotein, which subsided later on. The other case, in contrast, had only mild disturbance of liver function. The initial blood spot citrulline level in the former case was only 22.1 μM (cutoff 17.55 μM). Since the detection of citrin deficiency by screening may not be complete, clinical alertness for NICCD should be maintained. More efforts are also required in the treatment of the more severe cases, especially CTLN2.

WS-8-5

HEPATOCELLULAR CARCINOMA AND CHRONIC PANCREATITIS ASSOCIATED WITH CITRIN DEFICIENCY: CLINICAL AND PATHOLOGIC FINDINGS

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Citrin deficiency usually causes severe encephalopathy in adults that closely resembles that of hepatic encephalopathy. However, it has been recently noted that this disorder might develop as different clinical manifestations. We here report 3 patients with juvenile-onset chronic pancreatitis and 2 patients with hepatocellular carcinoma (HCC), all of whom were shown to have a citrin deficiency. In all 3 patients recurrent episodes of pancreatitis preceded the appearance of encephalopathy, and their radiological and histopathological findings of pancreas were well consistent with those of chronic pancreatitis. Two patients with HCC had no history of hepatitis viral infection, and one male patient had never shown any neurological symptoms before partial hepatectomy including a tumor. Histological examinations of the liver in both patients showed severe steatosis, but no cirrhotic changes were seen. We will review the clinical features of the patients with chronic pancreatitis and/or HCC causally related to citrin deficiency.

WS-8-6

NOVEL DIAGNOSTIC APPROACH TO CITRIN DEFICIENCY: ANALYSIS OF CITRIN IN LEUKOCYTES

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Objective: Deficiency of citrin in liver, which caused by SLC25A13 mutations, induces two clinical features of neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) and adult-onset type II citrullinemia. Hypercitrullinemia is the most characteristic feature, whereas there are noncitrullinemic individuals and period in citrin deficiency. Diagnosis of citrin deficiency is performed by gene analysis, however the mutations of both alleles have not been identified in 15–20% of cases. Thus we aimed to establish an alternative diagnostic method to examine citrin protein in leukocytes using peripheral blood. **Methods**: Subjects were 38 children having an episode of cholestatic liver dysfunction in infancy, 8 heterozygotes, and 11 healthy individuals. Western blot was used to detect citrin protein in leukocytes. All subjects were also evaluated for 12 known SLC25A13 mutations. **Results**: Citrin was detected in 16 of 38 children with cholestatic liver dysfunction, and no mutations were identified. Citrin was absent in 22 of 34 children. Among these, gene analysis could diagnose citrin deficiency in 18 patients. Two patients were revealed to be citrin deficiency with novel mutations later. In the remaining 2 patients, who exhibits the clinical features of NICCD, a known mutation was detected in one allele but unidentified in another allele. Citrin was also detected in 8 heterozygotes and 11 healthy individuals. **Conclusions**: Citrin was deficient not only in liver but also in leukocytes among patients with citrin deficiency. Analysis of citrin is useful to diagnose citrin deficiency even if without known mutations or hypercitrullinemia.

WS-9-1

THE LONG-TERM INTERNATIONAL SAFETY EXPERIENCE OF IMIGLUCERASE THERAPY FOR GAUCHER DISEASE

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Gaucher disease is a lysosomal storage disorder resulting from a deficiency of the lysosomal enzyme glucocerebrosidase. Since approval by the FDA in 1994, enzyme replacement therapy with Cerezyme® (imiglucerase for injection) has been the standard of care for the treatment of Gaucher disease. The objective of this study was to review the long-term international safety experience of imiglucerase from 1994 through 2004. All spontaneous adverse event reports captured in the pharmacovigilance database for imiglucerase from 1994 through 2004 were analyzed. All adverse events were classified using the current version of the Medical Dictionary for Regulatory Activities (MedDRA). Patients without prior exposure to imiglucerase from 1994 through 2005 were assessed for the development of antibodies to imiglucerase as detected by enzyme-linked immunosorbent and radioimmunoprecipitation assays. Analysis of the long-term safety experience with imiglucerase therapy demonstrates a stable and low rate of adverse events and seroconversion from 1994 through 2005. The majority of frequently reported adverse events related to imiglucerase were infusion-associated reactions which were predominantly self-limiting in nature and did not require discontinuation of treatment. Between 1994 and 2005, IgG antibodies were detected in approximately 15% of treatment-naœve patients. The long-term stability of reported events and seroconversion is a reflection of the well-characterized cell expression system and a mature quality-controlled manufacturing process used to produce imiglucerase. Imiglucerase is a safe therapy for the treatment of Gaucher disease with a stable and low rate of reported adverse events and seroconversion.

WS-9-2

TREATMENT OUTCOME AND SAFETY OF AGALSIDASE ALFA IN FABRY DISEASE: DATA FROM FOS – THE FABRY OUTCOME SURVEY

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FOS is a comprehensive international database of patients with Fabry disease, an X-linked lysosomal storage disorder caused by deficiency of α-galactosidase A. Data from FOS were analysed to determine the safety and treatment outcomes of enzyme replacement therapy (ERT) with agalsidase alfa. At the time of analysis, 436/752 patients were receiving ERT. Estimated glomerular filtration rate (eGFR) remained stable in those patients who started ERT with an eGFR of 30–90 ml/min/1.73m^2. ERT produced improvements in cardiac function ($p<0.05$) and regression of left ventricular (LV) hypertrophy ($p<0.05$), with a mean reduction in LV mass of 15–20 g/m$^{2.7}$ in year 2. Improvements in health-related quality of life and neuropathic pain (both $p<0.05$) were also observed. Since the launch of FOS in 2001, 139 adverse events have been recorded in 76/436 patients. Most were mild infusion-related reactions (prevalence 0.9%), which diminished over time. Agalsidase alfa has a good safety profile and has demonstrated clinical benefits in the long-term treatment of patients with Fabry disease.

WS-9-3

AGALSIDASE BETA (RECOMBINANT HUMAN α-GALACTOSIDASE A) THERAPY IN PAEDIATRIC PATIENTS WITH FABRY DISEASE

Wraith JE on behalf of the AGAL-016-01 Study Investigators

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Fabry disease is an X-linked disorder of globotriaosylceramide (GL-3) metabolism, secondary to a deficiency of the lysosomal hydrolase, α-galactosidase A. To evaluate the safety and efficacy of agalsidase beta replacement therapy in paediatric patients, 14 males and 2 females ranging from 8–16 years of age (mean = 12) were enrolled in an international, open-label study. A 12-week observation period to collect baseline data preceded the 48-week treatment period during which agalsidase beta was infused intravenously at 1 mg/kg every 2 weeks. Treatment was generally well-tolerated, with a safety profile similar to that of adult patients; 1 patient discontinued treatment due to a serious adverse event. Before treatment, plasma GL-3 concentrations were abnormal in the 14 males (range = 9.8–20.9 μg/ml), but normal in both females (<7.03 μg/ml). Plasma GL-3 was restored to normal in 6 males by Week 4 and in all males by Week 20 (range = 2.5–6.8 μg/ml). Skin biopsies were obtained for histological assessment of GL-3 accumulation in dermal capillary endothelial cells. Before treatment, 12 males presented with moderate or severe accumulation; by Week 24, no detectable accumulation was observed in any patient. Although clinical benefit assessments were exploratory and many patients exhibited only mild symptomatology during the observation period, treatment appeared to improve or stabilize renal, gastrointestinal, pain, and quality-of-life measures, particularly in those patients with significant pre-treatment symptoms. Overall, these results provide support for agalsidase beta as a safe and viable therapeutic option for paediatric patients and suggest that early intervention may prevent development of the complications associated with Fabry disease.

WS-9-4

A PHASE 3 EXTENSION STUDY OF ALDURAZYME® (LARONIDASE) IN MUCOPOLYSACCHARIDOSIS I (MPS I)

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Objective: To confirm the long-term safety and efficacy of enzyme replacement therapy with Aldurazyme in patients with MPS I. **Methods**: Forty-five patients from a 26-week double-blind, placebo-controlled study received Aldurazyme (100 U/kg, 0.58 mg/kg) IV weekly for an additional 182 weeks. Efficacy, safety, and antibody levels were assessed periodically. Most patients were Caucasian (82%), male (56%), and had Hurler-Scheie syndrome (82%). Mean patient age at baseline was 15.5 years (range 6–43). **Results**: Forty patients completed the study. Improvements occurred in several areas, including the 6-min walk test, apnea-hypopnea index, joint range of motion, and the CHAQ/HAQ disability index. By the end of the study, nearly all patients had normal liver volumes and one-third had normal urinary GAG levels. Although there was a slight decrease in percent predicted FVC, absolute lung volumes continued to increase in pediatric patients. Aldurazyme was well-tolerated. Infusion-associated reactions occurred in approximately half the patients, were generally mild and easily managed, and decreased in frequency over time. One patient discontinued because of an anaphylactoid reaction to laronidase, and one patient died of causes unrelated to Aldurazyme. Nearly all patients developed IgG antibodies to laronidase, but by the end of the study, approximately half had no detectable antibodies by ELISA. There was no apparent impact of antibodies on clinical efficacy or safety. **Conclusion**: Aldurazyme is a safe and effective long-term treatment for patients with MPS I

WS-9-5

CLINICAL BENEFIT OF ENZYME REPLACEMENT THERAPY (ERT) IN MUCOPOLYSACCHARIDOSIS II (MPS II, HUNTER SYNDROME)

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MPS II is an X-linked lysosomal storage disease caused by iduronate-2-sulfatase deficiency. In this double-blind trial, ERT with idursulfase, produced in genetically engineered human cells, was tested for its ability to improve function in MPS II. Patients (n = 96) were randomized to placebo or idursulfase at a dose of 0.5 mg/kg, IV, administered either weekly or every other week (EOW) for 1 year. After 1 year, patients in the weekly and EOW group exhibited significant improvements compared to placebo in the primary efficacy outcome, a composite endpoint comprising change in percent predicted forced vital capacity (%FVC) and distance walked in 6 min (p = .0049, weekly; p = .0416, EOW). Compared to placebo, the weekly dosing group increased the distance walked by 37 m (p = .0131) and the absolute FVC by 160 ml (p = .0011). Mean responses were meaningfully larger in the weekly vs EOW dosing groups for these and other secondary endpoints. Idursulfase was well tolerated. IgG antibodies were detected in 46.9% of patients in each idursulfase group, but did not appear to influence efficacy of ERT. These results support the use of weekly infusions of idursulfase in the treatment of MPS II.

WS-9-6

LONG TERM BENEFIT AND SAFETY WITH RECOMBINANT HUMAN ARYLSULFATASE B (rhASB) ERT FOR MPS VI

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MPS VI is a rare, fatal lysosomal storage disease. ERT with rhASB has shown positive results in 3 clinical studies and received FDA approval as galsulfase (Naglazyme™). Efficacy and safety are reported for the Phase 3 randomized, double-blind, placebo-controlled 24 wks study followed by 72 wks open-label, extension study. Data from patients receiving enzyme from the beginning of the study or receiving placebo for the first 24 wks are combined and change from baseline to 96 wks reported ($n = 38$). Patients improved a mean of 150 ± 20 m from baseline in the 12MWT ($p < 0.0001$). Similar improvements in 3MSC were noted with patients increasing a mean of 12.0 ± 1.5 stairs/min from baseline ($p < 0.0001$). All patients had rapid, sustained decline in urinary GAGs by a mean of 70%. Height and forced vital capacity (FVC) improved in 95% and 70% of patients, respectively. Five patients had delayed puberty at baseline and all showed progression. Most patients developed anti-rhASB antibodies, but these antibodies were not associated with IARs or lack of clinical benefit. In conclusion, rhASB supports improved endurance, height, pulmonary function, pubertal development and urine GAGs, and has an acceptable safety profile.

WS-9-7

DEVELOPMENT OF ENZYME REPLACEMENT THERAPY (ERT) FOR MUCOPOLYSACCHARIDOSIS IVA (MPS IVA) BY USING BONE TARGETING SYSTEM

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Enzyme replacement therapy is less effective to the bone lesion. Previous studies have found that the acidic amino acid oligopeptides bind specifically to the hydroxyapatite in bone. In addition, an osteoporosis drug, estradiol tagged with specific acidic oligopeptides has been more efficient in improving the bone density with fewer side effects on osteoporosis mice. This led us to believe that it could be used as an effective approach to treating systemic bone diseases like MPS IVA deficient in N-acetylgalactosamine-6-sulfate sulfatase (GALNS).

The purified untagged and tagged (the small acidic peptide-six glutamines were tagged to N-terminus of mature protein) GALNS enzymes derived from CHO cell lines were administered intravenously to MPS IVA mice at a single dose. The tagged enzymes had 10 times more prolonged clearance of half life time in blood circulation. Tagged enzymes retained longer in bone and bone marrow, keeping 4 times higher enzyme activity in bone in 24 h compared to the untagged enzyme. To see the effectiveness of clearance of storage and reversal of phenotype, the 250 units/g dose of each enzyme was given through tail veins weekly for 12 weeks on MPS IVA mice. The pathological findings in mice treated with tagged enzymes showed more clearance of the storage materials in bone and interestingly, cornea as well, compared to those with untagged enzymes.

These findings indicate the feasibility of using tagged enzyme to enhance the delivery and clinical effectiveness to bone in MPS IVA mice, suggesting potential clinical application of ERT in MPS IVA patients.

WS-9-8

CARBOHYDRATE-REMODELED ALPHA-GLUCOSIDASE WITH HIGHER AFFINITY FOR THE MANNOSE 6-PHOSPHATE RECEPTOR DEMONSTRATES IMPROVED DELIVERY TO MUSCLES OF POMPE MICE

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Clinical studies of enzyme replacement therapy for Pompe disease have indicated that relatively high doses of recombinant human acid alpha-glucosidase (rhGAA) may be required to reduce the abnormal glycogen storage in cardiac and skeletal muscles. This may be due to inefficient cation-independent mannose 6-phosphate receptor (CI-MPR)-mediated endocytosis of the enzyme by the affected target cells. To enhance the delivery of rhGAA to the affected muscles in Pompe disease, the carbohydrate moieties on the enzyme were remodeled to exhibit a high affinity ligand for the CI-MPR. This was achieved by chemically conjugating onto rhGAA a synthetic oligosaccharide ligand bearing mannose 6-phosphate residues in the optimal configuration for binding the receptor. The resulting modified enzyme (neo-rhGAA) displayed near normal specific activity and significantly increased affinity for the CI-MPR. Uptake studies using L6 myoblasts showed neo-rhGAA were internalized approximately 50-fold more efficiently than the unmodified enzyme. Administration of neo-rhGAA into Pompe mice also resulted in improved clearance of glycogen from all the affected muscles when compared with the unmodified rhGAA. Comparable or greater reductions in tissue glycogen levels in the heart, diaphragm and skeletal muscles of Pompe mice were realized using 5-fold lower dose of neo-rhGAA than the unmodified enzyme. These results demonstrate that remodeling the carbohydrate of rhGAA to improve its affinity for the CI-MPR represents a feasible approach to enhance the efficacy of enzyme replacement therapy for Pompe disease.

WS-10-1

***IN VIVO* IMAGING OF ADENO-ASSOCIATED VIRUS VECTOR DISTRIBUTION FOLLOWING NEONATAL GENE TRANSFER**

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Adeno-associated virus (AAV) vectors are the most promising vehicles to deliver therapeutic genes to the organs targeted in metabolic diseases. We developed AAV vectors for classical phenylketonuria (PKU) and successfully corrected hyperphenylalaninemia in a mouse model of PKU. Based on the achievement with adult PKU mice, we further questioned whether the neonatal gene transfer provides additional benefits such as prevention of brain damage and immunotolerance to the introduced enzymes. We systematicaly evaluated the biodistribution of AAV vectors depending on the viral capsid and the route of administration. AAV vectors (serotype 1, 2, 3, 4, 5 and 8) were given to newborn mice by intravenous (IV), intraperitoneal (IP) or intramuscular (IM) route. AAV biodistribution was visualized by the vector-encoded firefly luciferase expression with the *in vivo* imaging system. (1) In general, AAV1, AAV5 and AAV8 vectors showed better transgene expression than other serotypes. (2) AAV1 specifically transduced muscles. In addition to IM-injected AAV1 that was effective in adult mice, IP-injected AAV1 in newborns was equally effective by transducing proximal muscles. (3) IP-injected AAV5 mainly transduced peritoneum, much more strongly in newborns than in adults. (4) In adult male mice, AAV8 showed a remarkable tropism to the liver regardless of the route of infusion, whereas female livers were poorly transduced. In neonates, IP- and IM-injected AAV8 stayed at the site of infusion, while IV-injected AAV8 transduced the liver. (4) No gender-specific difference was observed following neonatal injection. These findings gave valuable implications for gene therapy targeting inborn errors of metabolism.

WS-10-2

IDENTIFICATION OF DIFFERENT MODES OF VIRAL TRANSPORT IN THE NON-HUMAN PRIMATE BRAIN AFTER CONVECTION-ENHANCED DELIVERY OF AAV SEROTYPE VECTORS

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Recombinant adeno-associated virus (AAV) serotypes-1 and -2 encoding human acid sphingomyelinase (hASM) were administered into multiple structures of the cynomolgus monkey brain using convection-enhanced delivery. Each monkey was injected with either AAV2-hASM (n = 2) or AAV1-hASM (n = 2) into the striatum, thalamus, motor cortex and hippocampus, and euthanized at 28–31 days post-injection. AAV1-treated monkeys received additional injections into the occipital cortex and cerebellum. Brain tissue sections from all 4 monkeys showed robust transduction in the injected structures, as well as in regions not targeted by surgery such as the medulla oblongata. The overall hASM expression pattern spanned 50–60 mm along the rostro-caudal axis of the brain. In situ hybridization demonstrated that this widespread transduction pattern was aided by movement of AAV through the perivascular space and by retrograde axonal transport in projection neurons that innervate the injection sites. Furthermore, the transduction pattern in the convoluted cortex suggests that both serotype vectors were widely dispersed by moving along the outside of the major white matter tracts. This study demonstrate that the topographical organization of the brain circuits, and the anatomical features of the Virchow-Robin space and white matter tracts may be exploited to achieve widespread gene/protein delivery in the primate brain including remote regions that are not accessible by surgery.

WS-10-3

AAV8-MEDIATED GENE THERAPY OF POMPE MICE NORMALIZES MUSCLE PATHOLOGY AND CORRECTS MOTOR FUNCTION DEFICITS

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Pompe disease, a lysosomal storage disorder, presents with accumulation of glycogen in the lysosomes of striated and smooth muscle due to a deficiency of the lysosomal enzyme acid alpha-glucosidase (GAA). To assess the utility of gene therapy for Pompe disease, we generated recombinant AAV8-based vectors encoding GAA under the transcriptional control of a liver-restricted promoter (DC190). Systemic administration of AAV8/DC190-GAA to Pompe mice generated sustained and high ($\sim$30 mg/ml) circulating levels of GAA. Treatment reduced the glycogen storage in the cardiac, diaphragm and skeletal muscles to near normal levels by 10 weeks. Treated Pompe mice, as well as vehicle-treated Pompe and B6129SF2/J normal control mice, were also tested for muscle strength and coordination using the accelerating and rocking Rota-rod, wire hang, and foot fault tests. The performance of the vehicle-treated Pompe mice declined over time when compared to wild type mice. In contrast, the performance of AAV8-treated Pompe mice was significantly improved over that of vehicle-treated Pompe mice, and was similar to that of normal mice. To model the treatment of more severely affected patient population, we administered AAV8/DC190-GAA to older (10 month-old) Pompe mice. Mice treated at this advanced stage of the disease showed a partial recovery of muscle function, but performance was not normalized despite high circulating levels of GAA. In summary, we have shown that systemic gene therapy using recombinant AAV vectors is capable of preventing degenerative myopathy and preserving motor function in Pompe mice, but is less effective in animals with extensive pre-existing pathology.

WS-10-4

CO-INJECTION OF AAV1-ASA AND AAV1-FGE VECTORS INTO THE BRAIN RESULTS IN WIDE SPREAD DISTRIBUTION OF ASA AND CORRECTION OF METACHROMATIC LEUKODYSTROPHY IN THE MOUSE MODEL

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Metachromatic leukodystrophy (MLD), inherited deficiency of arylsulfatase A (ASA), is characterized by deposition of sulfatide and demyelination in the central and peripheral nervous systems. Direct injection of viral vector into the brain is a possible therapeutic approach, but it is likely that repeated multiple injections are required for treatment of the whole brain. Recently, formylglycine generating enzyme (FGE) was shown to be essential for activation of ASA. In this study, we examined the utility of FGE co-expression in AAV type 1 vector (AAV1) mediated *in vivo* gene therapy of ASA knockout (MLD) mice. AAV1-ASA and AAV1-FGE were co-injected into a single site of the hippocampus. Enzyme assay and immunohistochemical analysis showed that ASA was distributed in both the injected and non-injected hemispheres 7 months after injection. A slight increase in ASA activity was also detected even in the cerebellum. Significant reduction of sulfatide was observed in the whole brain. Rotarod test and walking pattern revealed significant improvement of neurological functions. These results indicate that AAV1 mediated expression of ASA and FGE is highly efficient for long-term expression and secretion of functional ASA in the brain. Unexpectedly, ASA could be transferred to the brain areas distant from the site of gene transfer by axonal transfer and diffusion. Extensive distribution of ASA in the brain may rationalize direct gene therapy of MLD.

WS-10-5

LONG-TERM CORRECTION OF HYPERPHENYLALANINEMIA FOLLOWING LIVER-DIRECTED, AAV2/8-MEDIATED GENE THERAPY IN MURINE PHENYLKETONURIA

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Liver-directed gene therapy is a promising novel approach to the treatment of inborn errors of metabolism. We have developed a liver-specific, mouse phenylalanine hydroxylase (PAH)-expressing rAAV serotype 2 vector pseudotyped with serotype 8 capsid (rAAV2/8), and have administered this vector to *Pah*^{enu2} mice, a model of human PKU. Eight *Pah*^{enu2} mice (three female, five male) received 5×10^{11} vector genomes (vg) of rAAV2/8 via portal vein injection. Serum phenylalanine (PHE) levels decreased significantly by two weeks post injection in all rAAV2/8 treated mice (mean preinjection serum PHE $\pm$ SE = 2095 $\pm$ 80 μM vs. 146 $\pm$ 24 μM two weeks post injection; normal = 157 $\pm$ 32 μM), while serum PHE levels remained unchanged in four saline-treated controls. Likewise, phenylalanine clearance (evaluated with a parenteral PHE challenge) dramatically improved in all treated mice. These effects were stable in all mice out to at least 17 weeks post injection. Serum PHE remains normal (261 μM) in a single male animal that has been allowed to survive to now 36 weeks post-injection. Serum PHE in two treated female mice began to increase modestly after 20 weeks post injection. In the single female animal allowed to survive to 36 weeks post injection, serum PHE has increased to 969 μM but is still significantly lower than the preinjection PHE level (2256 μM). Liver-directed, rAAV2/8 vector-mediated gene therapy successfully corrects hyperphenylalaninemia in *Pah*^{enu2} mice, but the stability of expression and the incidence of long-term adverse effects must be further explored.

WS-11-1

THE NOT-QUITE-LEAVING-HOME MODEL OF MANAGING ADULTS WITH INBORN ERRORS OF METABOLISM IN SOUTH AUSTRALIA

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Aim: To describe the model of shared care developed to manage adults with inborn errors of metabolism (IEM) in an adult tertiary hospital. **Setting**: A state with 1.5 million inhabitants where, before 1996, care for IEM patients was provided only on an outpatient basis in a paediatric hospital. **Patients**: The Clinic has 5 patients with lysosomal storage disorders receiving enzyme replacement and other LSD patients under monitoring. The clinic sees a range of other conditions: mitochondrial disorders, adrenoleukodystrophy, aminoacidopathies, glycogen storage disorders. **Model**: An interested adult physician was co-opted to care for the adult IEM patients. The senior IEM paediatrician attends Adult outpatient clinics, continues to directly care for some patients and provides frequent input and advice to the 'adult' hospital physician and dietitian, as well as maintaining contact with former patients. IEM laboratory and genetic services continue to be provided by the paediatric hospital, with rapid communication of results to members of the adult team, who also receive education and training in these diseases through the paediatric centre. With increasing concentration of adults with IEMs in one centre, 'adult' medical, dietetic and nursing staff have acquired expertise in treating these diseases. **Conclusions**: A critical mass of patients has enabled facilities for enzyme infusions to be established, access to administrative support and participation in research studies. We believe the close relationship between paediatric and adult hospitals has been important in the success of this model, which has been accepted well by patients and could be considered by other medium-sized centres.

WS-11-2

PLASMA LONG-CHAIN POLYNSATURED FATTY ACIDS IN PKU PATIENTS ON DIET-THERAPY AND FREE-DIET GROUP IN ADOLESCENCE AGE

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Aim: to compare the fatty acid (FA) plasma levels in 59 adolescents with PKU (43 on diet-therapy mean age 14, SD 2 – and 16 on free-diet – mean age 13, SD 1.9) with 27 healthy, age-matched controls (mean age 13.5, SD 0.6). **Methods**: Measurements included plasma concentrations of phenylalanine and individual serum FA profiles. Plasma concentrations of phenylalanine were analysed by ion exchange chromatography. FA were measured with high resolution capillary gas-chromatography. FA are expressed as % of total FA. **Results**: In both groups the fatty acid analyses demonstrated reductions in the concentrations of long-chain polyunsaturated fatty acids (LCPUFA), including arachidonic acid (AA) (4.8 ± 1.2 and 4.9 ± 0.8, vs 6.5 ± 1.4, $p < 0.01$), eicosapentaenoic acid and docosahexaenoic acid (DHA) (0.6 ± 0.3 and 0.8 ± 0.2 vs 1.6 ± 0.5, $p < 0.01$) in PKU patients on diet and PKU patients on free-diet, respectively, vs controls. **Conclusion**: Since DHA and AA have relevant physiologic roles, including brain and retinal function, DHA and AA supplementations should be considered also for adolescents and adults on diet. While differences in the LCPUFA levels of PKU are generally explained on the basis of the nihil dietary intake, these results may suggest a role also for extra-dietary causes, maybe connected with some deranged metabolic pathways.

WS-11-3

A SCALE TO MONITOR PROGRESSION AND TREATMENT OF MITOCHONDRIAL DISEASE IN CHILDREN

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Mitochondrial diseases affect all age groups, but those with childhood onset of symptoms often seem to experience the greatest burden of disability. In some paediatric patients this can be explained by a cumulative disability acquired over many years. In others, additional factors, including the nature and severity of the molecular defect, must be considered. To date, no large-scale studies have attempted to document the natural history of paediatric mitochondrial disease. This is in part at least, because no assessment tool has been available to plot the temporal course of a disease with such a diverse clinical spectrum. We describe how a practical and semi-quantitative rating scale has been devised for children with mitochondrial disease, the Newcastle Paediatric Mitochondrial Disease Scale (NPMDS). The scale is multi-dimensional and reproducible, offering a tool through which mitochondrial disease progression can be objectively monitored. We anticipate that use of this tool will facilitate both longitudinal natural history studies and the assessment of future therapeutic interventions.

WS-11-4

NEUROLOGICAL OUTCOME IN A PATIENT WITH A *NULL* ORNITHINE TRANSCARBAMYLASE GENOTYPE FOLLOWING LIVER TRANSPLANTATION AT 6 WEEKS OF AGE

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We report the neurological outcome of neonatal liver transplantation in a 4 year old male with complete deficiency of ornithine transcarbamylase (OTC) due to a null mutation (R50X). He presented with poor feeding, respiratory distress and seizures. Peak plasma ammonium level was 984 μmol/L and peak glutamine 7814 μmol/L. On day 3 of life, he underwent hemodialysis plus intravenous Na^+ benzoate/Na^+ phenylacetate and arginine-HCl therapy. Plasma ammonium decreased to 204 μmol/L after 4 h of dialysis and he was placed on continuous hemofiltration until day 4. Because the patient was unable to tolerate 0.6 g protein/kg/day (supplemented with essential amino acid formula) while on Na^+ phenylbutyrate and suffered two subsequent hyperammonemic episodes, he received a cadaveric (half) liver transplant at 6 weeks of age. Upon institution of an unrestricted diet, the plasma ammonium never increased above 25 μmol/L. There were no seizures and brain MRI was consistent with a profound hypoxic injury. At 4 years of age, the child exhibits verbal dyspraxia, mildly delayed motor skills, attention deficit and hyperkinetic behavior. There is no evidence of spasticity, hyperreflexia, clonus or extrapyramidal motor findings. Receptive language is age appropriate. He is now maintained on sirolimus and L-citrulline supplementation (due to undetectable plasma citrulline). In conclusion, we report the second youngest patient with OTC to have undergone liver transplantation, and who exhibits no signs of severe MR/CP.

WS-11-5

EFFICACY OF BOTULINUM TOXIN FOR THE TREATMENT OF DROOLING IN HOMOCYSTINURIA

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We report two patients with non-responsive cystathionine-β-synthase deficiency. Both patients were diagnosed late in childhood (respectively at the age of 6 and 10 years) because of lens dislocation and intellectual impairment. Patients, one female (Pt. A) and one male (Pt. B), are now 30 and 32-year-old respectively. On methionine-diet restriction and betaine therapy (15 g/day), total plasma homocysteine is well controlled ranging from 40 to 100 μmol/L. Brain MRI, MR angiography of the intracranial vessels, EEG, EMG, nasopharyngeal fiberoptoscope examination (performed only for Pt.A because of suspected dysphagia episodes) were unremarkable. During the years Pt.A developed progressive dystonia of upper limb, myoclonic jerks of the right arm, oromandibular dystonia, very severe dysarthria, and sialorrhea. Anticholinergic drugs (trihexyphenidyl and biperiden) were tried with only initial benefit. Therefore, due to worsening of drooling, we decided to start botolinum toxin A (BTX-A) treatment for both patients. They received local injections of BTX-A (Botox; Allergan, Irvine, CA, USA) into the parotid glands on both sides. One week after the injection the patients reported an important decrease of drooling which lasted for two months. Each injection was performed after three months to avoid possible immunogenic reaction due to BTX-A. Patients were treated and followed-up for two years. We recommend the use of botulinum toxin for sialorrhea, unresponsive to anticholinergic therapy, in patients with homocystinuria.

WS-12-1

HIGH RISK SCREENING, CLINICAL AND LABORATORY STUDIES OF UREA CYCLE DEFECTS IN SOUTH CHINESE PATIENTS WITH HYPERAMMONEMIA

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In the past 10 years, 31 cases (10.4%) with urea cycle defects (UCDs) were detected from 297 patients with hyperammonemia in Peking University First Hospital. The etiological diagnoses were made by blood amino acids analysis and urinary organic acid analysis. 3 patients with citrin deficiency were further confirmed by liver pathological analysis and gene diagnosis. Among 31 cases of UCDs, 18 cases had ornithine transcarbamylase (OTC) deficiency, 5 patients were arginemia, 5 cases were citrullinemia type 1 and 3 were citrin deficiency. The patients had the onset from 3 days to 29 years and presented with varied symptoms such as coma, lethargy, psychomotor retardation, growth failure, vomiting, behavioral abnormalities, perceptual difficulties, recurrent cerebellar ataxia and headache. 4 patients (12.9%) were diagnosed postmortem. To control blood ammonia concentrations and prevent episodes of decompensation, protein restricted diet and medication (such as arginine, citrulline and sodium benzoate) has been given to the patient according to the disease condition. Clinical improvement was observed in 16 cases. 7 cases died of hyperammonemic encephalopathy. A boy with OTC deficiency received a partial liver transplant showed normal general condition for 4 years. UCDs are the most frequent causes of congenital hyperammonemia. Early diagnosis and adequate treatment can contribute a lot to improve the prognosis of the patients. Blood ammonia assay and further etiological analysis should be considered in the differential diagnosis of unexplained neurological and hepatic abnormality.

WS-12-2

VARIABILITY IN THE MANIFESTATIONS OF UREA CYCLE DISORDERS IN THE TAIWANESE POPULATION

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Eighteen patients with hyperammonemia underwent work-up for urea cycle disorders during the period from January 1993 to December 2005 inclusive. The diagnostic methods include plasma amino acids analysis, urinary orotic acid analysis, allopurinal loading tests, hepatocytes enzyme activities, and/or mutation analysis. 15 patients presented low citrulline levels initially. Twelve, 6 males and 6 females, had ornithine transcarbamylase (OTC) deficiency by the criteria of hypocitrullinemia and orotic aciduria. Among them, 5 belonged to the early-onset group (initial symptoms before or equal to age 28 days) and 7 belonged to the late-onset group (median onset age, 52 years; range, 8 months to 59 years). Analysis of the OTC genes detected 7 different mutations from 8 patients in 7 families. One mother presented hyperammonemia when delivering her second affected son. One male infant with hypocitrullinemia was proved to have carbamoyl phosphate synthetase deficiency by enzyme measurement upon living-donor liver transplantation at the age of one. The other two twin brothers with hypocitrullinemia but no orotic aciduria refused further diagnostic evaluation. Their onset age were 3 years and one brother died at the age of 7 years. Two in three adults with hypercitrullinemia carry the citrin gene SLC25A13 mutations. Neither of them survived to their fifth decades. In conclusion, both early-onset and late-onset cases of UCD were identified in Taiwan. All OTC mutations detected were different and most of them have not been reported in other populations, which may explain the variability of phenotypes in the Taiwanese patients.

WS-12-3

DIAGNOSIS AND MANAGEMENT OF INBORN ERRORS OF UREA CYCLE IN KOREA

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Eight inherited urea cycle related disorders have been well characterized. During past 10 years at our institution, we have experienced 25 unrelated OTC deficient patients, 14 AS deficient patients from 13 unrelated families, 6 CPS deficient patients from 3 families, 3 unrelated NICCD and 2 adult CTLN2 patients. The diagnosis has been made based on molecular characterization as well as biochemical profiling in all patients. Twenty four different mutations were identified in 25 unrelated families with OTC patients. The G100R, V323M, and R277W were correlated with late-onset phenotype in males. Six male OTC and 8 female OTC patients are alive. Two patients have been successfully liver transplanted. The patients with neonatal onset were managed with hemodialysis or continuous hemofiltration during acute metabolic crash. Long term management included Korean manufacturer-made special formula for UCD patients, sodium benzoate and citrulline supplementation. Ten prenatal molecular diagnoses including 1 preimplantation were carried out in 6 families at risk. Ten AS deficient (citrullinemia) patients were alive. Five different mutations were identified in all the families, 3 major mutations accounting for over 90% of mutated alleles. One patient was liver transplanted but succumbed to opportunistic infection. Two patients were identified by newborn screening by MS/MS before the onset of clinical symptoms, doing well so far. Nine prenatal molecular diagnoses were performed in 3 families at risk. Two CPS patients are alive, one neurologically handicapped and the other is fine.

WS-12-4

TREATMENT OF CITRIN DEFICIENCY: COMPARISON WITH OTHER UREA CYCLE DISORDERS

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We have enzymatically and genetically diagnosed more than 500 cases of urea cycle disorders including citrin deficiency. A deficiency of citrin, the liver-type mitochondrial aspartate/glutamate carrier, results in adult-onset type II citrullinemia with hyperammonemia (CTLN2) and neonatal intrahepatic cholestasis with multiple aminoacidemia, hypoglycemia and galactosemia (NICCD). A variety of the symptoms comes from loss of essential roles of citrin in supplying aspartate to the cytosol from mitochondria and transport of cytosolic NADH reducing equivalent into mitochondria as a member of malate/aspartate shuttle. We noticed that the hyperammonemia caused by citrin deficiency should be treated quite differently from those induced by the urea cycle enzyme deficiencies. A low protein and high carbohydrate diet is the basis of therapy for hyperammonemia. Such diet, however, is most probably harmful in the case of citrin deficiency, which may be concluded from several evidences: Blood ammonia levels of a CTLN2 patient had a circadian rhythm with a peak at evening, which disappears without meals (Yajima et al.). A CTLN2 patient fell into coma induced by hyperammonemia following hyperalimentation containing a high dose of glucose (Tamakawa et al.). Many CTLN2 patients with brain edema became deteriorated following treatment with glyceol containing glycerol and fructose (Yazaki et al.). We also showed that sucrose administration increased blood ammonia of human citrin deficiency model mice. Those coincide with much less carbohydrate intake of CTLN2 patients, strongly suggesting that a large amount of carbohydrate and compounds which increase cytosolic NADH should not be given for citrin deficiency patients.

WS-13-1

VERY-LONG-CHAIN FATTY ACID METABOLISM CORRELATES WITH PHENOTYPE IN X-LINKED ADRENOLEUKODYSTROPHY

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The phenotypical variation of X-linked adrenoleukodystrophy (ALD) ranges from severe cerebral form in childhood (CCALD) to mild adrenomyeloneuropathy (AMN) after 40 years and is not correlated with ABCD1 gene mutations. Up to now, no correlation has been demonstrated between the clinical phenotype and the accumulation of very-long-chain fatty acids (VLCFAs) in plasma or fibroblasts of ALD patients. In normal appearing white matter, however, levels of VLCFA correlates with phenotype. ALD fibroblasts exhibit reduced peroxisomal VLCFA β-oxidation activity but also enhanced elongation of VLCFA (Mol Genet Metab 84:144–51, 2005). In this study, we measured VLCFA content, oxidation of D_3-C24:0 (Clin Chem 50:1824-6, 2004) and de novo synthesis of D_3-C26:0 from D_3-C16:0 using stable isotope labeled fatty acids and a sensitive ESI-MS method in the fibroblasts of 6 CCALD, 9 mild AMN and 5 controls.

Peroxisomal β-oxidation activity as measured by the production of D_3-C16:0 from D_3-C24:0 which reflexes the flux state through peroxisomal β-oxidation was lower in CCALD than in AMN (1.16 ± 0.23 vs 1.45 ± 0.38; $p < 0.002$); C26:0/C22:0 was higher in CCALD than in AMN (0.21 ± 0.03 vs 0.17 ± 0.04; $p < 0.0001$) with no difference in de novo D_3-C26:0 synthesis. Culture of fibroblasts at 30°C, a condition known to stabilize the activity of instable peroxins did not increase the differences between CCALD and AMN fibroblasts. These studies demonstrate correlation between VLCFA metabolism in fibroblasts and ALD phenotype. Ongoing studies aim to optimize culture conditions to predict the phenotype of ALD in individuals cells.

WS-13-2

HIGH INCIDENCE OF HYPEROXALURIA IN GENERALIZED PEROXISOMAL DISORDERS

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The Zellweger Spectrum Disorders (ZSDs) are characterized by a generalized loss of peroxisomal functions caused by deficient peroxisomal assembly. Clinical presentation and survival are heterogeneous. Most peroxisomal enzymes are unstable in the cytosol of peroxisome-deficient cells of ZSD patients, but alanine:glyoxylate aminotransferase (AGT) is stable. Its deficiency causes primary hyperoxaluria type 1 (PH1, MIM 259900), characterized by hyperoxaluria, nephrocalcinosis and renal insufficiency. We observed ZSD patients with renal stones and hyperoxaluria. We aimed to determine the prevalence of hyperoxaluria in Zellweger Spectrum Disorders (ZSDs) and to find clinically relevant clues that correlate with the urinary oxalate load.

We analyzed urines of 31 Dutch ZSD patients with long survival (>1 year) and reviewed medical charts and renal ultrasounds in order to detect renal involvement. The relationship between the degree of neurological dysfunction, and urinary oxalate levels was also investigated.

In 23 living ZSD patients, hyperoxaluria was found in 19 (83%) and hyperglycolic aciduria in 14 (64%). Renal involvement with urolithiasis and nephrocalcinosis was present in five of which one developed end-stage renal disease. The presence of hyperoxaluria, potentially leading to severe renal involvement, was statistically significant correlated with the severity of neurological dysfunction.

Hyperoxaluria with renal involvement is a frequent biochemical finding in patients with Zellweger Spectrum Disorders. These patients should be screened by urinalysis for hyperoxaluria and renal ultrasound for nephrocalcinosis in order to take timely measures to prevent renal insufficiency.

WS-13-3

REDEFINING FATTY ACID METABOLISM IN HUMANS

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The human acyl-CoA dehydrogenases (ACADs) family consists of 11 evolutionarily conserved flavoenzymes. ACADs aree involved in the mitochondrial β-oxidation of fatty acids or branched chain amino acids, however, the substrate specificity and physiologic function of several members remain unclear. Multiple sources of investigation indicate that short-, medium- and very-long chain-acyl-CoA dehydrogenases are primarily involved in generating energy from straight chain saturated fatty acids, while isovaleryl-, short/branched-, and isobutyryl-CoA dehydrogenases are active in leucine, isoleucine and valine metabolism, respectively. We have recently shown that ACAD-9 plays a role in the β-oxidation of unsaturated fats, but it is also likely to have other novel roles. Moreover we have identified alternative forms of many of these enzymes and suggested that each fine tunes the function of the enzyme for a specific substrate. Recently, we have identified a new catabolic pathway for unusual branched chain fatty acyl-CoAs that appears to involve the newly identified family members, ACAD-10 and ACAD-11, as well as isovaleryl-CoA and possibly long chain acyl-CoA dehydrogenases. Gene expression and immunologic studies indicate novel tissue and cellular locations for many of the ACADs suggesting involvement in cellular functions other than energy metabolism. Our findings necessitate fundamental review of the role of mitochondrial fatty acid β-oxidation in intermediary metabolism, implicating involvment of ACADs in a wider variety of metabolic processes than previously recognized.

WS-13-4

REYE-LIKE SYNDROME RESULTING FROM NOVEL MISSENSE MUTATIONS IN MITOCHONDRIAL MEDIUM- AND SHORT-CHAIN L-3-HYDROXY-ACYL-CoA DEHYDROGENASE

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Medium- and short-chain L-3-hydroxy-acyl-CoA dehydrogenase (M/SCHAD) deficiency is a recessively inherited disorder of mitochondrial fatty acid oxidation. Currently, only 4 patients from 3 families have been fully reported in the literature. All of these patients presented with hypoglycemia associated with hyperinsulinism (HI). This association suggests that there is a unique role for M/SCHAD in regulating the pancreatic secretion of insulin. We present a fifth patient whose presentation was with hepatic dysfunction similar to Reye syndrome, a feature in common with most of the previously recognized disorders of fatty acid oxidation. There was no clinical indication to measure insulin levels at presentation and no subsequent documented history of hypoglycemia. Diagnosis was indicated by a hypoketotic 3-hydroxydicarboxylic aciduria and confirmed by measurement of reduced enzyme activity in skin fibroblasts (35% of normal). Sequencing of the *HAD1* gene revealed compound heterozygosity for two novel missense mutations, 170A>G, resulting in D45G, and 676T>C, resulting in Y214H.

The mutant enzymes were expressed and subjected to kinetic analysis. Y214H has no detectable activity, whilst D45G, which resides in the cofactor-binding pocket, has an altered K_m for NADH (96 μM versus 24 μM for the wild-type). This represents the first kinetic M/SCHAD mutant, and explains the high residual activity in skin fibroblasts. The lack of obvious HI in this patient may be related to the high residual activity and indicates that HI associated with M/SCHAD deficiency may only be present with complete deficiency. The spectrum of M/SCHAD phenotype should be broadened to include acute liver disease.

WS-13-5

TEN NEW PATIENTS WITH MALONYL-COENZYME A DECARBOXYLASE (MCD) DEFICIENCY

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MCD activity controls intracellular levels of malonyl-CoA, a key regulator of several carnitine acyltransferases. We report ten new patients with malonic aciduria associated with enzyme confirmed MCD deficiency in eight. Clinical details were available on eight, and molecular genetic characterization was obtained for seven. As for 15 previously described patients, cardinal clinical manifestations included developmental delay and cardiomyopathy; conversely, metabolic perturbations (e.g. acidosis) and seizures were infrequent in our patients. For all, detection of elevated malonic acid in urine (±increased C3DC acylcarnitine by tandem mass spectrometry) led to pursuit of enzyme studies. MCD activities (nmol/hr/mg protein) revealed: control (n = 22), 16.2±1.8 (SEM; range 5.7–46.2): patients (n = 8, assayed in duplicate), 1.7±0.3 (range 0.6–2.8; $p<0.0001$). Characterization of PCR-amplicons revealed six novel mutations: 1 nonsense, 4 missense, 1 large genomic deletion, 1 frameshift mutation, and 2 previously described mutations. For one additional patient, molecular characterization remains in progress. Our findings increase the number of enzyme confirmed MCD-deficient patients by ~40%, and expand the phenotypic and molecular heterogeneity of this rare disorder.

WS-13-6

GENOTYPE-PHENOTYPE CORRELATIONS IN MCADD

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Objective: To investigate genotype-phenotype correlations in children diagnosed with MCADD by expanded newborn screening (ENBS). **Methods**: Cross sectional chart review of all children diagnosed with MCADD from three New York ENBS follow-up centers. Infants were classified in three groups: eleven K304E homozygotes, ten with variant genotypes (one or no copies of K304E), and three with atypical MCADD (C8 levels of >30 with brittle fasting intolerance or sibling death, but not homozygous for K304E). **Results**: The mean C8 level on ENBS was 14.9 (range 0.99–31.7) in the K304E homozygotes, compared to 6.0 (0.86–23.2) in the neonates with variant genotypes ($p<0.2$). Organic acids were diagnostic in six of seven K304E homozygotes, but only one of seven with variant genotype ($p<0.01$). Follow-up C8 acylcarnitine levels were 5.2 (1.9–11.3) in the homozygous and 2.1 (0.53–5.3) in the variant infants ($p<0.01$), and follow-up hexanoylglycine levels were 24.7 (12.7–49.3) in the homozygous infants and 9.1 (3.8–23.2) in the variant infants ($p<0.001$). Several variant infants required both acylcarnitine and acylglycine analyses and/or DNA to clarify their affected status. **Conclusions**: Infants homozygous for the K304E mutation are more likely to have higher levels of diagnostic metabolites. The extent to which this predicts clinical outcome merits further study, however the 3 'atypical' infants with profound fasting intolerance and C8 levels >30 suggest a relationship between ENBS C8 level and vulnerability to metabolic stress. Infants with variant genotypes typically have normal organic acid analyses, and some may require both plasma acylcarnitine and acylglycine or DNA analysis to separate affected from carrier infants.

WS-13-7

ENDOGENEOUS CORRECTION OF EXERCISE-INDUCED CARDIAC CARNITINE DEPLETION IN VLCAD-DEFICIENT MICE

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Patients with defects of fatty acid oxidation present with low free carnitine in blood, termed 'secondary carnitine deficiency'. To correct low free carnitine concentrations, carnitine supplementation has often been discussed. We measured free carnitine and long-chain acylcarnitines in blood, heart muscle, skeletal muscle and liver from very long-chain acyl-CoA dehydrogenase (VLCAD)-deficient (n = 5) and wildtype mice (n = 5) under non-stressed conditions and after physical exercise. For the exercise test, the mice had to run for 1 h on a treadmill at a constant running speed of 16 m/min. One group of mice (n = 5 from each genetic variant) was sacrificed immediately after the exercise test, the second group rested for 24 h after the exercise test was terminated before sacrifice. Immediately after exercise, long-chain acylcarnitines were significantly increased in heart and skeletal muscle from VLCAD-deficient mice. Concurrently, free carnitine was significantly decreased, especially in heart muscle. Cardiac free carnitine decreased from 500 nmol/g under non-stressed conditions to 300 nmol/g after exercise, in comparison to wildtype mice with concentrations of 800 nmol/g before and after exercise. After a 24-h rest, long-chain acylcarnitines in heart and skeletal muscle completely normalized as compared to non-stressed conditions. Importantly, also free carnitine in heart muscle was fully replenished to concentrations prior to exercise, whereas blood free carnitine remained reduced. We observed a significant increase in carnitine production in liver. This observation gives prove that liver carnitine biosynthesis can completely compensate for the carnitine depletion in heart and skeletal muscle within 24 h after exercise stress.

WS-13-8

IDENTIFICATION OF A MOUSE MODEL OF HUMAN LONG-CHAIN 3-HYDROXYACYL-CoA DEHYDROGENASE DEFICIENCY (LCHAD)

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Using the metabolomics-guided screening coupling with ENU mutagenesis, we identified mice with elevated blood long chain acyl carnitines (C16-OH, C18-OH, and C18:1-OH carnitines). Those mice had poor weight gain but survived to adulthood despite a shorter life span. The same phenotype was observed in 1/4 of F2 offsprings indicated this phenotype is heritable and transmitted as autosomal recessive trait. Whole genome homozygosity mapping using about 300 SNPs showed 100% homozygosity in 5 consecutive SNPs on chromosome 5 spanning from 34 Mb to 63 Mb where candidate genes, Hadha and Hadhb, coding for mitochondria trifunctional protein α and β subunit, are located. Direct sequencing revealed normal α subunit, and detected a nucleotide T to A transversion in exon 14 (c.1210T>A) in both β subunit, resulting in a missense mutation of methionine to lysine (M404K). Complete blood cell counts (CBC), echocardiography (ECHO), electrocardiogram (ECG), functional brain MRI and tissue pathology were performed to characterize the disease phenotype of these mutant mice. Histopathology changes in the hadhb$^{-/-}$ mouse revealed enlarged fatty drops in white and brown adipocytes and hepatocytes indicating fatty acid accumulation. Trichorme stain of the heart revealed myocardium fibrosis. ECHO and ECG showed prolonged PR interval, conduction defects and cardiomyopathy. This is the first viable late-onset animal model of LCHAD deficiency due to Hadhb mutation. These mice could provide an important animal model for study of long chain fatty acid metabolism, investigating the pathogenesis of LCHAD deficiency and development of more effective therapies for this disease.

WS-14-1

HISTOPATHOLOGICAL AND BEHAVIORAL IMPROVEMENT OF MURINE MUCOPOLYSACCHARIDOSIS TYPE VII BY INTRACEREBRAL TRANSPLANTAION OF NEURAL STEM CELLS

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The therapeutic efficacy of neural stem cell transplantation for central nervous system (CNS) lesions in lysosomal storage disorders was explored using a murine model of mucopolysaccharidosis type VII (MPS VII). We used fetal neural stem cells derived from embryonic mouse striata and expanded *in vitro* by neurosphere formation as the source of graft materials. We transplanted neurospheres into the lateral ventricles of newborn MPS VII mice. The transplanted donor cells migrated far beyond the site of injection within 24 h, and some of them could reach the olfactory bulb. A quantitative measurement indicated that the lysosomal beta-glucuronidase (GUSB) activity in the brain was 12.5 to 42.3 and 5.5 to 6.3% of normal activity at 24 h and 3 weeks after transplantation, respectively. In addition, histological analysis revealed a widespread decrease in lysosomal storage in the recipient's hippocampus, cortex, and ependyma. A functional assessment with novel-object recognition tests confirmed improvements in behavioral patterns. These results suggest that intra-cerebral transplantation of neural stem cells is feasible for treatment of CNS lesions associated with lysosomal storage disorders.

WS-14-2

CELL THERAPY FOR THE BRAIN INVOLVEMENT IN LYSOSOMAL STORAGE DISEASE

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Lysosomal storage disease (LSD) is a group of genetic diseases caused by deficiency of an enzyme for the catabolism in lysosome. Most of them show systemic accumulation of undigested substrates in lysosomes, which cause progressive diseases including the brain. Bone marrow transplantation and enzyme replacement therapy are clinically available as effective therapy for some LSDs. But the brain involvement is an exception. We transplanted fetal brain cells, cultured neuronal cells, or bone marrow derived mesenchymal stem cells into the ventricle of neonatal mouse brain to deliver the deficient enzyme and protect the brain from the disease, and studied the migrating area of the grafted cells and their viable period. β-Galactosidase knock-out mouse (GM1-gangliosidosis model mouse) and transgenic mouse expressing human β-galactosidase were used as the recipient and the donor, respectively. The cells of $4\text{-}8 \times 10^4$ were injected into the right-side ventricle on the 2nd day after birth. The treated mice were examined histologically by β-galactosidase activity staining (X-Gal) and *in situ* hybridization of β-galactosidase mRNA to see the grafted cell distribution, and ganglioside-GM1 immunostaining to examine the therapeutic effect. The injected cells were grafted on the surface of the right-side of ventricle one week after the injection. They migrated into the deep area of both hemisphere, and the number of X-Gal positive cells became 10–20 fold two weeks after the injection. Brain transplantation of some special cells would have a considerable potential for permanent cure of the brain in LSDs.

WS-14-3

BRAIN TRANSPLANTATION OF GENETICALLY MODIFIED STEM OR PROGENITOR CELLS FOR CNS SYMPTOMS OF LYSOSOMAL STORAGE DISORDERS

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Objective: Mucopolysaccharidosis type VII (MPS VII) is one of lysosomal storage disorders (LSDs), caused by the lack of beta-glucuronidase (GUSB), resulting in the accumulation of glycosaminoglycans (GAGs) in various tissues including the brain. Here we showed the effectiveness of transplantation of genetically modified bone marrow stromal cells (BMSCs) into newborn ventricle or adult parenchyma of MPS VII mouse brain. We also tested effectiveness of transplantation of neural progenitor cells derived from embryonic stem cells (ESNPCs) into newborn ventricle of MPS VII. **Methods**: BMSCs retrovirally expressing human GUSB gene (G-BMSCs) were transplanted into newborn ventricle and adult left striatum of MPS VII mice. ESNPCs were transplanted into newborn ventricle. Assay of enzymatic activity, staining of enzymatic activity in section, measurement of GAGs contents, pathological evaluation and water maze test (WMT) were performed. **Results**: After transplantation of BMSCs into newborn ventricle, GUSB activity in treated mice was increased, and GUSB positive cells were found various regions of brain. No pathological lysosomal distension was observed in neuron and glia. GAGs contents decreased to almost heterozygous level. Brain function was significantly improved based on WMT. When BMSCs were transplanted into adult striatum, GUSB activity in treated hemisphere was increased. Some transplanted cells differentiated into neuron. About ESNPCs study, we successfully differentiated ES cells to ESNPCs *in vitro* (30% of cells were nestin positive). After transplantation of ESNPCs, some cells were found in the brain. **Conclusion**: Stem cell gene therapy is very promising methods to deliver therapeutic enzyme to the neuronal cells of LSDs.

WS-14-4

HEMATOPOIETIC CELL TRANSPLANTATION PREVENTS NEUROLOGICAL DISEASE IN ADRENOLEUKODYSTROPHY MOUSE

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X-linked adrenoleukodystrophy (ALD) is characterized by progressive demyelination and accumulation of very-long chain fatty acids (VLCFA) in the central nervous system. ALD has a wide spectrum of clinical manifestations, adrenomyeloneuropathy (AMN) being the most common (65%) form. AMN affects the spinal cord and leads to axonal degeneration that results in spastic paraplegia during adulthood. It is established that allogeneic hematopoietic cell transplantation (HCT) at an early stage of the human disease stabilizes or reverses cerebral demyelination. The effect of HCT to cure or prevent AMN is however unknown.The knock-out ALD mouse develops a late-onset phenotype that resembles AMN. We report that transplantation of bone marrow cells from normal mouse, known to contain progenitors of microglia, prevent the AMN phenotype of ALD mouse. The long-term follow-up to 3 patients with cerebral ALD transplanted at 8, 9 and 11 years shows that none of them have developed clinical or electrophysiological symptoms of AMN at 26 years.These data provide the first preclinical and clinical informations for the prevention of human AMN by hematopoeitic cell transplantation.

WS-14-5

MESENCHYMAL STEM CELLS (MSC) FOR CLINICAL APPLICATION

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MSCs are undifferentiated cells derived from bone marrow that can be isolated, expanded in culture, and characterized *in vitro* and *in vivo*. MSCs have the potential to differentiate to lineages of mesenchymal tissues, including bone, cartilage, fat, tendon and muscle. MSCs have immunological properties that allow them to be immuno-priviledged. Furthermore, MSCs can modulate the response of T cells, inhibit inflammation and secrete factors that stimulate tissue repair and engraftment of hematopoietic stem cells (HSC). Recent preclinical findings suggest that the infusion of MSCs may be a valuable treatment for a frequent, life-threatening complication of HSC transplantation, i.e. graft versus host disease (GVHD). Osiris Therapeutics Inc. demonstrated in a Phase I-study of the co-transplantation of HLA-identical sibling HSCs and HSCs donor-derived MSCs, that the MSC treatment was safe and it resulted in improvement of a 2-year survival. Subsequently, a Phase II-study which is designed to investigate the potential efficacy outcomes of 'universal' donor-derived MSCs administered at the time of GVHD occurring in patients who had undergone HLA-unmatched peripheral blood stem cell (PBSC) or bone marrow (BM) transplantation, is on going. Le Blanc et al., independently reported, a case of a 9-year old boy who had a grade IV GVHD after unrelated, haplo-identical PBSCT recovered rapidly following IV infusions of MSCs derived from his mother. In 2003, JCR entered into a licensing agreement with Osiris to develop its MSC technology for the use in the treatment for hematologic malignancy using HSCT in Japan. I would like to talk about immunological properties of MSCs elucidated from our studies and other laboratories. In addition, I would like to talk on JCR's engagement in assuring the quality and safety of MSCs.

WS-14-6

TRANSPLANTATION FOR INBORN ERRORS OF METABOLISM (IEM)

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Hematopoietic cell transplantation (HCT) is effective long-term treatment for selected IEM including Hurler syndrome, mannosidosis, globoid-cell (GLD) and metachromatic leukodystrophy (MLD), and cerebral X-adrenoleukodystrophy (X-ALD). Underlying principles include: (1) cross correction, (2) donor monocyte-derived macrophages, (3) immunosuppression and subsequent donor-derived immune system. Long-term survival with good quality of life can be achieved; however, HCT is generally not curative. Limitations of allogeneic HCT with marrow, peripheral or cord blood include: (1) time to achieve stabilization of developmental trajectory, (2) inability of HCT to halt neurocognitive decline in Hunter (MPS IIA), Sanfilippo (MPS III), symptomatic MLD and GLD infants, advanced stage cerebral X-ALD, Tay-Sachs, (3) inability to significantly impact myelin injury, neuronal cell damage, and resulting neurologic deficits, (4) progression of peripheral nervous system (PNS) demyelination (e.g. MLD), (5) persistence/progression of skeletal abnormalities,. Future clinical trials with alternative stem cells should address: (1) preparation and delivery of neural stem cells to repair cellular injury and/or provide healthy cells to the CNS and PNS, repair and/or replace damaged and dysfunctional myelin; (2) treatment regimens using mesenchymal stem cells to contribute healthy osteoblasts and chondroblasts to facilitate normal growth and development of bone and cartilage and repair abnormalities. Optimal therapeutic approaches to complex IEM may be multi-dimensional: hematopoietic, neural, mesenchymal stem cells (allogeneic or corrected autologous), systemic and/or CNS enzyme replacement, substrate depletion, chaperone therapy, etc. Greater understanding of stem cell biology and requirements for transplantation is needed to improve therapy and extend effective treatment to diseases currently not being ameliorated.

WS-15-1

AUDIOGENIC SEIZURES IN MURINE PHENYLKETONURIA

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The mechanisms underlying epileptic activity in untreated phenylketonuria (PKU) patients are not understood. Therefore, we studied the susceptibility of PKU mice (female BTBR, homozygous *Pah*^{enu2}) to audiogenic seizures (AGS). Serum phenylalanine (Phe) concentrations in PKU mice were more than 10 fold higher than in heterozygous carriers or wild-type BTBR mice. PKU mice were highly susceptible to AGS (118 dB, 10–20 kHz) with symptoms ranging from uncontrolled running to clonic and tonic convulsions, respiratory arrest, and death; carrier or wild-type mice showed no response to the stimulus. Seizure activity in PKU mice had a complex dependence on serum Phe concentrations. While mice with Phe levels of $\leqslant$1.8 mM exhibited the full pattern of AGS, mice with Phe levels of $\geqslant$2.2 mM were resistant to AGS. Serum Phe levels in the same mouse varied dramatically during the day/night period, averaging 0.4 mM. Depending on Phe levels, the same mouse could be either susceptible or resistant to AGS. Serum Phe could be lowered to normal, or increased to hyperphenylalaninemic within 12 h, by switching PKU mice from a high to low Phe diet, or vice versa. Susceptibility to AGS disappeared within 12 h after starting the low Phe diet. In contrast, it took more than 7 days on a standard diet for mice previously maintained on a low Phe diet for two weeks to regain susceptibility to AGS. The complex dependence of AGS on Phe levels suggests a role for Phe-induced depression of the glutamatergic and aminergic systems in the etiology of AGS.

WS-15-2

ELEVATED PHENYLALANINE LEVELS INTERFERE WITH NEURITE OUTGROWTH STIMULATED BY THE NEURONAL CELL ADHESION MOLECULE L1 *IN VITRO*

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Elevated levels of phenylalanine (Phe) as observed in patients with phenylketonuria interfere with proper neuronal development, leading to severe psychomotor deficits and mental retardation. We have analyzed the effects of Phe on neurite outgrowth *in vitro*. When expressed in fibroblasts, the neuronal cell adhesion molecules L1 and plexin B3 strongly increase the length of neurites emanating from cerebellar neurons in co-culture experiments. Elevated Phe blocks L1-mediated, but not plexin B3-mediated outgrowth, whereas tyrosine is ineffective. Elevated Phe also interferes with aggregation of fibroblasts overexpressing L1, suggesting that the pathological effect of elevated Phe occurs by interfering with L1-mediated cell adhesion.

WS-15-3

CEREBRAL ACCUMULATION OF DICARBOXYLIC ACIDS IN GLUTARIC ACIDURIA TYPE I – A BIOCHEMICAL RISK FACTOR

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Glutaric aciduria type I (GA-I) is a cerebral organic aciduria characterized by the accumulation of glutaric (GA) and 3-hydroxyglutaric acid (3-OH-GA). Both dicarboxylic acids are putative neurotoxins. However, cerebral concentrations and blood–brain barrier (BBB) permeability of these dicarboxylic acids are quite unknown. We investigated this issue in *Gcdh*$^{-/-}$ mice, hepatic *Gcdh*$^{-/-}$ mice, C57Bl/6 mice after intraperitoneal loading with d4-GA and d5-3-OH-GA, and using porcine brain capillary endothelial cells (BCEC), an *in vitro* model for the BBB. The major results of our study were as follows (1) an equimolar increase in *cerebral* and *hepatic* concentrations of GA and 3-OH-GA in systemic *Gcdh*$^{-/-}$ mice, (2) a discrepant increase (liver $>>$ brain) of GA and 3-OH-GA in hepatic *Gcdh*$^{-/-}$ mice and after intraperitoneal loading with d4-GA and d5-3-OH-GA, (3) a low permeability of BCEC for GA and 3-OH-GA (efflux $>$ influx), and (4) a small *trans*-stimulatory effect of *para*-aminohippuric acid, suggesting efflux stimulation via organic anion transporters (e.g. OAT3). These findings suggest that there is a cerebral *de novo* synthesis and subsequent trapping of GA and 3-OH-GA because of strongly limited BBB permeability causing a steep plasma-to-brain gradient. We hypothesize that cerebral trapping of GA and 3-OH-GA is an important risk factor for neurodegeneration in GA-I but should also be considered for other organic acidurias presenting with cerebral accumulation of dicarboxylic acids (e.g. MMA, D2- and L2-GA).

WS-15-4

TYPE I HYPERPROLINAEMIA ASSOCIATED WITH EPILEPSY AND MENTAL RETARDATION RESULTS FROM MUTATIONS OF THE PRODH GENE IN THREE SICILIAN CHILDREN

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Hyperprolinaemia type I (HPI) is an autosomal recessive disorder due to proline oxidase (POX) deficiency, that converts proline into pirrolin-5-carboxylate (P5C), with increased plasma and urine proline levels, and P5C absent in urine. Proline has been proposed as neurotransmitter in CNS. Patients with HPI and neurological phenotype, bearing mutations on PRODH gene on 22q11, have been reported. Different PRODH mutations were grouped into 3 categories by their effects on POX activity. Three unrelated Sicilian children with HPI presenting epilepsy, mental retardation and behavioural disorders were screened for PRODH mutations. The first patient was a compound heterozygote with a PRODH deletion on one allele and 3 missense mutations (Q19P, R453C, V427M) on the second allele. The second patient was a heterozygote with 3 missense mutations (Q19P, R453C, V427M). The third patient bore the A58T mutation. Clinical, metabolic and genetic correlations were attempted. The first patient had severe HPI (1883 μmol/L) and the worst phenotype; total PRODH activity was predicted to be null. The second child showed lower HPI (862 μmol/L) and a milder clinical phenotype. No explanation was provided for the last patient with milder clinical and biochemical (617 μmol/L) phenotype because A58T mutation is functionally unknown yet. Our study confirms that HPI may be related to a mild to severe neurological phenotype, correlating with the patients' genotype. Patients with epilepsy and cognitive/behavioural disturbances are worth to be investigated for HPI, although further studies are needed to elucidate the mechanisms by which HPI and PRODH genotype may determine neuropsychiatric disorders.

WS-15-5

N-ACETYLASPARTATE AND N-ACETYL ASPARTYL-GLUTAMATE IN A RAT MODEL OF CANAVAN DISEASE

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Canavan disease is an autosomal recessive leukodystrophy caused by aspartoacylase deficiency, which leads to accumulation of N-acetylaspartate (NAA) in brain and biofluids. In 1999, we reported an increase of N-acetylaspartylglutamate (NAAG), a NAA-derived dipeptide, in CSF and urine of Canavan's patients (Burlina AP, Eur J Pediatr 1999). More recently, Kitada et al. genetically identified a spontaneous rat model (tremor rat) of Canavan disease (Kitada K, J Neurochem 2000).

We decided to study the biochemical features of tremor rat brain at different ages. NAA and NAAG were measured, with capillary electrophoresis, in the following brain regions of the animals: cortex, hippocampus, basal ganglia, thalamus, cerebellum, pons, medulla oblongata, and spinal cord. Control (CTRL) and tremor (TM) rats (42 animals for each group) were sacrificed at 2, 3, 4, 6, 8, 12, and 24 weeks, and the brains immediately frozen. Tremor NAA content was significantly higher in all brain regions at different ages ($p < 0.0001$). Interestingly, tremor NAAG content was significantly increased in all brain regions in comparison with the controls ($p < 0.01$). Furthermore, a linear correlation was found between NAA and NAAG levels in spinal cord and medulla oblongata ($r = 0.73$ and $r = 0.81$ respectively).

WS-15-6

COGNITIVE FUNCTIONING AND PSYCHIATRIC DISORDERS IN CHILDREN WITH METABOLIC DISEASES

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Objective: To report on the intelligence scores and the psychiatric pathology of distinct groups of children with metabolic diseases. **Methods**: The study population consists of 53 children between 0 and 18 years of age. Diagnostic assessment included a semi-structured interview, self-report questionnaires and a standard intelligence test. **Results**: In 40% of the children older than 5 years, a child psychiatric diagnosis was made. While CBCL total and internalizing scores did not differ between metabolic disease groups, the CBCL externalizing scores for some groups did, especially in the 'intoxication group' with phenylketonuria as the most prevalent disorder. Two fifths of the children showed a below normal intelligence, while a performal-verbal IQ discrepancy was found in half of the children, especially in the 'intoxication group'. Of the school aged children almost one third attended a special needs school. **Conclusion**: In spite of the small sample size, the results suggest substantial psychiatric problems in children with a metabolic disease. Further study on larger groups is warranted, which should enable further comparison of patients affected by specific metabolic diseases.

WS-16-1

COMPARISON OF PHE MONITORING METHODS FOR DIETARY COMPLIANCE

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Monitoring plasma phenylalanine (Phe) is critical to the management of phenylketonuria (PKU) and requires frequent blood draws in the clinic setting. Recent methods, utilizing filter paper for blood collection, allow for home plasma Phe monitoring. The current study compared three different analytic methods for measuring plasma phenylalanine: tandem mass spectrometry (MS/MS), high performance liquid chromatography (HPLC), and amino acid analyzer (AAA). We studied 25 adolescent and young women with phenylketonuria (PKU), ages 12–48 who attended the Emory Metabolic Camp. All participants followed a low protein diet supplemented with a phenylalanine-free medical food. Diet regimens were individualized and compliance to the restrictions varied. Fasting blood samples were collected for each participant at baseline (pre-camp) and at the end of the week-long camp (post-camp). Blood samples were collected into heparinized tubes for analysis by AAA or were spotted directly onto filter paper for analysis by HPLC and MS/MS. Difference of least squares means determined that MS/MS and HPLC were not different from one another ($p = 0.2$), while AAA gave consistently higher measures % Phe ($p < 0.0001$) than either HPLC or MS/MS. The plasma phenylalanine values obtained from HPLC and MS/MS were on average 21% below those obtained from AAA. This study suggests that there are substantial differences in the results obtained from these analytic methods. When monitoring dietary compliance, clinicians should consider the method of determination used for plasma Phe.

WS-16-2

A 5-YEAR REPORT ON THE DIETARY MANAGEMENT AND OUTCOMES OF INBORN ERRORS OF METABOLISM AT KK WOMEN'S AND CHILDREN'S HOSPITAL, SINGAPORE

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A total of 32 patients were referred to the Department of Nutrition and Dietetics from March 2001 March 2006: 9 (28%) with organic acid disorders, 9 (28%) with amino acid disorders, 7 (22%) with disorders of carbohydrate metabolism, 5 (16%) with disorders of mitochondrial function, 1 (3%) with peroxisomal disorder and 1 (3%) unknown. Median age of referral was 11 months (range 0–8 years 3 months). Children with organic acid disorders were referred the earliest (median age of referral 2.75 months, range 0–34 months).

8 patients were lost to follow-up (deceased, returned overseas or defaulted after referral). Of the remaining 24 patients, 50% are regular with follow-ups; 79% are growing along their percentiles for weight and height; 71% are compliant to dietary advice; 63% use additives like glucose polymers/fats/oils to boost energy intake; 46% use specialized formulas; 21% are on multivitamins to supplement their intake from food and formula; and 75% have no feeding issues. Of the 5 children with failure to thrive, 80% have feeding issues, and 1 child had a percutaneous gastrostomy button inserted to boost his intake. 6 of the children are of school-going age, and the majority (83%) attend normal school.

The department has experience in dealing with a wide range of IEM, and is able to prescribe good dietary recommendations and follow-ups. More cases are anticipated when expanded newborn screening commences in July 2006, including new challenges like more cases of individual and rare IEM, lack of availability of resources like specialized formulas, and the dilemma of treating non-symptomatic patients.

WS-16-3

HOME ENTERAL TUBE FEEDING IN IMD CHILDREN: SAFETY ISSUES

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Many children with IMD, at risk of hypoglycaemia and metabolic decompensation, are dependent on long term home overnight enteral tube feeding (HOETF) but its safety issues have not been evaluated. **Aim**: To identify the safety and practical problems experienced in IMD patients on HOETF. **Methods**: 34 patients (median age 4.1 years: range 1.2–15.8 years) with IMD requiring overnight pump tube feeding were recruited. 12 had GSD; 8 fatty acid oxidation disorders; 9 organic acidaemias and 5 had other conditions. 56% were fed by gastrostomy and 44% nasogastric tube. A questionnaire addressed child safety issues, secondary carer training, equipment reliability, and carer night time disturbance. **Results**: The main safety issues identified were untrained secondary carers (71%); tube leakage (65%); faulty or misuse of pumps (50%) causing hypoglycaemia with hospitalisation in 2 children; equipment tampering by small children (29%) commonly causing disconnection of giving sets from feeding tube; and tube entanglement (71%) even around the neck. Carer sleep disturbance (100%), and tube blockages (45%) were common. **Conclusions**: There were significant safety risks for HOETF children. Procedures, training and equipment design need urgent attention to prevent potentially fatal complications resulting from HOETF in IMD.

WS-16-4

ADULT REFSUM'S DISEASE – DIET MODIFICATION RESULTS IN SUSTAINED REDUCTION IN PHYTANIC ACID AND ABSENCE OF ACUTE COMPLICATIONS

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Adult Refsum's Disease (ARD) is a rare autosomal recessive error of lipid metabolism with clinical features of retinitis pigmentosa, anosomia, peripheral neuropathy, deafness, ataxia, ichthyosis and bony abnormalities. The biochemical abnormality is defective peroxisomal alpha-oxidation of phytanic acid acid (3,7,11,15 tetramethyl-hexadecanoic acid) whereby this fatty acid accumulates in plasma and fat containing tissues. In uncontrolled ARD there may be acute neuropathy and cardiomyopathy. Treatment of ARD involves a diet low in phytanic acid. Data on the long term effects of diet on the biochemical and clinical progression of ARD is limited to isolated case reports. We reviewed the case notes of 14 patients with ARD who attended the Refsum's clinic for dietary reinforcement over a minimum of 10 (range 11–28) years follow-up. Clinical progression, highest and lowest annual plasma phytanic acid levels, body weight and dietetic details were recorded. At presentation, mean phytanic acid was 1648 (range 300–5888) μmol/l, and mean final follow-up phytanic acid was 171 (range 0-370) μmol/l. Weight loss ($n = 4$) and poor dietary compliance ($n = 2$) were the most common reasons for any transient increase in phytanic acid. Reductions of plasma phytanic acid – in some cases ($n = 2$) undetectable plasma levels – were achieved over a long period. There were no acute clinical exacerbations or hospital admissions over the extended follow-up period. Sustained adherence to an appropriate diet can prevent clinical relapses in ARD.

WS-16-5

PHENYLALANINE EXCHANGES IN PKU: SHOULD THEY BE WEIGHED?

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In PKU, many families would welcome a validated, non-weighed for estimating 50 mg phenylalanine exchanges (PE). **Aim**: A randomised, controlled study to compare 2 different non-weighed methods of estimating PE as an alternative to weighed food exchanges in PKU. **Methods**: 2 non-weighed methods for estimating PE ([A] common household measures using measuring cups and spoons and [B] digital photographs) were compared with weighing [C]. 51 normal healthy volunteers (age >14 years) measured/estimated one PE of each food using technique A, B and C on 3 separate days. This was for 8 food groups with 3 food items in each group. **Results**: Overall there was no difference between A and B, although the conventional weighing method was significantly more accurate than both A ($p<0.05$) and B ($p<0.05$). For the 3 groups: a median of 79% (6–100) (A), 82% (10–100) (B), and 96% (57–100) (C) of samples were within 20% of calculated weight but there was wide variation for individual foods. The normal healthy volunteers rated method B the easiest (63%), followed by A (33%) and C (9%). **Conclusions**: for estimating 50 mg PE for many foods (but not all) it is possible to use validated household measures or photographs for accuracy within 20% of calculated weights.

WS-16-6

MANAGING PKU PREGNANCIES USING A LOW PROTEIN DIET

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Aim: To review the use of our less restrictive protein counting system in pregnant women with PKU. **Methods**: Education on the new counting method (1 g of protein being equal to 50 mg phe) was provided by the dietitian over 2–3 visits. Phenylalanine, tyrosine, branched-chain amino acid and alanine levels are determined by tandem mass spectrometry, quantified against deuterium-labelled stable isotopes. Correction factors are used to relate the blood spot values to plasma. Our target Phe range prior to conception and throughout pregnancy is 75–150 μmol/L. During pregnancy, blood amino acid profile including phe is measured twice weekly from filter paper blood spots. **Cases**: Case 1: 34 year old lady who had not been on diet for many years was commenced on 10 g of dietary protein along with a non-phe protein supplement to achieve target phe levels. There was adjustment to her diet with $\frac{1}{2}$ – 1 g protein increases dependent on blood phe levels to final amount of 23 g of protein at the end of the pregnancy. Cases 2 and 3: Pregnancies currently continuing, both of these women have had previous pregnancies using unit counting system (1 unit being equal to 15 mg phe) to measure phe intake. Both report protein counting easier and have acceptable phe control. **Results**: Case 1 had phe levels of 20–197 μmol/L throughout pregnancy. She delivered a normal male infant. **Conclusion**: Protein counting is a safe and acceptable method of measuring phe intake in maternal PKU pregnancies.

O-1-1

MUTATION UPDATE OF HUMAN MITOCHONDRIAL ACETOACETYL-CoA THIOLASE (T2) DEFICIENCY

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Mitochondrial acetoacetyl-CoA thiolase (T2) deficiency affects isoleucine and ketone bodies in the catabolic process. T2 deficient patients develop severe ketoacidotic crises but are asymptomatic between crises. We have 55 T2 deficient cell lines now and analyzed their mutations. Missense mutations and small in-frame mutations such as one amino acid deletion/insertion were further characterized by transient expression analysis of wild-type mutant cDNAs at 30°C, 37°C, and/or 40°C and characterized mutations. In addition, we recently determined crystal structure of human T2 tetramer and can speculate effect of mutation on T2 molecule using this structure. Now we have a catalogue of 52 T2 gene mutations: 2 initiator methionine codon mutations; 27 missense mutations, three of which results in aberrant splicing; 1 three base-insertion; 3 three base-deletions; 2 two amino acid substitutions; 3 nonsense mutations, one of which causes nonsense-associated altered splicing; 7 small deletions/insertions which cause frameshift; 3 splice donor site mutations; 2 splice acceptor site mutations; 1 68 bp deletion including splice acceptor site; 1 Alu mediated gene deletion including exons 2–4. Despite extensive analysis, mutations could not be detected in 7 of 110 mutant alleles. Genotype is well correlated with chemical phenotype such as excretion of tiglylglycine but not with clinical phenotype in T2 deficiency.

O-1-2

MUTATIONS IDENTIFIED IN *D-2-HYDROXYGLUTARATE DEHYDROGENASE* IN 14 OUT OF 36 UNRELATED PATIENTS WITH D-2-HYDROXYGLUTARIC ACIDURIA

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D-2-hydroxyglutaric aciduria is an autosomal recessive neurometabolic disorder with a heterogeneous phenotype. Increased levels of D-2-hydroxyglutaric acid in body fluids characterize the disorder. Recently, we identified disease-causing mutations in the *D-2-hydroxyglutarate dehydrogenase* (*D2HGDH*) gene in 5 unrelated patients. Our laboratory has now screened 36 unrelated D-2-HGA patients for the presence of pathogenic mutations, including the 5 previously reported patients. All 10 exons and the adjacent splice sites were analyzed by direct DNA sequence analysis. In 9 additional patients presumed pathogenic mutations were identified (9 missense variants/mutations, 1 frameshifts, 1 splice error, 1 nonsense mutation). In DNA of 22 patients no mutations (n = 21) or only one potential pathogenic mutation were detected (n = 1). In 10 of these mutation-negative cases we were able to further study the D2HGDH mRNA by RT-PCR. In none of these cases aberrant mRNA was identified, and in 6 cases we were able to show that both alleles are expressed by confirmation of the presence of a heterozygous polymorphism. Currently, we are collecting additional material of the families in whom we did not find a mutation sofar. In conclusion, in 41% of patients with D-2-hydroxyglutaric aciduria we could demonstrate the presence of pathogenic mutations in the *D2HGDH* gene. Whether mutations are missed or that another gene is involved is currently being investigated.

O-1-3

MUTATION UPDATE OF SUCCINYL-CoA: 3-KETOACID CoA TRANSFERASE (SCOT) DEFICIENCY

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Succinyl-CoA:3-ketoacid CoA transferase (SCOT) is the main determinant of the ketolytic capacity of tissues. Hereditary SCOT deficiency causes episodic ketoacidotic crises and no clinical symptom between them. We have fibroblasts from 15 SCOT deficient families and performed mutation analysis. In cases of missense mutations, we performed transient expression analysis of wild-type and mutant cDNAs at 30°C, 37°C, and/or 40°C and characterized mutations. Sixteen different mutations were identified; two nonsense mutations (R217X, 283X), 12 missense mutations (V133E, A215V, G219E, S226N, V221M, R268H, G324E, L327P, S405P, T435N, C456F) and two splicing mutations (IVS1+1 ~ 6 6-bp del, c671G > A). We also made a tertiary structural model of human SCOT molecules and the effects of mutations on the molecule were speculated on the model. Among these mutations, several missense mutations retained significant residual SCOT activity when examined by transient expression analysis of mutant SCOT cDNAs. We divided SCOT deficient patients into two groups; patients with 'severe' mutations who have null-mutations in either allele, and patients with 'mild' mutations who have a mutation with residual SCOT activity at least one of two mutant alleles. SCOT deficient patients with 'mild' mutation(s) would not always show pathognomonic permanent ketosis.

O-1-4

BIOCHEMICAL CHARACTERIZATION OF HUMAN 3-METHYL-GLUTACONYL-CoA HYDRATASE AND FORMATION OF 3-HYDROXY-GLUTARIC ACID IN GLUTARIC ACIDURIA TYPE I

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3-Methylglutaconic aciduria type I (MGA1) is caused by a defect of 3-methylglutaconyl-CoA-hydratase. It was shown previously that MGA1 patients with reduced or absent 3-methylglutaconyl-CoA-hydratase activites have mutations within the *AUH* gene ('AU-binding homolog of enoyl-CoA hydratase'). The present work was initiated to kinetically characterize the 3-methylglutaconyl-CoA-hydratase, the gene-product of *AUH*. Therefore, we overexpressed *AUH* in *Escherichia coli* and determined the enzyme activity with different CoA-substrates. As expected, 3-methylglutaconyl-CoA-hydratase had a high affinity for its 'natural' substrate 3-methylglutaconyl-CoA (K_m = 8.3 µM). This forward reaction was favoured by a factor of 20 over the reverse reaction, the dehydration of 3-hydroxy-3-methylglutaryl-CoA. Interestingly, we found highest activity for the conversion of glutaconyl-CoA (K_m = 2.4 µM) to 3-hydroxyglutaryl-CoA, a key metabolite of glutaric aciduria type I (GA-I). To further invetigate he formation of 3-hydroxygluataric aciduria in GA-I, we studied the conversion of glutaryl-CoA to glutaconyl-CoA by human medium-chain acyl-CoA-dehydrogenase (MCAD). Compared to the conversion of the 'natural' substrate octanoyl-CoA, the oxidation of glutaryl-CoA was less effective (only 0.4% of the conversion of octanoyl-CoA) but seems possible at very high intracellular coenentration of glutaryl-CoA like in GA- I. Our results confirm the central role of 3-methylglutaconyl-CoA-hydratase in the degradation of leucine. Furthermore, the results give a possible explanation for the formation of limited amounts of 3-hydroxyglutaric acid in patients with GA-I.

O-1-5

UREASE PRETREATMENT-GAS CHROMATOGRAPHY-MASS SPECTROMETRY IN THE SCREENING AND DIAGNOSIS OF METHYLMALONIC ACIDURIA

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Objective: To screen and diagnose methylmalonic aciduria by urease pretreatment-gas chromatography-mass spectrometry. **Methods**: From July 2005 to January 2006, 153 urine samples from patients at high risk of inborn errors of metabolism were collected, Samples decomposed with urease, heptadecanoic acid added as an internal standard, protein denaturated and precipitate removed, evaporation performed to dryness, the residue was trimethylsilylly derivatized and then analyzed with gas chromatography-mass spectrometry (GC-MS). **Results**: Using the UP-GC-MS procedure, 8 cases of methylmalonic aciduria were successfully diagnosed, with high concentration of methylmalonic acid detected and an obvious signal of its isomeric compound found in a vitamin B_{12}-unresponsive case. In TIC profile, retention time(relative retention time) of methylmalonic acid and the isomeric compound were 6.78 min (0.396) and 8.52 min (0.24), respectively. Clinical improvements of some patients were observed after diet therapy, medicine treatment and neurological rehabilitation. **Conclusion**: UP-GC-MS is a very useful method to screen and diagnose methylmalonic aciduria, and early diagnosis and treatment contribute a lot to improve the prognosis of such patients.

O-1-6

URINARY QUALITATIVE ORGANIC ACID ANALYSIS: DIFFERING ANALYTICAL APPROACHES AND PERFORMANCE

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A qualitative urinary organic acid EQA scheme started in the UK in 1993 now has 130 participants in 25 countries. The scheme organised jointly from Heidelberg (Germany) and Sheffield (UK), as part of ERNDIM, circulates nine genuine patient samples each year. Participants can gain a maximum score of 2 points per sample or 18 points per year for a fully correct return. While most laboratories demonstrate improving performance with time, some laboratories perform consistently well, Group A (mean score over 6 years = 17.1, SD = 1.4) and a small number perform badly and more erratically, Group B (mean score over 6 years = 8.9, SD = 4.4).

In 2005 a comprehensive survey covering 28 aspects of the methodological and interpretive approaches and evaluating the experience of the participants was arranged to assess the differences that could give rise to this variability of outcome. 94 of the 130 participants responded and the returns showed a surprising consistency of analytical approach, e.g. 91/94 using GCMS and 93/94 forming trimethylsilyl derivatives before analysis. Group A laboratories demonstrated a statistically greater annual workload, mean 1289 samples per year when compared with Group B laboratories, mean 454 samples per year and those laboratories who used an internal standard during analysis achieved a significantly greater mean score = 15.6 vs 14.4 than those who did not. These and similar findings may be used to guide practice and future strategic planning.

O-2-1

MULTIPLEX LSD ENZYME ASSAY: FROM RESEARCH TO NEWBORN SCREENING

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The transfer of a research assay to clinical labs and its subsequent qualification has always been a challenge. Effective transfer is a critical issue since it has a direct impact on how the assay will be adopted and utilized, and whether it will generate similar results around the world. A multiplex LSD enzyme assay was designed to screen for Gaucher disease, Fabry disease, Pompe disease, Krabbe disease and Niemann-Pick A/B disease in newborns by simultaneously measuring enzyme activity deficiencies in dried blood spot samples. Because these disorders are very rare, access to patient samples for assay validation and the development of quality control samples in which patient samples are used are very limited. In this presentation, we will discuss how we optimized the enzymatic reaction conditions, streamlined the sample preparation and clean-up procedure and made the assay feasible for use in the high throughput newborn screening environment. The results of a feasibility study in which samples from LSD patient samples are tested with hundreds of normal samples.

O-2-2

URINE SCREENING FOR LYSOSOMAL STORAGE DISORDERS BY TANDEM MASS SPECTROMETRY

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Mass spectrometric methods have been developed to quantitatively determine accumulated oligosaccharides in the urine of patients with a clinical suspicion of a lysosomal storage disorder [1,2]. Specimens were referred to the National Referral Laboratory for Lysosomal, Peroxisomal and Related Genetic Disorders at the Children, Youth and Women's Health Service. We have developed a single method suitable for the detection of the mucopolysaccharidoses (MPS) and the oligosaccharidoses. Urinary oligosaccharides were derivatised with phenyl methyl pyrazolone and analysed by electrospray-ionisation tandem mass spectrometry. Elevations in neutral oligosaccharides indicated an oligosaccharidosis, and elevations in sialylated oligosaccharides indicated a mucolipidosis, with the metabolite structure indicating the subtype. Specific sulphated oligosaccharide profiles were formulated that enabled the identification of all MPS patients and their respective subtypes. Mass spectrometric data were converted to comparative values by reference to a QC urine sample. During a six-month trial of the method, MPS II, IIIA, IIIB, IVA and VI patients were identified and confirmed by follow-up enzymology. The previous thin layer MPS screening methodology required subsequent analysis of several MPS enzymes to identify a specific disorder; this procedure reduced follow-up enzyme analysis to a single assay.

1. Fuller M et al. Paediatric Res. (2004) 56:733–8.
2. Ramsay S et al. Anal. Biochem. (2005) 345:30–46.

O-2-3

MEASUREMENT OF URINARY ALPHA-GALACTOSIDASE A PROTEIN USING ENZYME-LINKED IMMUNOSORBENT ASSAY AND GLOBTRIAOSYLCERAMIDE USING TANDEM MASS SPECTROMETRY: EVALUATION FOR NON-INVASIVE DETECTION OF FABRY DISEASE

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Background: Enzyme replacement therapy (ERT) in Fabry disease has been shown to reduce globotriaosylceramide (GL-3) storage in tissues and alleviate clinical symptoms in some patients. Early detection of this disease and initiation of ERT in children may be worthwhile to prevent irreversible tissue damage. Children are frequently afraid of having blood drawn, so we developed a non-invasive method for the detection of Fabry disease using urine. **Methods**: GL-3 and α-galactosidase A (α-gal A) protein were measured in urine from 432 healthy volunteers, 40 Fabry hemizygotes and 29 Fabry disease heterozygotes using tandem mass spectrometry and ELISA, respectively. **Results**: All 34 hemizygotes with classic Fabry disease could be distinguished from the controls by either method. Three and 6 cases of 25 classic Fabry disease heterozygotes could not be distinguished from the controls by the method of tandem mass spectrometry and ELISA, respectively. But, when used in combination with the measurement of GL-3, the α-gal A protein assays differentiated 24 out of 25 heterozygotes with classic Fabry disease from the controls. Similar results were obtained using urine from renal and cardiac variant Fabry disease hemizygotes. **Conclusion**: The measurement of α-gal A protein and GL-3 in urine from children may be suitable for early detection for Fabry disease. Early initiation of ERT for Fabry disease before irreversible tissue damage will be possible following patient identification using the procedures that we have developed.

O-2-4

A NOVEL GAG ASSAY IN BLOOD BY TANDEM MASS SPECTROMETRY: APPLICATION TO SCREENING FOR MUCOPOLYSACCHARIDOSES

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Glycosaminoglycans (GAGs) are accumulated in mucopolysaccharidoses (MPS). We have established the tandem mass spectrometry (TMS) method to assay KS, HS, and DS levels in blood to compare each GAG level between control and MPS groups.

Plasma samples were digested by keratanase, heparitinase, and/or chondroitinase to get disaccharides of KS, HS, and DS. Digested samples were assayed by TMS to measure KS, HS, and DS. The 66 samples from the patients (MPS I, 17; MPS II, 22; MPS IIIA, 5; MPS IIIB, 4; MPS IIIC, 2; MPS IVA, 12; MPS VI, 4) (age; 0.1–39.3 years) and 150 control samples (age; 0–51 years) were analyzed.

All MPS I, II, III, and VI patients had a significant elevation of plasma DS + HS (average 3301 ng/ml, min. 829 ng/ml; max. 29200 ng/ml), compared with the controls (average 506 ng/ml, $p < 0.0001$). Specificity and sensitivity was 100% if the cut off value is 800 ng/ml between control and MPS groups. Except one MPS I case, all MPS I, II, and III patients had a significant elevation of plasma HS (average 1041 ng/ml, min. 231 ng/ml; max. 6810 ng/ml), compared with the controls (average 120 ng/ml, $p < 0.0001$). Except one case, all MPS IVA patients had a significant elevation of plasma KS (average 8.8 μg/ml, min. 2.3 μg/ml; max. 12 μg/ml), compared with the controls (average 2 μg/ml, $p < 0.0001$).

These findings suggest measurement of HS, KS, and DS levels by tandem mass spectrometry is applicable to the screening for MPS I, II, III, IV, and VI patients.

O-3-1

IS^3: AN APPROACH TO SAFETY MONITORING IN DAILY PRACTICE

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The Intensive Safety Surveillance Scheme (IS^3) is a post-marketing surveillance in Gaucher disease type 1 (GD1) patients. It is a web-based tool collecting comprehensive baseline and ongoing information. At the end of February 2006, IS^3 included information on 80% of the GD1 patients prescribed miglustat in the EU (44 centres, 13 countries). Mean (SD) age was 44 ± 17 years (62% of females). One third of patients were naive to treatment at baseline. Mean (SD) duration of previous enzyme replacement therapy (ERT) was 6 ± 4 years. Eighty-six percent of GD1 patients were considered unsuitable for ERT. Twenty-nine percent of the 85% of GD1 patients with neurological assessment at baseline had neurological symptoms. One third of patients had bone pain at baseline and was reported in 30% of naive patients and in 35% of patients previously treated with ERT. Overall exposure to miglustat represents a cumulative experience of 75 patient-years in GD1 patients, with a mean exposure of 15 months. No adverse events (AEs) were reported in 53% of these patients. AEs lead to discontinuation in 18% of patients; 50% of cases occurring during the first 6 months. Diarrhoea has been reported in only 17% of patients due to implementation of effective diet recommendations. No cases of peripheral neuropathy have been ascertained. Only two cases of skeletal symptoms were reported in patients osteoporotic at baseline.

The safety profile emerging from IS^3 is consistent with previous clinical trials. IS^3 is an effective tool for monitoring safety of patients receiving Zavesca® in a clinical practice setting.

O-3-2

NEUROLOGICAL IMPROVEMENT OF HEMATOPOETIC STEM CELL TRANSPLANTATION ON LATE-ONSET KRABBE DISEASE; TWO YEARS' CLINICAL COURSE OF SIBLINGS

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Introduction: Krabbe disease, also known as globoid cell leukodystrophy, is caused by deficiency of lysosomal enzyme galactocerebrosidase. Hematopoetic stem cell transplantation (HSCT) has recently been reported to be effective on this disorder. We performed HSCT on two siblings of late-onset Krabbe disease and here report its effect on neurological symptoms. **Cases**: (Case 1) 12 years male. 5 years 7 months: visual loss and slowly decreased muscular strength. 9 years: walking disability, tremor and Krabbe disease was diagnosed. 9 years 11 months: underwent cord blood stem cell transplantation (CBSCT). (Case 2) 9 years male, younger brother of Case 1. 5 years: when elder brother received the diagnosis, he was also diagnosed as Krabbe disease without symptoms. 6 years: visual loss. 6 years 6 months: underwent bone marrow transplantation (BMT). **Conditioning of HSCT**: (1) fludarabine phosphate 30 mg/m^2/day × 6; (2) busulfan 150 mg/m^2/day × 4; (3) cyclophosphamide 50 mg/kg/day × 4. GVHD prophylaxis: tacrolimus hydrate, and short-term methotrexate 7.5 mg/m^2/dose × 3. **Clinical course**: (Case 1) Walking disability and visual loss had progressed before CBSCT. However, in two years' follow-up, slight improvement was recognized in walking, tremor and vision. (Case 2) Neurological symptom became worth just after BMT. However, symptoms have been stable thereafter. **Discussion**: We experienced a rapid progression of their symptoms, just after HSCT. However, in two years' follow-up after HSCT, spasticity, tremor, walking and vision are slowly improving and CSF protein is also decreasing. HSCT is applicable to late-onset Krabbe disease, if early diagnosis is possible.

O-3-3

CORRECTION OF THE ALPHA-GALACTOSIDASE A DEFICIENCY AND REDUCTION OF GLYCOLIPID STORAGE IN FABRY MICE RECEIVING TRANSDUCED BONE MARROW MESENCHYMAL STEM CELLS

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Fabry disease is an X-linked metabolic disorder caused by a deficiency of alpha-galactosidase A (α-Gal A). This enzyme deficiency leads to impaired catabolism of alpha-galactosyl-terminal lipids such as globotriaosylceramide (Gb3). Patients develop vascular occlusions that cause cardiovascular, cerebrovascular, and renal disease. Although bone marrow transplantation have shown promise in the Fabry mice, it is difficult to be applied to clinical trials because of the low efficiency of retrovirus-mediated gene transfer into human bone marrow cells. In this study, we investigate the feasibility of genetic correction in the Fabry mice through transplantation of α-Gal A genetically engineered bone marrow mesenchymal stem cells (MSCs). Wild-type mouse bone marrow MSCs cell line was transduced with a retrovirus encoding α-Gal A and intraperitoneally transplanted into Fabry mice once per week. Multiple intraperitoneal transplantations of transduced MSCs resulted in extended correction of the α-Gal A deficiency and reduction of Gb3 in some organs at different time points. Although the transplanted MSCs have survived at least for 2 weeks, substantial improvements in the survival and differentiated state of the transduced MSCs should be necessary before this approach may have utility to treat Fabry disease.

O-3-4

INTRACEREBRAL AAV5-MEDIATED GENE TRANSFER IN METACHROMATIC LEUKODYSTROPHY (MLD): PRECLINICAL DEMONSTRATION OF EFFICACY AND TOLERANCE IN MOUSE MODEL AND PRIMATE

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Metachromatic leukodystrophy (MLD) is a severe lysosomal storage disorder caused by arylsulfatase A (ARSA) enzyme deficiency that results in the accumulation of sulfatides in neurons and glia, progressive CNS and PNS demyelination and neuronal damage. The most frequent (50%) and severe form of MLD is the late infantile form. Enzyme replacement therapy may prove to correct PNS demyelination, but there is no efficient therapy to halt or reverse CNS demyelination and neuronal loss. Hematopoietic cell transplantation has limited effects only in the juvenile forms of MLD.

In MLD mice, we showed that intracerebral injections of AAV5 vector encoding ARSA resulted in widespread diffusion of the enzyme in the brain, and in the prevention of sulfatide storage, neuropathological abnormalities (Purkinje cell loss, astrogliosis and microglia activation), and motor behavioral abnormalities. Treatment of MLD after the onset of symptoms showed nearly similar capacity of AAV5-ARSA vector to correct all CNS parameters of the disease.

Towards clinical application in MLD patients, we have scaled-up our AAV-mediated gene transfer strategy in primate to determine if a limited number of brain injections is not toxic and allows diffusion and stable expression of the recombinant ARSA enzyme that would be compatible with clinical benefits in patients. Preliminary results show strong expression and activity of ARSA, diffusion at distance from injection sites and confirm the lack of toxicity of this approach, These encouraging data support the use of intracerebral injections of AAV5-ARSA vectors to cure the rapidly progressive and devastating infantile forms of MLD.

O-3-5

COMBINATION BRAIN AND SYSTEMIC INJECTIONS OF AAV RESULTS IN WHOLE BODY THERAPY AND EXTENSION OF LIFESPAN IN THE NIEMANN-PICK MOUSE

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The majority of lysosomal storage diseases contain both CNS and visceral pathology. Many experimental designs use either intracranial injection of viral vectors to treat the neurodegenerative phenotype of the disease, or systemic delivery to treat the viscera. In this study, we tested whether a combination of brain and systemic injections of adeno-associated virus (AAV) vectors could increase lifespan, provide whole body reversal of pathology, and improve motor and cognitive function in the acid sphingomyelinase knock out (ASMKO) mouse model of Niemann-Pick disease. ASMKO mice at 4 weeks of age were injected systemically with AAV8-hASM, and subsequently, the same animals were injected into the brain with AAV2-hASM. All the mice treated by combination injections survived to 54 weeks of age. This was a significant improvement over untreated ASMKO mice, animals that had been treated by systemic injection only or by brain injection only, which had median life spans of 34 weeks, 45 weeks, and 43 weeks, respectively. Thus the brain alone injections did not protect animals from dying. Animals treated by the combination therapy also displayed normal weight gain, and significant functional recovery on the rotarod (motor task) and the Barnes maze (cognitive task) throughout the time course of the study. These data support the contention that combination therapy can improve the quality of life before and within the time frame of extended survival.

O-4-1

UPDATING THE EAST ASIAN mtDNA PHYLOGENY: A PREREQUISITE FOR THE IDENTIFICATION OF PATHOGENIC MUTATIONS

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Knowledge about the world phylogeny of human mitochondrial DNA (mtDNA) is essential not only for evaluating the pathogenic role of specific mtDNA mutations but also for performing reliable association studies between mtDNA haplogroups and complex disorders. In the past few years, the main features of the East Asian portion of the mtDNA phylogeny have been determined based on complete sequencing efforts, but representatives of several basal lineages were still lacking. Moreover, some recently published complete mtDNA sequences did apparently not fit into the known phylogenetic tree and conflicted with the established nomenclature. To refine the East Asian mtDNA tree and resolve data conflicts, we first completely sequenced 20 carefully selected mtDNAs – likely representatives of novel sub-haplogroups – and then, in order to distinguish diagnostic mutations of novel haplogroups from private variants, we applied a 'motif-search' procedure to a large sample collection. The novel information was incorporated into an updated East Asian mtDNA tree encompassing more than 1000 (near-) complete mtDNA sequences. A reassessment of the mtDNA data from a series of disease studies testified to the usefulness of such a refined mtDNA tree in evaluating the pathogenicity of mtDNA mutations. In particular, the claimed pathogenic role of mutations G3316A, T3394C, A4833G, and G15497A appears to be most questionable as those initial claims were derived from anecdotal findings rather than association studies. Following a guideline based on the phylogenetic knowledge as proposed here could help avoiding similar problems in the future.

O-4-2

MITOCHONDRIAL RESEQUENCING MICROARRAY ANALYSES OF FIRST 100 PATIENTS WITH SUSPECTED OXPHOS DISORDERS

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OXPHOS disorders are present in 1 per 10 000 live births and are classified under the generic term 'mitochondrial disorders'. These disorders are genetically complicated due to maternal inheritance of mitochondrial DNA as well as biparental inheritance of nuclear DNA. It is believed that approximately 50% of such patients have defects in the mitochondrial genome. We used a mitochondrial DNA resequencing microarray to sequence the entire 16.5 kb mitochondrial genome including all 37 genes for patients with suspected OXPHOS disorders to identify mutations. Results of our first 100 unselected patients submitted to us for this analysis are presented. These patients' clinical findings were variable and included exercise intolerance, vision loss, and signs of encephalomyopathy and myopathy. We have detected eight nucleotide changes that are either known or possible mutations in seven patients. Numerous reported or unreported polymorphisms were detected in all patients. Four patients had known mutations, MELAS A3243G, LHON G11778A, LHON A4917G and T5814C. Three additional patients had previously unreported heteroplasmic nucleotide changes in their tRNA gene(s); A10018G, T4363C, and one patient with T10035C as well as C15925G. Detailed family studies are ongoing to determine whether they represent mutations or polymorphisms. Mitochondrial DNA microarray analysis is a powerful mutation detection method that is rapid, reliable and sensitive and may be considered in those patients with a suspected OXPHOS disorder before performing a muscle biopsy.

O-4-3

TWO NOVEL TWISTS IN MITOCHONDRIAL DYSTONIA

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In clinical practice mitochondrial disease is seldom considered until a variable combination of seizures, alteration in tone, muscle weakness and developmental problems is evident. Rarely, one symptom occurs in isolation and predominates the clinical phenotype. We report two families where dystonia was the principal feature and other clues to a mitochondrial aetiology were scarce or misleading. Two distinct mitochondrial pathologies were eventually identified: a novel homoplasmic *MTTC* mutation and a primary Leber's hereditary optic neuropathy (LHON) mutation. The 'mild' nature of both mutations has permitted very high levels of mutated mitochondrial DNA (mtDNA) to accumulate. Patients with the *MTTC* mutation have no wild type mtDNA detectable and although the LHON mutation is heteroplasmic (wild-type and mutated mtDNA co-exist) in the patients we report, it is commonly observed to be homoplasmic (no wild-type detectable). The mitochondrial aetiology identified in these patients emphasizes the pathological potential of homoplasmic mutations and has important implications for the investigation and genetic counselling of families where dystonia is the principal clinical feature. Pursuit of a mitochondrial cause for dystonia in patients with homoplasmic mutations may be challenging, but primary LHON mutations are easily identified and should be considered early in the course of clinical investigation for dystonia.

O-4-4

THE PREVALENCE OF PATHOGENIC MITOCHONDRIAL DNA MUTATIONS IN ADULTS

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Since the first description of pathogenic mutations of mitochondrial DNA (mtDNA) in 1988, it has become apparent that defects of this genome are an important cause of neurological disease. Prevalence figures for these chronic debilitating diseases are, for different reasons, directly relevant to clinicians, healthcare economists and biologists. However, accurate prevalence estimates have been hampered by the clinical and genetic heterogeneity associated with mtDNA diseases. In 2000 we published prevalence data based on diagnoses current in 1997. Since then, diagnostic techniques, family tracing and clinical awareness of mitochondrial disease have improved to an extent that we believed it was appropriate to re-evaluate these data.
Between 1990 and 2003, adults in the North East of England with suspected mitochondrial disease were referred to a single investigation unit. Pedigree analysis was performed in all cases where a pathogenic mtDNA mutation was confirmed. Data was collated for the mid-year period of 2001 and the minimum point prevalence of mtDNA disease was calculated for adults of working age (>16 and $<60/65$ for female/male patients respectively). Our results demonstrate a significant increase in prevalence over those previously reported, with clinically evident mitochondrial disease in 10/100 000 of the population. A further 19.3/100 000 adults below retirement age are at risk of developing mtDNA disease through their first-degree relationship with a clinically affected individual harbouring an inheritable mtDNA mutation. These figures reveal that mitochondrial disease has a direct impact on the lives of 29.3/100 000 of the population studied and is one of the commonest inherited neuromuscular disorders.

O-5-1

CLINICAL AND GENETIC STUDIES ON 228 CHINESE PATIENTS WITH MITOCHONDRIAL ENCEPHALOPATHIES

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Mitochondrial encephalopathies in children are clinically and genetically heterogeneous group of disorders caused by mutations in nuclear or mitochondrial gene. To investigate the clinical and genetic characteristics of mitochondrial encephalopathies in Chinese patients, 228 cases that were hospitalized from 1992 to 2005 came from Mainland China were reviewed. The diagnosis was based on both the clinical presentation, radiological features of cranial computed tomography scan or magnetic resonance imaging and neuropathologic findings. The patients had various clinical forms of metabolic encephalopathies. In 152 (66.7%) patients with Leigh syndrome, A8344G, A3243G, T8993G and T8993C mutations on mitochondrial gene were detected in 6 patients. SURF1 mutations associated with cytochrome c oxidase deficiency were identified in 25 patients (10.7%). Only 1 (%) patient had 214C>T mutation on pyruvate dehydrogenase E1 α subunit gene. Among 17 (7.5%) patients who presented with mitochondrial myopathy, encephalopathy, lactacidosis and stroke-like episodes (MELAS), 12 had A3243G mutation on mitochondrial gene. In 59 (25.9%) cases with other types of mitochondrial encephalopathies, A8344G mutation on mitochondrial gene was identified in 3 cases with myoclonus epilepsy associated with ragged-red fibers (MERRF) syndrome, T3271G. was found in 1 patient. The genotypes of 180 (78.9%) patients remain unknown. Therefore, the genetic study on mitochondrial encephalopathies in Chinese patients represents a persistent challenge to clinicians.

O-5-2

PYRUVATE DEHYDROGENASE DEFICIENCY DUE TO A MISSENSE MUTATION OF THE E1-BETA SUBUNIT (*PDHB* 106T>C, R36C)

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Pyruvate dehydrogenase deficiency is a major cause of primary lactic acidosis. Most cases result from mutations of the gene for the E1-alpha subunit (*PDHA1*), with fewer cases resulting from mutations in genes for E3, E3-binding protein, E2, and the E1-beta subunit (*PDHB*). We have found one *PDHB* mutation among 65 analyzed cases of PDC deficiency, which is the third reported case with a *PDHB* mutation. This male was born to consanguineous parents, with IUGR, absence of the corpus callosum, severe neonatal lactic acidosis (8–22 mM), and normal to high lactate/pyruvate ratio (14–33). A brother died in the first day of life with severe lactic acidosis and absence of the corpus callosum. Hospitalization at age 6 months resulted in initiation of a ketogenic diet (fat 80% of energy) with thiamine (25 mg/day) and carnitine (100 mg/kg/day). At age 5 years, he is short with severe microcephaly, nystagmus, truncal hypotonia, is unable to stand, and speaks few words. He has no more decompensations. PDC activity in fibroblasts was 9% of controls, with low E1 and normal E2 and E3 activities. No mutation was detected within *PDHA1* (cDNA and 11 exons). A homozygous 106T>C mutation was found in *PDHB*, with the predicted amino acid change R36C. This residue is at the beginning of the mature peptide and is highly conserved across species, but is not homologous to other alpha-keto acid dehydrogenases. The rare cases of E1-beta deficiency are similar to E1-alpha deficiency and may only be distinguished by autosomal inheritance and mutational analysis.

O-5-3

ACUTE FLACCID PARALYSIS AS INITIAL SYMPTOM IN 4 PATIENTS WITH NOVEL E1α MUTATIONS OF THE PYRUVATE-DEHYDROGENASE COMPLEX

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We report on 4 boys from 3 families presenting initially in infancy with an acute onset of flaccid tetraparesis and areflexia, resembling Guillain-Barré syndrome (GBS). However cerebrospinal fluid (CSF) protein was normal, while serum and CSF lactate were elevated. All patients had recurrent similar episodes, usually associated with infections. Brain MRI showed T2 hyperintensities in the basal ganglia in two boys, in one of them at the first clinical presentation, the other one had a normal brain MRI during the first episode. A third boy had a normal MRI twice but an increased lactate peak in the basal ganglia in 1H-MR spectroscopy. Nerve conduction velocities (NCV) were normal in all patients, nerval and muscle action potentials were reduced and denervation could be detected by EMG partially. Biochemical analysis of muscle tissue, performed in two patients, revealed a deficiency of the pyruvate dehydrogenase (PDH). Molecular genetic analysis of the X-chromosomal E1α subunit of PDH showed three new mutations in phylogenetical conserved areas of the protein: Glu358Lys in patient 1, Arg88Lys in patient 2 and 3 (brothers) and Leu216Ser in patient 4.

In conclusion, children with 'atypical GBS' should be evaluated for a mitochondrial disorder, including pyruvate dehydrogenase deficiency, even after a first episode.

O-5-4

METHYLMALONIC ENCEPHALOMYOPATHY IS CAUSED BY MUTATIONS IN THE *SUCLA2* GENE AND HAS A HIGH INCIDENCE IN THE FAROE ISLANDS DUE TO A FOUNDER EFFECT

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We have identified 12 patients with a novel autosomal recessive disorder that we have named methylmalonic encephalomyopathy. The disorder has a high incidence of 1 in 1700 in the Faroe Islands, and a carrier frequency of 1 in 33. The symptoms comprised severe hypotonia, muscle atrophy, dystonia, deafness and reduced life span. Urine and plasma methylmalonic acid (MMA) were elevated. CT/MRI showed demyelination, atrophy and basal ganglia affection, and some patients fulfilled the criteria for Leigh syndrome. Because of the high incidence, we assumed that the disorder is caused by a founder effect. We performed a genomewide search for homozygosity with the Affymetrix 10K SNP array. The analysis revealed a homozygous region on chromosome 13q14, harbouring ten genes, among these *SUCLA2*, which encodes a β subunit of the Krebs cycle enzyme succinyl-CoA ligase. Mutation analysis showed a novel splice site mutation in *SUCLA2*. The elevated MMA can be explained by an accumulation of succinyl-CoA, which affects the metabolism of MMA. We suggest that MMA is a marker of the disorder, which is supported by the fact that we have ascertained a patient with another *SUCLA2* mutation through elevated MMA.

O-6-1

EARLY SIGNS OF BENEFIT IN CHILDREN WITH LATE-ONSET POMPE DISEASE FOLLOWING TREATMENT WITH ENZYME REPLACEMENT THERAPY (rhGAA) IN AN OPEN-LABEL STUDY

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Introduction: Pompe disease is a rare metabolic myopathy caused by a deficiency of the intra-lysosomal enzyme acid alpha-glucosidase (GAA) leading to a clinical spectrum of disease with onset from early infancy to late adulthood. The benefits of recombinant human acid alpha glucosidase (rhGAA) on cardiac, respiratory and skeletal muscle have been demonstrated in clinical studies evaluating Pompe patients with onset of disease in early infancy. **Methods**: The safety and efficacy of rhGAA 20 mg/kg qow i.v. was evaluated in 5 (3 male/2 female) Caucasian patients (aged 5–15 years at treatment onset) with a confirmed diagnosis of Pompe disease in an open-label study. At Baseline, all patients were ambulatory and one required nocturnal non-invasive ventilation. **Results**: Following the first 6 months of treatment, a clinically meaningful (>11%) increase in % predicted forced vital capacity (FVC) compared to baseline was observed in 3 patients (2 had FVC <80% predicted at Baseline) as was a clinically meaningful increase (>37 metres) in the distance walked in 6 min (conducted at fast speed) in 3 patients. rhGAA was well tolerated by all patients and no infusion-associated reactions were observed. Four patients developed anti-rhGAA IgG antibodies (titers ranging from 1/100 to 1/800) between weeks 8 and 16 with no signs of an (*in-vitro*) inhibitory antibody effect. **Conclusion**: Administration of rhGAA to patients with later onset of symptoms of Pompe disease was well tolerated and led to early signs of benefit on skeletal and respiratory muscle function in the first 6 months of treatment.

O-6-2

EVIDENCE OF CLINICAL BENEFIT FOLLOWING ENZYME REPLACEMENT THERAPY (ERT) WITH rhGAA IN CHILDREN WITH POMPE DISEASE

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Pompe is a neuromuscular disease due to a deficiency of the lysosomal enzyme acid alpha glucosidase (GAA). Results from two ERT trials in 39 children treated with rhGAA are discussed. Study 1 enrolled 18 patients <6 months of age; Study 2 enrolled 21 patients >6–36 months of age. After 1 year of ERT with rhGAA 18/18 (100%) patients in Study 1 were alive and 15/18 patients (83%) were free of invasive-ventilator support. In Study 2, 16/21 patients (76%) were alive and 10/16 (62%) patients who were free of invasive ventilation at baseline, remained so. In Study 1, 13/15 patients (72%) acquired new motor milestones, in contrast to 10/21 patients (48%) of those treated in Study 2. Thirty five out of 39 patients (90%) developed anti-rhGAA antibodies. In general, administration of rhGAA to this large cohort of children resulted in measurable clinical benefit, even in those patients with advanced stage of the disease at onset of ERT (Study 2).

O-6-3

GAUCHER DISEASE AND ENZYME REPLACEMENT THERAPY, IRANIAN EXPERIENCE

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We reported the clinical and laboratory findings as well as the effects of enzyme replacement therapy (ERT) in 18 Iranian patients with Gaucher disease (GD). The main clinical signs were hepatosplenomegaly (100%), thrombocytopenia (31%), bone involvement (12.5%) and abnormal eye movement (6.25%). Neurological abnormalities including psychomotor delay were observed in 9 cases. Splenectomy were carried out in 2 patients. Five patients (type 1:1, type 3a:1, type 3b:3) have been treated with ERT at 60 U/kg/dose every 2 weeks. Hepatosplenomegaly are being reversed to normal size. The neurodevelopment in all patients with type 3b were significantly improved. The slow improvement of growth failure has been seen. The patient with type 3a showed the improvement in general conditions, however, no improvement of signs from upper neuron disease has been observed.

O-6-4

ENZYME REPLACEMENT THERAPY IN A MOUSE MODEL OF GLOBOID CELL LEUKODYSTROPHY

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Globoid cell leukodystrophy (GLD), also known as Krabbe's disease, is a devastating, degenerative neurological disorder. It is inherited as an autosomal recessive trait caused by loss-of-function mutations in the galactosylcerebrosidase (GALC) gene. Previously, we have shown that peripheral injection of recombinant GALC, administered every other day, results in a substantial improvement in early clinical phenotype in the twitcher mouse model of GLD. While we did detect active enzyme in the brain following peripheral administration, most of the administered enzyme was localized to the periphery. Given the substantial CNS involvement in this disease, we were interested in determining whether or not a single dose administration of the recombinant enzyme directly to the CNS would result in any substantial improvement. Following intracerebroventricular (icv) administration of GALC we noted a significant, 16.5%, reduction in the GALC substrate psychosine, which is believed to play a pivotal role in the CNS pathology observed in this disease. Moreover, GALC was found not only in periventricular regions but also at sites distant to the injection such as the cerebral cortex and cerebellum. Most importantly, animals receiving a single icv dose of the enzyme at postnatal day 20 survived to an average of 52 days which compares favorably to the control twitcher animals, which normally only live to postnatal day 42. These results indicate that even a single dose administration of the recombinant enzyme can have significant clinical impact and suggests that other lysosomal storage disorders with significant CNS involvement may similarly benefit.

O-7-1

EFFECT OF AGALSIDASE ALFA ON PAIN IN ADOLESCENTS WITH FABRY DISEASE: DATA FROM FOS – THE FABRY OUTCOME SURVEY

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Pain is an early symptom of Fabry disease (FD) and has a significant impact on quality of life. This study set out to quantify the extent of pain in adolescents (aged 10–20 years; $n = 69$) enrolled in FOS. Mean scores at baseline on the Brief Pain Inventory (BPI) were 2.2 ± 2.6 for pain on average and 2.6 ± 3.1 for pain at its worst, and 34 out of the 69 patients were receiving pain medication. Not surprisingly, those patients who were started on enzyme replacement therapy (ERT) with agalsidase alfa were more severely affected than those who were not treated; BPI scores for pain on average were 3.0 ± 2.8 and 1.5 ± 2.2, respectively. Similarly, BPI scores for pain at its worst were 3.8 ± 3.4 in treated and 1.7 ± 2.5 in non-treated patients. Preliminary data on 21 patients treated with agalsidase alfa for 12 months indicate a beneficial effect of ERT, with scores for pain at its worst and pain on average decreasing by 0.8 ± 0.8 and 0.5 ± 0.6, respectively. These data illustrate the burden of pain in adolescents with FD and suggest an improvement after early ERT with agalsidase alfa.

O-7-2

MANAGEMENT OF GLOBOTRIAOSYLCERAMIDE IN URINE FOR LONG TERM MONITORING OF FABRY PATIENTS TREATED WITH ENZYME REPLACEMENT THERAPY

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Background: Fabry disease is a X-linked deficiency of lysosomal alfa-galactosidase A (α-GalA) and results in the progressive accumulation of globotriaosylceramide (GL-3) in various organs leading to renal and cardiac failure and/or stroke eventually. Enzyme replacement therapy (ERT) with recombinant human α-GalA (rh-α-GalA) has been shown to reduce GL-3 deposition in kidney and alleviate clinical symptoms in some patients. We present the efficacy of long-term ERT in Fabry patients assessed by monitoring renal function and urinary GL-3 concentration. **Materials and Methods**: Patients were administered Fabrazyme 1 mg/kg biweekly for one to three years. GL-3 was measured periodically in random urine or 24 h urine sample from twelve Fabry hemizygotes and four symptomatic heterozygotes using tandem mass spectrometry. IgG antibodies against rh-α-GalA were measured by ELISA specific for rh-α-GalA. **Results**: In five hemizygotes who were negative for IgG antibodies, urinary GL-3 was elevated at baseline and fell impressively and stayed low level. In two hemizygotes, there was a transient increase in GL-3 concentrations to baseline levels in association with the presence of antibodies after several months of ERT. Antibodies have been presence in high level since early phase of ERT in five males and GL-3 was reduced gradually. As we predicted, urinary GL-3 was less pronounced and IgG antibodies were negative during ERT in heterozygotes. **Conclusion**: In Fabry patients on ERT, change of urinary GL-3 seems to be related to presence of antibodies against rh-GalA. It will be needed further study if urinary GL-3 is good marker for efficacy of ERT.

O-7-3

ENZYME REPLACEMENT THERAPY IN FABRY DISEASE SHOULD BE STARTED IN PATIENTS WITH MICROALBUMINURIA

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Background: After the initiation of enzyme replacement therapy (ERT) for Fabry disease in 2000, patients with renal function below a certain point would still deteriorate in renal function regardless of ERT. In our previous study, the strikingly diverse response to the ERT, which correlated with serum, creatinine (Cr) levels and estimated GFR at the initial treatment of ERT was noted. **Purpose of study**: To evaluate the time point of starting ERT for preserve renal function. **Patients' population**: Four cases with isolated renal involvement of Fabry disease (renal variant) from 2 different families due to lack of acroparaesthesia, hypohidrosis, corneal dystrophy, lenticular opacity, angiokeratoma, hypertrophic cardiomyopathy, arrhythmia and cerebral insult. During a routine familial enzyme check, their serum creatinine levels were within normal range. Renal biopsy tissue showed the pathologic findings of Fabry disease. Decreased lymphocyte α-galactosidase A activity (3.0 nmol/h/mg protein, normal range 33.3–61.0 nmol/h/mg protein) with identified point mutation (R112H) in α-GalA gene further confirmed the diagnosis of Fabry disease in a family. **Methods**: Intravenous injection of recombinant human α-galactosidase (agalsidase beta, Fabrazyme®) 1 mg/kg/14 days was started. **Results**: After treatment with ERT for 2 years, the GFR was presented with normal renal function. No specific complaint or adverse effect was observed during infusion of recombinant human α-galactosidase. **Conclusion**: Early detection of patients with renal variant Fabry disease could be achieved and they could be treated before the point of no return.

O-8-1

UREA CYCLE DISORDERS IN INDIAN POPULATION

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Introduction: We present our experience at a referral centre in New Delhi, India. **Method**: The diagnosis was based on Tandem Mass Spectrometry, quantitative amino acid analysis (QAA) and molecular mutation analysis in a few. **Results**: We screened 839 symptomatic children from 2002 till date and 12 (1.43%) were diagnosed to have a urea cycle disorder. Six had citrullinemia, one citrin deficiency, two argininosuccinicaciduria deficiency (ASL),and three ornithine transcarbamylase, (OTC) deficiency. Mutations were identified in one case of citrullinemia and ASL deficiency. Prenatal diagnosis was done in two cases, ASL deficiency and citrullinemia. We did carrier screening in one case of OTC deficiency. **Conclusions**: There are few reports from the Indian subcontinent of urea cycle defects. Availability of technology in India has increased diagnosis of these disorders.

O-8-2

LONG TERM PROGNOSIS OF 18 CASES OF UREA CYCLE DISORDERS; RELATIONSHIP BETWEEN SURVIVAL RATE AND TREATMENTS

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In spite of recent advances in the diagnosis and treatment of congenital urea cycle disorders, the survival rate is still poor. As part of continuing efforts for a better prognosis of congenital urea cycle disorders, we evaluated the survival and neurological outcomes in 18 patients with these disorders in our hospital. Among the 18 patients, eight remain alive but ten have died. All but four neonatal onset cases died during winter.

Common infectious diseases including upper respiratory infections affect the frequency of hyperammonemia attacks. The availability of commercial-based arginine and partial living liver transplantation improved the patients' survival rate. Despite the progress of the hemodiafiltration technology, two patients died during CHDF treatment. One was a neonatal onset CPT-I case and the other was an infantile onset female case of OTCD who had survived to 9 years old. Neurological outcome of the late onset type in these disorders does not necessarily guarantee a better IQ than those of infantile onset cases. Collaboration of ICU physician, metabologist and dietician is essential in the management of congenital urea cycle disorders.

O-8-3

MENTAL RETARDATION WITH ARGININOSUCCINATE LYASE DEFICIENCY

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Argininosuccinate lyase deficiency (ASLD) is an autosomal recessive disorder affecting the urea cycle with substantial clinical and genetic heterogeneity. Most patients with the late onset form (or subacute form) developed mental retardation in childhood, although blood ammonia levels were well controlled by medication and nutritional manipulation. The biochemical basis of the neurological complication is still unclear. We therefore investigated the clinical course, enzyme activity, and mutation analysis in two ASLD patients without any hyperammonemic episodes. The patients were investigated for ASLD because of abnormal results in neonatal mass screening and liver function test. Plasma citrulline and argininosuccinic acid (ASA) were elevated and argininosuccinate lyase activity was extremely low in both patients (less than 3% of control). DNA analysis of ASL gene revealed compound heterozygous mutations (R146W/R168H) and a homozygous mutation of stop codon (X465Y), respectively. Blood ammonia level was mildly elevated in infancy (110–270 μg/dl), but well controlled by protein restriction, L-arginine and sodium benzoate (less than 70 μg/dl) after that. Nevertheless, mental retardation occurred and progressed gradually in childhood (IQ; 55 and 39, respectively). Epilepsy and other neurological abnormalities were not observed. L-arginine is universally used for ammonia detoxification in ASLD, however it leads to increase excretion of ASA in body fluid. We speculate that elevation of ASA and imbalance of various amino acids in CNS might disturb the function of neurotransmitter and/or normal development of neuron. Monitoring of ASA in blood and introduction of alternative pathway therapy (ammonia disposal via non-ASA) in early stage should be considered to minimize the effect of intermediary metabolites.

O-8-4

POTASSIUM RETENTION IN PATIENTS TREATED FOR ARGININOSUCCINATE LYASE DEFICIENCY

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Hypokalemia has been observed in patients with argininosuccinate lyase deficiency treated with sodium phenylbutyrate (SPB) or sodium benzoate (SB) to enhance nitrogen excretion. We hypothesized that these bulky anions decreased potassium (K+) reabsorption in the distal renal tubule. Three patients volunteered for balance studies in a clinical research center using a crossover study (on/off drugs preceded by washout period). Patients 1 and 2 received SPB (313 and 478 mg/kg/day, respectively) and patient 3 received SB (324 mg/kg/day). Plasma, 24-urine and stool, and food samples were analyzed daily for K^+ and Na^+ using flame photometry. Percent K^+ renal tubule reabsorption was normal while on/off drug in patients taking SPB (85.21 ± 0.83–96.89 ± 0.55%), but was reduced in patient taking SB. In this patient, K^+ reabsorption increased from 62.8 ± 1.72% to 81.90 ± 0.82% ($p = 0.005$) when SB was removed. K^+ supplementation failed to ameliorate SB inhibition as renal tubular K^+ reabsorption fell to72.70 ± 0.19%. Na^+ reabsorption remained normal in all patients on/off drugs (96.75 ± 4.44–99.83 ± 0.08%). Mean plasma K^+ concentrations on/off drug in patients on SPB were normal (3.6 ± 0.3–4.5 ± 0.7 mmol/L). In patient on SB, mean plasma K^+ concentrations normalized when off drug (2.6→3.3 mmol/L, $p = 0.007$) and while on drug and K^+ supplement (2.6→4.2 mmol/L, $p = 0.017$). Mean plasma aldosterone and Na^+ concentrations did not differ while on/off drugs. In conclusion, SB decreases K^+ reabsorption in distal renal tubule. K^+ supplementation normalizes plasma K^+ concentrations without affecting renal K^+ malabsorption in patients receiving SB.

O-8-5

LONG-TERM NEUROLOGIC FOLLOW UP OF HHH SYNDROME IN 4 CASES

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Hyperammonemia–hyperornithinemia–homocitrullinemia (HHH) syndrome is an autosomal recessive disorder where impairment of ornithine transport across the mitochondrial membrane causes accumulation of ornithine in the cytoplasm. The resulting ornithine deficiency in the mitochondria leads to reduced clearance of ammonia through the urea cycle. HHH syndrome has been treated with protein restriction since1969 when the first case was reported. However, there has not been any long-term follow up reported.

The four cases included in this study were followed up for 11 to 40 years. Diagnosis was made by plasma and urine amino acid analysis using ion exchange chromatography, cultured fibroblast ^{14}C ornithine incorporation assay. All four cases were confirmed by finding reduced ^{14}C ornithine incorporation of 0.023–0.062 (N: 0.595 ± 0.104). In all cases, ammonia was controlled by protein restriction. The neurologic outcome included memory loss, low IQ, spasticity of extremities and abnormal gait for all cases. Imaging study revealed subcortical, cerebral and cerebellar atrophy sparing basal ganglia. Individual clinical examination showed pyramidal signs, cerebellar signs, paraplegia, movement disorder, dystonia, and epilepsy. One case had 3 pregnancies – one with intrauterine growth retardation and the other two with normal outcomes. All four HHH cases showed progressive serious neurologic outcome despite control of hyperammonemia.

O-9-1

ISOLATED INCREASE OF TRISIALOTRANSFERRIN IN THE SERUM OF CDG PATIENTS WITH EVIDENCE FOR A DEFECT IN DEMANNOSYLATION

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Congenital disorders of glycosylation (CDG) are genetic diseases caused by defects in biosynthesis of the glycan moiety of glycoconjugates. N-glycosylation defects are screened for by isoelectrofocusing of serum transferrin (Tf IEF) or capillary zone electrophoresis (CZE) of total serum. Two major patterns are distinguished: type 1 with increased asialo- and disialotransferrin in patients with a defect in assembly of the glycan (CDG-I) and type 2 with variable increases in a-, mono-, di- and trisialotransferrin in patients with a defect in processing of the N-glycan (CDG-II).

The group of patients with an aberrant pattern on Tf IEF or CZE without knowledge of the primary defect is rapidly expanding (CDG-x). Structural glycan analysis of serum glycoproteins in case of CDG-IIx can point to defects in branching, demannosylation, galactosylation, sialylation and fucosylation. In some patients more than one defect is detected.

We compared the serum transferrin glycan structures with the patterns on Tf IEF and CZE in 18 patients with CDG-IIx and tried to identify groups with similar Tf pattern. We found that the 4 patients with structural evidence for a demannosylation defect had the same pattern characterized by high trisialotransferrin (27–35%; normal range 1.3–8.7%) and normal a-, mono- and disialotransferrin. Among these 4 patients, 3 had mild psychomotor retardation and dysmorphy and 1 had isolated liver disease.

O-9-2

CLINICAL FEATURES AND LINKAGE ANALYSIS IN A LARGE CONSANGUINEOUS TURKISH PEDIGREE WITH CDG TYPE IK

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Objective: Identification of a candidate gene in a large consanguineous Turkish pedigree with CDG Ix and phenotypic characterisation of affected individuals. **Methods**: A CDG type I pattern on isoelectric focusing of transferrin was identified in 5 individuals (2 m/3 f; 3–22 years; 1 deceased) in a consanguineous family of East-Turkish origin. CDG types Ia-Ii have been excluded by enzyme and/or mutation analysis respectively. A genome-wide SNP scan strategy was employed with the Affymetrix GeneChip 10K 2.0 in order to identify putative disease loci. **Results**: Common symptoms include psychomotor retardation (5/5), variable mild dysmorphic facial features (5/5), alternating strabismus (4/5), and febrile seizures (4/5). Less common symptoms include intractable seizures (2/5), muscle hypotonia (2/5), reduced deep tendon reflexes (2/5), cerebellar signs (2/5), failure to thrive (1/5) and gastrointestinal symptoms (1/5). One affected boy suffered from recurrent haemorrhages and died at the age of seven secondary to Budd Chiari Syndrome and stroke like episodes. A genome-wide SNP scan demonstrated a LOD score of 5.5 between SNP_A-1516465 and SNP_A-1517315 on chromosome 16p13.3. Within this region is the locus of the ALG1 gene, which was recently identified to be the underlying cause for CDG type Ik. ALG1 mutation analysis in our family is still ongoing. **Conclusions**: The intra-familial clinical phenotype of CDG type Ik in this large Turkish family is highly variable and associated with signifcant morbidity and mortality. Linkage to the ALG1 locus was found.

O-9-3

OVEREXPRESSION OF GAMT RESTORES GAMT ACTIVITY IN PRIMARY GAMT-DEFICIENT FIBROBLASTS

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Guanidinoacetate methyltransferase deficiency (GAMT; MIM 601240) is an autosomal recessive disorder of creatine biosynthesis. The clinical hallmarks are mental retardation and epilepsy; laboratory hallmarks are cerebral creatine deficiency, increased levels of guanidinoacetate, impaired GAMT activity in cultured cells and pathogenic mutations in the *GAMT* gene. Treatment with creatine and ornithine supplementation (combined with arginine restriction) results in clinical improvement and restoration of the cerebral creatine pool ($\approx$70%). So far, there are 27 patients and a total of 16 different mutations reported.

In order to provide final proof that GAMT deficiency is caused by a primary defect of the GAMT protein, we transfected the wild-type GAMT open reading frame (ORF) into primary GAMT-deficient fibroblasts (c.59G>C, p.Trp20Ser), and tested GAMT expression as well as GAMT activity. Wıld-type GAMT ORF was cloned into a pEGFP-N1 expression vector. This construct was then stably transfected into primary GAMT-deficient fibroblasts. Subsequently, the cells were harvested and GAMT assay was performed using stable isotope labeled substrates. Transfection of primary GAMT-deficient fibroblasts with wild-type GAMT results in the restoration of GAMT activity, in contrast to mock transfectants. Moreover, the expression of the GAMT-EGFP fusion protein was analyzed by Western blot, confirming the presence of GAMT fusion protein.

We provide definitive proof that mutations in the *GAMT* gene are responsible for GAMT deficiency. Furthermore, this model will be used for functional analysis of variants of unknown consequence (i.e., missense mutations).

O-9-4

IMPAIRED ISOPRENOID METABOLISM IN SJÖGREN-LARSSON SYNDROME

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Background: Sjögren-Larsson syndrome (SLS) is a rare neurocutaneous disease caused by mutations of the *ALDH3A2* gene coding for microsomal fatty aldehyde dehydrogenase (FALDH). FALDH oxidizes long-chain fatty aldehydes to acids. In SLS, fatty alcohols accumulate and fatty acids are not produced. So far, no single alcohol/aldehyde pathway has emerged as a satisfactory candidate to explain SLS clinical symptoms. **Hypothesis**: Isoprenoid alcohols such as farnesol (FOH) and geranylgeraniol (GGOH) are intracellular mevalonate derivatives formed during isoprenyl-PP metabolism and converted to aldehydes and acids by unidentified microsomal dehydrogenases. Isoprenols are bioactive metabolites regulating cell growth and apoptosis, neuronal Ca^{2+} signaling and skin maturation. We hypothesized that isoprenyl aldehydes were FALDH substrates and isoprenol metabolism was impaired in SLS. **Methods and Results**: Five SLS and 5 control fibroblast cell lines were incubated in DMEM containing either [1-^{14}C]-FOH or [1-^{14}C]-GGOH. Cells and media were analyzed by TLC and autoradioagraphy for the presence of farnesoic acid (FA) and geranylgeranoic acid (GGA). Synthesis of both FA and GGA was reduced by $\sim$80% in SLS cells as compared to controls ($p<0.007$). SLS cells incubated with GGOH further showed diversion of GGOH toward unidentified, more polar metabolites. FA synthesis was also measured in liver microsomes prepared from *aldh3a2*$^{-/-}$ gene knockout and wild-type mice. FALDH-deficient microsomes showed reduced FA synthesis ($\sim$20% of wild-type) and accumulation of both farnesal and unmetabolized FOH. **Conclusion**: FALDH control isoprenol metabolism in rodents and humans, and isoprenoid metabolism is impaired in SLS. Abnormal isoprenoid metabolism may play a role in SLS pathogenesis.

O-9-5

PROGNOSTIC FACTORS INDICATING THE OUTCOME OF GLYCINE ENCEPHALOPATHY: A FOLLOW-UP OF 40 CHILDREN

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Objective: Glycine encephalopathy (GE) is an autosomal recessive error of glycine degradation resulting in severe encephalopathy. An early prediction of the long-term outcome is yet not possible. **Methods**: With regard to the long-term outcome we retrospectively compared the clinical and biochemical aspects of 40 children with a severe neonatal, mild neonatal, severe infantile or mild infantile course of the disease. **Results**: Clinical symptoms at manifestation consisted of the trias muscular hypotonia, seizures and coma. Initial determination of CSF and plasma glycine concentrations were not useful in differentiating mild and severe outcome. 21% of those children presenting during neonatal period and 50% of those presenting in infancy made substantial developmental progress. Several parameters correlate with a poor outcome: early onset of spasticity (88% severe versus 0% mild outcome), frequent hiccuping (95% versus 22%), typical EEG patterns (77% versus 13%), microcephaly (73% versus 33%), congenital and cerebral malformations, mainly corpus callosum hypoplasia (57% versus 0%). Hyperactivity (23% severe versus 100% mild outcome) and choreiform movement disorders (12% versus 89%) are associated with a mild outcome. **Conclusion**: Prediction of the outcome of GE may be facilitated by searching for selected clinical parameters. Furthermore, early neuroimaging may be a valuable tool in prognosticating the outcome of GE.

O-10-1

UNREGULATED INSULIN SECRETION BY PANCREATIC β-CELLS IN HYPERINSULINISM/HYPERAMMONEMIA SYNDROME

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The hyperinsulinism/hyperammonemia (HI/HA) syndrome is caused by 'gain of function' of glutamate dehydrogenase (GDH). Several missense mutations have been found; however, cell behaviors triggered by the excessive GDH activity have not been fully demonstrated. This study was aimed to clarify electrophysiological mechanisms underlying the dysregulated insulin secretion in pancreatic β-cells with GDH mutations. GDH kinetics and insulin secretion were measured in MIN6 cells overexpressing the G446D and L413V. Membrane potentials and channel activity were recorded under the perforated-patch configuration that preserved intracellular environments. In mutant MIN6 cells, sensitivity of GDH to GTP was reduced and insulin secretion at low glucose concentrations was enhanced. The basal GDH activity was elevated in L413V bearing a mutation in the antenna-like structure. The L413V cells were depolarized without glucose, often accompanying by repetitive Ca^{2+} firings. The depolarization was maintained in the presence of ATP and disappeared by depleting ATP, suggesting that the depolarization depended on intracellular ATP. In L413V cells, the K_{ATP} channel was suppressed and the nonselective cation channel (NSCC) was potentiated, while sensitivity of the channels to their specific blockers or agonists was not impaired. These data suggest that the L413V cells increase the intracellular ATP/ADP ratio, which in turn causes sustained depolarization not only by closure of the K_{ATP} channel, but also by opening of the NSCC. The resultant activation of the voltage gated Ca^{2+} channel appears to induce hyperinsulinism. The present study provides evidence that multiple channels cooperate in unregulated insulin secretion in pancreatic β-cells of the HI/HA syndrome

O-10-2

DIAGNOSING STEROID ENZYMATIC DEFECTS PROFILING URINARY STEROIDS BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GCMS)

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This presentation intends to demonstrate that GC-MS profile of 39 urinary steroids has the ability of diagnosing every enzymatic derangement of steroid metabolism, through the presentation of this system in 4 cases of specific steroid enzyme deficiencies. Pt 1 was born as a normal girl, found to have testes in both groins, which were removed, and a provisional diagnosis of androgen insensitivity syndrome was made in infancy. Twenty-five years later, she asked for a more definitive diagnosis of her condition. Pt 2 was born with clitoral enlargement and a urogenital sinus. Pt 3 presented with hypospadias, micropenis, and pubertal gynecomastia. Pt 4 was a 6 years old with gynecomastia, and hyperpigmentation. **Results**: The GC-MS Profiling of Steroids in these patients revealed: Pt 1: decreased ratios of 5α/5β metabolites in the four pairs of 5α/5β isomers, characteristic of 5α-reductase deficiency; Pt 2: increased urinary 17HP, PT, and the pathognomonic PTone (11-Oxo-pregnanetriol), typical for 21-hydroxylase deficiency; Pt 3: increased THB, αTHB and THA, a decrease of THF, αTHF, and THE, and absent An and Et, all characteristic of combined 17OHase/17,20-lyase deficiency; Pt 4: rise in THS, THDOC, and 6-OH-THS and low THF, αTHF, and THE, diagnostic of 11β-hydroxylase deficiency. **Conclusions**: The profile provides an at-glimpse analysis of all steroidal pathways, and the results we show illustrate that GC-MS profile of steroids is a very powerful tool for the diagnosis of steroid enzymatic defects, which represent the more frequent inborn errors of metabolism.

O-10-3

EARLY EMBRYONIC LETHALITY ASSOCIATED WITH TARGETED DISRUPTION OF THE MURINE MEVALONATE KINASE GENE

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Mevalonate kinase (MK; OMIM 251170/260920) catalyzes the first committed step in cholesterol and isoprene synthesis. Human MK mutations associate with diverse disorders, from hyperIgD/periodic fever syndrome to severe mevalonic aciduria. We ablated the murine MK gene employing a gene-trap disrupted ES cell line (Lexicon Genetics, Woodlands, TX, USA). Chimeric mice were generated by ES cell injection into blastocysts from strain C57BL/6, followed by implantation of injected embryos into pseudopregnant females for development to term. Male chimeras showing extensive ES cell-derived agouti coat color were bred with C57BL/6 females for germline transmission and identification of heterozygous $MK^{+/-}$ mice. Sequential $MK^{+/-}$ matings yielded no $MK^{-/-}$ animals; small litter sizes were dominated by $MK^{+/-}$ animals. Genotyping of 12 embryos (gestational day (E)14) revealed only $MK^{+/-}$ mice. Successful targeting was verified by RT-PCR and liver gene-dosage [MK/control enzyme: $MK^{+/+}$ (n = 4), 5.04+1.66 (SD); $MK^{+/-}$ (n = 18), 2.54+1.02; p = 0.001]. Blood chemistries ($MK^{+/-}$ (n = 5) mice) revealed normal cholesterol, elevated SGPT (50–1550; nl <35), SGOT (262–1290; nl <45), phosphorous (13.4–17.7; nl <8.5) and K^+ (12.9–24; nl <5.8), and low creatinine (0–0.2 (nl 0.4–1.0), reminiscent of the leptin-deficient (obese) mouse (OMIM 164160). In addition to body weight regulation, leptin (an adipocyte-derived cytokine) influences hematopoiesis, reproduction, and immune/inflammatory responses. Early embryonic lethality implies a key role for MK in mouse embryogenesis. Murine models in which MK is depleted will be useful to explore the biological role of MK in intracellular signaling related to tumorigenesis, hormone regulation, inflammatory processes, and development.

O-10-4

DYSREGULATION OF THE RAS/MAPK PATHWAY CAUSES COSTELLO SYNDROME AND CARDIO-FACIO-CUTANEOUS (CFC) SYNDROME

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Costello syndrome is a rare, multiple congenital anomaly syndrome characterized by coarse face, mental retardation, cardiomyopathy and predisposition to tumors. The molecular basis of the disease has been unknown. Mutations in tyrosine phosphatase SHP-2 (*PTPN11*) have been identified in approximately 40% of patients with Noonan syndrome [1], which phenotypically overlaps with Costello syndrome. We hypothesized that the causative gene(s) for Costello syndrome and Noonan syndrome without *PTPN11* mutations is a functionally upstream or downstream molecule(s) of SHP-2 in the RAS-RAF-ERK pathway. We sequenced the entire coding regions of the four RAS genes (*K-RAS*, *H-RAS*, *N-RAS* and *E-RAS)* in genomic DNA from 13 individuals with Costello syndrome and 28 individuals with *PTPN11*-negative Noonan syndrome. We identified four heterozygous *de novo* mutations of *H-RAS* (G12V, G12A, G12S and G13D) in 12 of 13 affected individuals, all of which have been previously reported as somatic and 'oncogenic' mutations in various tumors. Fibroblasts established from patients were hypersensitive to growth factor stimulation as compared with control fibroblasts. Only a mutant allele was expressed in the ganglioneuroblastoma tissue surgically isolated from an individual with Costello syndrome despite the biallelic expression in her fibroblasts. Furthermore, we recently reported that oncogenes *KRAS* and *BRAF* were mutated in patients with CFC syndrome, other Noonan-related syndrome characterized by distinctive facial appearance, heart defects, ectodermal abnormalities and mental retardation. Our observations strongly suggest that dysregulation of the RAS-RAF-ERK pathway is a common molecular basis for the three developmental disorders, Noonan, Costello, and CFC syndrome.

O-10-5

ENHANCEMENT OF DRUG DELIVERY TO BONE: CHARACTERIZATION OF HUMAN TISSUE-NONSPECIFIC ALKALINE PHOSPHATASE TAGGED WITH AN ACIDIC OLIGOPEPTIDE

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Hypophosphatasia is caused by deficiency of activity of the tissue-nonspecific alkaline phosphatase (TNSALP), resulting in a defect of bone mineralization. Enzyme replacement therapy (ERT) was attempted but little clinical improvement was achieved.

Attaining clinical effectiveness with ERT for hypophosphatasia requires delivering functional TNSALP enzyme to bone. We tagged the C-terminus-anchorless TNSALP enzyme with an acidic oligopeptide (a six or eight residue stretch of L-Asp), which markedly increased affinity for hydroxyapatite abundant in bone. We compared the biochemical properties of the purified tagged and untagged enzymes derived from Chinese hamster ovary cell lines.

The specific activities of the purified enzymes tagged with the acidic oligopeptide were the same as the untagged enzyme. *In vitro* affinity experiments showed the tagged enzymes had 30-fold higher affinity for hydroxyapatite than the untagged enzyme. Lectin affinity chromatography for carbohydrate structure showed little difference among the three enzymes. Biodistribution pattern from single infusion of the fluorescence-labeled enzymes into mice showed the amount of tagged enzyme retained in bone was 4-fold greater than the untagged enzyme. The tagged enzymes demonstrated longer retention in bone and were present in higher concentration continuously up to one week. *In vitro* mineralization assays with each of three enzymes in the presence of high concentrations of pyrophosphate provided evidence of bone mineralization with the bone marrow from a hypophosphatasia patient.

These results show the anchorless enzymes tagged with an acidic oligopeptide are delivered efficiently and function bioactively in bone mineralization, suggesting the potential use of these tagged enzymes in ERT for hypophosphatasia.

O-11-1

DIET COMPLIANCE IN PATIENTS WITH PHENYLKETONURIA (PKU) – INFLUENCING FACTORS

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Background: The success of the PKU treatment is based on diet compliance. **Objective**: The aim of the study was to assess patients'/parents' attitude to the disease and basic knowledge concerning the PKU diet. **Methods**: We send out the questionnaire to 160 people: 40 patients (10–15 years) + 40 their parents, 40 patients (>15 years), 40 parents of patients (<10 years). The questionnaire is composed of 25 questions concerning social and environmetal factors, PKU and PKU diet knowledge, influence of disease acceptance on PKU diet compliance.

Results:

- Don't accept disease: patients: 40% (10–15 years), 35% (>15 years); parents: 25% (<10 years), 30% (10–15 years).
- Don't know their daily phe tolerance: patients: 62% (10–15 years), 40% (>15 years); parents: 30% (<10 years), 20% (10–15 years).
- Feel lost because of constant necessity of controlling their diet: patients: 28% (10–15 years), 52% (>15 years); parents: 10% (<10 years), 15% (10–15 years).
- Feel ashamed because they can't eat all products: patients: 30% (10–15 years) and 40% (>15 years)

Conclusion: The results of this trial have revealed the necessity of improvement of educational programs and the necessity of strict cooperation between patients and their families with psychologists.

NUTRICIA RESEARCH FOUNDATION sponsored the study.

O-11-2

ANALYSIS OF CURATIVE EFFECT FOR HYPERPHENYLALANINEMIA

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Objective: Jinan City is in north of China, where has a pretty high incident of 1:6937 for hyperphenyllalaninemia (HPA). Jinan newborn screening center has diagnosed 61 cases of HPA in over 420 thousand newborns since 1996. The incident of classical PKU and BH_4 deficiency is 93.4% and 6.5% of HPA. This article mainly analyzes the curative effect of HPA. **Method**: Grouping the HPA patients according to different type and diagnosed time. Group 1 is untreated HPA patients, whose average age is 1-year-old, including classical PKU (group 1a), and BH_4D (group 1b). The patients have not been diagnosed in time by newborn screening system, which appeared clinical symptom of HPA. Group 2 is the same amount of HPA patients diagnosed by newborn screening, including classical PKU (group 2a) and BH_4D (Group 2b). This group got the correct diagnosis in time and behaves no HPA clinical symptom. Evaluate the curative effect between the four groups by body development, intelligent development and leukodystrophy by MR spectroscopy. **Results**: (1) Most of patients in group 1 have severe mental retardation and leukodystrophy. Though the DQ increased after a period of treatment, 50% of them still have mental retardation. Patients in group 2 have no clinical symptom when they got diagnosed and develop very well after proper treatment, 90% of them have normal intelligence. (2) BH_4 patients have better compliance for treatment than classical PKU patients. **Conclusion**: Early diagnosis and early treatment are the key factors for the prognosis of HPA, while patients' compliance for treatment is also very important.

O-11-3

VARIABLE RESPONSE TO TETRAHYDROBIOPTERIN (BH_4) OF PATIENTS WITH PHENYLKETONURIA (PKU)

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We have investigated the response to tetrahydrobiopterin (BH_4) in 36 patients with phenylketonuria (PKU), ages were from 6 to 50 years. A decrease in blood phenylalanine (phe) above 30% was considered as a significant positive response. All patients were genotyped and the response to BH_4 was correlated to the genotype. Tetrahydrobiopterin was given orally in a single dose 10 mg per kg per day for 8 days. Blood phe was determined every 48 h for the period of 8 days. Prior to the trial, baseline phe was established on three separate blood samples. Patients with clinical classification of classical PKU were included in the study. Among those patients who were considered classical, one mild allele was found and in one patient, two mild alleles were found and the determination of classical PKU was given to that patient. Some of these patients, the clinical classification was established in the newborn period, which was not accurate as the patients got older. Some of the patients with the two mild alleles required higher doses of BH_4 to show significant lowering of blood phe levels with BH_4. Of interest, eight patients who did not respond to BH_4 and who were unrelated, 7 had IVS12nt1g>a. Some of those patients required higher dose of BH_4. Genotyping can be predictive in most cases of PKU and their potential response to BH_4. However, different dosages of BH_4 need to be experimented with when a responsive allele is present.

O-11-4

EFFECTS OF A SIX MONTH-SUPPLEMENTATION OF A NEW PHE-FREE POWDERED AMINO ACID PREPARATION ON BLOOD AMINO ACID PROFILE AND LEVELS OF ALBUMIN, PROTEIN AND TRANSFERRIN IN PKU PATIENTS

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Aim: To evaluate the effects of a six months supplementation with a new slowly-released (3 h) Phe-free amino acid powder in PKU patients. **Patients and methods**: Thirteen PKU patients (7 females, 6 males, mean age 14 years, range 5–26) were randomised to be supplemented for a 6 month period with either 100% (group 1) or 80% (group 2) the nitrogen daily needs with a new mixture. Plasma amino acid profile (ion exchange chromatography) and plasma total proteins (colorimetric method), albumin and transferrin (standard immune-enzymatic methods) were measured at baseline (t0) and at the end of supplementation period (t1). **Results**: The compliance with the new mixture was 100%. At t0 amino acid profiles and blood levels of proteins were comparable between groups. In group 1, plasma total protein and albumin levels (mg/dl) increased from t0 to t1 (mean, SD: 7.3, 0.2, vs 6.9, 0.3, $p = 0.02$; and 4.5, 0.2 vs 4.3, 0.1, $p = 0.04$, respectively); the amino acid profile showed an increase in methionine, in lysine and arginine concentrations ($p = 0.02$ for all the three parameters). In group 2, no significant differences were found as far as both the blood amino acid profile and blood protein levels. Comparison between the groups at t1, showed a difference in tyrosine (μmol/L) (group 1: 80, 18, vs 47, 17, group 2; $p = 0.001$). **Conclusions**: An increase of blood albumin and protein levels was observed in patients receiving the new Phe-free amino acid preparation, as covering 100% nitrogen daily need, maybe due to a more balanced amino acid absorption and availability (particularly for tyrosine) and consequent improvement of anabolic processes.

O-11-5

EFFECTS OF LARGE NEUTRAL AMINO ACID SUPPLEMENTS IN PKU: AN MRS AND NEUROPSYCHOLOGICAL STUDY

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Objective: To determine the effects of large neutral amino acid (LNAA) supplements on brain and plasma phenylalanine levels and neuropsychological performance in early treated patients with PKU. **Methods**: Prospective double blind cross over study with four phases in 16 subjects with classical PKU. Each 2 week phase consisted of either LNAA supplement or placebo and either usual PKU product or not, with a one month washout period between phases. Subjects were instructed to maintain their usual phenylalanine restricted diet and energy intake. Proton magnetic resonance spectroscopy, plasma amino acids and neuropsychological performance were measured at the end of each phase. **Results**: There were no significant differences in brain phenylalanine across the phases and a correlation with plasma levels only when on placebo and no PKU product. However some subjects did show a decrease in brain phenylalanine whilst on LNAA supplements. Plasma phenylalanine was lower when on usual PKU product and decreased with LNAA supplementation when not taking usual PKU product. LNAA supplementation had a specific impact on executive function particularly in verbal generativity and flexibility with the latter also associated with lower brain phenylalanine levels. Measures of attention were better on PKU product, with or without LNAA supplements. **Conclusion**: For individuals already complying with diet and PKU product, additional LNAA appear to be of limited value. LNAA supplementation may be of benefit to those unable to comply with taking PKU product.

O-12-1

TURKISH EXPERIENCE ON THE EPIDEMIOLOGY OF ORGANIC ACIDURIAS

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We report our experience on organic acidurias from five metabolic centers in Turkey after the establishment of GC/MS analysis in 1996. 805 cases of organic aciduria (160 methylmalonic aciduria, 148 maple syrup urine disease, 82 alkaptonuria, 81 propionic acidemia, 77 L-2-hydroxyglutaric aciduria, 64 late-diagnosed biotinidase deficiency, 62 glutaric aciduria type I, 33 isovaleric acidaemia, 29 HMG, 23 Canavan disease, 16 beta-ketothiolase deficiency, 9 glutaric aciduria type II and some rare allied disorders) will be reported. Active surveillance of symptomatic children with organic acidurias in the last decade 339 children were recorded from two reference metabolic centers in Istanbul. If we compare the total diagnosed patients with organic aciduria to all newborns (n: 1 138 307) from 1996 to February 2006 in this metropolitian, where 1/10 of the newborns of the country were born, the overall incidence for all organic acidurias were appraised to be 1: 3358. The result of a pilot study on newborn screening with Tandem MS/MS in Istanbul will be reported. Consanguinity (a mean rate of 26.7%) is an important factor for the high incidence of inborn errors of metabolism in Turkey. In a thesis on organic aciduria the consanguity rate (n: 199 patients) was found as 70.9%. In 21 patients with L-2-hydroxyglutaric aciduria from 15 Turkish families, 14 of them consanguineous, 9 mutations in the L2HGDH gene were found. L2HGDH, which encodes a putative mitochondrial protein dubbed the authors 'duranin' shows homology to FAD-dependent oxidoreductases.

O-12-2

DILATED CARDIOMYOPATHY IN PROPIONIC ACIDEMIA PATIENTS

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Objective: Dilated cardiomyopathy is now a well known complication of propionic acidemia (PA) even if it has been reported in only a small number of PA patients. We reviewed PA patients for evidence of cardiomyopathy and we described follow up and management of these patients. **Methods**: Five patients with PA had the diagnostic criteria of cardiomyopathy and were treated with standard therapy. For each patient we detailed clinical follow up, age at the onset of cardiomyopathy and medical management. Endomyocardiac biopsy (EMB) were performed for mitochondrial respiratory chain investigation (two patients). **Results**: Dilated cardiomyopathy occurred at age height ($n = 1$), six ($n = 1$), nine ($n = 1$), and five ($n = 2$). All patients had neonatal onset of PA (neonatal coma) excepted one (10 months). Complex III deficiency was identified for one patient and complex II deficiency for the other. Liver transplantation (LT) was performed for two patients. The first was transplanted thirteen years ago. Cardiac echography was normal one year after the transplantation. The second child was transplanted one week ago and shortening fraction was 28 % before LT. **Conclusion**: Dilated cardiomyopathy in propionic acidemia is a severe complication with unknown mechanism. Oxphos deficiency probably participate in the physiopathology of the cardiac defect, probably by a toxic accumulation of metabolites excreted in PA.

O-12-3

L-2-HYDROXYGLUTARIC ACIDURIA AND BRAIN TUMORS: REPORT OF TWO PATIENTS WITH MEDULLOBLASTOMA AND GLIOBLASTOMA

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L-2-hydroxyglutaric aciduria (L2HGA) is a rare autosomal recessive neurodegenerative disorder characterized by psychomotor delay, cerebellar and extrapyramidal signs and subcortical leuko-encephalopathy with basal ganglia and dentate nuclei involvement. The gene (*C14orf160/duranin/L2HGDH*) is identified recently on chromosome 14q22.1. In our cohort of 30 patients with L2HGA two of them developed medulloblastoma and glioblastoma (stage IV) during the course of their disease. The first patient was diagnosed at age 23 months with L2HGA and at age 3 years with medulloblastoma. Gross total tumor resection from the roof of the fourth ventricle was achieved; he died within six months with seeding throughout the craniospinal axis. The second patient was an 11-year-old boy who presented with left-sided weakness, progressive cerebellar symptoms and hemiparesis. He had an initial diagnosis of acute disseminated encephalomyelitis. He developed a right glial mass with cystic and hemorrhagic component and the mass was partially removed. He is on a chemotherapy and radiotherapy protocol. The relationship between brain malignant tumors and L2HGA is difficult to explain but may be due to DNA replication errors during demyelination and remyelination process. The combination of L2HGA and brain tumors is defined in anectodal cases. Mutational analysis of these patients will be discussed in parallel with other patients reported and may highlight the underlying pathogenic mechanism of this rare relationship.

O-13-1

FINDINGS OF BRAIN IMAGING (CT OR MRI) IN 52 CASES OF METHYLMALONIC ACIDEMIA

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Objective: To study children affected with methyl malonic acidemia referred between 1994–2005, to recognize findings of brain CT and MR images, the effect of therapy and to correlate the MRI and CT scan features according to severity of the disease. **Method**: 72 patients (age 8 months – 18 years) were included into a cross-sectional study in a referal center, for inborn errors of metabolism in Iran. Fifty two brain images of the patients including 47 MR and CT images were reviewed and reported by an expert radiologist of Harvard Medical University. Clinical course and outcome were evaluated by standardized questionnaires. **Results**: Fourteen patients had normal brain images. The most common findings of the images were ventricular dilation, diffuse cortical atrophy, periventricular white matter changes, corpus callosum thinning, subcortical white matter lesions, local cortical atrophy, gyral dilation, cerebellar atrophy respectively. Basal ganglia calcification was seen in 2 patients both presented in neonatal period. Treatment duration seems to have an improving effect on the severity of ventricular dilation. Children presented with psychomotor delay had usually multiple radiological findings while few findings were seen among those presented with loss of consciousness and those detected by neonatal screening. **Conclusion**: in addition to previously published brain imaging findings; corpus callosum thinning, brain stem thinning, focal infarct signal change, bright signal in centrum semioval and putamen calcification should also be considered. Treatment with adequate duration may have improving effects or may prevent some structural changes in CNS.

O-13-2

LATE-ONSET COMBINED HOMOCYSTINURIA AND METHYLMALONIC ACIDURIA (*cblC*) AND NEUROPSYCHIATRIC DISTURBANCE

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We report the case of a 36-year-old Hispanic female with a spinal cord infarct, who was subsequently diagnosed with methylmalonic aciduria and homocystinuria, *cblC* type, (*cblC*). Mutation analysis revealed c.271dupA and c.482G>A mutations in the *MMACHC* gene. Ten other reported late onset cases with similar presentations are also reviewed with updated molecular characterization. The patient had a past medical history significant for joint hypermobility, arthritis, bilateral cataracts, unilateral hearing loss, anemia, multiple miscarriages, frequent urinary tract infections and mental illness including psychosis and depression. There was no significant past history of mental retardation, failure to thrive and seizure disorder as reported in classic cases of *cblC*. Prior to the thrombotic incident, the patient experienced increased paresthesia in the lower extremities, myelopathy and impaired gait. The c.482G>A (p.R161Q) mutation occurs at a well conserved residue in MMACHC. Although c.482G>A has not been observed in homozygous state, it was identified in heterozygous state in three additional patients with late-onset disease. In addition, c.482G>A seems exclusive to patients of Hispanic descent. Due to unawareness of the late-onset presentation of *cblC*, the delay in establishing the diagnosis lead to significant neurological sequela and the patient remains wheelchair bound. We would like to emphasize the recognition of a neuropsychiatric presentation in late-onset *cblC*, and suggest that the evaluation of thrombotic events should include testing for this rare, but treatable disorder, regardless of age of presentation.

O-13-3

THE EVALUATION OF ELECTROENCEPHALOGRAPH TO MMA CURATIVE EFFECT

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Objective: To study change of electroencephalograph after therapy of MMA patients. **Method**: We analysis electroencephalogram result in 26 MMA patients with 16 lead Vıdeo-EEG. **Result**: (1) Result of EEG: 16 cases of MMA that have been seen accompanied by hypsarrhythmia, slow activity, spike and slowly discharge. (2) Convulsion spasm: 12/16 cases whose EEG were abnormal are convulsive.They were mainly presented as tonic-clonic seizures and spasms for one month to four years. (3) Prognosis: 11/12 cases convulsion free and their EEG were improved. one case died and his EEG changed from hypsarrhythmia to low voltage. After convulsion were controlled for 2–6 months,patients stopped antiepileptic drugs, and they had not convulsed for 1–5 years. Among four cases whose EEG were abnormal but had no clinical convulsion,three maintained normal and one died with EEG persisted low voltage. Among ten cases whose EEG were normal, only one had spike in his EEG but no clinical symptoms. **Conclusion**: (1) It is important to make causal diagnosis for patients with refractory epilepsy combined with mental retardation. (2) EEG is one of the impersonality guidelines to know cerebral function. The patients of MMA whithout convulsion should take EEG test. (3) EEG is one of the impersonality guidelines to evaluate treatment effectness. (4) Taking causal treatment to MMA patients with epilepsy also should take antiepileptic drugs. The period of treatment is individual.

O-13-4

AMINOACYLASE 1 DEFICIENCY REVISITED: ADDITIONAL FAMILIES AND FURTHER CHARACTERIZATION

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After the report of the first five individuals with genetically proven aminoacylase 1 (ACY1) deficiency at the 2005 SSIEM symposium awareness for this inborn error of metabolism has increased. We now present children with ACY1 deficiency from Norway (boy of Romani origin) and from the United Kingdom (girl of Arab ethnicity). Analysis of urinary organic acids was performed because of convulsions and yielded increased concentrations of several *N*-acetylated amino acids as the specific abnormality. ACY1 activity tests were performed in homogenates prepared from cultivated fibroblasts and EBV transformed lymphoblasts, respectively, and yielded less than 10% of the corresponding control values. Mutation analysis revealed homozygosity for two missense mutations. In the Norwegian child we identified the known R353C variant. In the boy from the United Kingdom we found the novel R197W mutation, which was absent in 200 control chromosomes. At the age of 4.5 years the patient from Norway exhibits retarded psychomotor development with epilepsy, while the girl appears free of physical and neurological problems at the age of 3.5 years. In addition, we report analytical data on a healthy sibling of a previously diagnosed ACY1-deficient individual indicative for ACY1 deficiency. At this point we cannot state whether ACY1 deficiency has pathogenic significance with pleiotropic clinical expression or is simply a biochemical variant. Awareness of this new genetic entity may help both in delineating its clinical significance and in avoiding erroneous diagnoses.

O-14-1

OUTCOME OF EXPANDED NEWBORN SCREENING REFERRALS IN UPSTATE NEW YORK

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Objective: To investigate the outcome of referrals for abnormal expanded newborn screening (ENBS) in upstate New York. **Methods**: Cross sectional chart review of all referrals from the state ENBS program. **Results**: Since November, 2004 we have received 75 emergent referrals for abnormal ENBS. Indications for referral include 3-MCC deficiency (15), CPT1 (12), PKU/hyperphe (10), IVA (6), MMA/PA (6), MCAD (5), CPT2 (4), SCAD (4), galactosemia (3), citrullinemia (2), carnitine deficiency (2), MADD (2), glutaric acidemia I (2), tyrosinemia (1), argininemia (1), SCHAD (1), methionine (1) (two premature infants had double indications). The overall predictive value of an emergent referral was 17%; an inborn error of metabolism was confirmed in approximately 1/3035 births. Confirmed diagnoses included MCAD (2), primary carnitine deficiency (1), SCAD (1), PKU (5), hyperphe (3), and galactosemia (1). Two disorders (3-MCC and CPT1) constituted 36% of all referrals, but no cases of these were confirmed (although three mothers were diagnosed with 3-MCC deficiency). Among the 3-MCC referrals one infant required enzyme assay; C5OH levels on the others normalized within months. Infants with persistent elevations in ethylmalonic acid (2) and citrulline (1) remain under study. All 12 CPT1 referrals were premature LBW infants. **Conclusions**: ENBS demonstrates that inborn errors are collectively frequent; however the predictive value of screening varies considerably between disorders and can be affected by prematurity. Our data suggest that mothers of infants referred for 3-MCC should be tested for this disorder, and that in unaffected infants the C5OH levels can be expected to resolve over months.

O-14-2

FOLLOW UP OF C5-HYDROXY ACYLCARNITINE (C5OH) ELEVATIONS DETECTED IN NEWBORN SCREENING (NBS) BLOOD SPOTS

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Acylcarnitine profiling in NBS allows for the detection of elevated C5OH concentrations. Although often considered to be only indicative of 3-methylcrotonyl-CoA carboxylase deficiency (3MCC), the differential diagnosis of C5OH elevations includes deficiencies of 3-hydroxy-3-methylglutaryl-CoA lyase (HMG), beta-ketothiolase, 2-methyl-3-hydroxy butyryl-CoA dehydrogenase, 3-methylglutaconic hydratase (MGA), biotinidase (BIOT), holocarboxylase synthase (HCS), and biotin. Additionally, maternal 3MCC and HCS have been uncovered by C5OH elevations in their newborns. Phenotypes range from asymptomatic individuals to acute, life-threatening episodic acidosis and death. We reviewed a 20-month period (n = 163 460; false positive rate, FP: 0.10%) for infants with isolated C5OH elevations. Forty-five infants (0.03%) were followed for elevated C5OH. Ten true positive (TP) cases included 3MCC (4 infant, 2 maternal), partial biotinidase deficiency (2), MGA (1), and HMG (1). We reviewed NBS data (C5OH, C5OH/C8, C5OH/C5, and C5OH/C16) to determine whether it is possible to differentiate FP (0.02%) from TP (0.01%) cases. Significant overlap was observed between TP and FP for all parameters. Although NBS for C5OH is warranted because of the clinically significant conditions that can be identified, it is not possible to avoid follow up testing in the approximately 1 in 5000 newborns with a false positive result for C5OH.

O-14-3

SCREENING FOR X-LINKED ADRENOLEUKODYSTROPHY BY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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X-linked adrenoleukodystrophy (XL-ALD) is the most common peroxisomal disorder affecting approximately one in 21 000 males. It is a progressive and fatal disorder that affects the nervous system, the adrenal cortex and the testis. Adrenal insufficiency can be totally prevented by presymptomatic therapy, while the cerebral phenotype, affecting 40% of all male patients, can be prevented by dietary therapy and hematopoietic transplant. However, those interventions are ineffective in patients already symptomatic. On these bases, the need for a screening method able to identify presymptomatic patients is compelling. Using a liquid-chromatography MS/MS (LC-MS/MS) method, we have measured three lipid classes, sphingomyelins, ceramides and lysophosphatidylcholine (lyso-PC) on blood spots and plasma sample form 25 male patients with XL ALD, and 9 patients with peroxisomal biogenesis disorders (PBD). Data were compared with 19 controls. The profile and relative abundance of lyso-PC appeared to be more suitable for discriminating affected patients from control. Concentrations of lyso-PC fraction containing hexacosanoic acid (C26:0) were distributed as follows (range, median): ALD 1.90–24.76 ng/ml, 6.69 ng/ml; PBD 14.42–84.68 ng/ml, 31.47 ng/ml; control 0.19–1.46 ng/ml, 0.63 ng/ml. The C26:0 lyso-PC to C20:0 lyso-PC ratio was as follows (range, median): ALD 0.54-6.98, 1.33; PBD 2.62–19.58, 4.30; control 0.03–0.41, 0.17. These preliminary data appear extremely promising and strongly indicate the suitability of this method as a screening tool for XL-ALD and other peroxisomal disorders.

O-14-4

NEWBORN SCREENING FOR MSUD: INCREASED SPECIFICITY BY ADDITION OF 2ND-TIER ASSAY FOR ALLOISOLEUCINE BY LC-MS/MS

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Alloisoleucine (Allo-Ile) is an intermediate of the L-leucine degradation pathway and increased Allo-Ile levels are pathognomonic for Maple-Syrup Urine Disease (MSUD). Newborn screening includes the measurement of branched-chain amino acids (BCAA), leucine (Leu), isoleucine (Ile), and valine (Val), which are suggestive of MSUD when elevated. Increased levels of BCAA are also frequently observed in newborn screening when the infant is receiving total parenteral nutrition, results which often trigger follow-up investigations. We developed a new LC-MS/MS method for the specific detection of Allo-Ile in a single dried blood spot (DBS) punch. Allo-Ile and other BCAA are extracted from DBS with Methanol/H_2O, dried under nitrogen, and reconstituted into mobile phase. Chromatographic separation of Allo-Ile from its isomers, Leu and Ile, is achieved within 10 min. Column re-equilibration is not required. The inclusion of isotopically labeled Ile (^{15}N-Ile) as an internal standard provides specificity for the quantification of Allo-Ile, Leu, and Ile. The assay is calibrated for BCAA concentrations up to 1250 μmol/L ($y = 3586x+30048$; $R^2=0.9919$). Retrospective analysis of a newborn screening DBS from an infant with MSUD demonstrated an Allo-Ile quantity of 136 μM (controls: <2). In 2005, 0.1% of newborns screened at the Mayo Clinic required follow up due to multiple AA abnormalities including BCAA elevations. Allo-Ile detection in DBS performed as a second-tier assay when elevated BCAA are encountered could be used to reduce the number of false-positive cases requiring unnecessary follow-up evaluation. In addition, this method could resolve an apparent elevation of Ile/Leu due to an accumulation of hydroxyproline.

O-14-5

MATERNAL PRIMARY CARNITINE DEFICIENCY IDENTIFIED BY NEWBORN SCREENING

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Newborn screening has been revolutionized by the use of tandom mass spectrometry (MS/MS) and primary carnitine deficiency, a disorder of fatty acid oxidation, is now detectable. This disorder is caused by mutations in the *SLC22A5* gene encoding the high-affinity carnitine transporter, OCTN2. Affected individuals classically present with hypoketotic hypoglycemia, hepatic encephalopathy, hypotonia, cardiomyopathy, or sudden death. Diagnosis is based on the identification of low free carnitine levels in plasma and is confirmed by measurement of carnitine transporter activity in skin fibroblasts. Treatment is with oral carnitine. Newborn screening using MS/MS detects low levels of free carnitine (C0). In affected infants, carnitine supplementation (500–100 mg/kg/day) causes a slow rise in plasma carnitine to near normal levels. In some infants with low free carnitine levels, initially identified through several newborn screening programs, a rapid rise in plasma free carnitine levels upon initiation of treatment was found. We report a series of cases in which the uncharacteristically rapid rise in plasma carnitine to normal levels in newborns, with initial carnitine levels of 2.5–8 μM/L, caused us to suspect maternal disease. Carnitine levels in the mothers ranged between 1–5 μM/L. Carnitine uptake in the fibroblasts of these mothers ranged from 2–17% of normal. To confirm the diagnosis further, the *SLC22A5* gene was sequenced in several mothers. The diagnosis and subsequent treatment of these mothers represent an unexpected and important added benefit of newborn screening programs and defines a much wider clinical spectrum for this condition than had previously been suspected.

O-14-6

COMPARING THE EFFICACIES OF SOME SUPERVISED MACHINE LEARNING ALGORITHMS FOR SCREENING INBORN ERRORS OF METABOLISM

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Inborn errors of metabolism (IEMs) are a large group of rare but often serious metabolic disorders. Due to the complexity of the collected experimental data, machine-learning approaches provide a promising approach in unearthing discriminatory rules that form the basis of expert clinical diagnostic systems for inborn metabolic disorders. The objective of this study is to apply data mining techniques to excavate the best discriminatory rules or classification model for building a clinical expert diagnostic system for IEMs. The database for IEMs has a large number of features. Feature selection techniques like gain ratio and relief are applied to select the most relevant features. Three machine-learning algorithms have been investigated for their accuracy focusing on five IEMs, namely fructosemia, galactosemia, isovaleric acidemia, propionic acidemia, and mucopolysaccharidoses. The machine learning algorithms used were decision trees, logistic regression analysis and artificial neural networks. The performance of the algorithm was verified on both the original database as well as with reduced feature database. All the algorithms were obtained from the WEKA machine-learning package. Logistic regression analysis, Multilayer perceptron and ID3 decision trees led to highly accurate classification rules with specificity (92–98%) and sensitivity ($>=97\%$). From the performance point of view ID3 decision algorithm outscores all other algorithms and can be used to build the rule base for an clinical expert diagnostic system for metabolic disorders. Such decision support systems may be incorporated successfully into automated screening programs for IEMs.

O-15-1

CACT DEFICIENY AND ACUTE CARDIAC ARREST IN NEWBORN

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A well newborn patient presented with acute cardiac arrest within 24 h after birth. He was successfully resuscitated by neonatal intensive care team of a regional private hospital in Hong Kong. After initial stabilization and aggressive treatment of ventricular arrhythmia, the patient was transferred to regional public hospital for workup of suspected IEM disease. The diagnosis of CACT deficiency was confirmed by molecular diagnosis and chromatography. Both were identified to be recessive carriers of the gene and genetic counselling and referral to prenatal diagnosis was made. **Conclusion**: There is an urgent need to establish clinical and pathological management pathways for treatment of acute cardiac arrest in newborn for risk management and training.

O-15-2

A NEW DISORDER IN FATTY ACID β-OXIDATION

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The acyl-CoA dehydrogenases (ACDs) are a family of multimeric flavoenzymes that catalyze the α,β dehydrogenation of acyl-CoA esters in fatty acid β-oxidation and amino acid catabolism. Defects have been identified in most of the ACDs. ACAD9 is a recently identified ACD that demonstrates maximum activity with unsaturated long chain acyl-CoAs. We now report three cases of ACAD9 deficiency. Case 1 was a 14 year old apparently healthy boy who died of a Reye-like episode and cerebellar stroke triggered by a mild viral illness and ingestion of aspirin. Case 2 was a 10 year old girl who presented with episodes of fulminant liver failure and hypoglycemia from 17 months of age, with otherwise minor illnesses. Case 3 was a 4.5 year old girl who died of cardimyopathy, whose sibling died of similar cardimyopathy at 21 months of age. All cases manifested marked defects in ACAD9 at the protein and mRNA level. The clinical variability is reminiscent of VLCAD deficiency. Despite significant overlap of substrate specificity between ACAD9 and VLCAD, it would appear that these proteins are unable to compensate for each other. Our studies of the enzymatic activity, tissue distribution, and gene regulation of ACAD9 and VLCAD identify the presence of two independently regulated functional pathways for long chain fat metabolism in various tissues. Our studies fundamentally redefine the roles for fatty acid β-oxidation in human intermediary metabolism.

O-15-3

PERIPHERAL NEUROPATHY AS A PRESENTING SYMPTOM IN MITOCHONDRIAL TRIFUNCTIONAL PROTEIN (MTP) DEFICIENCY

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LCHAD and mitochondrial trifunctional protein (MTP) deficiency are long chain fatty acid oxidation (FAO) defects that can be associated to signs of peripheral neuropathy and myopathy along with the frequent signs of non-ketotic hypoglycemia, liver failure and rhabdomyolysis. However, very few patients where the presenting symptom is a chronic neuromuscular phenotype have been reported so far. We present here two patients, (G. male and S. female) diagnosed at age of 15 with MTP deficiency revealed by neuromuscular symptoms. Both patients displayed early signs of motor deficit, walking impairment and amyotrophy and chronic muscle pain. EMG showed signs of sensorimotor neuropathy for both patients leading to the erroneous diagnosis of Charcot-Marie-Tooth neuropathy. A metabolic work-up including organic acid analysis and plasma acycarnitines was compatible with LCHAD or MTP deficiency. Enzymatic assays showed a very low activity for the 3 enzymes of the MTP confirming the diagnosis in both patients. Molecular analysis in patient G. revealed a R235W homozygous mutation in exon 8 of the α sub-unit of the MTP protein. Patient S., displayed a splicing site IVS7+2T>C homozygous mutation in the α sub-unit as well. Both patients have been treated with diet and L-carnitine supplementation. No significant improvement has been observed so far after 3 years of follow-up. In conclusion the exclusively neuropathic phenotype of our patients highlights the important place that hold these FAO disorders in the differential diagnosis of neuropathy in children and young adults. Our molecular data may contribute to understanding the mechanisms responsible for these particular phenotypes.

O-15-4

PIVALOYLCARNITINE ANALYSIS WITH HPLC/ESI-MS/MS IN A CARNITINE DEFICIENT PATIENT WITH HYPOGLYCEMIC CONVULSION INDUCED LONG TERM ADMINISTRATION OF PIVALATE-GENERATING PRODRUG

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Pivalate is used as a prodrug to increase oral absorption of therapeutic agents. The administered pivalate is excreted as pivaloylcarnitine into the urine and hypocarnitinemia is observed even in a short term therapy of pivalate prodrugs. During a long term administration, side effects such as tiredness, behavioral problems and weakness have been reported in children. Here we report pivaloyl- and other acylcarnitine profiles of a case with pivalate induced hypocarnitinemia using HPLC-ESI/MS-MS, CASE: A normally developed 19 months old boy was hospitalized because of vomiting and afebrile convulsion. His blood sugar level was 11 mg/dl and urine keton body was slightly increased (1+). Close questioning revealed that he had been administered pivalate-generating antibiotics because of otitis media for about 6 months. **Method**: Urine and serum samples were collected from the patient. Acylcarnitine profiles were examined the HPLC-ESI/MS-MS method with solid phase extraction. **Result**: On admission, instead of the extremely low level of free carnitine (0.41 μmol/l), pivaloylcarnitine was detected in the serum sample (1.6 μmol/l). After the carnitine infusion (100 mg/kg/day, three days), his serum free carnitine levels increased to about 150 μmol/l with the pivaloylcarnitine level of 14.2 μmol/l. Serum pivaloylcarnitine disappeared immediately after the stopping of carnitine infusion, but urinary excretion of pivaloylcarnitine continued about one month. **Discussion**: Administered pivaloylcarnitine remained very long period even the serum pivaloylcarnitine is not detected. This suggests that accumulated pivalate is not excreted other than carnitine conjugated from the body. Sufficient carnitine supplementation should be considered to those patients.

O-15-5

EXPRESSION AND CHARACTERIZATION OF PATIENT MUTATIONS IN VERY LONG-CHAIN ACYL-CoA DEHYDROGENASE (VLCAD) USING A PROKARYOTIC SYSTEM

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VLCAD catalyzes the intra-mitochondrial rate-limiting step in the beta-oxidation of fatty acids 14 to 20 carbons in length. Genetic deficiency of VLCAD manifests either in a severe, early onset form or a milder, later onset form. Characterization of the effect of patient mutations in the VLCAD gene on enzyme function has been limited by difficulties with expression of VLCAD. We have for the first time expressed and purified recombinant human VLCAD in *E. coli*. Additionally, we have generated a three-dimensional molecular model of the enzyme useful for predicting structural perturbations caused by patient mutations. We have used these techniques to study 6 VLCAD patient mutations: the two most common ones (T220M, V243A), a point mutation leading to a severe phenotype (R429W), and three mutations in the long C-terminal domain which shares no homology to other acyl-CoA dehydrogenase family members (A450P, L462P, and R573W). Crude *E. coli* extracts were tested for enzyme activity. Mutations associated with the severe phenotype (R429W and R573W) had no detectable activity toward palmitoyl-CoA. T220M and V243A had 5% and 18% of wild type VLCAD activity, respectively, which correlates well with a previous characterization of these mutants using a eukaryotic system. Surprisingly, A450P and L462P *E. coli* extracts were as active as wild type VLCAD. Molecular modeling places both of these residues on a surface of the enzyme that is postulated to interact with the inner mitochondrial membrane. These two enzymes have been purified for further investigation of enzyme kinetics, substrate specificity, and membrane binding.

O-15-6

MOLECULAR EVOLUTION OF THE ACYL-CoA DEHYDROGENASE (ACD) FAMILY, A SUPPLEMENTARY TOOL FOR SUBSTRATE PREDICTION

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Mammalian ACDs constitute a family of flavoproteins that catalyze the α,β-dehydrogenation of acyl-CoA thioesters to corresponding trans 2,3-enoyl-CoA products. Eleven members of this family have been described, however, the substrate specificity of two remains undetermined. Combining bioinformatic and phylogenetic approaches we investigated the evolutionary history of the ACDs with particular focus on substrate specificity prediction for the unknown enzymes. The origin of the ACD family can be traced back more than 2 billion years ago to the origin of the *Archaea*, *Bacteria*, and *Eukaryota*. At least two primordial ACDs were already present at this time, one of which was the ancestor of glutaryl-CoA dehydrogenase. The emergence of the ACD family is marked by several rounds of gene duplication at or early after the divergence of the three domains. The rise of the archaeal domain is marked by gene duplication early after its divergence producing ACDs specific to the archaeal lineage and by consecutive rounds of gene amplifications specific to extant archaeal taxa. *Bacteria* and *Eukaryota* emerged already equipped with 6–8 ACD homologs and there were only occasional lineage-specific duplications towards extant taxa. Two additional duplications occurred in parallel before speciation of higher eukaryotes, particularly *Coelomata*, about 400–600 mya. The most parsimonious scenario suggests that the most ancestral ACD had branched-chain specificity and that ACDs with straight-chain specificity derived later at the root of *Bacteria* and *Eukaryota*. Furthermore, our analysis suggests that the two undetermined ACDs will likely have specificity towards long branched-chain fatty acid substrates.

O-15-7

SHORT-TERM *IN VIVO* CORRECTION OF VERY LONG-CHAIN ACYL-CoA DEHYDROGENASE (VLCAD) DEFICIENCY IN THE VLCAD KNOCK-OUT MOUSE MODEL

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VLCAD deficiency is a disorder of fatty acid beta-oxidation with variable phenotype including cardiomyopathy, skeletal myopathy, and fasting nonketotic hypoglycemia which often results in significant morbidity and mortality. Treatment of VLCAD deficiency includes avoidance of fasting, a diet low in long-chain fatty acids, and vitamin supplementation. Nevertheless, a risk of precipitous metabolic decompensation during acute illness remains. The development of a safe, durable, and effective VLCAD gene delivery system for use in pre-symptomatic individuals identified with VLCAD deficiency through newborn screening could significantly improve quality of life.

A recombinant adeno-associated virus serotype 8 (AAV8) was prepared using a well characterized hVLCAD expression cassette under the control of the CMV promoter, given its ability to rapidly transfect muscle and hepatocytes. VLCAD-deficient mice were injected with either 1×10^{11} genome particles of AAV8-CMV-hVLCAD or saline. Ten days after injection the mice were fasted 24 h and blood and liver were collected for acylcarnitine analysis, Western blotting, immunohistochemical staining, and glucose determination.

In comparing pre- and post-treatment VLCAD deficient mice, those treated with AAV8-CMV-hVLCAD showed robust hVLCAD expression on Western analysis and immunohistochemical staining. Fasting hypoglycemia and elevations of long-chain fatty acids present in VLCAD deficient mice were corrected to normal levels. Together, these studies demonstrate short-term *in vivo* correction of VLCAD deficiency in a murine model using the human VLCAD gene. Further investigation of long-term transgenic expression of hVLCAD and its effects on the complex pathophysiology of VLCAD deficiency are being pursued.

O-16-1

THE R40H ALLELE OF ORNITHINE TRANSCARBAMYLASE IS TRANSMITTED MORE FREQUENTLY THROUGH MATERNAL LINEAGE THAN PATERNAL LINEAGE

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The R40H mutation is the most frequent one among those that are found in male patients with late-onset presentation. Father-to-daughter transmission of the mutant allele was demonstrated in some families. The aim of the present study is to determine whether or not there is a parental preference of transmission of the mutant allele. A total of 16 pairs of a parent and its offspring in 8 discrete families were studied. The genotype could be determined in 13 parents of the 16 pairs. The age at onset of the proband in each family ranged from 6 through 58 years of age (median; 16.5 years). Whether or not an individual carries the R40H mutation was determined by the mutational analysis, concentration of orotic acid in urine or the family tree analysis. There were two symptomatic female patients in those studied. The analysis results indicated that there were 3 cases of father-to-daughter transmission, 9 cases of mother-to-son transmission and one case of mother-to-daughter transmission. These results reveal that the mutant allele is three-fold more frequently transmitted through the maternal lineage than the paternal lineage. This may be accounted for by a higher reproductive loss rate in the males than in the females carrying this mutant allele. The apparent dominance of the mother-to-son transmission over the mother-to-daughter transmission (9 vs. 1) is likely to be due to a possible underestimation of asymptomatic female heterozygotes.

O-16-2

ESTIMATING THE MUTATIONAL UNIVERSE IN ORNITHINE TRANSCARBAMYLASE (OTC) DEFICIENCY. HIGH VALUE OF THE OTC STRUCTURE FOR IDENTIFYING DISEASE-CAUSING MUTATIONS

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OTC deficiency (OTCD), the X-linked, most frequent urea cycle error, results from mutations in the *OTC* gene encoding a 354-residue polypeptide. 231 clinical OTC mutations, including 150 missense single nucleotide polymorphisms (mSNPs), have been tabulated (Hum Mutat 19:93, 2002). Searching for OTCD mutations is laborious and expensive. If all OTCD-causing mutations were known, mutation screening might be simpler. We estimate the total number of causative mutations from the finding in 23 new OTCD patients of 22 different mutations, 12 of them novel, including 6 novel mSNPs. The number of causative mutations is estimated to be <843 (upper limit for 95% CI), including <361 mSNPs. Thus, $\sim$90%-sensitive diagnosis based on known-mutations screening may be attained in <5 years. Since OTCD-causing mSNPs represent $<20\%$ of all the possible (2064) *OTC* gene mSNPs, discrimination between causative and trivial mSNPs is crucial. We demonstrate with our novel mutations the value of the OTC crystal structure for pathogenicity assessment: P305R and S96F are justified to trigger complete deficiency, and D41G, E122G, L179F, P220T and delE273 to trigger partial deficiency. Five non-mSNP novel mutations (G71X, 7- and 10-base duplications and deletions in exon 5, G>A changes at +1 and +5 of IVS 4 and 9) and 10 previously reported mSNPs (N161S, H302R, R26Q, R40H, H202Y, A208T and K88N, E52K, T178M and R129H) found in our cohort are discussed also.

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O-16-3

THE CRYSTAL STRUCTURE OF BACTERIAL GLUTAMATE-5-KINASE (G5K) PROVIDES INSIGHT INTO THE HYPERAMMONAEMIA CAUSED BY PYRROLINE 5-CARBOXYLATE SYNTHASE (P5CS) DEFICIENCY

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Urea cycle operation requires adequate ornithine supply. In mammals, ornithine is synthesized from glutamate via glutamate 5-phosphate and glutamate 5-semialdehyde by the enzymes G5K and γ-glutamyl phosphate reductase (GPR), the two components of the bifunctional single-polypeptide enzyme P5CS. Ornithine is then produced by ω-transamination to glutamate 5-semialdehyde, which, in its cyclic form pyrroline-5-carboxylate, is also a precursor of proline. P5CS deficiency (OMIM 138250) is a rare cause of hyperammonaemia with low ornithine, citrulline, arginine and proline levels. Mutations in the G5K and G5PR moieties of P5CS have been identified in this deficiency. We have determined using X-ray diffraction the X-ray crystal structure of recombinant *Escherichia coli* G5K, showing that this enzyme exhibits the characteristic amino acid kinase fold first identified in our laboratory in carbamate kinase. Given the existence of substantial (33%) sequence identity between the catalytic domains of human and *E. coli* G5K, we have modelled the structure of the human enzyme, clarifying substrate binding and catalysis, and setting the frame for understanding the effects of clinical mutations. Further, the structure suggests the mode of feed-back inhibition of G5K by ornithine (in humans) or proline (in *E. coli*), as well as the possible architecture of the G5K-G5PR complex that allows G5P channelling within P5CS.

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O-16-4

STROKE IN HYPERORNITHINEMIA-HYPERAMMONEMIA HOMOCITRULLINURIA SYNDROME: CLINICAL/BIOCHEMICAL CORRELATION AND REPORT OF A NOVEL MUTATION

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Hyperornithinemia–hyperammonemia–homocitrullinuria (HHH) syndrome, caused by mutation in *SLC25A15* that encodes a mitochondrial ornithine transporter, has variable presentation with episodic hyperammonemia, liver dysfunction, and chronic neurological manifestations. We report the findings of HHH syndrome in 3 Saudi siblings. The 3-year-old proband presented with recurrent Reye-like episodes and hypotonia. Investigations revealed hyperammonemia, coagulopathy, and elevated liver enzymes. Plasma ornithine, urine homocitrulline, and orotic acid were elevated at 465 μM, 108 mmol/mol creatinine (reference range < 10), and 1210 mmol/mmol creatinine (reference range: 0.05-6), respectively. MRI showed multiple supratentorial stroke-like white matter lesions of different ages. MRA and hypercoagulopathy work-up were normal. She significantly improved on protein restriction and benzoate with normalized ammonia, PT, PTT, ALT, and ornithine. Homocitrulline and orotic acid declined to 23 and 14 mmol/mmol creatinine, respectively. Her 13- and 7-year-old siblings have milder phenotype with protein intolerance and learning problems. Ornithine levels were 650 and 493 μM, respectively. In comparison to their sister, homocitrulline (13 and 21 mmol/mmol creatinine) and orotic acid (11 and 71 mmol/mmol creatinine), were only mildly elevated even before treatment. The three patients were homozygous for a novel mutation in *SLC25A15* with g.817G > 2A transition resulting in Gly220Arg change. Parents were heterozygous. The mutation was not found in 50 ethnically-matched controls and is predicted by PolyPhen database to be pathogenic. In view of the CNS lesions, we sequenced the entire mtDNA genome and no potential pathogenic mutations were detected. This report presents a new CNS finding in association with HHH syndrome, illustrates considerable clinical/biochemical correlation, and describes a novel mutation.

O-16-5

ARGININOSUCCINATE LYASE (ASL) DEFICIENCY: TWELVE NOVEL MUTATIONS AND IDENTIFICATION OF A NOVEL ASL PSEUDOGENE IN ITALIAN PATIENTS

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Argininosuccinic aciduria (ASA) is an autosomal recessive disorder of the urea cycle caused by a deficiency of the enzyme ASL. We sequenced the ASL gene in 11 patients (7 neonatal and 4 late-onset form) diagnosed on the basis of hyperammonemia and presence of argininsuccinic acid. Homozygous mutations were detected in two kindreds, while all other patients were compound heterozygotes. We found a total of 14 mutations, only two of which were previously reported (Q286R and V178M). Among the 12 novel mutations we found 8 single nucleotide changes (5 missense, 1 nonsense, 2 splicing) and 4 small insertion/deletions. Only the IVS7 c.524+2 T > G mutation was found in two different patients. We found no evidence of intragenic complementation, since the patients with the milder phenotype were compound heterozygous for a missense and a frameshift mutations. Interestingly the V178M mutation was associated to a mild phenotype in another study, however in our series it was found in a patient with the severe phenotype. Moreover, we identified a new pseudogene located 3 Mb centromeric to ASL, that is 90% identical to a 561 nt region encompassing ASL exon 4n. In conclusion, genomic DNA provides a rapid, simple and reliable tool for the study of ASL deficiency.

O-16-6

IN VITRO PRODUCTION OF RECOMBINANT RAT LIVER CARBAMOYL PHOSPHATE SYNTHETASE I (CPSI) ALLOWS TESTING OF THE EFFECTS OF THE MUTATIONS FOUND IN CLINICAL CPSI DEFICIENCY (CPSD)

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CPSD, a recessively inherited error of the urea cycle, causes life-threatening hyperammonaemia. CPSI is a multidomain 1500-residue liver mitochondrial matrix protein that is allosterically activated by acetylglutamate, and which synthesizes carbamoyl phosphate from ammonia but not from glutamine. The pathogenic potential and effects on CPSI activity, mechanism and stability, of a number of missense CPSI mutations identified in patients with CPSI deficiency, was characterized in an *in vitro* system using *Escherichia coli* CPS as a model of CPSI (Yefimenko et al., J Mol Biol 349:127, 2005). However, some clinical mutations could not be studied because they mapped in the 40-kDa N-terminal domain (mutations S123F and H337R), a CPSI domain of obscure function which corresponds to the *E. coli* CPS glutaminase subunit; or because the mutations (G1376S, L1381S and R1453Q) mapped in the C-terminal CPSI-specific domain which is involved in binding of the essential activator acetylglutamate. We now report the characterization of the effects of these mutations on CPSI by using recombinant CPSI purified from insect cells from a baculovirus vector, allowing site-directed mutagenesis of CPSI. Except the G1376S mutation, all the clinical mutations tested appear on these bases to be disease-causing. Further, the mutations T1391V, N1437D, L1438T, P1439V and N1449S have helped delineation of the acetylglutamate site.

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O-17-1

REGULATION OF METHIONINE METABOLISM IN PATIENTS WITH METHIONINE ADENOSYLTRANSFERASE (MAT) DEFICIENCY

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In methionine adenosyltransferase (MAT) I/III deficiency, clinical manifestations and the levels of intermediates of methionine cycle are variable from case to case. Genetic analyses of Japanese hypermethionic patients revealed that eight cases were heterozygous for an R264H mutation which was transmitted in an autosomal dominant manner, and three were compound heterozygotes (E145K/R292C, Y92H/R356P, and R292C/R356L). Clinical consequences of R264H cases were generally well, however individuals with compound heterozygous mutations had some neurological problems and brain demyelination detected by MRI. Two patients (E145K/R292C and Y92H/R356P) had elevated total homocysteine levels in plasma, which might be misdiagnosed as CBS deficiency in infancy. Genotyping of folate metabolizing enzyme – methylenetetrahydrofolate reductase (MTHFR) – showed that they were homozygous or compound heterozygous for 677C>T or 1298A>C, resulting in reduced activity and decreased flux of homocystine through remethylation pathway. Elevation of plasma folate (>200 ng/ml) was observed four out of eight R264H individuals. They were also homozygote or heterozygote of the two mutations in the MTHFR gene, leading to inactive folate metabolism and accumulation of tetrahydrofolate. These results indicate that elevation of homocysteine and folate is associated with SNPs of the MTHFR gene, which affects the flux through remethylation and transsulfulation pathway. Since the causative relationship between neurological problems and severity of MAT deficiency (or methionine concentration) is not universal, evaluation of homocystein and folate metabolism will help to elucidate the molecular mechanism of clinical variability.

O-17-2

CITRULLINAEMIA TYPE 2 OUTSIDE EAST ASIA – ISRAELI EXPERIENCE

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Introduction: Citrullinaemia type 2 is a rare disorder in non-Asians, caused by mutations in the citrin SLC25A13 gene. Two phenotypes are known, an adult one (CTLN2) and neonatal intrahepatic cholestasis due to citrin deficiency (NICCD). Many questions exist regarding their relationship, distinct pathogenesis, course and management. Almost all known cases have been reported in east Asians, mostly from Japan. The sole exception is an Israeli case reported in 2002. We report here 2 further unrelated Israeli cases, with a unique mutation. **Case reports**: Case 1, of Israeli Moslem descent, presented at age 40 days with liver disease. Severe anaemia, hypergalactosaemia, citrullinaemia and hypermethioneamia were present. A novel mutation L598R (T1793G) was identified. Resolution followed cessation of breast feeding and feeding with MCT, Pregestimil© and later soy formula. He is normal and healthy at age 4 without treatment. Case 2, of mixed Israeli Moslem/Circassian descent, presented at 42 days with liver disease. Laboratory disturbances were similar to case 1. The same mutation was also identified. The child is doing well on no treatment at age 2. **Discussion**: All reported cases of NICCD liver dysfunction responded to cessation of breast feeding, raising the possibility that relative intracytoplasmic aspartate deficiency, related to citrin deficiency, may be aetiologic. The need for dietary manipulation in childhood is unknown, since the relationship of NICCD to the adult form is unknown, although one case has been reported. These cases demonstrate that NICCD exists outside east Asia and should be considered in all cases of neonatal liver disease.

O-17-3

THE ACUTE MANAGEMENT OF A 25 YEAR OLD WOMAN WITH MSUD AND DIABETIC KETOACIDOSIS IN AN INTENSIVE CARE UNIT (ICU)

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The 49 kg female with known MSUD and insulin dependant diabetes mellitus was unconscious and ventilated in ICU following 3 days of vomiting. Blood glucose was 44 mmol/L, leucine (leu) 1700 mmol/L, isoleucine (isoleu) 380 mmol/L, valine (val) 780 mmol/L. She was commenced on a sliding scale insulin infusion and IV 10% dextrose at 40 ml/h (half maintenance) to avoid cerebral oedema. MSUD Aid III was commenced on day 2 and she tolerated 0.6 g/kg/day protein via nasogastric tube (NGT). As the leu was still 1600 mmol/L, energy was increased to 220 kJ/kg with 30% dextrose, 10% intralipid, Duocal and MSUD Aid III (1 g/kg/day protein). Blood glucose normalised by 48 h. On day 3 leu was 970 mmol/L, and val and isoleu were approaching normal so isoleu and val supplementation were commenced via NGT. On day 4 leu level was 490 mmol/L and she was able to be extubated. Natural protein (0.25 g/kg) was commenced via NGT and increased slowly on a daily basis. By day 6 the patient was fully conscious and was discharged to the ward on her usual oral carbohydrate exchange diet which is low in protein (1 g/kg/day) and oral supplementation of MSUD Aid III (0.7 g/kg/day protein), total energy 128 kJ/kg/day. On day 7 the patient's usual subcutaneous insulin was commenced. **Conclusion**: Both the MSUD and diabetes mellitus responded to increased energy getting into the cells. The extra energy requirements of the MSUD were readily accounted for by adjusting the insulin sliding scale resulting in good control of both disorders.

O-17-4

MOLECULAR MECHANISMS IN CYSTATHIONINE BETA-SYNTHASE DEFICIENCY: ROLE OF MISFOLDING AND ABERRANT SUBUNIT ASSEMBLY

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Abnormal folding and misassembly of mutants was demonstrated as a cause of enzyme deficiency in several inborn errors of metabolism including phenylketonuria and beta-oxidation defects. In our study we explored whether this mechanism plays role in homocystinuria due to cystathionine beta-synthase (CBS) deficiency. The propensity of untagged CBS mutants to fold and assemble correctly was studied in a prokaryotic expression system. We analyzed a series containing ~20% of all known patient-derived mutations representatively located in different enzyme domains. Western blot analysis of soluble and particulate bacterial fractions after incubation with urea/SDS showed that all 27 mutants were contained – to a different extent – in both compartments yielding a total CBS signal between 75 and 270% of the wild type enzyme. The soluble fractions – after expression at both 37°C and 18°C – were subjected to native western blot, heme detection and measurement of catalytic activity. About 70% of mutants formed heme-containing tetramers at either one of the two expression temperatures; of these about 70% exhibited CBS activity. In contrast, the remaining 30% of mutants in the series were unable to attain any ordered quaternary structure and lacked the enzymatic activity. Topology of mutations – rather than their nature – appeared to be the major determinant of proper folding/assembly and activity as demonstrated by formation of active tetramers in 12/16 surface exposed mutations and only 1/11 globule buried mutations, respectively. This study shows that one third of CBS mutations inherently predispose the enzyme toward misfolding/misassembly and suggests that these mechanisms may play role in CBS deficiency.

O-17-5

NEW DISORDER OF SERINE/GLYCINE BIOSYNTHESIS: PHOSPHOSERINE AMINOTRANSFERASE (PSAT) DEFICIENCY?

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We present two siblings with a novel serine /glycine (ser/gly) biosynthesis disorder. **Case 1** is male, born to healthy, unrelated parents. Initially well, he presented at 9 weeks with frequent, severe, intractable seizures. Head circumference dropped from the 9th centile at birth to <0.4th. Brain MRI revealed atrophy and a hypoplastic cerebellar vermis. Plasma and CSF ser/gly were low, (plasma: ser 51 μmol/L (60–300) and gly 121 μmol/L (140–420), CSF: ser 18 μmol/L (35–80), gly <1 μmol/L (0–10)). Oral serine and glycine treatment normalised plasma and CSF levels but had limited clinical effect. He died aged 7 months. **Case 2** is the sister of case 1. She was well at birth. Ser and gly were low in plasma on Day 1, and in CSF on Day 2 (ser 5 μmol/L, gly <1 μmol/L). Supplementation with 500 mg/kg ser and 200 mg/kg gly commenced on Day 2, normalising plasma and CSF levels. Cranial US at 10 days and MRI scan at 4 months showed no abnormality. She is now 2.5 years old and developing normally. Fibroblast 3-phosphoglycerate dehydrogenase (Case 1 and 2) and 3-phosphoserine phosphatase (Case 1 only) were normal. PSAT activity (Case 1 only) was equivocal. Mutational analysis revealed compound heterozygosity in both siblings (c.del G107 – frameshift, c.299A>C – missense, Asp>Ala) for mutations in the PSAT gene. Further work is required to confirm the pathogenicity of the missense mutation but these results indicate a presumptive diagnosis of PSAT deficiency. PSAT deficiency is a severe neurometabolic disorder; late treatment of the ser/gly deficiency is associated with a poor outcome, but outcome is good if treatment is started at birth.

O-18-1

2-HYDROXYISOBUTYRIC ACID AMONG CHILDREN BORN OUT OF ASSISTED REPRODUCTIVE TECHNOLOGIES AND ITS RELATION TO AUTISM

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Diagnosis of inborn metabolic disorders throws a unique challenge to laboratories in India, where only a handful of centres capable of such investigations exist. Autism has not only been a diagnostic challenge to laboratories, but also to the clinicians alike. Follow-up studies and management modalities are emerging everyday with a gradient against the affordability of people in the developing countries. A broad spectrum of assisted reproductive technologies has become available for couples with fertility problems. Results of follow-up studies of children born as a result of assisted reproduction have shown that neonatal outcome and malformation rates were not different from those of the general population. We present 23 cases of children between 3-9 years of age born out of assisted reproductive technologies presenting with autism spectrum disorder. They represent 28% out of a total of 81 cases seen with similar maternal history and assisted reproductive procedures. Autism in them was diagnosed by the DSM IV criteria. On approach to their metabolic parameters, we found significant elevations of 2-hydroxyisobutyric acid on HPLC analysis in their urine samples. Propionate, methylmalonate and 3 hydroxyisobutyrate were detected within the normal reference ranges. This pattern probably indicates deficiencies of acyl-Co A dehydrogenases in the metabolism of leucine, isoleucine, valine, lysine, short-chain fatty acids or sarcosine. We propose to extend this study to a larger population as a predictor of risk among children of children born out of assisted reproductive technologies.

O-18-2

GLUCOSE TRANSPORTER 1 DEFICIENCY SYNDROME (GLUT1-DS); THE QUEENSLAND EXPERIENCE IN 8 PATIENTS

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Glucose Transporter 1 deficiency syndrome (GLUT1-DS, OMIM #606777) was first described by De Vivo in 1991. The diagnosis is suggested by hypoglycorrhachia (CSF glucose value <2.2 mmol/L), with a CSF: plasma glucose ratio generally < 0.4, and can be confirmed by performing erythrocyte 3-O-Methyl-D-Glucose (3-OMDG) uptake studies and GLUT1-DS genotyping. The gene (SLC2A1) has been mapped to chromosome 1p34.2. We describe a case series of eight patients whose manifesting symptoms included combinations of ataxia, developmental delay and severe seizure disorders that were refractory to anticonvulsant medications. Their ages at the time of diagnosis ranged from 4 months to 17 years of age. The CSF:glucose ratio ranged from 0.2 to 0.39 with a median of 0.33. The 3-OMDG assays were performed in 5 patients with a range from 41% to 68%. SLC2A1 genotyping was performed in 5 patients identifying the following mutations; 516–517insTTGAG, 517-531delCTGGGCAAGT, 771delC, R330X. Several polymorphisms and an intronic insertion were found in one patient, the pathogenic significance of these polymorphisms in children with strong clinical features of GLUT1-DS remains uncertain at this time. All children were treated with the classical ketogenic diet (KD), consisting of a 4:1 ratio of fat:non-fat diet. There was no progression of the observed ataxia, lower limb spasticity, movement dyspraxia, dystonia or clonus while compliance with the KD was maintained. Complete withdrawal from aintconsulvants has been possible in half the patients, with the remaining half experiencing a significant reduction in seizure frequency and quantity of anticonvulsants required.

O-18-3

IDENTIFICATION AND CLONING OF A NOVEL SPLICE VARIANT OF THE CREATINE TRANSPORTER GENE *SLC6A8* IN PRIMARY FIBROBLASTS

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Mutations in the X-linked creatine transporter gene (*SLC6A8, CTR1*) lead to cerebral creatine deficiency which results in mental retardation, speech and language delay, autistic-like behavior and epilepsy. Insights in the mechanisms how the transporter is regulated, such as regulatory proteins (e.g. splice variants), could be of importance for the development of successful treatment strategies of SLC6A8 deficiency. Until now, only one splice variant of *SLC6A8* with unknown function was identified several years ago, CTR2. Probably due to a mistake in the sequence analysis, the published CTR2 mRNA contains one base deletion, which leads to a frameshift and likely does not result in a functional protein. We thought to investigate whether this mRNA, without the deletion, could encode a functional protein, e.g. a regulator of CTR1. Therefore, we investigated whether CTR2 mRNA was present in primary fibroblasts from different non-*SLC6A8*-deficient subjects. To this end, RT-PCR was performed using specific CTR2 primers. Surprisingly, we did not find the expected CTR2 mRNA, instead we found a shorter mRNA which we named CTR4. This mRNA contains intron 4 of the CTR1 sequence and is subsequently spliced like the CTR1 mRNA until the 3′ untranslated region. Using an open reading frame (ORF) finder program, a shorter ORF 100% homologous to CTR1 was found, which predicts a truncated CTR1 protein, comprising only 5 transmembrane domains of the CTR1 transporter instead of 12. We cloned CTR4 as a fusion protein with EGFP and currently we are investigating its endogenous function and its putative effects on CTR1.

O-18-4

THE DIAGNOSTIC YIELD OF METABOLIC TESTING IN A TERTIARY CARE CENTRE FOR PATIENTS WITH MENTAL RETARDATION

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Objective: To determine the yield of additional metabolic testing in a tertiary care centre for patients with mental retardation (MR). **Methods**: A retrospective analysis in 478 patients. All had a 1 day work-up including MRI of the brain, evaluation by neurologist, clinical geneticist, pediatrician metabolic diseases, psychologist, physiotherapist and eye specialist. Before referral, initial metabolic screening was already performed. **Results**: Of 478 patients (♀ 213, ♂ 265) (0.6–17.6 years, median 4.6 years), 17 (3.6%) were diagnosed with a metabolic disease. Diagnoses were made on clinical suspicion despite initial screening (MPS-3 and CDG-1a), a different test than in former initial routine screening (X-linked creatine transporter deficiency), another medium than initial screening (hyperoxaluria), other biological material than initial screening (GLUT-1 deficiency, tetrahydrofolate reductase deficiency, tryptophan hydroxylase deficiency in CSF and respiratory chain disorders in muscle). **Conclusion**: Additional focussed metabolic testing yields a surprisingly high amount of metabolic diagnoses in a tertiary care centre after initial screening.

O-19-2

OUTCOME OF TYPE IIIA GLYCOGEN STORAGE DISEASE IN JAPAN

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Introduction: Outcome of type IIIa glycogen storage disease (GSD) is thought to be poor, because this disorder has both liver and muscle involvement. However, incidence of type (a GSD is quite low, few follow-up study on type (GSD are reported up until now. We report here long-term prognosis of 10 cases with type IIIa GSD whose diagnosis was confirmed by enzyme assay in Japan. **Patients and Methods**: Ten cases were diagnosed as having type IIIa GSD by measuring glycogen debranching enzyme activity, and DNA analysis was carried out in 2 cases. The age at diagnosis were ranged between 1 and 36 years, and frequent meals with starch, dextrin, and glucose was given to patients who were diagnosed in childhood. Physical, blood as well as electro-physiological examination were made periodically to evaluate long-term complications of type IIIa GSD. **Results and Discussion**: At diagnosis, massive hepatomegaly was seen in all cases with splenomegaly in elder cases. Liver cirrhosis with esophageal varices, muscle dysfunction, ventricular hypertrophy and cardiac failure occurred in all adult cases, and 4 cases died from liver cirrhosis and cardiac failure in fourth decade of life. Liver cirrhosis as well as cardiac muscle dysfunction must play important roles in poor prognosis of Japanese type IIIa GSD. In conclusion, further approach for treatment of GSD III is requested to improve outcome of type IIIa GSD.

O-19-1

MOLECULAR CHARACTERIZATION OF EGYPTIAN PATIENTS WITH GLYCOGEN STORAGE DISEASE TYPE IIIA

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Introduction: Glycogen storage disease type IIIa (GSD IIIa) is an autosomal recessive disorder characterized by an excessive accumulation of abnormal glycogen in the liver and muscles. It is caused by a deficiency in glycogen debranching enzyme (AGL). The spectrum of AGL mutations in GSD IIIa patients depends on ethnic groups. **Aim**: To shed light on molecular characteristics of GSD IIIa in Egypt, where high rate of consanguinity and large family size increase the frequency of recessive genetic diseases. **Patients and Methods**: Three Egyptian GSD IIIa patients from unrelated families were investigated. The patients were confirmed as having deficient AGL activity in peripheral red blood cells. All patients showed both liver and muscle involvement and consanguinity was ascertained in all families. Genomic DNA was isolated from leukocytes and the AGL gene was sequenced. Point mutations identified in patients were verified using restriction fragment length polymorphism. Twenty-three polymorphic markers in the AGL gene were genotyped for haplotype determination. **Results**: We identified three different individual AGL mutations; of these, two are novel deletions [4-bp deletion (750-753delAGAC) and 1-bp deletion (2673delT)] and one the nonsense mutation (W1327X) previously reported. All are predicted to lead to premature termination, which completely abolishes enzyme activity. Three consanguineous patients are homozygotes for their individual mutations. Haplotype analysis of mutant AGL alleles showed that each mutation was located on a different haplotype. **Conclusions**: Our results indicate the allelic heterogeneity of the AGL mutation in Egypt. This is the first report of AGL mutations in the Egyptian population.

O-19-3

MUSCULAR GLYCOGEN STORAGE DISEASE WITHOUT (OBVIOUS) STORAGE OF GLYCOGEN IN MUSCLE

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The muscular glycogen storage diseases (GSD) comprise a wide spectrum of metabolic diseases with defects in the pathway of glycolyses. Even within one subgroup of GSD there is a wide clinical and biochemical heterogenity. Because of the muscular glycogen storage, muscle biopsies are usually performed and the pathologist is inquired about the observation of glycogen storage in the specimen.

During the past 10 years in 5 of our patients, later diagnosed with either proven M. Pompe (GSD II, $n = 2$) or McArdle's disease (GSD V, $n = 3$), muscle biopsies were performed for etiological studies for neuromuscular diseases. In all of these patients biopsies did not reveal specific abnormalities. Non specific myopatic changes, including vacuolisation were seen, without glycogen storage in muscle cells. In only 1 patient additional EM investigation showed glycogen storage in muscle cells (M. Pompe).

It is concluded that muscle biopsy is of limited diagnostic value when a muscular glycogen storage disease is suspected. Even in cases with severe muscle weakness and elevation of CPK concentrations in serum up to 10 000 U/l no glycogen storage in muscle cells could be detected. It remains unclear why this discrepancy between the clinical and biochemical parameters and the observations at pathologic evaluation exists. Nevertheless it seems rational to exclude muscle biopsy from the diagnostic work-up of muscular glycogen storage diseases. Enzyme analysis in fibroblasts and/or DNA investigations are sensitive in diagnosing M. Pompe and a McArdle function test and enzyme analysis in muscle will be necessary in order to confirm or exclude McArdle's diseaese.

O-19-4

PRELIMINARY DATA ON A STARCH TO IMPROVE TREATMENT OF HEPATIC GLYCOGEN STORAGE DISEASES (GSD)

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Background: Cornstarch has been used as the mainstay of therapy in GSD since the early 1980's. However, potential problems with it exist: duration of normoglycaemia of less than 4 h, poor palatability, abdominal bloating, diarrhea, and malabsorption. **Objectives**: To see if a newly developed physically modified cornstarch (WMHM20) has a longer duration of action and is better utilized than cornstarch. **Methods**: A double-blind randomized trial with 21 patients (aged 3–49 years, 8 female) with GSD Ia (15), GSD Ib (4) and GSD III (2). Paired starch loads of cornstarch versus WMHM20 (2 g/kg) were given at an interval of greater than 3 days apart. Baseline preparation was similar before each starch load. Plasma glucose, lactate, insulin and breath hydrogen were monitored at baseline and hourly during the starch loads. The starch load terminated when the near-patient blood glucose was 3.0 mmol/L or less, if the participant had fasted for 10 h after starch administration or when the patient wished to do so. **Results**: Median duration of normoglycaemia was 9 h for WMHM20 versus 7 h for cornstarch, but only 6 patients taking WMHM20 ended the test with hypoglycaemia (9 patients with cornstarch). Glucose decreased slower ($p = 0.04$), lactate was suppressed at a faster rate ($p = 0.15$) and increased at a slower rate ($p = 0.17$) for WMHM20 versus cornstarch. Hydrogen excretion was significantly increased ($p = 0.05$) with cornstarch, with 8 subjects fulfilling criteria for malabsorption versus 2 for WMHM20. **Conclusion**: WMHM20 appears to have better efficacy with less malabsorption than cornstarch.

O-20-1

LONGITUDINAL AND CROSS-SECTIONAL ANALYSIS OF LANGUAGE IN CHILDREN WITH GALACTOSAEMIA

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32 children with galactosaemia have had regular, prospectively collected speech and language assessments, through a multidisciplinary clinic, since the introduction of newborn screening for galactosaemia 23 years ago. The cohort ages range from 15 months to 23 years. At 12 months of age, cross-sectional analysis showed all children with language development within normal limits. Language impairment was present in 11/23 at 2–3 years (4 severe, 2 moderate, 5 mild), 13/23 at 4–5 years (6 severe, 5 moderate, 2 mild), 13/19 at 7–8 years (8 severe, 3 moderate, 2 mild) and 10/15 at 10–12 years (5 severe, 3 moderate and 2 mild). There was a statistically significant correlation between both expressive and receptive language and IQ but not consistently for the cross-sectional ages tested. Both language and IQ declined over time in the longitudinal analysis. There were no significant differences between the various subsets of language development tested or between expressive and receptive language scores when compared at cross-sectional ages. There was no relationship between age at diagnosis, clinical severity at diagnosis, initial or subsequent galactose-1-phosphate concentrations. There was discordance for language development in both sets of siblings suggesting factors other than genotype to be important. These results highlight the need for close monitoring of language development in children with galactosaemia.

O-20-2

A SYNDROME OF GROWTH RETARDATION, DYSMORPHY AND EPISODES OF HYPERTHERMIA IN CHILDREN WITH A *COG7* MUTATION

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We describe a second family with a congenital disorder of glycosylation (CDG) due to a defect in COG7, one of the 8 subunits of the conserved oligomeric golgi (COG) complex (COG1-8). Two siblings presented with pre- and particularly postnatal growth retardation, dysmorphy (flat and narrow forehead, low-set ears, small mouth, retrognathia, short neck with loose wrinkled skin, adducted thumbs, simian creases and overlapping, long fingers), hypotonia, joint contractures, cardiac abnormalities (VSD and ASD II) and episodes of hyperthermia ($>41°C$). A combined disorder of N- and O-linked glycosylation with hyposialylation was detected. Western blot analysis of the oldest affected sibling showed a significant reduction in COG5 and COG7. COG6 and COG8 were decreased to a lesser extent. In both siblings a homozygous, intronic splice site mutation (c.169+4A>C) in *COG7* was identified. Disruption of the splice donor site and activation of a cryptic splice site lead to at least two different transcripts: one with a 19 basepair deletion and one with a 83 basepair insertion. The patients showed a similar phenotype except for a less pronounced liver involvement as the previously described CDG-IIe patients with the same mutation. **Conclusion**: COG defects are a growing subgroup of CDG. Any patient with an unexplained clinical syndrome and indication for a defect in N- and O-glycosyation should be checked for a defect in the COG complex.

O-20-3

SEDOHEPTULOSE AS MARKER FOR TRANSALDOLASE DEFICIENCY: QUANTITATIVE ANALYSIS OF HEPTULOSES IN URINE BY LC-MS/MS

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Transaldolase deficiency is a recently discovered disorder in the pentose phosphate pathway and is associated with liver dysfunction and a multi-organ involvement including kidney, hearth, cutis laxa and dysmorphia. In 6 patients with transaldolase deficiency we found a high amount of an unknown heptose sugar which we suspected to be sedoheptulose. Here, we present a method using liquid chromatography tandem mass spectrometry for quantitative measurement of the heptuloses sedoheptulose and mannoheptulose using a stable isotope labeled internal standard.

Urine samples were desalted by anion and cation exchange and an internal standard ($^{13}C_4$-erythritol) was added. Separation of the heptuloses was achieved by liquid chromatography using an Aminex HPX-87C column. Detection of sedoheptulose and mannoheptulose was carried out by negative ionization electrospray tandem mass spectrometry under multiple reaction monitoring conditions.

Age-related reference ranges for these heptuloses in urine were established. Patients with transaldolase deficiency where shown to have highly elevated urinary concentrations of sedoheptulose and slightly elevated concentrations of mannoheptulose. It was also shown that mannoheptulose is excreted in large amounts after eating avocado. This method could be used in the diagnosis of transaldolase deficiency and in elucidating the metabolism of heptuloses in humans, of which knowledge is very limited.

O-20-4

SCREENING NEWBORNS FOR GALACTOSEMIA USING A BREATH TEST

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Many inherited disorders of metabolism such as galactosemia produce irreversible damage to infants before mail-based screening of dried blood on filter paper is completed. We are developing a method for detecting newborns before nursery discharge using total body oxidation of ^{13}C-labeled substrates to $^{13}CO_2$ in expired air. 11 normal newborns were tested for $^{13}CO_2$ enrichment of expired air before and after oral administration of 7 mg/kg of ^{13}C-D-galactose. The ratios of $^{13}CO_2$ to $^{12}CO_2$ were quantitated by a dual isotope gas mass spectrometer at 0, 30, 45, 90, 100, 110 and 120 min and expressed as the cumulative percent dose recovered (CUMPD). The mean CUMPD of normal newborns at 2, 24 and 48 h of age was 4.31 ± 3.52, 5.74 ± 2.52, and 4.07 ± 2.19 respectively (p = NS). Their mean CUMPD values at 2 months of age had increased to 11.18 ± 4.83 ($p < -0.044$). Four children with classic galactosemia had a mean CUMPD of 0.69 ± 0.41. Their mean CUMPD compared to normal infants was significantly different ($p = 0.003$). We conclude that total body oxidation of galactose increases at least two fold in normal newborns between 2 and 60 days of life, that galactosemic children have significantly less oxidative capacity than normal newborns and that newborn breath testing before discharge from the nursery may be an effective tool for prediction and prevention of morbidity and mortality due to classic galactosemia.

O-20-5

GENERATION OF A CHIMERIC MOUSE LACKING GLYCEROL KINASE IN THE LIVER: UNDERSTANDING THE PATHOGENESIS OF GLYCEROL KINASE DEFICIENCY

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Glycerol kinase deficiency (GKD) is an X-linked defect of glycerol metabolism. The glycerol kinase (Gyk) knock-out (KO) mouse is a murine model of GKD that exhibits hyperglycerolemia, hypoglycemia, metabolic acidemia and death by 2–3 days of age. We hypothesize that hepatic Gyk activity is primarily responsible for the metabolic abnormalities in Gyk KO mice. To test this hypothesis, we created a chimeric mouse lacking Gyk in the liver, but with Gyk present in all other organs using fumarylacetoacetate hydrolase (FAH) KO mice. Gyk deficient hepatocytes were isolated from the livers of male Gyk KO mice pups. $3–8 \times 10^5$ hepatocytes were injected into spleens of FAH KO mice. To induce liver repopulation with Gyk deficient hepatocytes, the protective drug (NTBC) was withdrawn from recipient FAH mice. Non-transplanted FAH mice uniformly died within 4 months after drug withdrawal, but some transplanted mice survived. Two months after transplantation, the surviving mice were sacrificed and tissues were harvested. Hepatic Gyk activity was approximately 40% of non-transplanted FAH mice. However serum and urine glycerol concentrations were the same as non-transplanted FAH mice. These results suggest the Gyk deficient hepatocytes have a selective growth advantage in FAH mice livers and half of Gyk activity in liver is sufficient to maintain normal metabolism of glycerol. These results also suggest that the surviving mice had residual FAH^-/Gyk^+ hepatocytes. We are using an NTBC cycling protocol to improve survival and obtain mice with $< 5\%$ of normal hepatic Gyk activity equivalent to that in KO mice.

O-21-1

PREVENTION AND MANAGEMENT OF GENETIC DISORDERS IN THE MUSLIM WORLD

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We are at a time of unprecedented increase in knowledge of rapidly changing technology. Such biotechnology especially when it involves human subjects raises complex ethical, legal, social and religious issues. A WHO expert consultation concluded that 'genetics advances will only be acceptable if their application is carried out ethically, with due regard to autonomy, justice, education and the beliefs and resources of each nation and community"

Public health authorities are increasingly concerned by the high rate of births with genetic disorders especially in developing countries where Muslims are a majority. Therefore it is imperative to scrutinize the available methods of prevention and management of genetic disorders.

Islam is a religion which encompasses the secular with the spiritual, the mundane with the celestial and hence forms the basis of the ethical, moral and even juridical attitudes and laws towards any problem or situation.

Islamic teachings carry a great deal of instructions for health promotion and disease prevention including hereditary and genetic disorders, therefore we will discuss how these teachings play an important role in the diagnostic, management and preventive measures including: genomic research; population genetic screening, including premarital screening, pre-implantation genetic diagnosis; assisted reproduction technology; stem cell therapy and genetic counselling.

O-21-2

PRE-IMPLANTATION GENETIC DIAGNOSIS (PGD) FOR GENETIC DISORDERS IN SAUDI ARABIA

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Saudi Arabian culture is highly consanguineous, with the first cousin marriages accounting for 60–70% of all marriages. Given the difficulties in management of genetic disorders, preventive measures for the suffering families from autosomal recessive disorders by doing pre-implantation genetic diagnosis is undertaken. Almost 20 cases are successfully prevented by PGD. The first of these disorders is Sanjad-Sakati Syndrome (SSS) OMIM# 24140, which is characterized by congenital hypoparathyroidism, growth and mental retardation with a unique 12 bp deletion. The second is Niemann Pick Disease type B (NPD-B) OMIM# 257200,) (acid sphingomylinase (ASM) deficiency) with more than 70 mutations have been reported in (SMPD1) gene, which presents with severe phenotype in Saudi Arabia. Four unique mutations are found in our Saudi families. A family with (W533R) mutation in the (SPMDI) gene suffering from a severe phenotype underwent PGD. The third disorder is Morquio's disease (MPSIV) OMIM# 253000, with severe classic phenotype with N-acetyl galactosamine-6-sulftase deficiency (MPSIV-A). More than 20 different mutations in (GALNS) gene have been reported in (MPSIV-A). A family with three affected siblings with severe classic (MPIV-A) with detected W195C mutation in the (GALNS) gene underwent PGD. In all these three families PGD was undertaken using fluorescent PCR(F-PCR) and/or nested PCR with sequencing on a single cell. A singleton pregnancy ensure after transfer of one heterozygous and one normal embryo and prenatal diagnosis by CVS confirmed a normal pregnancy. This is the first report of successful PGD in different genetic disorders in Saudi Arabia.

O-21-3

INBORN ERRORS OF METABOLISM IN THE NETHERLANDS: A CLINICAL REGISTRY

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Objective: To facilitate research on incidence, natural course and treatment of inborn errors of metabolism in the Netherlands in order to improve quality of life and long-term outcome for patients with inborn errors. **Methods**: Retrospective registry and since 2002 prospective registry of all patients diagnosed in all clinical metabolic centers. Metabolic diagnosis has to be confirmed, depending on the disease by metabolites, enzymatic studies or mutation analysis. **Results**: At present, more then 2000 patients are registered with 180 different diseases, including phenylketonurie (PKU), which is currently the only metabolic disease detected by neonatal screening in the Netherlands. Since there is underregistration, the registry offers the minimal incidence of inborn errors of metabolism in our country. The overall incidence is 40 per 100 000 live births with PKU 27.5%, respiratory chain disorders 13%, fatty acid oxidation disorders 10.5%, glycogen storage diseases 7.5%, galactosemia 6%, organic aciduria 4%, mucopolysaccharidosis 3%, homocystinuria 3% urea cycle disorders 2%, congenital disorders of glycosylation 1.5%, other disorders 22%. **Conclusion**: Our registry provides a minimal estimate of inborn errors of metabolism incidence in the Netherlands and is a useful tool for further research.

O-21-4

AN ANALYSIS OF TREATMENT EFFICACY IN INBORN ERRORS OF METABOLISM

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Objective: In the past years, much progress has been made in understanding inborn errors of metabolism and in designing treatments for these diseases. In order to measure these progresses, 65 randomly selected inborn errors of metabolism were analyzed in 1983 and 1993 in terms of their phenotype and their response to treatment. We once again engaged in the task of analyzing the response to treatment of these 65 inborn errors of metabolism. **Method**: Diseases were scored in a database according to 7 phenotypic parameters. We used the medical literature found in OMIM, OMMBID, and over 250 recent publications. **Results**: We observed significant improvements in the response to treatment in these disorders. The proportion of conditions for which there is no response to treatment progressively decreased; from 48% in 1983, to 31% in 1993, to 29% in 2006. Concomitantly, there was an increase in the proportion of conditions which fully respond to treatment; from 12% in 1983 and 1993, to 17% in 2006. The conditions for which there was a partial response also respond better to the treatments. **Conclusions**: Larger clinical studies and registries now permit a better assessment of the natural history and the response to treatment for rare inborn errors of metabolism. Reasons for improvements in treatment response include new small molecules, new enzyme replacement therapies, and further disorders treated by liver or bone marrow transplantation. We believe our analysis constitutes a robust and reliable assessment of treatment efficacy in inborn errors of metabolism.

O-22-1

BONE MINERAL DENSITY IN TYPE 1 GAUCHER DISEASE: PREVALENCE OF FRACTURES AND EFFECT OF TREATMENT WITH IMIGLUCERASE

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We determined the effect of enzyme replacement therapy (ERT; Cerezyme, imiglucerase) on bone mineral density (BMD) in type 1 Gaucher disease (GD). The GD population included adults (males 18–70 years, females 18–50 years) enrolled in the International Collaborative Gaucher Group (ICGG) Gaucher Registry with lumbar spine BMD measurements. BMD data with up to 8 years of follow-up were analyzed for 160 patients not on ERT (untreated) and 342 patients treated with ERT. BMD was assessed by dual energy X-ray absorptiometry (DXA) of the lumbar spine. Z-scores were compared to a reference population. DXA Z-scores for GD patients in the untreated group were significantly below normal (y intercept = –0.80 Z-score units, $p < 0.001$) and remained approximately one standard deviation below the reference population over time (slope = –0.010 Z-score units per year, $p = 0.68$). DXA Z-scores for GD patients on ERT (dose of 60 U/kg/2 week) were significantly lower than the reference population at baseline (y-intercept = –1.17 Z-score units, $p < 0.001$), but improved significantly over time (slope= +0.131 Z-score units per year, $p < 0.001$). A significant dose-response relationship was noted for the ERT group, with the slopes for the three main dosing groups of 15 U/kg/2 week, 30 U/kg/2 week, and 60 U/kg/2 week of +0.088, +0.109, and +0.131, Z-score units per year, respectively. The BMD of ERT-treated GD patients increased to within 0.5 standard deviations of the mean of the reference population after approximately 8 years of ERT at the reference dose (60 U/kg/2wk). ERT with imiglucerase significantly improves BMD in patients with GD.

O-22-2

THE SUCCESSFUL TREATMENT OF SOME TYPE I GAUCHER ADULT PATIENTS WITH VERY LOW DOSE ENZYME REPLACEMENT THERAPY

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Enzyme replacement therapy is now well established as the treatment of choice in Type I Gaucher disease. While higher rather than lower dose regimens are likely to be more clinically effective the extraordinary cost of the therapy means there are economical benefits of the later.

Twelve type I adult patients were commenced on the very low dose of 7.5 units of alglucerase/imiglucerase per kilogram per fortnight. Follow-up five year data reveal a good visceral and haematological response with outcomes consistent with recently published guidelines. The liver size decreased on average by 21% during the first 1–2 years of treatment and by 39% by year 3–5. By years 2–5 the average decrease in spleen size was 57% (range 37–77%). All patients reduced and maintained their spleen volume to <2–8 times normal.

Satisfactory clinical and radiological skeletal improvement was also demonstrated in most patients. Three patients had an inadequate overall skeletal response to therapy and will require a higher treatment dose. Biomarkers also steadily improved although perhaps not quite at the same rate as that seen in higher doses.

Very low dose enzyme replacement therapy may be appropriate for adult type I Gaucher patients with mild skeletal disease. Close clinical, biochemical and radiological monitoring is essential and the dose should be increased if a poor response becomes apparent.

O-22-3

GENOTYPE/PHENOTYPE CORRELATION AND THE OUTCOME OF ENZYME REPLACEMENT THERAPY IN JAPANESE TYPE 3 GAUCHER PATIENTS

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Objective: Correlation between genotype and phenotype in Gaucher disease is often discussed. However, compared to type 1, data on the correlation in neuronopathic form is limited because of the small number of patients. In our study, we have analyzed the genotype/phenotype correlation and the outcomes of enzyme replacement therapy (ERT) in Japanese type 3 patients. **Methods**: Among the 124 Gaucher patients who have been diagnosed at our institute, clinical and laboratory parameters before/after ERT were analyzed for 42 type 3 patients from the medical charts and the questionnaires. Genotypes were detected by PCR, SSCP, and direct sequencing. **Results**: Of 42 patients, 16 patients whose diagnosis at onset was type 1 developed neurological symptoms, and their diagnosis were ultimately changed to type 3. L444P/L444P was the most common genotype in this group. The initial neurological symptoms were diverse, but laryngeal spasms were especially seen in many type 1→3 patients. **Conclusion**: Some patients developed neurological symptoms during the course of treatment, even when they were initially diagnosed as having type 1 Gaucher disease. Our study showed that homozygote or compound heterozygote with L444P and F213I were frequently seen in this group of patients. These genotypes are not specific in type 3 but seem to have a high prevalence, suggesting that a careful observation is required even when the initial diagnosis was type 1. As in other cases, ERT may not be effective for neurological symptoms.

O-22-4

PRODUCTION AND CHARACTERIZATION OF CYSTEINE VARIANTS OF GLUCOCEREBROSIDASE

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Deficiency of the lysosomal enzyme β-glucocerebrosidase results the accumulation of glucocerebroside in the lysosome leading to Gaucher disease. β-Glucocerebrosidase contains seven cysteines, arranged as two intra-chain disulfide bonds and three free residues. While the cysteine residues have been implicated in enzyme activity, they are not catalytic residues and the published crystal structure of β-glucocerebrosidase offers no insight into their role in the structure or function. We therefore, chemically and genetically modified the free cysteine residues to better understand the role of these residues in the molecule. Incubation of β-glucocerebrosidase at neutral pH or above resulted in rapid loss of activity that correlated with the generation of protein aggregates and was associated with loss of reactive thiols. Chemical modification of the free cysteine residues also resulted in loss of activity but prevented aggregation at neutral pH, suggesting two distinct mechanisms for loss of activity. To elucidate the role of each of the three free cysteine residues in the activity and stability of β-glucocerebrosidase, we generated a series of cysteine mutants replacing one, two or all three cysteine residues. Cysteine residues could be replaced with full retention of enzymatic activity. This allowed us to identify modified forms of β-glucocerebrosidase with retained enzymatic activity, improved neutral pH stability, and improved resistance to oxidation. These β-glucocerebrosidase variants may allow development of extended delivery formulations to reduce the frequency of enzyme replacement therapy.

O-23-1

ENZYME REPLACEMENT THERAPY FOR MPS VI WITH RECOMBINANT HUMAN N-ACETYLCALACTOSAMINE 4-SULPHATASE (rhASB) FROM 8 WEEKS OF AGE – A SIBLING CONTROL STUDY

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A male infant, the sibling of an affected girl, had the diagnosis of MPS VI confirmed by testing in the neonatal period by enzyme analysis of N-acetylgalactosamine 4-sulphatase in leukocytes and genotype (T69M/H393P compound heterozygote). Both siblings were started on intravenous therapy with rhASB, 1 mg/kg weekly, at 8 weeks and 3.5 years respectively. The male infant has not developed hepato-splenomegaly, has full range of movements of joints and has good growth. He has developed skeletal changes including pectus excavatum, rib flaring and an early gibbus, has mild corneal clouding and minimal mitral valve dysplasia. The older sibling has improved scoliosis (she was due to have rods inserted prior to starting therapy), reduction of hepatosplenomegaly and improved joint mobility since starting the enzyme. Both siblings have shown a significant reduction in urinary glycosaminoglycans (175 gm/mol down to 26 gm/mol in the male). There have been no safety issues. The infusions are given via an implanted vascular access port over 4 h. Approaching 3 years of age, the major benefit of the early commencement of therapy is the much better range of movement of joints, improved growth and the lack of scoliosis compared to his sister at a similar age.

O-23-2

EXPERIENCE WITH LARONIDASE TREATMENT IN 5 MPS I PATIENTS WITH VARIOUS PHENOTYPES AND INDICATIONS

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Patients: patients 1, 2 and 3 (14, 11, and 3 years old) presented with an attenuated Scheie phenotype. Patient 1, suffered visual loss due to optic nerve compression. Patient 4, a 2 years old boy with a severe Hurler phenotype presented with an impaired psychomotor development. ERT has been proposed while waiting for a bone marrow transplantation (BMT). Patient 5, a 15 years old, Hurler patient underwent successful BMT at age of 5, but displayed a severe progressive pulmonary disease with life threatening pulmonary hypertension and poor general condition. **Methods**: All patients have been treated by a weekly infusion of laronidase. Clinical outcome was assessed according a detailed protocol. **Results**: At this stage of treatment (30, 26 and 21 months for patients 1, 2 and 3; 9 months for patients 4 and 5), no adverse effects occurred. Urinary GAG levels were high and decreased for all patients except for patient 5 who had low GAG before treatment. Patients 1, 2 and 3 showed improved joint mobility and better exertion tolerance. Eyesight improved dramatically for patient 1. Patient 4 continued to decline neurologically. Patient 5 showed a better joint mobility and was able to stand and walk. Pulmonary hypertension improved. **Conclusion**: our data are in line with the reported efficacy of ERT in patients with the mild (Scheie) phenotype. Further follow-up is needed for assessment of ERT in the severe Hurler patients with neurological impairment and in transplanted patients with progressive symptoms in which ERT may have a positive effect.

O-23-3

RESTRICTION OF UPPER EXTREMITY RANGE OF MOTION (ROM) IN MPS I: NO RESPONSE TO 1 YEAR OF ENZYME REPLACEMENT THERAPY DESPITE IMPROVEMENT IN COMPLEX MOTOR FUNCTIONS

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In patients with the attenuated phenotype of Mucopolysaccharidosis type I (Hurler-Scheie and Scheie phenotype), quality of life is severely jeopardized by progressive joint stiffness with restriction of the range of motion of large joints. Previous studies have shown that Enzyme Replacement Therapy (ERT) with recombinant alpha-L-iduronidase (Aldurazyme®) can improve some of the clinical manifestions of the disease, such as respiratory function, physical capacity, hepatosplenomegaly and motor movement as assessed by goniometry. However, no improvement in ROM, again assessed by goniometry was observed in a double blinded, placebo controlled study.

To determine the effect of ERT on motor movement, we performed a longitudinal study on the efficacy of 12 months of ERT on the upper extremity ROM in six patients with the attenuated phenotype of MPS I, using three-dimensional video analysis, with blinded off line processing of the data. 3-D video analysis was used to allow for unrestricted movements, to avoid bias and to compensate for compensatory trunk movements. Six different movements were studied: shoulder abduction and anteflexion, elbow flexion and extension and pro- en supination.

No improvement in any of the studied ROMs was observed during the study. However, all patients reported improved suppleness of joint movements. In addition, some of the patients showed improvement in more complex motor functions, such as hair combing, after 12 months of ERT, significantly improving the quality of life. ERT in MPS I may stop progression of the restriction of ROM. Early initiation of therapy, before irreversible damage has occurred, may therefore be important.

O-23-4

LARONIDASE TREATMENT IN MUCOPOLYSACCHARIDOSIS I (MPS I) PATIENTS LESS THAN 5 YEARS OLD

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Objective: To evaluate the safety and efficacy of enzyme replacement therapy in young and severely affected children with MPS I. **Methods**: A 52-week, open-label study of laronidase was performed in 20 MPS I patients (16 Hurler, 4 Hurler-Scheie; mean age 2.9 year, range 0.5–5.1) All patients initially received 100 U/kg (0.58 mg/kg) laronidase IV weekly; 4 patients had the dose increased to 200 U/kg during the study. **Results**: Laronidase was well-tolerated at both doses. Two patients died of causes unrelated to laronidase. Seven patients (35%) experienced 33 infusion-associated reactions: most were mild and easily managed, and the highest patient incidences were pyrexia ($n = 6$) and chills ($n = 4$). One patient experienced 3 laronidase-related serious adverse events (none severe). All patients developed IgG antibodies to laronidase. Urinary GAG levels declined rapidly and were reduced by 61.3% at week 52. Liver volume normalized in 50% of patients and the liver edge was reduced by 69.6% in the 10 patients with palpable livers at Week 52. The proportion of patients with left ventricular hypertrophy decreased from 50% to 175%. Seven patients showed increased growth velocity Z-scores. Sleep studies were improved or stable in 67% (10/15) of evaluable patients. Cognitive function improved in the Hurler-Scheie and younger (<2.5 year) Hurler patients. Investigators reported overall clinical status as improved in 94% (17/18) of patients. More robust GAG reduction occurred in patients with low antibody titers and those treated at the higher dose. **Conclusions**: Laronidase is safe and clinically beneficial in young, severely affected MPS I patients.

O-24-1

CHARECTERISTICS OF 9 CHINESE CASES WITH CHILDHOOD ATAXIA WITH CENTRAL NERVOUS SYSTEM HYPOMYELINATION/ VANISHING WHITE MATTER

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We report the 9 Chinese patients with childhood ataxia with central nervous system hypomyelination/vanishing white matter (CACH/VWM) here. The onset of the disease occurred between 6 months to 3 years of age. Family history was positive in 5 cases. Almost all cases had normal psychomotor development before the onset of the disease. The initial symptom was usually movement disorder with predominant involvement of lower limbs. The onset or deterioration of the disease followed respiratory tract infection in 6 cases and followed minor head trauma in 3 cases. The course was progressive in 7 cases and with episodic deterioration in 4 cases. Mental abilities were relatively better preserved. Head circumference was normal in 7 cases. Positive upper motor unit signs were found in 8 cases and ataxia in 4 cases. Optic atrophy was found in 3 cases. MRI indicated the diffuse and symmetrical involvement of deep white matter which showed long T1 and T2 signal. Subcortical white matter was also involved with predominance in frontal and parietal lobes. FLAIR image showed symmetrical high signal intensity in cerebral white matter with low signal intensity even similar to CSF in partial area or low signal in most area of white matter with only meshwork of higher signal preserved. All the laboratory tests including the enzyme and biochemical test specific for some well-known leukoencephalopathy were normal. The clinical characteristics of Chinese CACH/VWM patients are consistent with the cases reported previously in other places. The genetic analysis of these patients is ongoing.

O-24-2

GFAP MUTATIONS IN 3 CHINESE INFANTILE ALEXANDER DISEASE PATIENTS

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Alexander disease is a rare fatal disorder of the central nervous system, causes progressive loss of motor and mental function. Since 2001, it has been shown that almost all cases of Alexander disease have a dominant mutation in one allele of the gene for glial fibrillary acidic protein (GFAP) that causes replacement of one amino acid for another. Only in very rare cases of the adult-onset form is the mutation present in either parent. We searched for GFAP mutations in 3 patients who had typical clinical symptoms of infantile Alexander disease (macrocephaly, psychomotor delay and later regression, spastic paraparesis, etc.) and characteristic cranial MRI abnormalities (extensive cerebral white matter changes with frontal predominance, etc.). Two type missense, heterozygous, de novo GFAP mutations were found in exon 1 for 3 suspected patients. Two patients carried the same arginine mutations (R88C), and one had tyrosine mutation (Y83H) which has not been reported yet according to our knowledge. These results suggested that Exon1 mutations of GFAP may be the 'hot spot' exon for Chinese infantile Alexander disease. That is similar with the results from USA. We will collect more Chinese patients to figure out the spectrum of the mutations for GFAP that will facilitate the molecular diagnosis of Alexander disease in China.

O-24-3

INITIALLY-NORMAL PLASMA CYSTINE IN A NEONATE WITH SEVERE SULFITE OXIDASE DEFICIENCY: A PITFALL IN DIAGNOSIS

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A term male presented at birth with progressive encephalopathy and lactic acidemia. Sulfite oxidase (SO) deficiency was considered, but the plasma amino acid profile at 2 days of age was unremarkable: specifically plasma cystine (32 μmol/L) was within reference range (26–71). Since plasma cystine has been reported to be very low or undetectable in all cases of SO deficiency, this normal result decreased suspicion. Priority was then given to other lines of investigation: all negative. The patient died at 15 days. Sample analysis was completed retrospectively. Plasma from day 9 showed undetectable cystine. Total homocystine was undetectable in plasma from day 2. Urine sulfocysteine was markedly elevated (days 2 and 10). These findings, with normal plasma uric acid and normal urine xanthine and hypoxanthine, implied isolated SO deficiency. SO enzyme activity was undetectable in fibroblasts. Two unambiguous null mutations were identified in the *SUOX* gene. The reason for the initially-normal plasma cystine appeared to be biological not iatrogenic: the infant was not on formula, TPN or any medications at that time. We hypothesise that there was residual cystine from transplacental transport. Early testing for SO deficiency is challenging: urine sulfite dipsticks suffer many false-negatives and false-positives, rapid urine sulfocysteine assay is not widely available; and we now show that routine plasma amino acid analysis may be uninformative in the first few days of life. Plasma homocystine may be depleted by reaction with sulfite more rapidly than is cystine: making assay of total homocystine a crucial first-line test for SO deficiency.

O-24-4

MATURATION ERRORS OF DYSTROPHIN PRE-mRNAS IN 26 CASES WITH MUTATIONS IN DYSTROPHIN GENE

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Splicing is the maturation process of pre-mRNA by which introns are removed and mRNA production is completed. Although mutations at splicing consensus sequences located at intron/exon boundaries have been reported to induce splicing errors, it is possible that other intronic or exonic mutations will result in abnormal splicing. Dystrophinopathy, the most common inherited myopathy, is caused by mutations in the dystrophin gene. So far, we have analyzed the dystrophin gene mutations of 358 dystrophinopathy cases, and 26 genomic mutations inducing splicing errors are detected. In 13 cases, single nucleotide substitutions were detected at splicing consensus sequence, in which 6 mutations induced the exon skipping, 6 mutations activated the cryptic splice sites, and both splicing errors were observed in one case. Seven nonsense mutations resulted in splicing errors, in which 5 mutations induced the exon skipping and the other two mutations induced both the exon skipping and the creation of new splice site. In two cases, single nucleotide changes deep in the intron sequence led to the formation of extra exons by creation of new splice sites, one in intron 2 and the other in intron 62. Exon skipping was also detected in four frame shift mutation cases, which included single nucleotide deletion of exon 27, 4-nucleotide deletion of exon 38, 52-nucleotide deletion of exon 19, and 608-nucleotide insertion of exon 44. Detail analysis of these cases makes it possible to clarify the function of exonic and intronic sequences for the pre-mRNA maturation mechanism.

O-24-5

UNVERRICHT-LUNDBORG DISEASE WITH SHORTER REPEATS EXPANSION DIAGNOSED BY RT-PCR

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Cystatin B (CSTB; also called stefin B) is a small protein that is a member of the superfamily of cysteine protease inhibitors, and its role is thought to be as a protector against the proteinases leaking from lysosomes. The Unverricht-Lundborg type of progressive myoclonus epilepsy (EPM1) is characterized by autosomal-recessive, stimulus-sensitive myoclonus, generalized tonic-clonic seizures, slowly progressive cerebellar ataxia and dementia. In EPM1 patients, the CSTB mRNA level was reduced in various tissues. The majority of these patients have an unstable 600–900 bp insertion in the 5′ untranslated region (5′-UTR) of the CSTB gene, consisting of large repetitive GC-rich dodecamer units. The number of repeats is usually more than 30, compared to 2–3 in normal people, and amplification of the dodecamer unit expansion is extremely difficult with conventional PCR method. These characters render gene diagnosis difficult, especially in cases with shorter expansions.

Here, we report a case of a 44-year-old Japanese male with EPM1. He had suffered from severe myoclonus since the age of 11 years. In a genetic study, PCR amplification of the 5′ untranslated region of CSTB was unsuccessful. However, a minor modification to Southern blotting protocol enabled us the analysis of a shorter dodecamer repeat expansion of exon 1 of the CSTB gene, and quantitative real time-PCR (qRT-PCR) successfully detected the lower level of CSTB gene expression in white blood cell. Although, this expansion appeared to be shorter than those observed in Caucasian EPM1 patients, these data demonstrate that qRT-PCR is suitable for the diagnosis of EPM1 with shorter expansions.

O-25-1

MUTATIONS IN THE REGULATORY DOMAIN OF PHENYLALANINE HYDROXYLASE (PAH) AND RESPONSE TO TETRHYDROBIOPTERIN (BH_4)

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Tetrahydrobiopterin (BH_4) responsive phenylketonuria (PKU) was studied and was reported earlier on 15 different mutations. This report deals with mutations in the regulatory domain of PAH, amino acids 1-142. We have identified 9 mutations in this domain with favorable response to BH_4. Eight are point mutations and one IVS mutation. Each of the patients had an allele in the regulatory domain. Patients were given oral BH_4, 10 mg per kg once daily. The response of individual patients was reduction in blood phe concentration of 23.2% to 72.3% from the baseline phe level. Patients had genotypes F39L/R408W, F39L/281L and R68S/R408W, and were thought to be classical PKU, when challenged with BH_4, blood phe level decreased by 44.3% to 58.4%. Review of the Phenylalanine Hydroxylase Locus Knowledgebase (PHAdb) revealed that the majority of mutations in the regulatory domain confer mild phenotype, suggesting that mutations in the regulatory domain are good candidates for BH_4 responsiveness.

O-25-2

BH_4 RESPONSIVE PHENYLKETONURIA IN KOREA

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Of the 108 patients who were diagnosed with and treated for classic PKU in the Department of Pediatrics, Soonchunhyang University Hospital from 1999 to 2005, 10 patients were BH_4 responsive PKU. Among these 10 patients, 6 patients were male and 4 patients were female.s On BH_4 loading tests, opposed to the fact that the phenylalanine level decreased to nearly normal range on PTPS (6-pyruvoyl-tetrahydropterin synthase) deficiency patients, that of BH_4 responsive classic PKU patients were decreased to 40.6% after 8 h. Four patients were treated only with BH_4 whereas the other 6 patients were treated with BH_4 and phenylalanine free milk. The gene mutations of these patients were as following: R241C/A259T (3 patients), R241C/T278I (1 patient), Y356X/R408Q (1 patient), R53H/R243Q (1 patient), and R241C/R243Q (2 patients).

As we report, many of the classic phenylketonuria patients respond to BH_4 loading test. Therefore, all patients that were detected on the neonatal PKU screening test, must be challenged by BH_4. Instead of the phenylalanine-restrict diet, BH_4 treatment would be expected to improve their quality of life substantially in BH_4 responsive PKU patients.

O-25-3

TETRAHYDROBIOPTERIN (BH_4) RESPONSIVE HYPERPHENYLALANINEMIA WITHOUT BH_4 DEFICIENCY IN JAPAN OVER THE PAST 10 YEARS

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Background: Between 1995 and 2001 twelve patients with mild PKU responsive to BH_4 were identified by neonatal PKU screening and treated with tetrahydrobiopterin (BH_4) in Japan. Half of these patients were treated with BH_4 alone without diet therapy. **Object**: To diagnose patients with BH_4-responsive HPA without BH_4 deficiency, we evaluated the responses of BH_4 loading in patients with HPA detected by neonatal PKU screening between 2002 and 2005. **Methods**: In all of the 83 patients with HPA detected by neonatal PKU screening, we examined biopterin metabolism by pteridine analysis, dihydropteridine reductase assay and single-dose BH_4 loading test. Four-dose, and 1-week BH_4 loading tests were conducted in patients who had normal BH_4 metabolism and decreases in plasma phenylalanine concentrations by over 20% in the single-dose test. **Results**: Among 83 patients, sixteen patients had normal biopterin metabolism, and their mean values of percentage decline in serum phenylalanine from initial values were 43, 43, 52 after single-dose, four-dose, and 1-week BH_4 loading tests, respectively. **Conclusions**: Sixteen patients with BH_4-responsive HPA without BH_4 deficiency were newly diagnosed between 2002 and 2005. A total of 28 patients were detected in the past 10 years and the incidence was about 20% of HPA in neonatal PKU screening in Japan.

O-25-4

SUCCESSFUL PREGNANCIES AFTER LOW PHENYLALANINE LEVELS IMPROVED SPERMATOGENESIS IN 2 MEN WITH PKU

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Aim: To describe the changes in sperm morphology and motility after institution of a low-phenylalanine diet in 3 infertile men with PKU. **Cases**: Case 1, born in 1961, was diagnosed with PKU at 2 years of age. Dietary treatment was poorly tolerated and ceased at 14 years. He has borderline intellectual disability. Infertility was recognized in 1998. Case 2, born in 1961, diagnosed at 11 months and treated with PKU diet until 11 years. Infertility recognized in 1992 but unable to cope with low phenylalanine diet at that time. Several attempts as assisted reproduction (*in vitro* fertilization) were unsuccessful. Case 3 was born in 1960 and diagnosed in infancy. He was on a low phenylalanine diet until 7 years. Infertility was recognized in 1995. **Methods**: Phenylalanine was quantitated by amino acid analyzer or tandem mass spectrometer according to standard methods. Sperm analysis was performed in specialized laboratories with total sperm count, morphology and motility recorded according to WHO strict criteria. **Results**: Cases 1 and 2 were able to achieve low phenylalanine levels (300–500) on the PKU diet, with gradual improvement in sperm number, motility and quality over 3 months. Both their wives conceived (at ages 26 and 39 years). Case 3 was not able to manage the diet and adopted a child. **Conclusion**: Male infertility has not been recognized as a long-term issue in males with PKU. These results suggest that the effects of high phenylalanine on sperm morphology are reversible.

O-25-5

EFFECT OF BH_4 ON SELECTIVE ENDOTHELIAL DYSFUNCTION OF CORONARY ARTERIES INDUCED BY CARDIOPULMONARY BYPASS

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We hypothesized that cardiopulmonary bypass induces a selective alteration of the coronary arterial endothelial cell signal transduction which could be explained by a state of depletion and/or decreased activity of endogenous tetrahydrobiopterin (BH_4). The aim of this study was to assess the effects of cardiopulmonary bypass and BH_4 on the endothelial function of epicardial coronary arteries in a swine model of cardiopulmonary bypass. Swine underwent 90 min of cardiopulmonary bypass with (n = 9) or without (n = 19) brief cardioplegic arrest $\pm$ *in vivo* BH_4 administration, followed by a 60 min period following weaning from cardiopulmonary bypass and were compared to a control group (n = 7). Endothelium-dependent relaxations of epicardial coronary artery rings were studied using standard organ chamber experiments in the presence or absence of *in vitro* BH_4 or superoxide dismutase (SOD) and catalase. Cardiopulmonary bypass caused a statistically significant reduction of endothelium-dependent relaxations to serotonin ($p<.0001$), bradykinin ($p<.001$), UK14304 ($p<.0001$) and calcium ionophore ($p<.01$) in epicardial porcine coronary arteries. *In vitro* and *in vivo* BH_4 supplementation improved endothelium-dependent relaxations to serotonin and bradykinin, which were left unchanged by SOD-catalase administration. Cardiopulmonary bypass was associated with a decrease in nitric oxide availability (p = .002) and increased oxidative stress ($p<.001$), which were both restored by *in vivo* BH_4 administration ($p<.001$). Treatment with BH_4 improves the endothelial dysfunction of porcine epicardial coronary arteries, restores nitric oxide availability and reduces the oxidative stress associated with cardiopulmonary bypass.

O-26-1

PRACTICAL ADVANTAGES OF ROUTINE USE OF BRAIN MAGNETIC RESONANCE SPECTROSCOPY IN PATIENTS WITH PHENYLKETONURIA

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Magnetic resonance spectroscopy was described as a method for assessment of brain phenylalanine concentrations. However, many researchers failed to detect the brain phenylalanine signal in their patients with phenylketonuria (PKU) although theoretical recommendations for this technique had been published previously. Therefore we aimed to define the practical value of routine use of the above method. **Methods**: We used a 1.5 T magnetic resonance scanner (PRESS technique, examination of the area of 25 cm^3 of brain, H_2O signal suppression, relaxation/echo time 1500/30 m/s, 512 acquisitions – as recommended). 104 PKU-patients and 10 healthy controls were examined. We measured the brain phenylalanine signal intensity (BrainPhe) as the ratio: phenylalanine peak integral / the sum of integrals of the major peaks of the spectrum. Each examination was performed twice. Blood phenylalanine concentration (BloodPhe) was assessed parallelly in participants. **Results**: BrainPhe in the majority of patients in whom BloodPhe was below 1.2 mmol/l did not exceed the background signal intensity observed in the spectra acquired in healthy volunteers. However, in patients with BloodPhe >1.2 mmol/l the signal was detectable and its intensity was proportional to the BloodPhe. The repeatability of two subsequent measurements of BrainPhe in the same patient varied by up to 20%. **Conclusion**: Detection of the brain phenylalanine signal in PKU patients with blood phenylalanine >1.2 mmol/l is possible and informative. On contrary the possibility for exact calculation of the molar brain phenylalanine concentration seems to be questionable in spite of low repeatability of spectroscopic spectra acquired.

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O-26-2

EVALUATION OF PLASMA LEVEL OF VITAMIN A, E, COENZYME Q10 AND OX-LDL TO THE PARAMETERS OF LIPID METABOLISM IN POLISH CHILDREN WITH PHENYLKETONURIA (Preliminary Report)

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Phenylketonuria (PKU) is a congenital metabolic disease of autosomal recessive inheritance, resulting from deficiency of a hepatic enzyme phenylalanine hydroxylase (PAH). The low-phenylalanine diet caused the reductions of total cholesterol (t-chol), LDL- and VLD-cholesterol, beta-lipoprotein in PKU patients. The value of HDL-cholesterol is within the norm, while the level of triglycerides is elevated. A question arises whether lipid metabolism disorders lead to the increase in lipid peroxides and LDL (ox-LDL) peroxidation products in PKU, and if there is a correlation between lipid metabolism parameters and the levels of antioxidants: coenzyme Q10, vitamins E and A.

The study involved 42 PKU children aged 5–12 years, referred to consultation in the Metabolic Out-Patient Department from 6 cities in Poland. Healthy children (n = 24) were the controls. Serum total cholesterol, triglicerides,HDL-cholesterol were measured with with enzymatic methods. LDL-cholesterol was calculated according to Friedewald's formula and ox - LDL by enzyme immunoassay. Retinol, tocopherol and coenzyme Q10 were determined by chromatography system (HPLC).

Our study shows that ox-LDL was reduced in PKU patients. The levels of retinol and and coenzyme Q10 were comparable to healthy children but vitamin E level was found decreased. Our preliminary data may suggest that those PKU children need giving supplementation of vitamin E.

The study was sponsored by Nutricia Research Fundation.

O-26-3

RESTING ENERGY EXPENDITURE IN CHILDREN WITH PHENYLKETONURIA: NO AGREEMENT BETWEEN INDIRECT CALORIMETRY AND PREDICTION EQUATIONS

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Hyperphenylalaninemia (HPA) is caused by a deficiency of phenylalanine (Phe) 4-monooxygenase. Patients affected by severe forms (PKU), if not treated present mental handicap. Treatment is based on a low-Phe, low-protein diet supplemented with Phe-free amino acids. To correctly evaluate the appropriateness of the diet with respect to growth, it is very important to consider resting energy expenditure (REE), the largest component of total daily energy expenditure. The gold standard to measure REE is indirect calorimetry. **Aim**: To assess the degree of agreement between indirect calorimetry and equations commonly used to predict REE in a cohort of PKU patients. **Methods**: In 42 PKU patients (25 females, 17 males; mean age 13.5 ± 6.6 years) REE was measured (MREE) with an open circuit indirect calorimetry under standardized conditions. In all subjects REE was predicted (PREE) using equations from FAO/WHO/UNU, Maffeis, Harris-Benedict and Schofield. **Results**: Only PREE derived from Maffeis equations showed non-statistic differences when compared to MREE (p = 0.684) but it is reported that Maffeis equations underestimate REE and are performed with data obtained only from children aged 6–10 years. Interestingly MREE in PKU patients show a statistically significant reduction in REE ($p<0.001$) compared to healthy controls PREE. **Conclusions**: Indirect calorimetry should be used to correctly evaluate REE in PKU patients. Our results show a low REE in PKU patients. Nevertheless many authors reported overweight in PKU population but further investigations are necessary to explain these data and to elucidate any role of the diet.

O-26-4

INHIBITION AND ACTIVATION OF TETRAHYDROBIOPTERIN ON *IN VIVO* PHENYLALANINE HYDROXYLASE WITH PHENYLALANINE BREATH TEST

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Tetrahydrobiopterin (BH_4)-responsive phenylalanine hydroxylase (PAH) deficiency is characterized by reducing the blood phenylalanine level after a BH_4-loading. Phenylalanine breath test (PBT) quantitatively can measure the conversion of L-[1-^{13}C] phenylalanine to $^{13}CO_2$ and clarified the increase of phenylalanine oxidation in BH_4-responsive PAH deficiency. However, the mechanism underlying BH_4 responsiveness still remains unknown. Here, we studied the effects of BH_4 and phenylalanine on *in vivo* PAH activity of normal controls (n = 4) using PBT. Phenylalanine oxidation rate was calculated as $^{13}CO_2/^{12+13}CO_2$ ($\Delta^{13}C$, ‰) and cumulative recovery rate during 120 min (CRR_{120}, %; total amount of $^{13}CO_2$/the administered dose of ^{13}C-phenylalanine). In normal blood phenylalanine (54.4–70.2 μmol/l), the administration of BH_4 reduced $\Delta^{13}C$ peak from 49.6 to 24.4 ‰ and also reduced CRR_{120} from 16.9 to 10.2%. In high blood phenylalanine (177–218 μmol/l), in other words after phenylalanine loading, a peak value of $\Delta^{13}C$ in BH_4+Phe loading group (57.9 ‰) was significantly higher than phenylalanine loading group (36.6 ‰), but CRR_{120} was approximately similar (21.1 and 19.9%, respectively). From these results, most important regulator of *in vivo* PAH was phenylalanine, which served as a substrate for the enzyme and as an activator. Over dosage of BH_4 inhibited PAH in normal phenylalanine level and activated PAH in high phenylalanine level. The regulation system is built for maintaining suitable phenylalanine level in a human body. The appropriate BH_4 supplementation must be reviewed in BH_4 responsive PAH deficiency.

O-26-5

A COMPARISON BETWEEN SIMPLE PHE AND COMBINED PHE+BH_4 LOADING TEST IN PHENYLKETONURIA

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In order to investigate the responsiveness of PKU to BH_4 oral administration, 7 patients (3–22 years) were enrolled in this study. On the basis of their biochemical phenotype and genotype, 2 patients were assigned to the severe, 3 to the mild, and 2 to the benign class of PAH deficiency. All causal mutations carried by selected patients had been previously reported as BH_4 responsive. After normalization of plasma Phe levels obtained by adjusting the diet to the Phe dietary tolerance, three types of oral 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. Duration of the tests was extended to 24 h after Phe administration, and patients had a protein-free, normocaloric diet. The comparison between the different tests showed in all patients an identical course of plasma Phe and Tyr concentrations, irrespective of BH_4 administration. Following both simple and combined loading tests, only patients belonging to the benign class of PAH deficiency showed a Phe reduction higher than 30% and a consistent increase of plasma Tyr, so questioning BH_4 effectiveness in PKU.

O-27-1

EARLIER CLINICAL DIAGNOSIS AND TREATMENT OF TETRAHYDROBIOPTERIN(BH_4) DEFICIENCY

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Objective: To evaluate the clinical situation, diagnosis and treatments of tetrahydrobiopterin(BH_4) deficiency and to provide evidence for its earlier diagnosis and treatment. **Method**: Analysis the clinical situation, diagnosis and treatments of seven Case BH_4 deficiency patients who were diagnosis by neonatal screening in QingDao from 1998 to 2002. **Results**: Seven case BH_4 deficiency patients were diagnosed 6-pyruvoyl tetrahydropteiin synthase (PTPS) deficiency by uropterin analysis and BH_4 stress test. **Conclusion**: The screening of BH_4 deficiency in all hyperphenylalaninaemia patients is necessary, at least, the symptoms of nervous system must be observed carefully in order to earlier diagnosis and treatment.

O-27-2

CLINICAL DIAGNOSIS AND GENE ANALYSIS OF TETRAHYDROBIOPTERIN DEFICIENCY

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Objective: To understand the significance of the identification of tetrahydrobiopterin deficiency (BH_4D) for all patients with hyperphenylalaninemia (HPA). **Method**: (1) The clinical data, urinary pterin assay and BH_4 loading test of four suspicious BH_4D patients were analyzed. (2) The mutation analysis of *PTS* gene was performed by sequencing. (3) By artificial introduction of restriction sites (AIRS), the common mutations N52S, P87S and D96N of *PTS* were detected by PCR-RFLP analysis. **Results**: (1) Based on the clinical date, three patients were diagnosed as BH_4D during 6–20 months of age. They were diagnosed as HPA after birth by newborn screening and developed a mental retardation although treated by the lower Phe diet. (2) Four *PTS* gene mutations (N52S, P87S, D96N and L127F) were identified without the mutation L127F reported ever before. The genotypes of three PTS deficiency families were confirmed: N52S/L127F, P87S/D96N and N52S/D96N. N52S and D96N were the common mutations in our study. **Conclusion**: The results of *PTS* gene analysis were consistent with the clinical diagnosis. It is important that all the patients with HPA should be differentiated between PKU and BH_4D. The early diagnosis could ensure the patients treated rightly with improvement of their intelligence and life quality.

O-27-3

MOLECULAR ANALYSIS OF THE *PAH* GENE IN PHENYLKETONURIA (PKU): CORRELATION WITH BH_4 RESPONSIVENESS

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Objective: To screen a cohort of PKU patients for mutations in the PAH gene, and to correlate these results with BH_4 responsiveness. **Method**: An extended BH_4 load study in a group of Australian patients with PKU showed that up to half of them may be BH_4-responsive (Mitchell et al., Molec Genet Metab, 2006: 86; S81–5), and we are currently screening the PAH gene in these and other individuals from our PKU Clinic. **Results**: To date we have obtained DNA samples from 43 PKU patients, and have completed molecular analysis for 11 of them. As expected, results so far indicate that most individuals are compound heterozygotes for previously identified mutations, including mutations which according to the BIOPKU website (*http://www.bh4.org*) could potentially be BH_4 responsive. Interestingly, we have found two patients with the same genotype, p.R408W/p.F39L, who appeared to show very different BH_4 responsiveness. During the course of BH_4 therapy conducted over a week, one patient's phenylalanine rose from 450 μmol/L to 800 μmol/L, whereas the other appeared to show a partial response, the level dropping from 730 μmol/L to 400 μmol/L. According to the PAH Locus Knowledgebase (http://www.pahdb.mcgill.ca), this combination of mutations is associated with classical PKU. Homozygosity for the p.R408W mutation is associated with classical PKU. **Conclusion**: These preliminary data suggest that *in silico* analysis and/or residual enzyme activity are of limited value in the prediction of BH_4 responsiveness, and that at present a clinical/biochemical evaluation is necessary.

O-27-4

RATIO OF OMEGA-6 TO OMEGA-3 FATTY ACIDS IN PLASMA AND ERYTHOROCYTE MEMBRANES OF ADULT WITH PHENYLKETONURIA COMPARED TO METABOLICALLY HEALTHY CONTROLS

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The patients with phenylketonuria (PKU-P) must take a low phenylalanine diet from the neonatal periods. This restriction may be influenced on the nutritional condition. In this study 48 PKU-P on a low phenylalanine diet and 60 healthy controls were tested for cholesterol (CH), triglyceride (TG), HDL, LDL and Omega6 (Om6) /Omega3 (Om3). The BMI of PKU-P differed from that of controls significantly ($p = 0.003$). HDL level of PKU-P was significantly lower than that controls ($p < 0.001$). There was no statistical differences between PKU-P and controls with respect to the level of CH, TG and LDL in plasma. And Om6 and Om3 levels, and their ratio in plasma and erythrocyte membranes (EM) of PKU-P were almost same as those of controls. Neither PKU-P nor controls had an ideal Om6/Om3 ratio, which was especially relevant for the EM. The results were independent on the use of phenylalanine-free amino acid mixture contained Om6 and Om3 supplements at the ratio of 4:1.

O-28-1

GENE THERAPY OF PKU IN A MOUSE MODEL BY ECTOPIC EXPRESSION OF PAH AND ITS BH_4-COFACTOR GENES IN SKELETAL MUSCLE BY A TRIPLE-CISTRONIC AAV2-BASED PSEUDOTYPE 1 VECTOR

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Phenylketonuria (PKU) is an autosomal recessive inborn error of metabolism with a deficiency of the hepatic phenylalanine hydroxylase (PAH) leading to toxic accumulation of circulating phenylalanine (Phe) in blood, resulting in growth failure, microcephaly, seizures, and mental retardation. PAH catalyzes the hydroxylation of Phe to tyrosine and requires molecular oxygen and tetrahydrobiopterin (BH_4) as cofactor, the latter being synthesized in liver but not in skeletal muscle. BH_4 biosynthesis requires the consecutive action of the enzymes GTPCH and PTPS, with dihydroneopterin triphosphate as the first intermediate. Here we aimed at expressing the PAH system in skeletal muscle to degrade serum Phe in PKU patients. However, BH_4 is abundant in liver but scarce in skeletal muscle, as the cofactor-synthesizing enzyme GTPCH is absent in muscle tissue, and PTPS is expressed at low levels. We first demonstrated that transgenic PKU mice that had no liver PAH and expressed coordinately PAH along with GTPCH in skeletal muscle tissue accumulated dihydroneopterin triphosphate and remained hyperphenylalaninemic unless synthetic BH_4-cofactor was supplied. Thus, PTPS activity is limiting in skeletal muscle to synthesize BH_4 and to support Phe hydroxylation. Next, a recombinant triple-cistronic AAV2-based pseudotype 1 vector expressing PAH along with GTPCH and PTPS was generated and injected into the M. gastrocnemius of the hind legs of the PKU mouse, leading to long-term clearance of blood Phe and therapeutic treatment of PKU, including complete phenotypic reversion This non-invasive application is the basis to develop an efficient therapy for PKU using a triple-cistronic gene transfer into skeletal muscle.

O-28-2

LIVER DIRECTED ENZYME REPLACEMENT THERAPY – A NEW APPROACH FOR PKU TREATMENT

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Phenylketonuria (PKU) is an inherited error of metabolism caused by a deficiency in the liver enzyme phenylalanine hydroxylase (PheOH). The disease is characterized by the accumulation of phenylalanine in the plasma, which leads to neurological damage. Today most PKU patients rely on a strict low protein diet in order to maintain low plasma phenylalanine concentration. We suggest a novel approach for the treatment of PKU by enzyme replacement therapy using fusion proteins based on the wild type human PheOH enzyme. Fusing this enzyme to a targeting sequence will enable its delivery to the liver, thus restoring the impaired activity of the endogenous mutant enzyme. Restoring the PheOH activity in the liver will decrease the high levels of phenylalanine in the blood of PKU patients through the normal metabolic pathway. We have constructed PheOH based fusion proteins. We show that these proteins can be delivered into a variety of human liver cell lines and retain the PheOH activity after internalization. We also show that plasma phenylalanine levels were lowered in mice treated with PheOH based fusion proteins after IV administration. Based on these findings we suggest an alternative concept for the treatment of PKU.

O-28-3

ANALYSIS OF THE PHENYLALANINE HYDROXYLASE (PAH) GENE IN HYPERPHENYLALANINEMIC PATIENTS IDENTIFIED BY NEWBORN SCREENING USING HIGH-RESOLUTION DNA MELT PROFILING

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Defects in the PAH gene can lead to phenylketonuria. Assessing the PAH gene is becoming routine to evaluate candidate patients identified by newborn screening. High-resolution melt profiling provides a rapid and sensitive means to identify sequence variants in a PCR product. Each coding region of the PAH gene is included in a single PCR product in addition to a minimum of 25 nucleotides upstream and 15 nucleotides downstream of the 5′ and 3′ splice junctions respectively. All fragments function with a common thermal cycling condition. The following polymorphisms: IVS4--22 C/T, Q232Q, V245V, L385L, and Y414Y were frequently identified by the melt-profiling panel. To triage these polymorphisms, multiplex, site-specific assays were developed. Dried blood spots were obtained from 25 newborns having elevated phenylalanine and these were used to as a source of template DNA for genotype analysis. Melt profiling determined the region(s) containing sequence variants by their generating atypical melt profiles. Samples that generated atypical melting profiles were selected for DNA sequencing. The site-specific assays triaged atypical profiles generated by common polymorphisms such that no further evaluation was required. Identifying the region(s) of PAH having sequence variation required $\sim$3 h. Rapid assessment of PAH is critical to identify candidate patients and may help identify those that may be responsive to BH_4 therapy.

O-29-1

GASTROINTESTINAL SYMPTOMS IN FABRY DISEASE: DATA FROM THE FABRY OUTCOME SURVEY

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Background: Little is known about gastrointestinal (GI) manifestations of Fabry disease (FD) and effects of enzyme replacement therapy (ERT) on such symptoms. **Method**: We performed a retrospective analysis of data from 752 patients enrolled in the Fabry Outcome Survey (FOS) to evaluate the nature and prevalence of GI symptoms in FD and the effects of ERT with agalsidase alfa. **Results**: In total, 342 patients (71 children) had GI symptoms. GI symptoms were present in a higher proportion of children than adults (60.8% vs 49.8%) and in a higher proportion of females than males (54.2% vs 48.9%). The most prevalent GI symptom was abdominal pain (32.5% of GI symptoms), followed by diarrhoea (20.5%). More males than females suffered from diarrhoea (25.9% vs 16.7%). After 12 months of ERT, abdominal pain was improved in 12.9% of patients. Only 6.2% of patients developed abdominal pain as a new symptom under ERT. Males showed greater improvements in GI symptoms than females ($p<0.05$) and children showed more improvements than adults ($p<0.05$). Diarrhoea was improved after 1 year of ERT in 50% of the patients with diarrhoea at baseline. Males showed greater improvements than females. After 2 years of ERT, the prevalence of abdominal pain in male patients decreased from 45.5% to 36.4%, while the prevalence of diarrhoea decreased from 28.1% to 21.1%. **Conclusion**: In conclusion, there is a high prevalence of GI manifestations in patients with FD. GI symptoms improved significantly after 12 and 24 months of ERT with agalsidase alfa.

O-29-2

THE FABRY REGISTRY DEMONSTRATES HETEROGENEITY OF RENAL PROGRESSION IN 833 MALES AND FEMALES WITH FABRY DISEASE

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Introduction and Methods: Fabry disease may cause progressive renal damage. The pattern of expression and disease progression is less clear, particularly in females. A cross-sectional analysis of adult patients not on dialysis or transplanted (376 males, 457 females) from the Fabry Registry was performed to better characterise renal disease. **Results**: 21% of males and 13% of females had estimated (e) GFR <60 ml/min/1.73 m^2 (chronic kidney disease – CKD stages 3–5) and 26% of males and 41% of females had eGFR 60–89 ml/min/1.73 m^2 (CKD 2). Mean patient age was higher in females at each stage. Age range at CKD 4/5 was wide both in males (22–67) and females (36–74). There was a negative linear relationship between eGFR and 24 h proteinuria. However, 11% of males and 33% of females with CKD 3–5 had proteinuria <300 mg/24 h. Conversely, a significant proportion of patients with eGFR >90 ml/min/1.73 m^2 had proteinuria. The proportion of patients with BP $>130/80$ mmHg was 23% in CKD 2/3 and 12.5% in CKD 4/5, not different between males and females. This is lower than expected for CKD 3-5 patients. **Conclusions**: The wide age range at advanced CKD stage suggests that progression of renal dysfunction is more heterogeneous than thought. Proteinuria is an early and progressive complication, but absence of proteinuria does not rule out progressive renal dysfunction. Hypertension is not prevalent, not even in advanced CKD stages. This analysis, which for the first time includes more females than males, confirms that a significant proportion of females suffer moderate to severe renal disease.

O-29-3

THE FABRY REGISTRY, A SCIENTIFIC TOOL TO OUTLINE THE NATURAL COURSE OF FABRY DISEASE

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Introduction: Fabry disease (FD) is a multi systemic disorder due to the deficiency of the lysosomal enzyme α-galactosidase A. While early symptoms present in the peripheral and autonomous nervous system, severe late complications affect heart, kidneys and cerebrovascular system and contribute to premature death. Although the inheritance is classically considered as X-linked recessive, female patients may develop symptoms. **Methods**: The Fabry Registry is the largest global assessment programme collecting clinical data related to the onset and progression of FD. As of January 2006, 1863 patients (53% males, 47% females) have been enrolled. Among both genders, 88% of patients were adults (18 years of age or older). **Results**: Data in the Fabry Registry of patients who received enzyme replacement therapy, show median ages at onset of symptoms of 10 years in males and 13 years in females. However, there was an 11-year difference in the median ages at diagnosis (27 and 38 years, respectively). The ages at onset of first renal, cardiac, and cerebrovascular events were similar in males and females. However, males tended to experience their first cardiac event earlier than females (41 versus 49 years). **Conclusions**: Through the Fabry Registry, the medical community has access to data that may contribute to increased awareness of this under-diagnosed disorder resulting in earlier diagnosis and intervention. Ultimately, this will lead to optimization of patient care.

O-30-1

PHENOTYPE PREDICTION IN ASYMPTOMATIC METACHROMATIC LEUKODYTROPHY

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Metachromatic leukodystrophy (MLD) is a neurodegenerative disease with a reported incidence of 1:92 000 live birth in Australia. The primary defect results from the decreased catalytic action of arylsulphatase A (ASA) on sulphatide, resulting in the accumulation of sulphatide and other lipids in the lysosomal compartment of affected cells. MLD is classified into three clinical forms based on the age of onset: late-infantile (1–2 years), juvenile (3–16 years) and adult (16 years and above). Bone marrow/stem cell transplantation, when performed before the onset of clinical symptoms, has shown positive results. Pre-symptomatic detection of MLD may be possible in the near future through the introduction of a newborn screening program. However, clinicians and families will require accurate information on disease severity and rate of progression to make informed decisions about therapy options. We have developed a strategy to predict clinical phenotype in MLD patients through the determination of residual ASA protein/activity in cultured skin fibroblasts (SF) using sensitive immune-based assays, and lipid profiling (including sulphatide) in both SF and patient urine using electrospray ionisation-tandem mass spectrometry. Measurement of residual enzyme protein/activity in SF was able to differentiate unaffected controls, ASA-pseudodeficiency (ASA-PD), ASA-PD/MLD compound heterozygotes and MLD patients, but failed to distinguish the different MLD phenotypes. However, tandem mass spectrometric quantification of sulphatide and other secondarily altered lipids, including phosphatidylglycerol/lysobisphosphatidic acid, in urine and SF from MLD patients enabled further differentiation of the late-infantile form of the disorder from the juvenile and adult forms.

O-30-2

SIX NOVEL MUTATIONS DETECTED IN THE GALC GENE IN 17 JAPANESE PATIENTS WITH KRABBE DISEASE, AND NEW GENOTYPE-PHENOTYPE CORRELATION

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Krabbe disease is one of autosomal recessive leukodystrophies. It is pathologically characterized by demyelination in central and peripheral nervous systems and the0accumulation of globoid cells observed in brain white matter. It is caused by deficiency of galactocerebrosidase (GALC) activity. We investigated mutations of GALC gene in018 Japanese patients with Krabbe disease, the largest subject number of Japanese patients to date, and found 32 mutations. Of these mutations, six were novel including two nonsense mutations, W115X and R204X, two missense mutations, and S257F and L364R, the small deletion, 393delT, and the small insertion, 1719-1720insT. With our findings, taken with reported mutations in the Japanese patients, we confirmed several mutations common to Japanese patients. The two most frequent mutations, 12Del3Ins and I66M+I289V, accounted for 39% of all mutant alleles. Along with two additional mutations, G270D and T652P, up to 57% of genetic mutations in Japanese patients may be accounted for. Distribution of the mutations within the GALC gene indicated some genotype-phenotype correlation. I66M+I289M, G270D and L618S contributed to a mild phenotype. Screening for these mutations may provide a tool with which to predict the clinical phenotype.

O-30-3

ARYSULFATASE A MUTATION IN FOUR CHINESE PATIENTS WITH METACHROMATIC LEUKODYSTROPHY

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Objective: Metachromatic leukodystrophy (MLD) is an autosomal recessive inherited disorder and a severe metabolic, neurodegenerative disorder in which deficiency of arylsulftase A(ARSA) was caused by mutations in the ARSA gene. Up to now, the investigation of the ARSA gene mutation has not been reported in MLD patients in China. In this study, ARSA mutations in four Chinese patients with MLD were identified. **Methods**: Four Chinese patients (patient 1, 2, 3 and 4) with juvenile MLD were included in this study. Genomic DNA samples were extracted from peripheral bloods of all patients. All 8 exons and exon-intron boundaries of ARSA gene were amplified by PCR and followed by direct DNA sequencing. **Results**: Patients 1 and 2 both were with compound heterozygous mutation that is one allele with the G296T (G99V) mutation in exon 2 and the other allele with the G251A (R84Q) mutation in exon 2. Patient 3 were also with compound a heterozygous mutation that is one allele with the C862T (R288C) mutation in exon 5 and the other allele with the 1337-1338insC frameshift mutation in exon 8. Patient 4 was only found 177-180insCA frameshift mutation in exon1. **Conclusion**: Five alterations of ARSA gene were identified in four Chinese patients with MLD. Three were known missense mutation (R84W, G99V, and R288C), and two novel frameshift mutations (177-180insCA and1337-1338ins C). This is the first report about ARSA mutations in MLD patients in China. The two novel frameshift mutations are the first mutations reported in those two exons.

O-30-4

THE NATURAL HISTORY OF NIEMANN-PICK DISEASE TYPE C IN FRANCE

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Niemann-Pick C disease (NPC) is a neurovisceral lipid storage disorder due to mutations in either the *NPC1* or *NPC2* gene. The corresponding proteins are involved in cellular transport of cholesterol, glycolipids and other molecules, but their precise functions and relationship remain unclear, and their primary substrate unknown. Our survey covered 123 families diagnosed with NPC in France. The diagnosis was assessed by filipin staining and LDL-induced cholesterol esterification in all but 4 families (liver lipids only). Genetic complementation analysis and/or genotyping revealed 8 families (11 patients) with mutations in the NPC2 gene. I1061T constituted 28% of the studied NPC1 mutant alleles. The clinical presentation and follow-up of 154 patients was reviewed. NPC presented as neonatal cholestatic liver disease in nearly half of the cases, isolated splenomegaly was the initial symptom in quarter of the cases, while another quarter had a neurological or psychiatric (3%) presentation. With the exception of 20 cases who died before 1 year of age from perinatal hepatic or respiratory fulminant forms, the severity of the disease was determined by the neurological involvement, with an age at death varying from 2 to 49 years. Our cohort was remarkable by the quite large proportion of cases with an infantile and late infantile neurological onset, but also included 13 patients with adult neurological onset. With emerging therapies, a better knowledge of the natural history of NPC should help in defining a pragmatic algorithm for optimal management of patients.

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O-31-1

MAGNETIC RESONANCE EVALUATION OF CEREBRAL PERFUSION CHANGE IN PEDIATRIC PATIENTS WITH LEIGH SYNDROME

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Purpose: To detect the change of cerebral perfusion in pediatric patients with Leigh's syndrome (LS) by using MR perfusion technique. **Methods**: Twelve patients with Leigh's syndrome and thirteen normal children were scanned with the sequence of flow-sensitive alternating inversion recovery exempting separate T1 measurement (FAIREST). Their relative cerebral blood flow (CBF) values were obtained in regions of bilateral basilar nuclei and bilateral thalami. Student t-test was used to compare them between the two groups and ROC curve analysis was carried out. **Results**: Statistical analysis revealed significant difference between two groups in the regions of bilateral basilar nuclei and right thalamus ($p < 0.05$). The rCBF values for LS group and control group were 0.432 ± 0.158 and 0.619 ± 0.125 for right basilar nuclear, 0.478 ± 0.186 and 0.621 ± 0.123 for left basilar nuclear, 0.630 ± 0.189 and 0.833 ± 0.160 for right thalamus, respectively. The areas under the ROC curves were 0.833 and 0.756 for the rCBF of right and left basilar nuclear, respectively. **Conclusion**: Relative CBF maps may reveal changes of cerebral blood flow in some specific brain regions in patients with Leigh's syndrome. It can provide additional information to the clinicians in the evaluation of the disease.

O-31-2

CALORIE UTILISATION AND WEIGHT GAIN IN MITOCHONDRIAL RESPIRATORY CHAIN DEFECTS

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Background: Feeding difficulties and failure to thrive are among the presenting symptoms of children diagnosed with mitochondrial respiratory chain defects (MRCD). Nutritional interventions (NI) such as enteral feeding (EF) with/without high fat diets may be prescribed to improve nutritional status and debilitating symptoms. The effect of NI in children with MRCD is reported. **Method**: Patients diagnosed with MRCD who had NI (EF and 'high fat diet' $>50\%$ energy for varying periods) ($n = 6$) were followed up for 4–36 months. Weight, intake, feeding difficulties, body temperature and gastrointestinal symptoms were monitored. Estimated energy requirements (EER) were determined using basal metabolic rate (BMR) predictive equations. Retrospective anthropometric and feeding data were collected for sex- and diagnosis-matched controls ($n = 6$) who had no NI. **Results**: The median increase in body weight (expressed as percentage of ideal body weight) of patients who had NI was 15% (range 0–58) in a median of 9 months (range 0–36). EER decreased from an initial median of BMR $\times$ 1.35 (range 1.2–2.0), to a median of BMR $\times$ 1.0 (range 0.6–1.6). Children without NI had severe FTT due to feeding difficulties that increased prior to death. In all patients, body temperature was not increased except during acute infections. Thyroid function tests were normal. **Conclusion**: The accelerated weight gain in these children cannot be explained by immobility, body temperature or thyroid function alone and further research is needed to explain these observations. Equations used to predict BMR and EER may not be appropriate for these children.

O-31-3

MITOCHONDRIAL PHOSPHATE CARRIER DEFICIENCY – A NOVEL DISORDER OF THE OXIDATIVE PHOSPHORYLATION

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We report on two sisters presenting with muscular hypotonia and cardiomyopathy after birth. Both had lactic acidosis, usually 4–8 mmol/l, and growth retardation. They died with 6 and 7 months of age, respectively. Enzymatic investigation of the muscle biopsies showed normal activities of the respiratory chain. Substrate oxidation of functionally intact fresh muscle mitochondria revealed a decreased activity with all substrates when stimulated by ADP, but normal activity in the presence of the uncoupler CCCP (ratio CCCP-/ADP-respiration: 8.5; normal: 1.0 ± 0.1). This result clearly pointed to a defect within the mitochondrial ATP synthesis machinery, including the ATP synthase, the adenine nucleotide translocator (ANT) and the mitochondrial phosphate carrier (mPC). No aberrations were found after: (1) genetic screening of *ATP6/8* and *ANT1* genes, (2) blue native electrophoresis test of ATP synthase protein content in muscle and (3) ATP synthesis assay in fibroblasts, thus pointing to a tissue specific defect. Finally, sequencing of the mPC *SLC25A3* gene revealed a homozygous missense mutation in both patients at a highly conserved position in the alternatively spliced exon 3A. This alternatively spliced isoform is predominant in heart and muscle. Introduction of the homologous mutation in the mPC Mir1p of *S. cerevisiae* resulted in a growth deficiency on non-fermentable glycerol medium. To our knowledge this is the first report on a mitochondrial phosphate carrier defect.

O-31-4

CORRECTION OF TOXIC ACCUMULATION OF THYMIDINE AND DEOXYURIDINE IN A MITOCHONDRIAL NEUROGASTROINTESTINAL ENCEPHALOPATHY (MNGIE) PATIENT BY HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Deficiency of thymidine phosphorylase (TP) results in progressive fatal MNGIE disease, clinically defined by gastrointestinal dysmotility, cachexia, peripheral neuropathy, white-matter changes in brain MRI. The cause for this disease is believed to be the toxic accumulation of thymidine and deoxyuridine which generate imbalanced mitochondrial deoxyribonucleoside triphosphate pool, which in turn is responsible for the mitochondrial DNA (mtDNA) mutagenesis and depletion. **Hypothesis**: Therapeutic strategies to reduce these nucleosides concentration in patients may be effective. The appeal of TP deficiency as a candidate disease for curative hematopoietic stem cell transplantation (HSCT) arose from the clinical observation that infusions of platelets, which contain abundant functional TP, reduced circulating levels of thymidine and deoxyuridine in three patients. **Methods and results**: We treated a 10 year old patient with a moderate T-cell-depleted HSCT from her HLA identical mother. Concomitant with engraftment, 12 days post transplantation, the serum levels of thymidine and deoxyuridine declined markedly, and became undetectable 27 days following transplantation. After 6 months she gained 9 kg. **Conclusion**: These results constitute a proof of concept that restoration of TP activity by HSCT can ameliorate biochemical imbalances that cause MNGIE. Clinical follow-up is needed to evaluate long-term, multi-systemic efficacy of this treatment.

O-31-5

PEROXISOMAL PROLIFERATOR ACTIVATED RECEPTOR AGONISTS AS A POTENTIAL THERAPY FOR MITOCHONDRIAL RESPIRATORY CHAIN COMPLEX (RCC) DEFECTS

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Peroxisome proliferator activated receptors (PPARs) are a family of ligand-activated transcription factors known to regulate fatty acid metabolism by controlling the expression of specific target genes. PPARα is preferentially expressed in tissues with high fatty acid utilization like heart and muscle, while PPARγ is found at high levels in adipocytes and other cell types involved in lipid synthesis and storage. Recent data highlighted the potential of fibrates, PPARα agonists, in the correction of inborn errors of fatty acid oxidation. We investigated the effects of Bezafibrate, a common hypolipidemic drug acting as a PPARα agonist, Pioglitazone and Rosiglitazone, PPARγ agonists for type II diabetes on mitochondrial RCC capacities in complex I and IV deficient cell lines. Skin fibroblasts from patients with established defects of complex I, IV and normal controls were incubated for 72 h with 500 μM Bezafibrate, 100–400 nM Rosiglitazone and Pioglitazone, respectively. Fibroblasts were harvested and assayed for complex I, II + III, IV and citrate synthase. The complex IV enzyme activities in two definite complex IV deficient cell lines remained unchanged after the treatment even at very high concentration. In contrast, complex I enzyme activities in partial deficient cell lines increased up to nearly 50% after the PPARγ treatment. However, the changes in definite complex I deficiency were minimum. Mild elevation of citrate synthase (up to 32%) was observed after the treatment suggesting mitochondrial proliferation. Further studies on variable deficient cell lines would provide the foundation for possible therapeutic intervention and pharmacological correction of mitochondrial disorders.

O-32-1

NEUROPSYCHOLOGICAL ASPECTS OF MUCOPOLYSACCHARIDOSIS TYPE II WITH MILDER PHENOTYPES

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The neuropsychological aspects of patients with mucopolysaccharidosis type II (Hunter disease) with milder phenotypes were investigated to delineate their psychosocial problems and improve the quality of life of such patients. Wechsler intelligent tests (WAIS-R, WISC-III), the Yatabe-Guilford personality test and the Baum test were performed on 9 adults and 3 children with Hunter disease with milder phenotypes. The average Full-scale intelligence quotient (FIQ), Verbal intelligence quotient (VIQ) and Performance intelligence quotient (PIQ) of the 12 patients were 76.2 (range: 53–106), 78.3 (53–108) and 82.0 (62–103), respectively. The subscale scores of the IQ suggested that their abilities for short-term memory, visual recognition and motor coordination were relatively high, whereas comprehension and similarities were relatively low. The Yatabe-Guilford personality test results indicated that 11 of the 12 patients had standard personalities, while 1 adolescent patient showed a psychosis pattern. The Baum test results suggested that the patients had difficulties in forming relationships with others or the community, and the same adolescent patient was also suspected of having psychosis. Therefore, patients with Hunter syndrome may have risks for psychological problems. Understanding their personalities, improving their social environment and early treatment should be very important.

O-32-2

UTILITY OF SERUM HEPARIN COFACTOR II-THROMBIN COMPLEX IN MPS DISEASES

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The clinical heterogeneity in the MPSs makes prognostication of clinical course and evaluation of treatment regimes difficult. The identification of MPS disease biomarkers that objectively reflect disease severity and therapeutic response would be invaluable to patient care. Using differential proteomics in the murine MPS I model we have found increased serum heparin cofactor II-thrombin complex (HCII-T) in affected animals. Analysis of human samples revealed significant differences in HCII-T levels in affected MPS I individuals compared to normal. Untreated MPS I patients (n = 15) have levels of 16 300 to 209 000 pM, while normals average 238 pM (SD: 154, n = 47); severe MPS I individuals display the highest concentrations. HCII-T appears to be responsive to treatment. Attenuated patients (n = 11) receiving ERT show reduced HCII-T; one MPS IH patient who has had combined ERT and BMT showed a reduction from 182 000 to 22 000 pM after 5 weeks of ERT, and further reduction, 6000 pM after BMT. Only one treated MPS I patient has post-treatment levels in the normal range. One patient, receiving long-term ERT (132 weeks), initially showed a marked reduction in levels but subsequently showed a gradual return to pretreatment levels within 132 weeks; this contradicts urinary GAG excretion, which remains low. Increased HCII-T has been found in the study of a single Sanfilippo A patient. We propose that HCII-T levels accurately reflect the burden of MPS disease and may be a useful biomarker in all MPSs. In addition these studies indicate that serpins may play a role in the pathophysiology of the MPSs.

O-32-3

MURINE MODEL (*Galns*$^{tm(C76S)slu}$) OF MPS IVA WITH A MISSENSE MUTATION AT AN ACTIVE SITE AMONG SULFATASE PROTEINS

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Mucopolysaccharidosis IVA (MPS IVA) is an autosomal recessive disorder caused by a deficiency of N-acetylgalactosamine-6-sulfate sulfatase (GALNS), required for degradation of keratan sulfate and chondroitin-6-sulfate.

To study a missense MPS IVA, we produced a p.C76S (an active site replacement, corresponding to p.C79S in humans) knock-in mouse by introducing a Cys76 to Ser (p.C76S) in the endogenous murine *Galns* by targeted mutagenesis. Homozygous *Galns*$^{tm(C76S)slu}$ mice has no detectable GALNS enzyme activity. At 3 months old, lysosomal storage is present primarily within reticuloendothelial cells such as Kupffer cells and cells of the sinusoidal lining of the spleen. Vacuolar change is observed in the visceral epithelial cells of glomeruli and cells at the base of heart valves but it is not present in parenchymal cells. In the brain, hippocampal and neocortical neurons and meningeal cells show lysosomal storage. Radiographs reveal no change in the skeletal bones of mice up to 12 months old. Thus, the *Galns*$^{tm(C76S)slu}$ mice show visceral storage of GAGs but lacks the skeletal features. The quality of mRNAs are no different between the mutant and normal mice. No secondary reduction or increase of other sulfatases is observed.

Overall, the *Galns*$^{tm(C76S)slu}$ mice display less pathological findings compared to the previously reported mice who are tolerant to human enzyme (*Galns*$^{tm(hC79SmC76S)slu}$) with substantial reduction of enzyme activities of other sulfatases.

This knock-in *Galns*$^{tm(C76S)slu}$ mouse affecting solely GALNS activity without reduction in other sulfatases should be useful in studying pathogenesis of MPIVA due to an active site point mutation and developing a new treatment.

O-32-4

CLN3P DEFINES A NOVEL PALMITOYL-PROTEIN Δ-9 DESATURASE, WHICH IS DEFICIENT IN BATTEN DISEASE

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Batten disease, CLN3, is the most common recessively inherited, untreatable, neurodegenerative disease of man. It is characterized by progressive neuronal loss and displays intraneuronal proteolipid storage. Although the gene for the disorder was cloned over a decade ago, the function of the encoded protein, CLN3P an integral membrane-bound lipid raft component, is so far undefined. We performed protein sequence analysis of CLN3P using the Pfam server and matched a possible fatty acid desaturase domain with a low stringency. We tested the CLN3 protein for fatty acid desaturase activity using various substrates including free fatty acids and acyl-coenzyme A's. Palmitate and myristate were noted to be substrates for desaturation but not acyl-CoA's. The kinetics of desaturation of free palmitate (k_m = 950–1370 nm) indicated that this was not a physiological substrate for CLN3P activity. Therefore, we evaluated other potential membrane-associated substrates for the reaction. We chose the S-palmitoylated H-Ras protein, which is the substrate that was used to define the palmitoyl-protein thioesterase deficiency in CLN2 and found kinetics that suggested this is a natural substrate for the desaturation reaction (k_m = 0.121–0.124 pM). We synthesized an artificial substrate, palmitoyl-cysteine, which demonstrated high affinity in the reaction (k_m = 12.4–15.0 pM). Desaturase activity was measured in pancreas obtained from the *cln3*$^{-/-}$ mouse using palmitoyl-cysteine. This demonstrated an 80% reduction, 0.69 compared to 3.4 nmol/min/mg protein in the wild type. This experiment further confirms that S-palmitoyl-protein desaturation is the specific function of CLN3P and that deficiency of this novel membrane-protein modification enzyme results directly in CLN3.

O-32-5

CATHEPSIN D DEFICIENT NEURONAL CEROID LIPOFUSCINOSIS: A NOVEL NEURODEGENERATIVE DISEASE OF CHILDHOOD

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Cathepsin D is a ubiquitously expressed lysosomal protease that is involved in proteolytic degradation, cell invasion and apoptosis. In mice and sheep cathepsin D deficiency is known to cause a fatal neurodegenerative disease.

We have discovered a novel and so far undescribed cathepsin D associated human neurodegenerative disorder that manifests in childhood. Two missense mutations, F229I and W383C, were identified in a child that suffered from early blindness and progressive psychomotor disability. Patient fibroblasts showed a markedly reduced enzymatic activity and diminished amount of cathepsin D. The skin biopsy revealed granular osmiophilic deposits in the Schwann cells of the patient. Expression of human wild-type and mutant cathepsin D in cathepsin D –/– mouse fibroblasts demonstrated a significant residual enzymatic activity for mutant F229I, but an almost complete loss of function for mutant W383C. The later mutant also showed a disturbed posttranslational processing to the mature peptidase and an intracellular mistargeting to non-lysosomal compartments. Kinetic analysis of mutant F229I expressed in cathepsin –/– cells disclosed a reduction in maximal enzyme velocity explaining the decreased enzymatic activity of this mutant. The functional importance of F229I is underlined by the strict conservation of this residue among members of the pepsin family of peptidases. The structural effects of cathepsin D mutants were anticipated by computer modeling suggesting larger structural alterations for W383C than for F229I. Our studies indicate a new type of human neuronal ceroid lipofuscinosis and add further insight into the cellular functions of human cathepsin D.

O-33-1

MUTATIONAL ANALYSIS OF THE GNPTA GENE IN JAPANESE PATIENTS WITH I-CELL DISEASE (MUCOLIPIDOSIS II)

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Leroy's I-cell disease (mucolipidosis II) is characterized by progressive psychomotor deterioration accompanied by bone/joint deformities or cardiac involvements. The disease presents very early in life, and fibroblasts of the patients have diminished activity of GlcNac-1-phosphotransferase, which is responsible for the correct intracellular targeting of multiple lysosomal enzymes. The incidence of this disease has been believed to be high in Japan possibly due to the presence of common mutations. In order to address this question, in this study, we have performed a genome-wide linkage analysis of 7 Japanese families with I-cell disease as well as a mutational analysis of the alpha subunit of the GlcNac-1-phosphotransferase (GNPTA) gene of the family members. The results of the linkage analysis showed that, in all families, the disease maps to chromosome 12q, excluding the possibility of genetic heterogeneity. We have identified 9 different mutations in these 7 families, most of them being nonsense or frameshift mutations. Except for the 3565C>T mutation which was found in two families, other mutations were private to each of the families. Thus, at least by this study, we could not find any evidence of the presence of a common GNPTA mutation in Japan.

O-33-2

NOVEL INTERACTION OF HEAT SHOCK COGNATE PROTEIN 70 (HSC70) WITH MUCOLIPIN-1, THE PROTEIN RESPONSIBLE FOR MUCOLIPIDOSIS TYPE IV

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Background: Mucolipidosis type IV (MLIV) is a lysosomal storage disorder that is caused by mutations in the *MCOLN1* gene, which encodes the protein mucolipin-1 (MLN1). MLN1 is a novel member of the transient receptor potential (TRP) cation channel gene family, and it has been shown to function as a novel Ca^{2+}-permeable channel. The goal of the current study was to identify proteins that interact with MLN1, which will shed light on the normal function of MLN1. **Methods**: Cell culture, yeast-two hybrid system 3, *in-vitro* and *in-vivo* co-IP, Western blot, gene sequencing, cloning, transfection, protein transport assay. **Results**: We identified the constitutively expressed 70kDa heat-shock cognate protein (Hsc70) and heat-shock protein 40kDa (Hsp40) as strong MLN1 interactors. *In vitro* and *in vivo* co-immunoprecipitation experiments using full-length Hsc70, Hsp40 and MLN1 have confirmed the interaction. The interaction with Hsc70 is enhanced following treatment with ionomycin, a calcium ionophore. Further, we have shown co-localization of Hsc70 and MLN1 in CHO cells using immunofluorescence. Hsc70 is involved in endocytosis and is a member of a chaperone complex that also includes HIP, HOP, Hsp90 and BAG-1. This complex is associated with the lysosomal membrane and is involved in protein translocation across the membrane. It is likely that MLN-1 plays a role in this protein transport process, as we can also co-immunoprecipitate Hsp90 and HOP with MLN-1. Understanding how MLN-1 interacts with this chaperone complex will help us to obtain a better understanding of the pathogenesis of mucolipidosis type IV.

O-33-3

RADIOLOGICAL AND BIOCHEMICAL FEATURES OF 5 CHINESE PATIENTS WITH GM1 GANGLIOSIDOSIS

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GM1 gangliosidosis is a rare autosomal recessive lysosomal storage disorder. Cranial magnetic resonance imaging (MRI) and skeletal radiographs are an important method to evaluate the pathological damage in the patients. Our study aimed to review the radiological and biochemical features of 5 Chinese patients with infantile GM1 gangliosidosis. The patients (4 girls and 1 boy) came from 5 unrelated families. They showed psychomotor retardation from the neonate to infantile period and hospitalized at the age of 9 to 27 months. Their beta-galactosidase activities in peripheral blood leukocytes were markedly decreased (1.4 to 3.9 nmol/h/mgñprotein vs normal control 88 to 204 nmol/h/mgñprotein). Macrocephaly, hypotonia, respiratory obstruction, intention myoclonus, hepatomegaly, liver dysfunction, mild hyperammonemia and lactic acidemia were found in all patients. Macular cherry-red spots were not present on their eyes. Skeletal radiographs revealed wide hypoplastic wedge-shaped metacarpals and anteriorly beaked thoracolumbar vertebrae. The T2-weighted magnetic resonance (MR) images of the brain revealed certain characteristic features, including delayed myelination and abnormal appearance of the subcortical white matter, internal capsule, and basal ganglia. In a girl with the late infantile form of galactosialidosis, extensive hyperintense lesions involving bilateral basal ganglia were observed.

O-33-4

IMPAIRMENT OF TRK SIGNALING IN G_{M1}-GANGLIOSIDOSIS MICE BRAINS

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G_{M1}-gangliosidosis is an autosomal recessive lysosomal lipid-storage neurodegenerative disorder caused by deficiency of lysosomal β-galactosidase, resulting in progressive neural and visceral accumulations of G_{M1} ganglioside and its derivatives. Little is known about the molecular mechanisms of neurodegeneration in this disease. Cerebellar granule cells from postnatal day 8 β-galactosidasedeficient mice showed abnormal distribution of G_{M1} ganglioside in plasma membrane, which was accompanied by lysosomal G_{M1} accumulation. Since G_{M1} locates in lipid rafts and it is known to regulate Trk neurotrophin receptor-mediated signal transduction, we investigated its behavior in mouse brain. By immunoprecipitation assay, the association of G_{M1} with Trk was found to be defective and the phosphorylation of Trk A protein was significantly decreased in the cerebral cortex, midbrain and cerebellum of affected mice compared to wild type littermates. Consistent with this, downstream signaling from Trk A was also impaired. These results suggest that impairment of Trk signaling may cause the onset of neurodegenration in G_{M1}-gangliosidosis.

O-34-1

IDENTIFICATION OF THE CYTOCHROME P450 ENZYMES RESPONSIBLE FOR THE OMEGA-HYDROXYLATION OF PHYTANIC ACID: IMPLICATIONS FOR REFSUM DISEASE

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Phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) is a 3-methyl-branched-chain fatty acid originating from dietary sources. Patients suffering from Refsum Disease have a defect in the omega-oxidation pathway required for the degradation of 3-methyl-branched-chain fatty acids. Consequently, the patients accumulate phytanic acid in plasma and tissues. Our previous studies have shown that phytanic acid is also a substrate for the omega-oxidation pathway. In this paper we show that omega-hydroxylation of phytanic acid in human liver microsomes is strongly inhibited by 17-ODYA (IC50 $<$400 nM), while diethyl-dithiocarbamate and ketoconazole showed minor inhibitory effects, indicating that one or more members of the cytochrome P450 family 4 class are responsible for phytanic acid omega-hydroxylation. This was confirmed by incubations of microsomes containing individually expressed human cytochrome P450 enzymes prepared from baculovirus infected insect cells (Supersomes™) with phytanic acid, which revealed that multiple cytochrome P450 enzymes of the family 4 class are able to omega-hydroxylate phytanic acid with the following order of activiy: CYP4F3A > CYP4F3B > CYP4A11 > CYP4F2. The enzymes identified in this study are potential therapeutical targets for Refsum disease as induction of these cytochrome P450s may lead to an increased breakdown of phytanic acid via omega-oxidation.

O-34-2

OMEGA-OXIDATION OF VERY LONG-CHAIN FATTY ACIDS IN HUMAN LIVER MICROSOMES: IMPLICATIONS FOR X-LINKED ADRENOLEUKODYSTROPHY

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X-linked adrenoleukodystrophy (X-ALD) is a severe neurodegenerative disorder biochemically characterized by elevated levels of very long-chain fatty acids (VLCFA). Excess levels of VLCFAs are thought to play an important role in the pathogenesis of X-ALD. Therefore, therapeutic approaches for X-ALD are focused on the reduction or normalization of VLCFAs. In this study, we investigated an alternative oxidation route for VLCFAs, namely ω-oxidation. The results described in this paper show that VLCFAs are substrates for the ω-oxidation system in human liver microsomes. Moreover, VLCFAs were not only converted into ω-hydroxy fatty acids, but they were also further oxidized to dicarboxylic acids via cytochrome P450 mediated reactions. High sensitivity towards the specific P450 inhibitor 17-octadecynoic acid suggested that ω-hydroxylation of VLCFAs is catalyzed by P450 enzymes belonging to the CYP4A/ F subfamilies. Studies with individually expressed human recombinant P450 enzymes revealed that two P450 enzymes, i.e. CYP4F2 and CYP4F3B, participate in the ω-hydroxylation of VLCFAs. Both enzymes belong to the cytochrome P450 4F subfamily and have a high affinity for VLCFAs. In summary, this study demonstrates that VLCFAs are substrates for the human ω-oxidation system and for this reason stimulation of the *in vivo* VLCFA ω-oxidation pathway may provide an alternative mode of treatment to reduce the levels of VLCFAs in patients with X-ALD.

O-34-3

PEROXISOMAL BRANCHED CHAIN THIOLASE (SCPx) DEFICIENCY: IDENTIFICATION OF A NEW PEROXISOMAL DISORDER IN A PATIENT WITH LEUKOENCEPHALOPATHY WITH DYSTONIA AND MOTOR NEUROPATHY

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We report the identification of a new peroxisomal disorder, caused by mutations in the SCP2 gene, which codes for the peroxisomal enzyme branched-chain 3-oxo-acyl-CoA thiolase (SCPx). The latter enzyme plays a key role in the beta-oxidation of the branched chain fatty acids pristanic acid and di- and trihydroxycholestanoic acid, which are intermediates in the production of the primary bile acids cholic acid and chenodeoxycholic acid, respectively. The patient involved, a 45-year old Caucasian male, was admitted with a 28-years history of dystonic head tremor and spasmodic torticollis. Nerve conduction studies of the lower extremities showed a predominantly motor and slight sensory neuropathy. Cranial MRI showed a leukoencephalopathy and involvement of the thalamus and pons. Furthermore, nistagmus, hyposmia and azoospermia were found. Analysis of peroxisomal parameters in plasma revealed normal plasma very-long-chain fatty acids, whereas plasma pristanic acid was markedly elevated. Phytanic acid and the bile acid intermediates di- and trihydroxycholestanoic acid were also elevated, although less markedly.

These data pointed to a defect in the peroxisomal beta-oxidation of branched-chain fatty acids. Detailed enzymatic studies revealed the specific deficiency of the peroxisomal enzyme branched-chain 3-oxo-acyl-CoA thiolase, also named SCPx. This was confirmed by subsequent mutation analysis, showing a homozygous one-nucleotide insertion, which leads to a frame shift and premature stop codon.

O-34-4

EX VIVO LENTIVIRAL TRANSDUCED AUTOLOGOUS HEMATOPOIETIC CELL GENE THERAPY FOR CHILDHOOD CEREBRAL ADRENOLEUKODYSTROPHY: PHASE I/II CLINICAL TRIAL DESIGN

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Children with cerebral X-linked adrenoleukodystrophy (ALD) suffer from severe demyelination that starts at 5–10 years of age and leads to vegetative state or death within 2–5 years. Allogeneic hematopoietic cell transplantation (HCT) is the only curative treatment. The beneficial effect of HCT is likely due to the capacity of myelo-monocytic precursors to penetrate into the brain and differentiate into microglia expressing normal ALD protein. However, allogeneic HCT is associated with marked morbidity/mortality risk, and less than 50% of ALD boys who are candidate for HCT can beneficiate from the procedure due to the lack of related or unrelated HLA-matched donor. We have provided pre-clinical data demonstrating safety and efficacy of ALD gene transfer into CD34+ cells with a HIV-based vector. (1) No abnormal hematopoiesis *in vitro* or in secondary transplanted ALD mice due to vector-mediated insertional mutagenesis; (2) no replication competent lentivirus (RCL) in transduced $CD34^+$ cells; (3) limited number (1–3) of vector integrated copies per cell; (4) no modification in brain microglia differentiation due to ALD transduction; (5) no adverse effects to overexpression of ALD gene; (6) ALD transgene is not silenced in HSC-derived microglia. Based on these results, a clinical protocol was approved by the AFSSAPS french regulatory agency. The aim of this phase I/II clinical trial is to evaluate the safety and efficiency of autologous transplantation of ALD $CD34^+$ cells corrected with a lentiviral vector. This protocol will be proposed to patients with cerebral ALD, who are candidate for HCT but have no matched donor.

O-35-1

PIPECOLIC ACID CONCENTRATIONS AND MOLECULAR ANALYSIS OF THE ANTIQUITIN (ALDH7 A1) GENE IN PATIENTS WITH PYRIDOXINE-DEPENDENT EPILEPSY

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Following the description of elevated pipecolic acid (PA) as a diagnostic marker of pyridoxine dependent epilepsy, the molecular background has been identified by mutations of the antiquitin (ALDH7A1) gene on chromosome 5q31 recently. We report on biochemical and molecular findings of 16 patients with probable or definite pyridoxine-dependent epilepsy. Samples were collected while on pyridoxine-HCl. PA was determined by GCMS. Mutation analysis of the ALDH7 A1 gene was performed by direct sequencing of all 18 exons and flanking introns. Detected mutations were confirmed by restriction fragment polymorphism analysis. We found homozygous or compound heterozygous mutations in 10 patients, while in 3 patients only one mutation and in 3 patients no mutation of the ALDH7 A1 gene could be identified so far. We have found elevated PA (3.4–16. 8 µmol/l, normal <2.46) in all patients with mutations on both alleles and in 2 patients with one mutation identified. PA in plasma was normal in 3 patients with no mutation identified and in one heterozygote, while her affected sibling did not carry the mutation. PA in plasma seems to be a reliable marker of pyridoxine-dependent epilepsy caused by mutations of the ALDH7 A1 gene.

O-35-2

PYRIDOXINE-DEPENDENT SEIZURES

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Pyridoxine-dependent seizures are a rare recessively inherited condition with an unclear molecular background. 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. Recently isolated pipecolic acid elevations in the plasma and/or CSF have been described as a biochemical marker in this disorder. We would like to present seven patients (2 girls, 5 boys) between the ages of 2 months and 6 years, with pyridoxine dependent seizures in whom the biochemical tests and mutation analysis will be presented. Clinical findings are seizures beginning in the neonatal period followed by afebrile seizures in the infancy. Three of them had megalencephaly, delayed in speech and walking. They had mild ataxic gait. All patients respond to pyridoxine treatment. In one of the patients there is additional cortical neuronal migration disorder and in the other there is a history of siblings lost with neonatal adrenoleukodystrophy. These additional findings indicate that diagnosis of pyridoxine-dependent seizures may be challenging for the pediatric neurologist in the presence of complex family history of another neurometabolic disease and cortical dysplasia. We conclude that pyridoxine-dependency should be included in the differential diagnosis of patients presenting with seizures in the neonatal and infancy period. Urinary eleveation of pipecolic acid will enable clinicians for early recognition of VITB6 dependent epilepsy patients.

O-35-3

A *GIF 290 T>C* UNFREQUENT POLYMORPHISM IN INHERITED INTRINSIC FACTOR DEFICIENCY

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Inherited intrinsic factor (IF) deficiency is a genetic cause of pernicious anemia. We describe one case of a 3-year old child with a megaloblastic anemia, a deficient secretion and normal molecular phenotype of IF and an heterozygous 290T>C substitution in exon 3 (Met79Thr) of *GIF* (Gastric Intrinsic Factor). The substitution was also found in the mother, but not in another case of familial Addisson-Biermer anaemia. The mother had a low plasma level of vitamin B_{12} and no clinical sign of vitamin B_{12} deficiency. This IF phenotype was also found in another adult carrier with low serum B_{12}, hyperhomocysteinemia at 30 µmol/L who presented a pulmonary embolism and no anemia. The vitamin B_{12} supplementation was efficient in all cases. The genetic variant corresponded to an infrequent polymorphism (290 C allele frequency of 0.022, 95% exact confidence interval: 0.005–0.063, $n = 136$). In conclusion, this new variant of GIF associated with 2 cases of IF congenital deficiency provides an additional evidence of the complexity of the genetic and phenotypic traits of this rare disorder.

O-35-4

THREE CASES OF CONGENITAL FOLIC ACID MALABSORPTION

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Three female patients with congenital folic acid malabsorption (CFM) in two families were clinically investigated. Marriage to a cousin was observed in one family. Macrocytic anemia developed between the age of 2 to 4 months. Oral administration of high doses of folate improved hematological abnormalities. One shows retinitis pigmentosa. The other two cases have severe stomatitis which is resistant to high dose oral folate administration. Brain CT scan revealed calcification in basal ganglia during early childhood in one patient. Brain MRI studies showed delayed myelination in the two that are sisters. Although developmental deterioration was prevented in one patient who was administered folate intramusucularly (IM) at 26 months, severe retardation remained. In the other cases, who are sisters, neurological symptoms were milder than the aforementioned case. The elder sister who had been treated at an early stage, could not tolerate folate IM, therefore oral administration is being continued. Although the younger sister was under high dose folate administration from 3 months old, she developed epilepsy and mental retardation with hyperactivity resembling pervasive developmental disorder during childhood. Weekly high dose folate IM from 5 year old was effective to control of her seizures with co-administration of usual antiepileptics. High dose folate IM is effective for CFM.

O-35-5

CLINICAL PRESENTATION, DIAGNOSIS AND THERAPY IN TRANSCOBALAMIN II DEFICIENCY

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Introduction: Transcobalamin II (TC II) regulates the intestinal absorption of cobalamin via receptor mediated endocytosis and subsequent transport in plasma to tissues. Deficiency of TC II results in reduced bioavailability of cobalamin and subsequent pertubation of cobalamin dependent metabolic reactions. **Case report**: An 8 week old female infant was referred for further work-up of pancytopenia, recurrent vomiting and protein losing enteropathy. She was born as the first child to non-consanguineous, healthy parents. She has been well until 6 weeks of age when she started to vomit. Metabolic work-up demonstrated significant urinary excretion of methylmalonate, methylcitrate and 3-hydroxyisovaleric acid. The reminder of the work-up, including plasma homocysteine was unremarkable. She fully recovered within 2 weeks following initiation of 1 mg intravenous hydroxycobalamin per day. TC II deficiency was confirmed by ELISA. The results of the molecular analysis are still pending. At the last follow-up at 12 months of age she continues to receive 1 mg hydroxycobalamin twice weekly. Her psychomotor development remains completely unremarkable. **Conclusion**: TC II deficiency should be suspected in any infant with otherwise unexplainable pancytopenia and protein losing enteropathy. Early treatment with hydroxycobalamin will prevent long-term neurologic sequelae.

O-35-6

BIOTIN RESPONSIVE METABOLIC ACIDOSIS IN TWO SIBLINGS WITH NORMAL BIOTIN METABOLISM

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A six month old boy, born as the second child to healthy, non-consaguinuous parents was referred to our institution with severe metabolic acidosis during a viral infection. He was exclusively breastfed. On admission he was found to be tachypnoeic, but his clinical examen was otherwise unremarkable. Laboratory investigations showed severe metabolic acidosis (pH 6.9, HCO_3 1.8 mmol/l, BE –28), normal lactate 1.2 mmol/l, 3-hydroxybutyrate 12.1 mmol/l (norm <0.1), normal glucose 120 mg/dl, and normal ammonium 46 µg/dl. Organic acid analysis in urine showed massive ketonuria without evidence of multiple carboxylase deficiency. Despite aggressive therapy including bicarbonate and THAM infusions the clinical situation dramatically deterioated requiring mechanical ventilation. A trial with biotin (20 mg/d orally) was started. Within 6 h of the first dose of biotin, metabolic acidosis improved and mechanical ventilation could be discontinued after 24 h. The infant was discharged after 2 days. His clinical and neurological exam. were completely normal. The boy now at the age of 12 months continues on 20 mg of biotin/day without any additional episodes of decompensation. His older sister also presented with metabolic acidosis at the age of 18 months during a gastroenterits but was treated promptly with oral biotin. Extensive laboratory investigations including analysis of biotinidase activity, molecular analysis of intestinal biotin transporter (SLC19A3) were within normal limits. **Discussion**: Although known disorders of biotin metabolism and transport as well as nutritional causes have been ruled out, biotin therapy should be considered in any infant with sudden metabolic acidosis and ketosis.

O-36-1

ACUTE INTERMITTENT PORPHYRIA: NEW MUTATIONS FOUND IN THE PORPHOBILINOGEN DEAMINASE GENE IN CZECH AND SLOVAK PATIENTS

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Acute intermittent porphyria (AIP, MIM 176000) is an inborn error of heme metabolism. It results from the half-normal activity of porphobilinogen deaminase (PBGD, EC 4.3.1.8), one of the enzymes of the heme biosynthetic pathway. AIP is manifested by acute, life-threatening attacks of abdominal pain, gastrointestinal dysfunction, neurological disturbances, and accumulation of porphyrin precursors in the urine. To date, circa 300 various mutations in the PBGD gene have been found. Molecular analysis of DNA of AIP patients from seven Czech and Slovak unrelated families revealed eight mutations, including three novel mutations (610 C>A, 675 delA, 966 insA), and five previously reported mutations (76 C>T, 77 G>A, 518 G>A, 771 +1 G>T, 973 insG). Of particular interest was one patient who had two mutations, 518 G>A and 610 C>A, both located in the same allele of exon 10. Mutation screening was performed by PCR, denaturing gradient gel electrophoresis (DGGE), and DNA sequencing. To establish the effect of 518 G>A, 610 C>A, 675 delA, and 966 insA mutations, we prepared mutant constructs and characterized them enzymatically. **Conclusion**: Three novel mutations were identified in seven unrelated AIP patients. These mutations provide insight into the molecular heterogeneity of AIP, and facilitate molecular diagnosis in AIP families.

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O-36-2

ACUTE INTERMITTENT PORPHYRIA PRESENTING AS PERVASIVE DEVELOPMENTAL DISORDER AND MENTAL RETARDATION

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Introduction: Acute intermittent porphyria (AIP) is associated with a wide variety of symptoms but may present solely with psychiatric morbidity. The known spectrum of phenotypes includes psychosis, personality disorders and severe neurotic disturbance. Childhood pervasive developmental disorder (PDD) associated with mental retardation (MR) has not been previously reported. **Case Report**: A 14-year-old adolescent girl with PDD and MR was referred for investigation. She had been treated over the years with a variety of medications with poor results. Extensive investigations for known genetic and metabolic causes of PDD and MR failed to reveal a diagnosis. The parents were healthy and family history was negative. After the onset of menstruation, her behaviour deteriorated. Regulation of menstruation with oral contraceptives was expected to improve control but unexpectedly led to severe deterioration requiring hospitalisation. This observation led to the discovery of AIP. **Results**: Urinary excretion of porphyrin precursors was borderline normal in the proband and her mother, but porphobilinogen deaminase activity in erythrocytes was reduced by ~60%, in both. Mutation analysis revealed G532A (D178N) heterozygote status in proband and her mother. **Discussion**: The association of AIP and PDD/MR reported here extends the known phenotypic spectrum of AIP. AIP may present at a younger age than usually described. Patients with PDD/MR with no diagnosed cause, who fail to respond to treatment or who deteriorate unexpectedly, should be investigated for neuroporphyria.

O-36-3

CURATIVE BONE MARROW TRANSPLANTATION IN ERYTHROPOIETIC PROTOPORPHYRIA

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We report successful reversal of phenotype by bone marrow transplantation (BMT) in a man with erythropoietic protoporphyria (EPP). Our patient had severe photosensitivity since infancy. In 2003 he developed EPP-related liver failure with markedly elevated serum bilirubin and ALT as well as porphyrin concentrations in erythrocytes, serum and urine. To halt progression and prepare for liver transplantation, treatment aiming to intervene at several steps in the likely pathophysiology of EPP liver disease was started. Within eighty days all serum liver function tests were normalized and porphyrin biochemistry was approaching habitual concentrations. The situation offered an opportunity to attempt correction of the erythroid tissue, the main site for protoporphyrin overproduction in EPP, aiming to reverse phenotype and to avoid future liver affection. A BMT with a high-resolution HLA-identical unrelated donor was performed with reduced conditioning. Unfortunately the patient had autologous recovery. Another unrelated donor was identified and the patient underwent a second BMT, this time with heavier conditioning. The transplant course was uncomplicated. The EPP genotype is no longer present in peripheral blood cells, porphyrin biochemistry and liver function tests are normal and there are no symptoms of photosensitivity. Whether BMT prevents liver complications is unknown. Our report suggests that liver failure in EPP is reversible by medical treatment and provides strong evidence that the bone marrow is the target organ for EPP. It is reasonable to believe that future liver complications to EPP are prevented in this patient but longer follow-up is needed to provide a conclusive answer.

O-36-4

MUTATION SPECTRUM OF THE HUMAN *ATP7B* GENE OF KOREAN PATIENTS WITH WILSON DISEASE AND PREDICTION OF WILSON DISEASE INCIDENCE IN KOREAN POPULATION BY MAJOR MUTATION SCREENING IN NEWBORN FILTER PAPER

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Wilson disease (WD) is an autosomal recessive disorder of copper transport, probably one of the most common inherited metabolic disorders in Korea. The product of WD gene is a copper transporting P-type ATPase (*ATP7B*). Efforts have been made to identify novel mutations and investigate an allele frequency of each mutation in Korean patients with WD. Also, the neonatal screening of four major mutations (p.R778L, p.N1270S, p.A874V and p.L1083F) was undertaken in 476 newborns (952 alleles) to estimate the incidence of WD in Korea. Mutation scanning of entire coding regions including intron-exon boundaries was performed in 193 unrelated Korean WD patients by PCR-direct DNA sequencing. Function of the mutant has been characterized by yeast complementation assay and confocal microscopic evaluation after transient expression in mammalian cell system. Newborn screening method was based on multiplex PCR and SYBR Green I based real-time PCR with amplicon melting curve analysis using an ARMS method. Molecular defects of the *ATP7B* gene have been characterized in 77.5% of 386 WD alleles, accounting for 40 different mutations. The p.R778L is the most common mutation with 36.5% of allele frequency. The other common mutations are p. A874V, p.N1270S and p.L1083F. Their allele frequencies are 9.3, 6.2, and 4.4% respectively. Carrier frequency and incidence of WD in Korean population were estimated as 1/100 and 1/36 000 respectively, based on newborn screening. In conclusion, WD mutation spectrum is diverse and incidence is same as in other ethnic groups.

O-36-5

PROTECTIVE EFFECTS OF CURCUMIN ON LIVER INJURY OF COPPER-OVERLODING RATS

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Wilson's disease (WD) is an inherited disease of copper metabolism. Deposition of copper generates free radicals and plays major role in the pathogenesis of liver injury. The aim of the present study was to explore the protective effect and mechanism of curcumin (diferuloylmethane), a plant-derived polyphenol, on liver injury in copper overloading rats. Using an established copper-overloading Wistar rat model, curcumin was administered orally either 50 mg/kg or 200 mg/kg for 2 weeks and 4 weeks in the treatment groups. At the end of the experimental periods, blood and liver samples were taken for the measurement of malondialdehyde (MDA), glutathione (GSH) and glutathione peroxidase (GSH-px) levels. Apoptosis of liver cells was observed by electron microscope (EM). Fas/FasL were examined by immunohistochemistry. Liver mRNA levels of TNF-α, IL-Iβ and IL-8 were evaluated by reverse transcription polymerase chain reaction. Curcumin prevented the increase in MDA levels and the decrease in GSH and GSH-px levels. Observations by EM showed copper could induce liver cell apoptosis. Histological findings showed a protective effect of curcumin on liver injury in copper-overloading rats. The expressions of FasL/Fas in curcumin groups were significantly lower than untreated groups. The administration of curcumin inhibited the expression of TNF-α and IL-Iβ and IL-8 mRNA. Our data suggest that curcumin exerts antioxidant and anti-apoptosis activity in this model.

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P-1-1

SUCCESSFUL EARLY MOLECULAR SCREENING OF MSUD ON TWO AT-RISK NEWBORNS IN WISCONSIN NEWBORN SCREENING PROGRAM

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Maple syrup urine disease (MSUD) is an autosomal recessive disorder caused by mutations in genes BCKDHA, BCKDHB, DBT, and DLD. Although rare in most populations, MSUD has an incidence as high as 1 in 200 births in the Old Order Mennonite population. BCKDHA mutation Y438N is believed to be the only common mutation in this population. We have developed a reliable, rapid, low cost, and easy to use assay to detect this mutation using tetra-primer ARMS-PCR (Amplification Refractory Mutation System) technology. Genomic DNA was extracted from a 0.32 cm dry blood spot, and underwent a single PCR reaction followed by agarose gel electrophoresis. The mutant and wild type alleles were identified by their different-sized amplification products. The entire process took only 4 h. Two Mennonite families with parents known to be heterozygous for Y438N mutation agreed to participate in the study. Blood samples were collected from each newborn at 1 h after birth, and screened for Y438N mutation. One baby was identified as homozygous for Y438N mutation within 10 h of birth, and the treatment was initiated immediately. The child is now 8 months old, and has not experienced a metabolic crisis. Another baby was found to be unaffected based on the presence of two wild-type alleles. **Conclusions**: Tetra-primer ARMS-PCR procedure is a reliable, rapid, low-cost, and easy to use method for targeted mutation analysis on newborn screening specimens. This methodology can expedite the identification and management of maple syrup urine disease.

P-1-2

NEWBORN SCREENING PILOT STUDY FOR CITRIN DEFICIENCY: NO ABNORMAL AMINO ACID PROFILES IN SOME NEWBORN PATIENTS

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The patients with citrin deficiency due to SLC25A13 gene mutations are reported to experience neonatal intrahepatic cholestasis (NICCD). Biochemical features of this condition are the elevated blood levels of several amino acids including citrulline, together with galactose and bile acids, during early infancy. However, it is not clear what percent of the patients show NICCD or have abnormal amino acid profiles during neonatal period. We tested our cut-off values of screening indices, such as citrulline, methionine, phenylalanine, tyrosine and arginine, in newborn screening pilot study by tandem mass spectrometry, and have found 4 patients in 300 000 newborns, while 3 patients with negative results experienced NICCD later in early infancy. Among these 3 patient, one had jaundice and gray stool at 1 month of age and received surgical confirmation for biliary atresia because of ambiguous imaging results, and the other received systematic investigation for galactosemia because of mildly elevated galactose levels. No false-positive case has been recorded. Retrospective analysis of newborn dried blood spots of 12 patients, who experienced NICCD, revealed that 4 patients had index amino acid levels lower than the cutoff values; in a patient among 4, the galactose level in newborn blood spot was elevated, and in another, a mild increase of citrulline level together with markedly increased ratio of citrulline to serine in blood spot during NICCD was just a clue to citrine deficiency. All of 7 patients with negative results were not homozygotes for the IVS11+1G>A mutation.

P-1-3

GENETIC TESTING FOR GAUCHER DISEASE: SIMULTANEOUS DETECTION OF COMMON POINT MUTATIONS AND PSEUDOGENE-DERIVED COMPLEX ALLELES BY REVERSE-HYBRIDIZATION

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Gaucher disease (GD), the most frequent lysosomal storage disorder, is an autosomal recessive disease characterized by glucocerebrosidase (GBA) deficiency due to mutations in the GBA gene. Accumulation of glucocerebroside, mainly within cells of the monocyte-macrophage lineage, eventually leads to splenomegaly, hepatomegaly, thrombocytopenia, bone marrow suppression, or bone lesions. The disease is panethnic and has been divided into three major types on the basis of the absence (type 1) or the presence and severity of neurologic manifestations (types 2 and 3). The most common variant is type 1 GD which is particularly prevalent among Ashkenazi Jews with a carrier rate of 1 in 15. Enzyme replacement therapy is available for type 1 GD patients. We have developed a reverse-hybridization assay for the simultaneous detection of eight common point mutations (84GG, IVS2 (+1)A, 1226G, 1297T, 1342C, 1448C, 1504T and 1604A) and two multiply mutated alleles (RecNciI, RecTL) derived from rearrangements between the structural gene and the GBA pseudogene. The test is based on two multiplex DNA amplification reactions and ready-to-use test strips presenting a parallel array of oligonucleotide probes for each wild-type and mutated allele. The entire genotyping procedure from blood sampling to final result requires less than 6 h, and hybridization/detection may be automated using robotic equipment.

P-1-4

SEMI-AUTOMATED DETECTION OF MULTIPLE MUTATIONS ASSOCIATED WITH HEREDITARY SUGAR INTOLERANCE

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A variety of genetically determined enzyme and transporter deficiencies may cause hereditary intolerance to common dietary sugars. Lactose intolerance (adult-type hypolactasia, lactase non-persistance) is an extremely frequent autosomal recessive condition causing diarrhea, nausea and flatulence. It is highly associated with two mutations located upstream from the lactase-phlorizin hydrolase (LPH) gene locus. Hereditary fructose intolerance is an autosomal recessive disorder caused by mutations in the aldolase B gene (ALDOB). Affected subjects suffer from severe abdominal pain, vomiting, hypoglycaemia, and unless fructose-containing food is strictly avoided may even die from irreversible damage of the liver and kidney. We have developed a reverse-hybridization assay for the rapid and simultaneous detection of two mutations (–13910 C/T, –22018 G/A) upstream of the LPH locus and four mutations (Δ4E4, A149P, A174D, N334K) in the ALDO B gene. The test is based on a single multiplex DNA amplification reaction followed by the hybridization of biotinylated PCR products to a test strip presenting a parallel array of allele-specific oligonucleotide probes for each mutation. The entire procedure from blood sampling to the identification of mutations requires less than 6 h, and hybridization/detection may be carried out manually or essentially automated using robotic instrumentation.

P-1-5

INBORN ERRORS OF METABOLISM AND THE ORPHAN DRUG ACT

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A list of all designated orphan products, granted since the US Orphan Drug Act was enacted, was screened against a list of inborn errors of metabolism. A total of 54 designations for inborn errors of metabolism were found. This compares with 1554 total orphan designations for all diseases. Of these 54 orphan designations, twenty, or 35%, have already led to products approved by the FDA for marketing, compared with a total of 284 marketed orphan products. While orphan designations related to inborn errors of metabolism comprise only 3.5 % of total orphan designations, they comprise 7.0% of all orphan products approved for marketing. On average there have been 2.35 orphan designations granted per year related to inborn errors of metabolism. The 54 orphan designations noted above comprise 49 unique products. There has been intense research interest in certain diseases, leading to multiple orphan designations for these indications. Gaucher's Disease, Fabry's disease, Wilson's disease, urea cycle disorders, cystinosis and acute intermittent porphyria all have received more then one orphan designation. In total the 54 orphan designations relate to treatments for 27 different inborn errors of metabolism. On average nearly one orphan product a year has been approved for inborn errors of metabolism over the lifetime of the office. Advances such as enzyme replacement therapies and 'pegylated' proteins have found wide applicability and have led to standard of care changing clinical advances both within and outside of the rare disease community.

P-1-6

PHENYLKETONURIA IN SERBIA AND MONTENEGRO

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The incidence of phenylketonuria (PKU) in Serbia and Montenegro is 1:12 300 newborns. This is the first report on phenylalanine hydroxylase (PAH) gene mutations and genotype-phenotype correlation in PKU patients from Serbia and Montenegro. According to pretreatment serum phenylalanine level, 34 unrelated patients were assigned to classic PKU (65%), mild PKU (35%) and MHP (0%). By using both PCR-RFLP and 'broad range' DGGE/DNA sequencing analysis, 19 mutations were identified (13 missense, 3 nonsense, 2 splice and 1 frameshift-del). Mutation detection rate was 97%. The most frequent mutations were: L48S (21%), R408W (18%), P281L (9%), E390G (7%) and R261Q (6%), accounting for 60% of all mutant alleles. The remaining ones (R158Q, I306V, IVS12+1G>A, Q20X, R111X, V177L, P225T, R261X, L15/S16fsCTdel, S231F, R252Q, R297H, IVS10-11G>A and R413P) occurred at frequency less then 5%. Calculated homozygosity value was rather low (0.10), indicating the heterogeneity of population. This finding reflects numerous migrations over the Southeastern Europe. The genotype-phenotype correlation was studied in homozygous and functionally hemizygous patients. The phenotypic inconsistency of the mutation L48S, the most frequent one in Serbia and Montenegro, was reported in previous European studies. To the contrary L48S was exclusively associated with the classical PKU phenotype in our study. Consistent severe phenotype of L48S could be a consequence of independent modulatory genetic factors specific for the population. The characterization of PAH mutations created the base for molecular diagnostics and genetic counseling of phenylketonuria in Serbia and Montenegro.

P-1-7

SCREENING FOR INBORN ERRORS OF METABOLISM IN KUWAITI POPULATION BY TANDEM MASS SPECTROMETRY: A PILOT STUDY

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Background: Clinical studies have shown high rates of inborn errors of metabolism (IEMs) among Arabian populations in the Gulf region. **Objectives**: The aims of this study were to indicate the frequency of IEMs among Kuwaiti population and to highlight the importance of tandem mass spectrometry in pediatric practices for IEMs screening of amino acids, organic acids, fatty-acid oxidation (FAOs) and urea cycle disorders. **Methods**: More than 1520 samples collected from Kuwaiti newborns and symptomatic infants from different clinics in Kuwait were analyzed in the period May 2005–March 2006, by in-house tandem mass spectrometry (Micromass) using NeoLynx program for Neonatal Screening. This program permits profiling of the diagnostic markers and automatic detection of abnormalities. The results and demographic data of patients were built in our database. Interpretation and consultation processes were based on reviewing the profiles and reports generated by the program. **Results**: In our screening, the following were identified, five patients with propionic acidemia, three patients with methylmalonic acidemia, two patients with GA-I, one patient with IVA, two patients with MCAD, one patient with Tyrosinemia I, one patient with PKU, one patient with MSUD, one patient with homocystinuria, four patients with citrullinemia, sixteen patients with FAOs and one patient with Canavan's disease. SIDS cases due to FAOs were also diagnosed. **Conclusions**: IEMs are prominent among Kuwaiti population. High percentages of FAOs defects and organic acids disorders were detected. The study indicates the importance of screening of IEMs in newborns and symptomatic infants using tandem mass technology in Kuwait.

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P-1-8

DEVELOPMENT OF A PKU PATIENT SELF-CONTROL SYSTEM. PRODUCTION OF L-PHENYLALANINE SENSOR

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Most PKU patients have been well treated owing to the newborn mass-screening program and Phenylalanine (Phe)-restricted diet. However, some degree of neurological impairment were inevitable in cases with poor dietary control. Precise and frequent measurement of Phe levels in blood is difficult at home because of laborious steps in the ordinary system. Therefore we developed a high sensitive Phe sensor for point-of-care testing (POCT) for PKU patients.

The Phe sensor consists of sensor-tip and sensor-meter. In the sensor-tip, a working electrode and a counter electrode were formed by the screen-printing method. Reagents, such as phenylalanine-dehydrogenase, coenzyme and diaphorase, were fixed by dropping and drying the solution containing the reagents on a working electrode. Furthermore, the sensor-tip was built by arranging the membrane, which was applied to tetrazolium salt and buffer, on both electrodes. The sensor-meter has functions of applying a potential to the sensor-tip and measuring a response current.

Using this sensor, we have succeeded in measureing Phe at concentrations from 0.5 mg/dL to 50 mg/dL in standard solution. Good linearity was obtained in the correlation curve between the data by the enzymatic chemical method and the data by the sensor.

These results proved that our Phe sensor can be used as a tool of POCT for PKU patients. Clinical examination is being performed on many PKU patients. The final purpose of this study is to construct a useful Phe sensor which can support the Quality-of-life (QOL) for every PKU patient to avoid the occurrence of the disease.

P-1-9

ANALYSIS OF THE RESULTS OF PILOT SCREENING BY TANDEM MASS SPECTROMETRY (MS/MS) IN NEWBORN WITH LOW AND VERY LOW BIRTH WEIGHT IN POLAND

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Tandem mass spectrometry (MS/MS) represent a powerful method for detection of many inborn errors of metabolism (IEM) and is now widely used in the neonatal screening. Although it is applied for all neonates, there is still problem with preterm and low birth weight (LBW) and very low birth weight (VLBW) infants because the normal ranges of aminoacids and acylcarnitines for them are not yet established.The present study aimed to determine the birth weight-related changes in aminoacids (AA)and acylcarnitines (AC) profiles in newborn screened for IEM.We analysed samples from 7077 neonates born between 2005 and 2006 weighted $\leqslant$2500 g (560 g–2500 g). Analysis was done by Tandem Mass Spectrometry (Perkin Elmer) in dried blood using published methods. **Results**: Re-sampling was requested on 403 neonates (6%) of whom 197 (48.8%) had an abnormal AA profile, 201 (49.8%) an abnormal AC profile and 11 (1.4%) low free or total carnitine concentrations. The most often abnormalities included high concentration of methionine (139), arginine (52) and isovelerylcarnitine (171). In all cases, the recall investigations after a suspicious first screening tests were normal. Analysis of normal results showed that mean concentrations of most AA as well as free carnitine, acylcarnitines C4, C5 and C8 were higher in neonates $\leqslant$2500 g as compared to respective mean concentrations in AGA neonates. Mean concentrations of total carnitine were lower in VLBW neonates 560–1000 g and higher in neonates $>$1000 g to 2500 g as compared to AGA neonates. **Conclusion**: introduction of weight related cutoffs for AA and AC in MS/MS screening seems to be necessary in order to eliminate false positive/negative results.

P-1-10

CALIBRATION STANDARDS FOR DICARBOXYLIC ACYLCARNITINES

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Dicarboxylic acylcarnitines are diagnostic metabolites for a number of inherited diseases, and their quantification has become part of the neonatal screening program in many countries.

A drawback in the quantification of dicarboxylic acylcarnitines is the lack of specific (labeled and unlabeled) standards. Although dicarboxylic acylcarnitines have been prepared chemically, yields were poor and products were impractical to handle since no crystalline compounds could be obtained.

Common practice in acylcarnitine measurement is a derivatisation step to transform carboxyl groups into ester functions, methyl or butyl esters finding broad application. For dicarboxylic acylcarnitines, this results in the formation of a diester derivative: one ester function at the carnitine part of the molecule, the second at the unsubstituted part of the dicarboxylic acyl moiety.

We found that mono-esterified dicarboxylic acids with various chain lengths, coupled to carnitine, provide acyl-esterified dicarboxylic acylcarnitines in good yields. Products are stable solids, easy to handle and obtainable in a high degree of purity. When applied in the usual sample preparation procedure including esterification, they are transformed into the known diester derivatives.

A number of mono-esterified dicarboxylic acylcarnitines has been prepared, to make them available as calibration standards for tandem MS quantification of the parent compounds in different matrices.

P-1-11

ON-LINE CHEMICAL DIAGNOSIS OF INBORN ERRORS OF METOBLISM USING GAS CHROMATOGRAPHY MASS SPECTROMETRY WITHIN NETWORK LABORATORY

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Since 1997, we started overseas service of 'chemical diagnosis of inborn errors of metabolism' by analyzing urinary metabolites using gas chromatography mass spectrometry (GC/MS) method to Asian countries. Urine samples were collected from sick neonates or infants suspected to have a metabolic disease by the doctor, and received by airmail within 5–7 days, which was the time required for bedside urine collection. The result was reported by e-mail to the referring doctor in 2 days after the sample was received. However, many sick neonates or infants died before their final diagnosis, despite the therapy given. To avoid the death of patients due to late diagnosis, the most effective speedy analysis and on-line data analysis report process was developed to save sample posting time. To achieve this task, a 'Network Laboratory' was established in the selected hospital where sample preparation and GC/MS analysis were done on the same day of urine sample collection; the GC/MS analysis data could be accessed by data analysis center. The data analysis process and result reporting was done on-line between Japan and Network Laboratory. The samples of 3400 patients were analyzed during May 2005 to March 2006, and 336 patients were discovered with no death during this period. Through this program, it is now possible to give the result within 1 to 2 days of sample collection, thus saving the precious time. Since it is very rapid and accurate, we recommend this program to be expanded to other hospitals to reduce mortality and morbidity in children.

P-1-12

SCREENING AND DIAGNOSIS FOR INHERITED METABOLIC DISEASES BY GAS CHROMATOGRAPH/MASS SPECTROMETER

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Objective: Using the technology of gas chromatograph /mass spectrometer to screen and diagnose inherited metabolic diseases for high risk children, in order to discover type of diseases and evaluate their incidence. **Methods**: 182 urine samples from high risk cases of inherited metabolic diseases were analyzed by gas chromatograph/mass spectrometer. Most patients had convulsion, slow development, malnutrition, mental retardation, muscle hypotonia, hepato-splenomegaly, vomiting and acidosis. **Results**: Nineteen of 182 patients were positive and the incidence was 10.4%. Twelve kinds of diseases were concerned in 19 cases, including six cases with methylmalonic academia (one with homocysteinemia), two with hyperglycerolemia, two with lactic acidmia, one with propionic academia, one with glutaric aciduria type I, one with dicarboxylic aciduria, one with fructose-1,6-diphosphatase deficiency, one with tyrosinemia, one with maple syrup urine disease, one with pheylketonuria, one with multiple carboxylase deficiency and one with β-aminoisobutyric acid. **Conclusions**: It is difficult to diagnose inherited metabolic disease only by using common biochemical methods and other auxiliary examination. However, GC/MS is one of important and useful methods for diagnosing inherited metabolic diseases. Simultaneously, it could be used to study the metabolic changes in the course of disease.

P-1-13

EXPANDED NEWBORN SCREENING: LONG TERM FOLLOW UP OF 25 PATIENTS WITH ORGANIC ACIDEMIAS AND UREA CYCLE DISORDERS

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There are quite different recommendations which genetic metabolic diseases should be investigated using tandem mass spectrometry in newborn screening programs. As a contribution to this discussion we report on the long term follow up of 25 patients (12 patients with organic acidemias and 13 with urea cycle disorders) found in the newborn screening program in Germany. 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. 2 Children died in spite of early treatment at 4 and 16 months of age. 23 infants were detected by expanded newborn screening. 5 newborns were symptomatic before results of screening tests were available. 11 children showed metabolic changes where clinical relevance is not clear. In 13 children treatment was indicated, in 9 of them treatment was initiated on the basis of screening test results only. Thus 9 out of 25 patients (36%) had direct benefit 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.

P-1-14

***WWW.RAMEDIS.DE*: DATABASE FOR LONG TERM FOLLOW UP OF PATIENTS WITH RARE METABOLIC DISEASES**

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A database for patients with genetic metabolic diseases accessible via the internet was developed. The aims were the following: to create an electronic publishing tool for case reports with direct database input and long term follow up of treated patients and to provide a tool for multicenter studies. Including molecular genetic, clinical and biochemical data genotype/phenotype correlation should be possible. For data input controlled vocabulary of 366 different diseases, 713 laboratory parameters and 636 clinical terms is included. Direct input of data is possible using the input and edit function. In addition a short abstract with free text can be included. For long term follow up of patient case report forms for some diseases were developed. As an example long term follow up of 10 patients with B_{12} non responsive methylmalonic aciduria and chronic renal failure is demonstrated. Data confidentiality is assured by anonymous data input which is only possible by the corresponding author. Simple search functions can be performed by all users. So far data from 735 patients with more than 25 000 laboratory values are included. To make this approach more effective more data should be included. In addition as for other publication forms a review process should be implemented.

P-1-15

***WWW.METAGENE.DE*: ONLINE KNOWLEDGE BASE FOR INBORN ERRORS OF METABOLISM**

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There is a rapid increase in numbers and complexity of inborn errors of metabolism (IEM). Where as the clinician has the problem to decide which tests are necessary, the laboratory expert often lacks clinical experience or gets only little information about the clinical symptoms. Consulting excellent textbooks is best, but time consuming. Databases on the internet contribute to a faster diagnoses and treatment of these disorders. As result on our approach to give diagnostic support in IEM we developed the reference book METAGENE online (*www.metagene.de*), also available for Desktop PC and Pocket PC. So far 415 diseases and differential diagnoses, more than 1400 laboratory findings and clinical symptoms, mass spectrometry results, pictures of patients, 2500 references and multiple links to OMIM, Expasy and PubMed have been implemented. METAGENE is helpful for teaching younger and less experienced colleagues working in the metabolic unit. For clinicians, health professionals and parents groups links to international societies and patient support groups are implemented. In contrast to OMIM special attention is made on the typical metabolic profile of diseases. So far, there is a free access for all users who would like using METAGENE.

P-1-16

TWO CASES OF CITRIN DEFICIENCY DETECTED BY NEWBORN SCREENING IN KOREA

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Adult-onset type 2 citrullinemia (CTLN2), citrin deficiency, is an autosomal recessive disorder that has been increased the detection by using MS/MS in newborn screening. We report two citrin deficiency cases detected by newborn screening program in Korea. Both cases have been ascertained by elevated levels of citrulline, ornithine, phenylalanine and tyrosine with normal galactose. Their clinical manifestations include prolonged jaundice, hepatomegaly, and failure to thrive. Laboratory investigation indicated mild liver dysfunction AST 76, 142 U/L (N: 15–37 U/L), ALT 24, 73 U/L (N: 30–65 U/L), mild hyperammonemia 103–112 μg/dl (N: 12–66 μg/dl), and markedly increased direct bilirubinemia 3.8, 4.2 mg/% (N: 0.1–0.2 mg/%), and alkalinephosphatase 1693–2946 U/L (N: 50–136 U/L). Plasma amino acid analysis showed a raised citrulline 360, 557.2 nmol/ml (N: 10.0–45.0 nmol/ml), arginine 156, 418.1 nmol/ml (N: 6.0–140.0 nmol/ml), and tyrosine 187, 397.7 nmol/ml (N: 550.0–147.0 nmol/ml). Abdominal sonogram displayed moderate fatty liver in both cases. Molecular study in one case revealed compound heterozygote with IVS11+1G>A and IVS16ins3kb. Citrin deficiency should be considered as a differential diagnosis in the babies with citrullinemia, direct hyperbilirubinemia, high alkalinephosphatase and mild liver dysfunction.

P-1-17

CPT-1A P479L VARIANT IDENTIFIED IN ALASKA NATIVE INFANTS BY EXPANDED NEWBORN SCREENING

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The Northwest Regional Newborn Screening Program (NWRNSP) performs comprehensive newborn screening by MS/MS for the states of Alaska, Hawaii, Idaho, Nevada, and Oregon. Since initiating tandem MS screening for Alaska in July, 2003, we have detected 28 cases of carnitine palmitoyltransferase-1A (CPT-1A) deficiency in Alaska Native infants. All of the infants have subsequently been confirmed to be homozygous for a CPT-1A c.1436C→T sequence variant that results in a proline to leucine change at amino acid 479 (P479L). CPT-1A activity in cultured skin fibroblasts from seven of these infants is reduced to approximately 20% of normal, but importantly this amino acid substitution eliminated sensitivity of the CPT-1A enzyme to its inhibitor, malonyl-CoA. Classical CPT-1A deficiency is a rare inherited disorder of metabolism associated with fasting or illness induced hypoketotic hypoglycemia, liver dysfunction and an increased risk of sudden infant death. To date, infants who are homozygous for the P479L substitution have reportedly been healthy, but the associated phenotype has yet to be carefully studied. The high incidence of this sequence variant in the Alaska Native population is unlikely to have occurred by random chance; rather it suggests a positive selection within the population, which may represent an adaptive mechanism to the high fat, high protein diet and harsh temperatures of the arctic region experienced by Alaska Native peoples through history. Further studies will be required to determine the full impact of this common sequence variant on the health of the Alaska Native population.

P-1-18

NEWBORN SCREENING FOR GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY: HIGH INCIDENCE IN SAUDI INFANTS

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency is genetically heterogeneous disorder. The most common clinical manifestations are neonatal jaundice and acute hemolytic anemia. **Aims**: To determine the incidence of G6PD deficiency in Saudi infants screened at birth and to determine the incidence of neonatal jaundice requiring phototherapy in G6PD-deficient infants. **Method**: All Saudi infants born at Saad Specialist Hospital in AlKhobar, Saudi Arabia, were screened for G6PD at birth. Peripheral blood samples were taken and analyzed using ultraviolet quantitative kinetic method. Reference range according to manufacturer was 120–240 mU/RBC in million. Cases were classified according to WHO classification into severe (<10% of lower reference range) and moderate (10–60%). **Results**: 1366 Saudi infants were born in the hospital between January and December 2005, all of them were screened. 156 infants had G6PD deficiency (incidence 114 per 1000 live birth). Two thirds of G6PD-deficient infants had severe enzyme deficiency. One third of the G6PD-deficient infants were females. Two thirds of G6PD deficient females had severe enzyme deficiency. 35 (22%) of G6PD-deficient infants developed neonatal jaundice requiring phototherapy. **Conclusion**: The incidence of G6PD deficiency was high in the screened infants, with high incidence of severe G6PD deficiency in females. Significant number of G6PD-deficient infants developed neonatal jaundice.

P-1-19

DETECTION OF NEONATAL HAEMOCHROMATOSIS BY TANDEM MASS SPECTROMETRY

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We report two neonates with neonatal haemochromatosis detected by abnormal Tandem Mass Spectrometry (MSMS) amino acid profile at newborn screening. **Case 1**: 3080 g 2 day old male, born at term, developed hypoglycaemia requiring dextrose infusion, was breast fed and discharged on day 6. **Case 2**: 3240 g 3 day old male, born at term to hepatitis C positive Mother. The baby was initially well, developed lethargy and hypotonia on day 3 and found to have hypoglycaemia requiring a dextrose infusion, was gavage fed until breast feeding was established and was discharged on day 7. **Follow up**: On notification of the abnormal amino acid profile with elevated tyrosine and methionine levels suggestive of liver disease each case was reviewed. Each baby was reviewed on day 13 and case 2 was referred to a tertiary hospital for further evaluation on day 17. Both had elevated Ferritin levels; Case 1: 3586 μg/L, Case 2: 8750 μg/L (NR 10–120) consistent with neonatal haemochromatosis. Both babies were treated with desferrioxamine, selenium and acetyl cysteine and oral vitamins C and E, FFP and albumin and intramuscular vitamin K. Both remain well. **Conclusion**: The early recognition of an abnormal amino acid profile, suggesting liver disease, by MSMS facilitated the early intervention and treatment in two infants with neonatal haemochromatosis. Newborn screening programmes should be aware that there is a need for notification of results suggesting organ dysfunction as well as recognizable inborn errors.

P-1-20

A DIRECT, NON-RADIOACTIVE HPLC ASSAY OF HEMOLYSATE GALACTOSE-1-PHOSPHATE URIDYLYLTRANSFERASE ACTIVITY

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Conventional UDP-glucose consumption or radioactive galactose-1-phosphate uridylyltransferase (GALT) assays have limited accuracy for low enzyme activity. We aim to apply a sensitive, direct, and non-radioactive HPLC assay of hemolysate GALT activity for clinical laboratory setting. Hemolysates were prepared as described previously, except that endogenous metabolites were removed by spun-column separation prior to analysis. GALT enzymatic reactions were performed as described previously. Substrates and products of the reaction were separated and quantitated using a DX600 HPLC system, as described previously for yeast and mammalian cell samples. Patient samples that have been confirmed both biochemically and genetically in our lab were analyzed; results of the HPLC method were compared to those derived using the traditional radioactive method; the correlation was 0.93 (15 N/N, 11 D/N and LA/N, 11 G/N, 11 D/G and 8 G/G). We also studied the reproducibility of UDP-Galactose formation in these assays. Preliminary data demonstrated that the % CV (intra-assay) was 4.4–5.3 for N/N, 8.3–14.4 for D/G and 24–25 for G/G respectively; % CV (inter-assay) on selected samples was 12.1 for N/N (n = 6), 10.8 for D/G (n = 4) and 37.7 for G/G (n = 4), respectively. It is understandable that as the GALT enzyme activity in a sample decreases, the degree of detected variation of UDP-Galactose formation increases. We conclude that this new method is feasible and more quantitatively reliable. Further validation studies are needed to compare the sensitivity and reproducibility of this approach to conventional methods.

P-1-21

BIOMONITORING OF 21 TRACE ELEMENTS IN URINE OF WILSON DISEASE TREATED WITH D-PENCILLAMIN BY ICP-MS

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Objective: Wilson disease is an autosomal recessive disease that has abnormal copper metabolism. Metal-chelator D-pencillamin is one of the most prescribed drugs for treatment of Wilson disease in China. However, whether long term D-pencillamin therapy induces metabolic abnormality of necessary trace elements remains unclear. This study measured 21 trace elements in 24h urine of Wilson disease treated with D-pencillamin by using a novel high sensitive and stable method ICP-MS. **Methods**: 40 Wilson disease patients received low copper diet and D-pencillamin therapy were included in our study. D-pencillamin treatment duration varies from 3 months to 14 years. 12 healthy children were studied as control. 24 h total urine were collected and 21 trace elements including Be, Mg, Al, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Cd, Sb, Ba, Ti, Pb, Th, and U were measured by ICP-MS. **Results**: Urine copper and arsenicum were significantly increased in patients with Wilson disease compared with healthy children ($p < 0.01$). Higher urine concentrations of necessary trace elements like Calcium and Zincum were also showed in Wilson disease patients ($p < 0.05$). **Conclusion**: This study indicates that D-pencillamin has chelate effect not only on harmful Cu and As, but also on necessary trace elements like Calcium and Zincum. Our study underscores an important role for biomonitoring urine trace elements in long term D-pencillamin therapy of Wilson disease.

P-1-22

BLOOD GLYCINE LEVELS IN NEWBORNS WITH LATER DIAGNOSIS OF NON-KETOTIC HYPERGLYCINAEMIA

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Non-ketotic hyperglycinemia (NKHG) is a devastating metabolic condition which usually presents with seizures and encephalopathy in infancy. Glycine levels are raised in both plasma and cerebrospinal fluid with an increased CSF/plasma ratio. Blood glycine levels can now be measured in neonates by routine tandem mass spectrometry newborn screening. Early diagnosis of such patients would allow for early institution of therapy, which might improve the neurological sequelae of those patients with post-neonatal onset.

From April 1998 to March 2006, 733 527 babies were screened by MSMS. The median, upper 95th and 99.5th centiles for dried blood spot glycine were 280, 549, and 827 μmol/L respectively. Eight of those babies were subsequently diagnosed with NKHG. Blood glycine levels on newborn screening were 242–1210 μmol/L. Two out of the eight NKHG patients had glycine levels above our action level of 1000 μmol/L on newborn screening. Those two and two others were symptomatic within 72 h of life, and diagnosed quickly. The remaining patients could not have been diagnosed by newborn screening without a recall rate for glycine alone of 0.3 to 72% of babies born. We conclude that babies with NKHG do not usually have a sufficiently elevated blood glycine level at 48–72 h of life for identification by current newborn screening strategies.

P-1-23

5 YEAR AUDIT OF NEWBORN SCREENING IN AUSTRALIA

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Newborn screening (NBS) in Australia is publicly funded but not mandatory. All babies are offered screening for a range of treatable inborn errors of metabolism at one of 5 state reference centres. Tandem mass spectrometry screening started from 1998, but was not universal until 2005. Between Jan 2000 and Dec 2004 there were 1 252 425 babies born, and we audited results for phenylketonuria (PKU) congenital hypothyroidism (CH), cystic fibrosis (CF) and galactosaemia (Gal). There were 890 refusals (0.07%). Most samples were collected between days 2–4 of life (89%). Repeat collections were requested from 8921 babies (0.71%) for unsuitable samples and 3909 babies for abnormal results. To ensure consistent reporting, definitions for inclusion have been determined. There were 86 babies diagnosed with PKU (1:14 563) with an elevated phenylalanine level on NBS, negative test results for pterin disorders, and for whom a low phenylalanine diet has been prescribed. A further 2 babies were diagnosed with a pterin disorder and 35 with persistent hyperphenylalaninaemia. Primary congenital hypothyroidism was confirmed in 447 babies with a further 8 diagnosed clinically (1:2753). Only PKU and CH were screened for in all babies. Cystic fibrosis was detected in 385/1 227 332 with 24 diagnosed clinically (1:3000 live births). Galactosaemia due to galactose-1-phosphate uridyl transferase was confirmed in 19/949 675 babies (1:49 983) plus 2 were diagnosed with galactokinase deficiency. There were no missed cases of PKU or galactosaemia. In 2006, all Australian babies are screened for PKU, CH, CF, and by tandem mass spectrometry for 30+ inborn errors of metabolism.

P-1-24

EARLY MSUD DIAGNOSIS BY PCR-BASED MUTATION SCREENING OF AN E2 FOUNDER DELETION

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Background: Maple syrup urine disease (MSUD) is a rare autosomal recessive disorder caused by defective enzyme activity of the branched chain alpha-ketoacid dehydrogenase enzyme complex. Early diagnosis and management of MSUD are imperative in preventing permanent neurological insult. In the Philippines, a 4.7 kb deletion in the E2 gene has been reported as a common mutation. Molecular analysis to immediately screen for the founder mutation commonly found in affected Filipino patients was done to provide early diagnosis and effective treatment prior to development of any clinical insult. **Methods**: Two neonates born to unrelated carrier couples for MSUD were enrolled. Exon 11 and the junction fragment containing the 4.7 kb deletion of the E2 gene were both examined by PCR amplification. **Results**: Electrophoresis results revealed one patient to be homozygous and the other to be a carrier of the E2 deletion. One patient was found to be homozygous for the E2 gene deletion thus treatment for MSUD was provided. The other was found to be a carrier thus normal feeding was started. **Conclusion**: Examination of the E2 founder mutation facilitated early MSUD diagnosis and is thus beneficial for provision of proper treatment.

P-1-25

NEWBORN SCREENING SYSTEM IN JINAN

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Objective: Jinan is a capital city of north China, which has a population of 5.6 million and 60 000 to 70 000 newborns every year. This article discusses how to establish a successful mode for newborn screening system in Jinan and improve the quality of population by degrading the birth defect. **Methods**: Set up a screening center which in charge of administration, organization and actualize of disease screening for the whole city. Founding the screening system includes specimen collection, delivery, laboratory test, diagnoses, treatment and follow up. This system proved to be effectively which has covered 99.7% of the birth population. In 2001, we bring hearing screening system into disease screening system, setting up synchronous administration mode for newborn disease screening and hearing screening. We have screened five diseases by the system: PKU, CH, CAH, G-6-PD and hearing loss. **Results**: We screened over 410 000 newborns and diagnosed 256 cases of congenital disease which includes 182 cases of CH, 65 cases of PKU, 2 cases of CAH and 7 cases of G-6-PD(For CAH and G-6-PD,we screened 20 000 newborns). We also diagnosed 300 cases of hearing loss from over 90 000 newborns. All the patients got the early diagnosis and proper treatment. **Conclusion**: Newborn screening is an effective way to protect newborns from the harm by congenital metabolic diseases. In our developing country, we should set up an effective and economic system for newborn screening.

P-1-26

CITRIN DEFICIENCY DETECTED BY TANDEM MASS SPECTROMETRY USING DRY BLOOD SPOTS

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Citrullinemia type 2, a defect in citrin a mitochondrial aspartate-glutamate carrier protein is a recently described cause for neonatal intrahepatic cholestasis. It has been found to be more common in Asian population especially the Japanese. We described here 2 babies, both Chinese in origin, presented with liver impairment in late neonatal period associated with cholestatic jaundice and elevated liver enzymes. Both patients had positive urine reducing sugar but non-glucose in origin. Serum ferritin levels were elevated but lower than those found in neonatal hemochromatosis. Dry blood spots for high risk screening were carried out for both babies using tandem mass spectrometry. Both revealed marked elevation of citruline and methionine as well as tyrosine and ornitine. These were further confirmed by plasma amino acids revealing characteristic elevation of citrulline, methionine and tyrosine, lysine, threonine and ornithine with low aspartic acid Urine organic acid revealed only metabolites found in liver impairment. Rapid diagnosis resulted in early medical treatment using lactose free soy milk milk and fat soluble vitamins. Both recovered from their cholestatic jaundice and remained healthy. Citrin deficiency is another inborn errors of metabolism detected by newborn screening.

P-1-27

A NEW ASYMPTOMATIC CASE OF METHYLMALONIC ACIDEMIA (MMA) IDENTIFIED BY MS/MS NEWBORN SCREENING

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Tandem mass spectrometry (MS/MS) newborn screening has revealed a number of asymptomatic subjects even if affected by metabolic disorders. We report such a condition in a child affected by MMA who was detected by the analysis of derivatized acylcarnitines in blood spot (PerkinElmer MS2 Kit). The sample collected at 3 days of life showed an elevation of propionylcarnitine (C3: 4.38 μM) slightly below the cut-off (4.60 μM) and a C3/C2 ratio higher than cut-off (0.20, cut-off 0.18). In a second sample (at the age of 28 days) a marked increase of C3 (7.5 μM) and C3/C2 ratio (0.64) was found. Urine methylmalonic acid excretion was increased and was not reduced after hydroxycobalamin administration, suggesting a defect of methylmalonyl-CoA mutase (EC 5.4.99.2). Direct sequencing of the 13 exons and intron-exon boundaries of methylmalonyl-CoA mutase gene (c.DNA NM.00025; g.DNA NT.007592) revealed that the patient was compound heterozygote for two already described mutations, N219Y (c.655A>T) and R694W (c.2080C>T), which were compatible with a mild (mut^0/mut^-) biochemical phenotype. The boy, now 8 months old, has been examined regularly since the first month of life when the specific dietary treatment was introduced. A very mild dystonic posture of upper limbs, observed during the first months of life, disappeared afterwards. His psychomotor development remained normal. **Conclusion**: (a) C3/C2 ratio is an important diagnostic marker for early MMA detection; (b) since, in the present case, the molecular analysis results are consistent with a mild form with infantile onset, the absence of symptoms could be the results of early treatment.

P-1-28

ACUTE METABOLIC CRISIS INDUCED BY VACCINATION IN SEVEN CHINESE PATIENTS

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Seven Chinese patients (5 boys and 2 girls) with vaccination-induced acute metabolic crisis were reported. Only 1 boy with 21-hydroxylase deficiency had been diagnosed prior to his vaccination. In the remaining 6 patients, the pre-existing diagnoses were not confirmed before the vaccination. Acute metabolic crisis occurred in 7 patients between 3 and 12 h after the administration of Japanese encephalitis, diphtheria and tetanus toxoids and acellular pertussis, hepatitis B or measles vaccines. **Case 1 and 2** displayed acute adrenal insufficiencies at the ages of 5 years and 3 months, respectively. **Case 3** had presented with mild motor retardation previously. **Cases 4 to 7** were previously healthy, but suffered from fever, seizures, coma, acidosis and hypoglycemia after being vaccinated. Glutaric aciduria type 1 was found in case 4. Leigh syndromes were found in cases 5, 6 and 7. They all died from respiratory failure before 2 years of age. Symmetrical foci, cystic cavitations with neuronal loss and vascular proliferation were observed by autopsy. Among the 7 patients, although the vaccines were not the primary cause of the acute metabolic crisis, the severe acute episodes occurred coincidentally. A detailed etiological investigation before vaccination administration is paramount.

P-1-29

CONGENITAL TOXOPLASMOSIS WITH NEONATAL HEPATITIS PRESENTING AS AN ELEVATED C16 AND C18:1 ON EXPANDED NEWBORN SCREEN BY TANDEM MASS SPECTROSCOPY

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A newborn came to the attention of the Wisconsin Newborn Screening Program because the initial newborn screen performed at 64 h of life showed an elevated C16 of 10.2 μmol/L (normal <8.7 μmol/L) and C18:1 of 5.2 μmol/L (normal <2.8 μmol/L). A repeat sample was collected at 10 days of age and continued to show elevated long chain acylcarnitines: C16 of 9.0 μmol/L, C18:1 of 4.6 μmol/L and C18:2 of 1.0 μmol/L (normal <0.9 μmol/L). These values triggered a report of probable carnitine palmitoyltransferase II (CPT II) deficiency. The infant was evaluated at the regional metabolic center and found to have cholestatic jaundice and massive hepatomegaly. Further metabolic testing did not reveal a primary metabolic disorder. This included normal CPT II activity in the patient's fibroblasts. An evaluation for congenital infections revealed markedly elevated toxoplasmosis IgG and IgM. Subsequent evaluations showed cerebral calcifications and bilateral chorioretinitis. These finding in conjunction with the serology confirmed the diagnosis of congenital toxoplasmosis. The patient was treated for congenital toxoplasmosis and in follow up at 12 months of age has had complete resolution of his liver disease, normal growth and development and only mild residual visual impairment. The elevated acylcarnitine profile in this newborn was likely a reflection of primary liver dysfunction rather than a marker of metabolic disease. This report raises the question of whether further evaluations for liver dysfunction should be considered in infants with elevations of long chain acylcarnitines on expanded newborn screening as part of the second tier testing.

P-1-30

GLUTARIC ACIDEMIA TYPE I DETECTED BY CT IMAGING: RETROSPECTIVE MEASUREMENT OF NEONATAL MASS SCREENING GUTHRIE CARD USING MS/MS

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The patient was a 1-year-old girl with no family history of enlargement of the head circumference. Her parents were not consanguineous. No abnormality was pointed out during pregnancy and the neonatal periods. Right dislocation of the hip was detected at 1 month old and her legs were fixed by a band. At 3 months old, she was introduced to our hospital due to head enlargement. At 7 months old, she was admitted to our hospital. We observed the enlargement of a cistern like 'winged bat sign' in cranial CT imaging. By using tandem mass spectrometry, abnormality of the serum carnitine concentration, free 21 nmol/ml (36–74), and glutaryl 3.36 nmol/ml (<0.3), were detected. Urine glutaric acid and 3-OH glutaric acid were also increased. Her glutaryl-CoA dehydrogenase activity was decreased in lymphocytes. We measured the concentration of carnitine using dried blood filter paper taken for neonatal mass screening 4 days after birth. Her glutaryl carnitine has already increased to 2.63 nmol/ml. Adding glutaric acidemia screening to the neonatal screening system is expected to detect glutaric acidemia earlier and to improve the prognosis.

P-1-31

NEWBORN SCREENING OF GALACTOSEMIA BY TANDEM MASS SPECTROMETRY (MS/MS): A PILOT STUDY

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The aim of this study was to set up a MS/MS method for the determination of Total Hexose Monophosphates (HMPs) for newborn screening of galactosemia. Dried blood spots were extracted with acetonitrile/water solution containing [1,2-$^{13}C_2$]Gal-1-P as internal standard. A MS2 PerkinElmer mass spectrometer was used in negative ionization. Multiple reaction monitoring of the transitions m/z 259→79, m/z 259→97 for HMPs and m/z 261→79 for internal standard was applied. An experimentally determined correction of 1.63% (closed to theoretical value of 1.85%) on the internal standard signal was used to take into account the overlap of the M-H+2 isotope peak of HMPs with the [1,2-$^{13}C_2$]Gal-1-P signal. The method was linear up to 4 mmol/l. The limit of detection was 0.03 mmol/l. The mean recovery was 101±9%. The mean within and between days variations was 3.8% and 3.2% respectively. This method was used to retest the samples positive to the analysis of GALT activity (Neonatal GALT Kit, PerkinElmer), currently employed in our laboratory for newborn screening. A reduction of recall rate from 0.81% (using only GALT activity) to 0.09% (HMPs cut-off 0.30 mmol/L corresponding to 98% of reference distribution, n = 985) was obtained. We detected a newborn affected by a variant form of galactosemia showing a reduced GALT activity (1.95 U/gHb; cut-off 3.5) and an increased HMPs concentration (1.01 mmol/l). Molecular genetic analysis detected a N314D mutation and a novel sequence variation G299D, both confirmed in parents. In conclusion, this method is a valid tool to increase sensitivity and specificity of screening of galactosemia.

P-1-32

METABOLIC, NUTRITIONAL AND ARTIFACTUAL SOURCES OF CHANGES IN URINARY AND PLASMA AMINO ACIDS: A COMPREHENSIVE APPROACH

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The determination of amino acids in plasma and/or urine is a key for diagnosis and follow-up of enzymatic deficiencies in amino acid metabolism or disorders in their transport. Clinical interpretations may still be hindered by ambiguity in the sources of some urinary amino acids as well as in the relationship between their excretion and inborn errors of metabolism (IEM); to arrive at diagnosis, amino acid data should be correlated with clinical aspects. This study is intended to assist the interpretation of amino acid profiles because, in addition to IEM, it also refers to other pathologic causes and to physiologic, nutritional, drug therapy and artifactual sources. Relevant data have been compiled from several years of experience in selective screening by two-dimensional thin layer chromatograms (Wadman et al., 1980) with major references on the topic and bibliographic searches. We illustrate with particular aspects analytical interferences with ninhydrin-positive spots and for some primary and secondary disturbances of amino acids profiles dues to non-IEM disease, drug therapy [anticonvulsivants (Valproate-family, Vigabatrin (γ-vinyl-GABA), carbamazepine), antibiotics (beta-lactamics), supplemets as arginine-sorbitol, folate], diet or physiologic conditions (with respect for age-specific distribution of amino acids in a pediatric population). Some preanalytical issues, including possible misinterpretations, are reviewed with regard to IEM; poor preservation of samples will lead to nonenzymatic conversion of some amino acids. On the other hand, metabolic decompensation (lactic acidosis, ketosis, or liver failure) gives rise to an abnormal excretion of some acids (aromatic acids) that are otherwise involved in particular IEM.

P-1-33

EVALUATION OF URINARY ACYLCARNITINES FOR DIFFERENTIAL DIAGNOSIS OF CASES SHOWING BLOOD CARNITINE DEFICIT IN TANDEM MS SCREENING

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Objectives: We evaluated urinary acylcarnitines of cases who showed blood free carnitine deficit, to determine the utility in differential diagnosis. **Methods**: Seven cases which was revealed to be carnitine deficiency (free carnitine <20 μM) by tandem MS analysis were investigated. Urine sample was diluted with methanol 1:10 (v/v), and then centrifuged. 10 μl of supernatant was analyzed according to the preparation method of routine acylcarnitine screening. **Result and Discussion**: Three cases of carnitine transporter deficiency, and 4 cases of secondary carnitine deficiency, follwing one each of VLCAD deficiency (VLCADD), valproic acid (VPA) treatment, poor nutrition, and a long time pivalate prodrug antibiotics treatment, could be indentified. The cases of carnitine transporter deficiency showed an increased excretion of free carnitine in urine despite an extremely low level in blood. No specific findings in urine acylcarnitine profile were observed in cases of VLCADD and VPA treatment. In a case of having pivalate prodrug antibiotics, urine acylcarinitine analysis showed decrease of free carnitine and marked increase in pivaloylcarniitine (C5) excretion. In a case of poor nutrition, acylcarnitine analysis in both urine and blood revealed to be biotin deficiency with secondary carnitine defict, showing an increase of 3-OH-isovalarylcarnitine as well as a low level of free carnitine. **Conclusion**: Urinary organic acid analysis by GC/MS is often used as a diagnostic support in the tandem MS screening. Evaluation of urinary acylcarnitine using tandem MS will also be useful in differential diagnosis in some cases, in particular of free carnitine deficit.

P-1-34

SELECTIVE SCREENING FOR METABOLIC DISEASES IN PUBLIC HEALTH SYSTEM IN BRAZIL

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Public health interest about genetics is still a growing process in developing countries but a first step of this movement was done by the Health Ministry in Brazil in 2001 with the creation of the National Newborn Screening Program (NNSP) – set at the Public Health System. Currently there are 26 states (out of a total of 27) working on this program in Brazil and phenylketonuria (PKU), congenital hypothyroidism (CH), sickle cell disease (SC) and cystic fibrosis (CF) are the diseases detected. One of the NNSP's objectives is covering 100% of the brazilian newborns (NB) but we have already huge differences among the states (47–99% of NB coverage) with a medium coverage in 2005 of 77.5% of the NB in our country. In 4 experiences years (2001–2005), 13 447 947 newborns were screened by NNSP and we found 1293 PKU patients, 7003 CH patients, 5661 SC patients (SC screening performed in only 10/26 states) and 254 CF patients (CF screening performed in only 3/26 states). All the detected patients have their treatment and follow-up supported by federal resources in Regional Reference Centers of the NNSP. After almost five years, NNSP enabled us to: (a) perform an epidemiologic study of the selected diseases; (b) educate a group of health workers and students (multidisciplinary team) for research, detection, prevention and treatment in this area; (c) give support treatment to the detected patients and genetic counselling to their families; (d) evaluate the need of neonatal screening for other diseases.

P-1-35

CONSTRUCTING A MODEL OF ASSESSMENT OF NEWBORN SCREENING PROGRAM

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Research directed toward the development of an instrument for assessment of the National Newborn Screening Program (NNSP) set at the Unified Health System (UHS) in Brazil in June/2001. On the basis of the construction of a logical model of NNSP and the identification (in literature and in reference brazilian newborn screening centers) of specific markers in newborn screening and public health, a preliminary matrix was elaborated and passed for the evaluation of NNSP federal managers and validation consensus of NNSP specialists. Selected 15 markers, thus distributed: (a) markers for the 'screening step': program coverage, neonatal age at the screening day, time taken to send the samples, results emission and the number of modified test results; (b) 'follow-up step' markers: percentage of patients' absences and the average time of return; (c) 'diagnosis step' markers: number of confirmed cases, pathologies prevalence and the rate of newborn's age at this moment; and (d) 'treatment and follow-up step' markers: patient's relationship with the Newborn Screening Reference Centers, patients' death rate and number of patients receiving treatment by the UHS. We believe that the selected markers will allow to evaluate NNSP as well as will serve as indirect appraisers of the quality, of the structure and of the NNSP formation process.

P-1-36

SPECTRUM OF IEMs IN INDIAN NICUs

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Objective: Ours is one of the major referral center for diagnosis and treatment of IEM in India. Aim was to determine the spectrum of common IEMs presenting in the first 90 days of life as critical illness, so as to develop recommendations for newborn screening tests at least in NICU. **Subjects and method**: We selected 316 critically ill babies admitted in various NICUs with clinical suspicion of IMD from a period 2000–2006. **Results**: All these 316 babies were subjected to a standard battery of tests to detect IEM – plasma ammonia, lactate, blood sugar level, ABG, anion gap, urine ketones, MRST, urine orotic acid, HPLC aminoacids of plasma and if required CSF, urine GC-MS for organic acids and MS/MS for carnitine/acyl carnitine and aminoacid profile. In addition to these specific diagnostic tests were performed in relevant cases. Incidence for various disorders was: galactosemia 22 (6.96%), NKHG 19 (6.01%), biotinidase deficiency 11 (3.48%), MSUD 11 (3.79%), OTC deficiency 8 (2.53%), tyrosinemia type I 8 (2.53%), citrullinemia 7 (2.22%), propionic acidemia 6 (1.89%), methyl malonic acidemia 6 (1.89%), GA type II 6 (1.89%), CAH 5 (1.58%), argininemia 4 (1.27%), fructose 1,6 diphosphatase Def. 4 (1.27%), CPT II Def. 4 (1.27%), cystic fibrosis 3 (0.95 %) and suspected mito-chondriopathies 9 (2.85%). **Conclusion**: It was evident that there are atleast 15 common IEMs (with incidence >1%) in NICU babies. Screening for galactosemia, biotinidase deficiency, MSUD and urea cycle defects should be recommended for all NICU babies in India.

P-1-37

SCREENING FOR HHH SYNDROME IN NORTHERN SASKATCHEWAN

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The most recent of five HHH syndrome patients from 3 communities in northern Saskatchewan (SK), identified at 1 year of age by DNA analysis, had normal amino acids during newborn screening. Ornithine (Orn) levels in all five children have remained high since diagnosis. 568 newborn bloodspots from babies from a region of SK with a high prevalence of HHH were screened for the ORNT1-F188Δ mutation, and for amino acids by tandem MS. 1014 blood spots from babies born in low risk communities from southern SK were analyzed as controls. 22 of 568 samples (3.9%) were heterozygous for ORNT1-F188Δ, 546 were homozygous normal. Of these 546 normals, 33 (6.04%) had high Orn ($\geqslant$120 μmol/L) compared to 0.30% (3 out of 1014) in controls from the south. The Orn/Cit ratio in heterozygotes, Normals from the north, and controls from the south were the same (3.71 ± 1.87 μmol/L, 3.55 ± 2.46 μmol/L and 3.69 ± 2.66 μmol/L, $p > 0.314$). The Orn/Arg ratio was lower in the northern group (normals) compared to controls from the south (4.36 ± 7.88 μmol/L vs 5.86 ± 10.44 μmol/L, $p = 0.005$). Orn levels were higher in the northern normals when compared to controls from the south (48.62 ± 42.44 μmol/L vs 38.16 ± 30.38 μmol/L, respectively, $p < 0.001$). Heterozygote frequency of 1/14 in the three northern communities was confirmed by PCR. Screening newborns for HHH syndrome by amino acid profiling by tandem MS is unreliable due to high false positives (6.04%), but may be useful later for clinical diagnosis. DNA testing for the ORNT1- F188Δ mutation is the most reliable method for early detection of HHH syndrome.

P-1-38

DIAGNOSIS OF INBORN ERRORS OF METABOLISM IN SERBIAN CHILDREN A REFERRAL CENTRE EXPERIENCE

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Diagnosis and treatment of inborn errors of metabolism (IEM) is very difficult in Serbia, mostly due to limitations in diagnostic resources and poor treatment access. Presently we are able to make a diagnosis of most organic academia, amino acid disorders, peroxysomal disorders and some of lysosomal disorders, like Gaucher and Fabry disease. Screening for phenylketonuria (PKU) has begun at our institution in 1967. Thanks to a good will of several European centres, we may occasionally have a few other inborn errors of metabolism diagnosed.

In the past 10 years by screening (massive neonatal or among patients with symptoms) we confirmed the diagnosis of following inborn errors of metabolism in 102 patients: phenylketonuria 23, glycogen storage disease type I 9, fructose intolerance 2, congenital hyperlactatemia (PDH deficiency) 3, alpha mannosidosis 1, Krabbe disease 2, GM1 gangliosidosis 3, Sandhoff disease 1, metachromatic leukodystrophy 2, mitochondrial disorders 5, MPS 10, MSUD 3, urea cycle disorders 3, Niemann-Pick Type B 1, methylmalonic acidemia 5, glutaric aciduria 1, other organic academias 5, tyrosinemia type I 1, ALD X linked 6, Zellweger and like disorders 1, non-ketotic hyperglycinemia 2, Gaucher disease 8, Wilson disease 8 (the results will be presented in table).

Unfortunately, there are more patients with IEM in Serbia waiting for a chance to be diagnosed, and even more need a proper treatment. Better prospects for these patients must rely on improved pediatricians' education for recognition of this group of disorders, and reasonable financial support for appropriate diagnostic and therapeutic procedures.

P-1-39

CONCENTRATION OF ACYLCARNITINES IN BLOOD COMPARTMENTS: SPECIFIC DISTRIBUTION PATTERNS FOR DIFFERENT ACYLCARNITINES.

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Introduction: acylcarnitines (AC) analysis for selective screening can be done on DBS or plasma. Previous studies suggested different partition patterns in blood for some AC. In normal children, most AC have very low concentration, therefore partition studies are difficult. **Aim**: We studied the partition pattern of various AC in patients with known metabolic disease. **Methods**: samples from patients (hematocrit >30%) with HMG-CoA-lyase, 3MCC, GA-I, MCAD, EPEMA, IVA, MMA, PA, and MAD, were studied simultaneously in whole blood and plasma. From another aliquot, plasma and blood cells were separated and volume was corrected with normal saline. AC were measured (MS/MS-Waters, USA). Results of the elevated AC markers for each disorder were examined. C16 levels were also considered for all samples as an index of comparison. **Conclusion**: Results revealed higher levels in plasma compared to whole blood (range %) for the following metabolites: C4 (175–186), C5 (126–154), C5DC (129–176), C6 (144–203), C8 (181–187), C10:1 (184–191), C10 (126–187), C14:1 (190) and C14 (134). In contrast, levels of C5OH (15–33) and C16 (0–68) were consistently lower in plasma. Levels of C2 and C3 tended to be higher in whole blood, though results were not uniformly consistent. Results of the partitioning of the AC between plasma and blood cells confirmed that C5OH and C16 are concentrated in blood cells, in contrast to all other AC. This study highlights the importance of measuring disease markers in the correct sample matrix for specific inborn errors.

P-2-1

MATERNAL PHENYLKETONURIA: AN EXPERIENCE FROM ITALY

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This report summarizes a review of our experience with maternal PKU during the last 20 years.Twenty pregnancies were analysed in 8 HPA women: 5 with classic PKU (Phe levels above 1200 μmol/L on normal diet) and 3 with mild PKU. The offspring were assessed according to the dietary treatment of the mother during pregnancy: group 1 not treated, group 2 treated late in the pregnancy and group 3 treated before conception. The first group includes 10 children of 2 mothers with PKU and 3 children of 1 mother with mild PKU: mental retardation was found in 10, microcephaly in 4. There were no abnormal physical or neurological findings in the 3 children born from woman with mild PKU. The second group includes 2 children from 2 mothers, one with mild PKU and the other with classic Pku who were treated with a low Phe diet but the diet was introduced at 13th week and at 11th week of gestation, respectively(Phe pre-treatment levels 640 and 2240 μmol/L) The first child was normal, within the second patient had microcephaly, congenital heart disease,cleft lip and palate,and mental retardation.

The third group includes 5 children from mothers with classic PKU (all diagnosed and in follow-up in our Dept.) who were treated before conception. In the mothers or group 2 and 3 Phe concentrations were keeped between 100 and 300 μmol/L and plasma Tyr concentrations between 50 and 100 μmol/L. The offspring from these pregnancies show normal neurological development with normal growth. Strict adherence to diet, close follow-up, and good obstetric care are needed during pregnancy in mothers with PKU.

P-2-2

TETRAHYDROBIOPTERIN-RESPONSIVE PHENYLALANINE HYDROXYLASE DEFICIENCY IN ITALY

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We describe the results of the BH_4 loading test performed in 57 Italian PKU patients. Patients with classical (26/57), moderate (8/57), mild (6/57) PKU and 17 subjects with MHP were orally given BH_4 20 mg/kg. Blood Phe and Tyr were determined at 0 h, 4 h and 8 h. Since 2004 our protocol has been modified with measurement of Phe and Tyr also after 24 h. From the 63 patients included in this study, 9 showed a positive response (reduction $>30\%$ at 8 h): 5 MHP patients, one with moderate, and 3 with mild PKU. In these 9 patients a defect in the synthesis or recycling of tetrahydrobiopterin was excluded by analysis of urinary pterins and DHPR activity (Dr N. Blau-Zurich).

One children with moderate and one with mild PKU are currently on BH_4 treatment (10 mg/kg/day in two doses). In the first patient combined BH_4 and dietary treatment is effective to keep Phe levels within the therapeutic range, in the second patient a good metabolic control is achieved with BH_4 therapy without any dietary restriction.

Our findings show: (1) the therapeutic potential of BH_4 in some patients with mild and/or moderate PKU; (2) the largest incidence of patients with biopterin-responsive phenylalanine hydroxylase deficiency in the group of the patients with mild PKU (50%); (3) the ineffectiveness of the pharmacological doses of tetrahydrobiopterin in patient with classical PKU.

P-2-3

PHARMACOKINETIC OF ORALLY ADMINISTERED BH_4 IN PATIENTS WITH PHENYLALANINE HYDROXYLASE DEFICIENCY

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Tetrahydrobiopterin (BH_4) was measured in dried blood spots from patients with hyperphenylalaninemia after oral administration according to following protocols: (A) 20 mg/kg BH_4 sampling times 0, 4, 8, and 24 h ($n = 64$); (B) 20 mg/kg BH_4 sampling times 0, 2, 4, 8, 12, 24, and 32 h ($n = 34$); (C) 100 mg/kg Phe+20 mg/kg BH_4 sampling times –3, 0, 4, 8, and 24 h ($n = 35$); and (D) 2×20 mg/kg BH_4 sampling times 0, 4, 8, 24, and 48 h ($n = 11$). Maximal BH_4 blood concentrations (C_{max}) were compared with Phe levels at different time points and responsiveness (% of Phe reduction) was calculated for each group of patients. BH_4 peaked at 4 h after BH_4 administration in 58/64 patient (median = 22.7 nmol/mg Hb), 6 patients had C_{max} levels at 8 h (median = 13.5 nmol/mg Hb). When compared with C_{max} at 4 h, BH_4 concentrations at 2 and 12 h were 28% and 74% lower, respectively. Almost normal BH_4 levels were reached 24 to 32 h after single BH_4 administration. Repeated administration of BH_4 (2×20 mg/kg at T_0 and T_{24}) resulted in a second BH_4 peak at 32 h (38% lower compared to 4 h). In the combined Phe/BH_4 loading test, BH_4 concentrations increased 3 h after Phe administration ($\sim 51\%$), probably due to induction of GTP cyclohydrolase, but BH_4 profile was not significantly different from those of other protocols. There was a significant reduction of blood Phe after a combined Phe+BH_4 challenge in all patients. There was no significant effect of BH_4 concentration on the outcome of the loading test in all protocols.

P-2-5

THE DIFFERENCE OF SEIZURE BETWEEN PATIENTS WITH TETRAHYDROBIOPTERIN (BH_4) DEFICIENCY AND PHENYLKETONURIA (PKU)

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Objective: To better understand the difference of seizure between patients with tetrahydrobiopterin (BH_4) deficiency and phenylketonuria (PKU). **Method**: The time of onset, clinical manifestation, electroencephalogram (EEG) and outcome of seizure were compared between two groups. **Results**: In the last twelve years, 98 PKU and 12 BH_4 deficient patients experienced seizure out of 391 late-treated patients with hyperphenylalaninemia, excluding other causes such as high fever, hypocalcemia and neonatal asphyxia. Seizure of the 98 PKU cases occurred at the age of 10.7 ± 2.6 ($4.5 \sim 27.1$) months, displaying various patterns, mostly infantile spasm (55/98). Of the 32 cases who had EEG detection, 31 were found to have epileptiform discharge and 15 have hypsarrhythmia. EEG follow-up after treatment further revealed that abnormalities were still present but less severe. Administration of sodium valproate and other anticonvulsant drugs to control seizure resulted in a long-term and tough course. In comparison, seizure of the 12 BH_4 deficient cases occurred at the age of 5.1 ± 1.9 ($2.7 \sim 11.0$) months, presenting paroxysmal spasm of limbs, sometimes with fixed eyes. EEG was carried out in 10 cases, among which 3 were found to have slight epileptiform discharge, and the other 7 have normal EEG. EEG tracing revealed that change with age after treatment was not specific. The symptom was under control immediately after the administration of levodopa. **Conclusion**: The onset time, clinical, electrographic manifestation and control of seizure in BH_4 deficiency were quite different from those in PKU. There should be substantial difference in the mechanism of seizure between BH_4 deficiency and PKU.

P-2-6

RELATIONSHIP BETWEEN GENOTYPE AND INTELLECTUAL PHENOTYPE IN UNTREATED PHENYLKETONURIC PATIENTS

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The aim of this study was to explore correlation between phenylalanine hydroxylase (PAH) genotype and intellectual phenotype in untreated patients with phenylketonuria (PKU). Genomic DNA was isolated from whole-blood samples of 165 untreated PKU patients. PAH gene mutation screening analyses were performed by polymerase chain reaction (PCR) of exon 3, 5, 6, 7, 10, 11 and 12, followed by single strand conformational polymorphism (SSCP) or denaturing high-performance liquid chromatography (DHPLC). The genotypes were determined by direct sequencing at last. Eighty-six untreated PKU patients whose two mutant alleles were both defined were involved in this study. The DQ/IQ of these patients were tested by Gesell development schedules. Genotypes were classified according to Guldberg's classification (Am J Hum Genet 63:71–79, 1998). Among 86 patients, 8 (9.3%) were mild retardation, 31 (36.1%) were moderate, the severe mental retardation accounted for 47 (54.6%). The relationship between genotype and intellectual phenotype in this group was examined. It was found that the intellectual phenotypes of 63 (73.3%) patients were compatible with genotypes and not well matched in 23 (26.7%) cases. The result indicates that the intellectual phenotype was well matched with genotype in untreated PKU patients. Genotype determination is useful in the prediction of clinical phenotype in PKU patients.

P-2-7

THE INCIDENCE OF TETRAHYDROBIOPTERIN SYNTHASE DEFICIENCY IN NORTHERN CHINESE POPULATION AND OUTCOME OF LATE-TREATED PATIENTS

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Objective: To get the incidence and to evaluate the treatment and outcome of late treated patients with tetrahydrobiopterin synthase (BH_4) deficiency in Northern Chinese population. **Methods**: From 1992 to 2005, a total of 889 patients with HPA were diagnosed in Northern China. After differential diagnosis, patients with BH_4 deficiency were treated with BH_4, levodopa and 5-hydroxytryptophane (5-HTP) immediately. Their blood phenylalanine levels, psychomotor and intelligence development were followed up. **Results**: A total of 61 cases were diagnosed as BH_4 deficiency, all of them were revealed as 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency. Among 569 cases of HPA detected by neonatal screening, PTPS deficiency was the cause in 41 patients giving a frequency of BH_4 deficiency as a cause of HPA of 7.2%. Their age at diagnosis were 2.1 months ~ 13 years. Due to the neonatal screening, the rate of late diagnosed BH_4 deficient patients were decreasing year by year. Forty-two patients were treated within 6 months and their development were normal or nearly normal. The rest 19 late-treated patients lived in community care home for people with learning disability before diagnosis. Even after treatment for 18–119 months, 2 (11%) were not fully ambulant, 7 (36%) were non-verbal, 13 (78%) had significant challenging behaviour, and up to 4 (21%) had epilepsy. **Conclusions**: The incidence of BH_4 deficiency among patients with HPA in Northern China is 7.2%, much lower than that in Southern China, and PTPS deficiency is the most common form of BH_4 deficiency. Development cannot benefit a lot from treatment in late-treated patients.

P-2-8

CHARACTERISTICS OF DIETARY TREATMENT OF PKU IN DIFFERENT REGION OF CHINA

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China is such a large country that each region is characterized with its own local natural food. Due to the versatile diet structure and economic restraint, it is very difficult for the patients from different areas to follow identical diet as in European countries. Even the same food, if cultivated in different area, could have different content of phenylalanine (phe) and other nutrient. Since 2000, 37 patients in good compliance but with higher phe levels were transferred to our department from other neonatal screening centers. **Objective**: To identify the contents of natural food available in different regions of China and their effects on plasma phe levels and body growth. **Method**: We remodeled the diet structure according to the specific contents of phenylalanine, other amino acids, lipids and carbohydrates of the food in each patient's own hometown. The plasma amino acids levels before and after treatment were determined by using the amino acid analyzer. Body and mental development were followed-up. **Results**: Plasma phe levels in 32 patients were controlled within 2–6 mg/dl after the adjustment of their daily diet. Significant decrease were observed on plasma phe levels before and after treatment (580.9 ± 178.6) μmol/L, (226.5 ± 75.4) μmol/L respectively ($p < 0.01$), while other necessary amino acids levels greatly improved ($p < 0.01$). These patients presented nearly normal physical and intellectual development after five-year follow-up. **Conclusion**: Exact measurements of phe should be done in natural food in different regions especially in patients with good compliance by higher phe levels. High plasma phe level inhibit absorption of other amino acids from digestive system.

P-2-9

QUALITY OF LIFE ASSESSMENT IN ADULT PKU-PATIENTS RETURNING TO LOW-PHENYLALANINE DIET

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Phenylketonuria (PKU) is the most common treatable disorder of amino acid metabolism in man. Treatment consists in the restriction of dietary phenylalanine intake. Low compliance or discontinuation of dietary therapy in majority of adults can lead to neuropsychological abnormalities and emotional problems. Thus, the aim of this survey was to assess the quality of life in patients who discontinued or significantly relaxed the diet, in a hope to collect data which could help to stimulate their diet resumption. **Methods**: Adult PKU-patients having classic phenylketonuria were tested by means of modified Psychological General Well-Being Index to assess their quality of life. The participants answered 21 questions concerning their anxiety, depressed mood, sense of positive well-being, self-control, general health and vitality and received 0–5 points for each answer. Cut-off points for the total score were 0–57 (severe distress), 58–68 (moderate distress) and 69–105 (positive well-being). **Results and conclusion**: 48 patients with PKU were included into the study. The results they achieved ranged from 30 to 94 points (severe distress in 16%, moderate distress in 37%, positive well-being in 47% of participants). Our results suggest, therefore, that interpersonal differences exist between the adult patients on relaxed diet, in part of whom quality of life often remains good, but who can also suffer from severe emotional distress. Returning to strict diet should be recommended especially for the last group of patients, despite typical compliance problems.

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P-2-10

PHENYLALANINE HAS DIFFERENT EFFECTS ON Rac1, Cdc42 AND RhoA EXPRESSION AND ACTIVITY IN CULTURED CORTICAL NEURONS

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Objective: Phenylketonuria (PKU) is characterized by high concentration of phenylalanine and mental retardation (MR). Pathologic changes in the brain of untreated PKU patients are reductions or abnormalities in axons, dendrites and synapses, which are thought to be due to toxic effects of phenylalanine and/or its metabolites. The mechanisms underlying impaired brain development and cerebral dysfunction by phenylalanine 'toxicity' remain unclear. Rho GTPases (Rac1, Cdc42, RhoA) are key signaling proteins that regulate neurite growth and synaptic connectivity. This study aimed to examine the role of Rho GTPases in neuronal injury induced by phenylalanine. **Methods**: Primary cultures of neurons were fixed and double-immunostained with anti-MAP2 and anti-tau1. The number of dendrites and spines were counted. Real time PCR and Western blot analysis were used to evaluate the Rho GTPases mRNA and protein expression. Rho GTPases activities were measured by GST pull down assay. **Results**: We had demonstrated that phenylalanine induced the reduction in number of primary dendrites and dendritic branches and spine density in cultured neurons. We discovered that phenylalanine down-regulated Rac1, Cdc42, RhoA mRNA and protein expression. Pull down assay showed that phenylalanine decreased Cdc42/Rac1 activity with time dependent, but increased RhoA activity. **Conclusions**: We had demonstrated that high concentration of phenylalanine effected the mRNA and protein expression and activity of Rac1, Cdc42 and RhoA. These results may provide important insights into the molecular mechanism underlying abnormality of dendrites and dendritic spines induced by phenylalanine, and probably are one of complicated mechanism of neuronal injury induced by phenylalanine.

P-2-11

LONG-TERM THERAPY OF PKU IN MICE BY AAV2 PSEUDOTYPE 8 GENE TRANSFER TO LIVER

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Phenylketonuria (PKU) is caused by a deficiency of the hepatic phenylalanine hydroxylase (PAH) leading to hyperphenylalaninemia and irreversible brain damage. We recently reported on the use of AAV2 pseudotyp 8 hybrid vectors expressing PAH under the control of the CMV promoter and delivered to the liver of PKU mice via single injections of portal vein ($5 \times 10e12$ vg) or tail vein ($2 \times 10e13$ vg; Ding et al., 2006, Gene Ther, in press). Whereas untreated PKU mice exhibit normal expression of mutant Pah-mRNA and PAH protein, but had no detectable enzyme activity and Phe levels around 2000 micromol/l, viral treated mice exhibited two weeks after delivery of AAV2/8 a complete phenotypic correction with blood Phe levels decreasing from high to normal values (<120 micromol/l). Here we report on the follow-up of this gene therapy approach during a period of over a year. For tail vein injection, one female started to rise Phe concentrations after 35 weeks, and at 56 weeks the two females had Phe blood levels of 1203 and 1453 micromol/l. For portal vein infusion, mice remained at therapeutic levels at least until week 61, and three weeks later the remaining two females had Phe values of 284 and 517 micromol/l. Expression analysis of PAH and parameter important for the enzymatic activity at different treatment intervals are under way. In conclusion, a 4-fold lower concentration of the AAV2/8 vector directly injected into the portal vein as compared to tail vein resulted in a more efficient long-term correction of serum Phe levels.

P-2-12

MATERNAL PKU IN THE MORAVIAN REGION (CZECH REPUBLIC)

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The objective of this study was to present a survey of maternal PKU in the Moravian region. The group consisted of 19 mothers aged 19 to 32 years, who had given birth to 27 live babies. Two of them suffered from atypical PKU with phenylalanine (Phe) levels of 10–20 mg/dl on an unrestricted diet and 17 with classical PKU. The IQ of the mothers varied from 43 to 114. Two of them were high-school graduates, 16 were apprenticed. Nine of them did not plan pregnancy, two were treated from 13th week. One child was born without any treatment. Pregnancy was terminated in 6 cases due to a high maternal level of blood Phe, and in 6 cases the abortion was spontaneous. High maternal Phe concentrations were associated with distinct syndrome: facial dysmorphism (6 cases), microcephaly (4), psychomotor retardation (1), subnormal intellingence (3), ADHD syndrome (5), and low birth weight (2). No child was born with a heart defect. One child was born with a supernumerary phalanx of the 5th toe, one child with ureter dilatation. The poor outcome of pregnancy depended on belated onset of treatment with low-protein diet, high maternal blood Phe, low socio-economic status of the mother, and decreasing IQ. The best results were obtained by close cooperation of attending obstetrician with metabolic team experienced in medical care of persons with PKU, and Phe levels of the mother's blood between 1–4 mg/dl.

P-2-13

EFFICACY OF BH_4 TREATMENT IN A TYPICAL PKU AFTER NEONATAL BH_4 LOADING TEST: 20 MONTHS OF FOLLOW UP

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Case report: The patient is the second child of non consanguineous parents. He is born after an uneventful pregnancy. Neonatal screening is positive for PKU with a Phe level at 600 µmol/L at Day 3 and at 1080 µmol/L at day 7. The patient mutations are Y414C and IVS10-11G>A, these two mutations are known to be BH_4 responsive. A BH_4 loading test is performed (20 mg/kg in one dose) and the Phe levels dropped dramatically (Phe: 60 µmol/L 8 h after the load). After discussion with the parents, the child is treated by BH_4 with a normal diet. We observed a dose/effect relation: Phe level increases as soon as the BH_4 dosage decreases under 18 mg/kg/d (one dose per day). Keeping the dose of 20 mg/kg, the metabolic control has been excellent; we compared it with the metabolic control of a girl with the same genotype born 8 years ago. Both controls were identically excellent. With BH_4 treatment, Phe tolerance is about 1450 mg/day (patient treated by protein substitute: 400 mg/d). Somatic and psychomotor developments at 20 months are normal. **Conclusion**: BH_4 treatment is efficient from neonatal period in BH_4 sensitive PKU patient. It allows a greater quality of life with an excellent metabolic control.

P-2-14

EVALUATION OF BH_4 LOADING TEST AFTER NEONATALSCREENING FOR HYPER PHENYLALANINEMIA

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We report our experience of BH_4 loading test on 10 children screening from 2003 to 2006 and we compared the outcome to the outcome of ten neonates screened from 1993 to 2002 for PKU. The BH_4 loading test (20 mg/kg) is begun the next morning after screening. Diet is unchanged for 24 h. Blood Phe levels are sampled on a Guthrie card at H0, H2, H4, H6, H8, H12 and H 24. We look at the BH_4 sensitivity (decrease of Phe $>$ 30% at H24) and we compared the quality of the management (time to reach Phe control) between the two groups. Results: we detect one PTPS deficiency and 5 BH_4 sensitive PKU patients. For the timing of management, neonates screened after 2003 are hospitalized 1 day before those born before 2003. In the loaded neonates, the Phe control is reached sooner for the sensitive patients (9.3 ± 1.6 days) compared to the non sensitive ones (18 ± 8.3 days, $p < 0.05$). The overall loaded group reached the Phe control (12.8 ± 6.7 days) sooner than the non loaded group (20 ± 8.3 days, $p < 0.05$). We did not observe any side effects. **Conclusion**: Neonatal BH_4 loading test is useful in the neonatal period; it allows detecting the BH_4 deficiency patients and the BH_4 sensitive patients. It also allows reaching the Phe control sooner compared to the non loaded group.

P-2-15

PRENATAL GENE DIAGNOSIS FOR HIGH RISK PHENYLKETONURIA

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Objective: About 10–20% mutations of phenylalanine-hydroxylase gene can not been found in Chinese PKU families according to formerly data. Therefore, to set up a method of prenatal gene diagnosis for analyzing the high risk fetus of phenylketonuria (PKU) family with only one identified mutation is very important. **Method**: Chorionic villi DNA were collected from 22 fetuses of 15 PKU families. The gene of phenylalanine hydroxylase (PAH) were detected using polymerase chain reaction (PCR) with single strand conformation polymorphism (SSCP) and short tandem repeats (STR) in intron 3. **Results**: Two mutations were found in 12 families. The other 3 families were found only one mutation. Total 4 fetuses were selected and diagnosed as PKU patients, 13 fetuses were assessed as heterozygote and 4 fetuses were concluded as normal fetuses. The other one was assessed as either heterozygote or PKU patients. **Conclusion**: The methods of combining SSCP and STR for diagnosing high risk PKU fetus can not only promote the accuracy of diagnosis for the family with two known mutations, but also help diagnosing the family with one known mutation. It is very suitable and useful for quick prenatal gene diagnosis, especially for the family found only one mutation.

P-2-16

ASIEM PKU HANDBOOK REVISION, 2005

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The Dietitians Working Party of ASIEM produced the first ASIEM PKU Handbook in 1996. This was later made appropriate for use in New Zealand. In 1998 The Handbook was reviewed by dietitians and by families of those with PKU in 2002. Findings were reported at ASIEM 2002 and 2003. A dietitian coordinator was appointed by ASIEM September 2004 to oversee the rewriting and production process which was completed by July 2005. Contributions from ASIEM dietitians, consultants, clinical nurse consultants and social workers from Australia and New Zealand were collated. This material was rewritten into a coherent whole by a professional copywriter and was professionally printed. Feedback from focus groups of families with PKU, and from raw copy by contributors, has ensured this revised Handbook contains current information which will be useful to consumers in both New Zealand and Australia. Essential financial support from ASIEM and metabolic formula companies enabled employment of the coordinator, copywriter and printing costs. Evaluation feedback to date from consumers and health professionals has been overwhelmingly positive. The Handbook can be read online on the website of The Children's Hospital at Westmead. In future this Handbook could be readily adapted for use in other disorders of protein metabolism.

P-2-17

BH_4 STUDY WITH 12 DANISH PATIENTS WITH THE Y414C MUTATION

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12 patients, age 8–29 years, diagnosed with PKU with the Y414C mutation were included in this BH_4 treatment study. Divergent responses have been reported for this mutation. We wanted to verify if uniform trial conditions could eliminate the varying results, to test for of BH_4 responsiveness with decreasing dosages of BH_4 on a diet with normal protein content. Each dosage was taken for one week. Throughout the trial natural protein was increased to 1 g/kg BW and the amino acid supplement omitted. Diet records were registered initially and during the test period, and S-phenylalanine, S-phe, was measured 4 times a week. A positive response was defined as a decrease in S-phe of at least 30%. Two patients were below this limit. Three patients homozygotic for Y414C showed decreases at 70–73%, 48–64% and 24–32% with 20, 10 and 5 mg of BH_4/kg/day respectively. Correspondingly, nine heterozygotics had decreases at 18–71%, 12–47% and 6–27%. Three patients showed decrease in S-phe but only after 48 h. A certain carry over effect of the former dosage was observed in most of the patients. No serious side effects were noted. This extended BH_4 test seems to be effective to detect even the late responders. The varying responses in spite of uniform trial conditions and genotypes could be due to differences in BH_4 absorption.

P-2-18

SERUM LEVELS OF IMMUNOGLOBULINS IN PATIENTS WITH PHENYLKETONURIA ACROSS THE FIRST TWELVE YEARS OF LIFE

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Hyperphenylalaninemia (HPA) is caused by a deficiency of phenylalanine (Phe) 4-monooxygenase. Patients affected by more severe forms (PKU), if not treated, present severe mental handicap. The treatment is based on a low-Phe diet. In HPA patients low IgG, IgA and IgM and high IgE levels, respectively, have been previously reported, and related to dietary treatment and/or a peculiar environmental care. AIM. To assess the serum levels of IgA, IgG, IgM, IgE in HPA patients on either dietary treatment or a free diet. **Methods**: 98 patients (57 PKU on diet and 41 mild HPA on a free diet) were followed from birth through 12 years of age. IgA, IgG, IgM and IgE levels were evaluated at ages 1, 3, 6, 9 and 12 years and compared to data from age-matched, healthy counterparts. **Results**: Both PKU and mild-HPA patients showed significantly lower IgG levels at 1, 3 and 9 years compared to controls, while no differences were present at ages 6 and 12 years as far as IgA levels. IgM and IgE levels were significantly higher in our patients at any age when compared to controls. Mild-HPA showed higher IgE levels than PKU after 9 years of age. **Conclusions**: We have found higher levels of serum Ig classes in HPA patients, irrespective of the genetic form, in particular IgM and IgE. Further investigations are needed to elucidate the associations between the serum Ig picture and development of and/or the protection from atopic symptoms in HPA patient, either treated or on a free diet.

P-2-19

WHICH BIOMARKERS ARE RELEVANT IN ORDER TO VALIDATE AND EVALUATE THE PKU TREATMENT?

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When the first PKU diet was created about four decades ago, the main focus was to remove as much phenylalanine (phe) from the diet as possible and supplement the loss of the rest of the amino acids (AA) with a drinkable powder mixture. Over the years the PKU nutritional therapy has been developed from only containing AA to today's complete nutrition supplement including extra supplement of carbohydrates, essential fatty acids, vitamins and minerals. In order to evaluate the efficacy of the diet, many parameters can be measured from blood samples: phe, tyrosine, fatty acid (FA) profile, vitamin and mineral status. But are the supplements complete? Intake, absorption and bioavailability are different from person to person and not all parameters are easy to measure. A survey was conducted over 'The Metabolic Dietician's Mail Server List' on the Internet. The following question was asked: Which biomarkers do you find relevant to measure in order to validate and evaluate the PKU diet? Data: Eight centres participated in the survey. Following parameters were measured: phe (8 centres = 100%), tyrosine (75%), AA profile (50%), CBC (25%), prealbumin (50%), vit./min. status (38%) and FA-profile (13%). **Conclusion**: Specific biomarkers are necessary in order to validate and evaluate the PKU diet. However, the choice of biomarkers varies a lot between the individual PKU centres. There is no consensus regarding this matter which could be relevant to investigate further in order to detect the most relevant biomarkers.

P-2-20

SERUM SELENIUM AND ZINC IN PHENYLKETONURIA PATIENTS

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Objective: Serum selenium concentrations in childhood display a significant age dependency. In children receiving a low protein diet serum selenium concentration does not increase with age as observed in the healthy pediatric population with unrestricted nutrition. Selenium deficiency may have several serious short- and long-term medical implications. We have studied the concentration of serum selenium and zinc in a group of patients with classical phenylketonuria. **Patients and methods**: 20 patients (age 8 to 25 years) on low protein diet (daily phenylalanine (Phe) intake below 500 mg, natural proteins below 10 g per day) were tested for serum selenium and zinc concentrations. Selenium was measured in serum by electrothermal atomic absorption spectrometry and serum zinc by atomic absorption flame photometry. Reference values for zinc and selenium in these age groups are determined for our population. The amino acids in plasma and vitamin B_{12} in serum were routinely measured in all patients. **Results**: Of the children and young adults on protein restricted diet only 10 % showed selenium concentrations within the reference intervals, but the others were deficient (normal mean value 1.08 micromol/l, mean value in patients 0.40 micromol/l). Zinc deficiency was present in 56% of the patients (normal mean value 13.3 micromol/l, mean value in patients 9.3 micromol/l). The patients do not show any clinical signs of selenium and zinc deficiency. All patients have normal vitamin B_{12} concentration and amino acids analysis (except Phe). **Conclusion**: The results confirm important selenium and zinc deficiency in patients on low protein diet. At present our patients get amino acid supplement without added selenium and with low zinc content for the treatment of PKU. The present study should urge the introduction of the advanced, selenium and zinc supplemented amino acid products.

P-2-21

MUTATION ANALYSIS OF PHENYLALANINE HYDROXYLASE (*PAH*) GENE IN LARGE MULTI-ORIGIN POPULATION

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Phenylketonuria (PKU), one of the most common autosomal recessive inborn errors of metabolism, is caused by mutations in the gene encoding the hepatic enzyme phenylalanine hydroxylase (PAH).More than 510 mutations have been described at PAH locus up to date. The object of this research was to define the epidemiology and mutations of PAH gene (and their prevalence) that cause phenylketonuria (PKU) in different ethnic groups among the Israeli population.

Denaturing high-performance liquid chromatography (DHPLC) is a sensitive and fast method for the detection of mutations. DNA samples were collected from 192 families and mutations screening were performed using the new DHPLC technique and sequencing the DNA alterations for characterization of the mutations. In screening the 13 coding Exons of the PAH gene, we found 5 new mutations and characterize the four most common mutations (IVS10–11G/A/N – 14%, A300S/N – 12%, A403V/N – 10%, R408W/N – 8%). About 10% of the patients were homozygous for missence mutations and 40% of the patients were found to be compound heterozygous, some with more then two mutations alleles as results from intermarried and match making. In this study we report the successful use of DHPLC to analyze rapidly the complete coding sequence of the PAH gene in a total of 192 patients from different origin. This study enables us to estimate the prevalence of the disease and the design of prenatal diagnostic tests for this disease targeted specifically to different population groups, as well as for the detection of BH_4-responsive mutations.

P-2-22

THE EFFICACY OF A NEW VITAMIN AND MINERAL TABLET IN PKU

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Some patients with PKU require complete vitamin and mineral supplementation but most supplements are unpalatable or incomplete. *Phlexy-Vits tablets* (SHS International) are a new supplement (dose: 5 tablets/daily) that provide the adult UK (1991) reference nutrient intake for vitamins and minerals. **Aim**: an open, interventional trial, investigating the *Phlexy-Vits tablets* acceptability and impact on biochemical and haematological micronutrient status. **Methods**: 11 subjects with PKU (median age 20 years: range 8–33 years) on dietary treatment, with no vitamins or minerals added to their protein substitute took 5 *Phlexy-Vits tablets*/daily for 4 months. All but one subject took alternative vitamin and mineral supplements before the trial. Fasting bloods were taken at baseline (– week 2 and at week 0) and 4 months for selenium, zinc, copper, glutathionine peroxidase, C-reactive protein, haemoglobin, MCV, ferritin, vitamin B_{12}, serum folate and vitamin A. **Results**: With the exception of serum folate, all analytes were within normal reference ranges. Folate was above the normal reference range in one pre-baseline blood in 3 subjects and high in 7 subjects at 4 months. Subjects scored *Phlexy-Vits tablets* higher on taste and acceptability than the previous preparations. Compliance was good. **Conclusion**: In the short term, *Phlexy Vits tablets* appeared efficacious, acceptable and popular with patients.

P-2-23

'COOKING 4 FUN': A USEFUL RESOURCE FOR LOW PROTEIN DIETS?

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Motivating children with IMD to cook with low protein foods is challenging. Any innovative teaching resource is welcomed but rarely evaluated. **Aim**: To assess the impact and practical application of a new 'fun' low protein DVD with accompanying recipe book aimed at children with IMD. **Methods**: All children and adults (n = 150) with IMD on low protein diets attending the hospital were given the DVD and recipe book. After 6 months, they were sent a questionnaire asking (1) if they had used the resource; (2) success of recipes; and (3) if the resource had increased their interest in cooking. **Results**: Only 47 questionnaires (from 33 families) were returned; a response rate of 22%. 84% were under 16 years of age. 87% of responders had seen the DVD/recipe book at least once with almost 50% watching the DVD 2–5 times. The majority had made some of the recipes (80% of carers; 77% patients). Almost all recipes were successful. More carers (75% book; 71% DVD) than patients (59% book; 50% DVD) said the resources were useful. Almost all (77% patients; 88% carers) reported that the resource motivated them to try new recipes. **Conclusions**: Although a useful and inspiring educational tool for some, it appeared to be used by the minority of families on low protein diets. Identifying a resource that will help and stimulate the majority of families to cook remains an enigma.

P-2-24

A CASE OF DIHYDROPTERIDINE REDUCTASE DEFICIENCY

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Tetrahydrobiopterin (BH_4) is the essential cofacter of phenylalanine (Phe), tyrosine (Tyr), and tryptophan (Trp) hydroxylases. BH_4 deficiency, a rare but severe type of hyperphenylalaninemia, is characterized by progressive neurologic symptoms despite early detection and treatment with a Phe-resrticted formula. It is caused by enzyme defects in the biosynthesis or regeneration of BH_4. We present a boy with hyperphenylalaninemia due to dihydropteridine reductase (DHPR) deficiency, DHPR is the enzyme necessary for recycling of the cofacter BH_4. Regeneration of BH_4 from quinonoid dihydrobiopterin (qBH2) derivative is catalyzed by DHPR. Some DHPR deficient patients could de misdiagnosed, because their serum Phe levels were not lowered after the BH_4 loading. An expensive dose of BH_4 is needed to keep with the Phe, Tyr, Trp hydroxylation reaction. Severe BH_4 deficiency is a naturally occurring model of cerebral catecholamine and serotonin shortage. Our patient could be reversed with BH_4 therapy,but the concentration of homovanillic acid (HVA) and 5-hydroxyindole acetic acid (5HIAA) in cerebrospinal fluid (CSF) remainded low. Administration of the neurotransmitter precursors L-dopa/carbidopa and 5-hydroxytryptophan appeared to be the most effective treatment and may prevent neurological damage if started early in life. The role of dopamine as a prolactin-inhibiting facter on both the pituitary and hypothalamic level was established. Serum prolactin profiles provide information on brain catecholamine states and may serve as a potentially useful and easy tool in estimating the catecholamine content in the CSF of DHPR deficiency patient.

P-2-25

OBSTACLES IN MANAGING PHENYLKETONURIA IN INDONESIA

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Introduction: Early diagnosis and good metabolic control is the hallmark of phenylketonuria (PKU) management. Therefore, it requires the infrastructure support, beginning from laboratory to food suppliers. This is the first PKU case reported in Indonesia which shows how to deal with limited infrastructure support. **Case description**: A three month-old girl from a Javanese family was referred from Japan to our hospital with the diagnosis of PKU. Her physical examinations were normal. To control her phenylalanine level, we managed the diet by combining the phenylalanine-free milk, breast milk, and regular infant formula with adequate composition of phenylalanine, protein and calories for the first three months. We do have screening test in Indonesia, but for the follow-up test we should deliver the dried blood spot to another country and wait two weeks for the result. Collaboration with medical food supplier was also required, but after one year the phenylalanine-free products were still not yet imported. The most important part was how to adjust the intake of local food products into good combination that fulfilled her requirements for growth and controlled her phenylalanine level. The expenses for this child were very high (1200 USD monthly; compared with income per capital of 60 USD in Indonesia). Despite every infrastructure limitation, the child is one and a half year by this March and develops normally proved by normal results of Denver Development Screening Test. **Conclusion**: It is very important to have a good infrastructure support before we begin with national screening policy.

P-2-26

COENZYME Q10 DEFICIENCY SEEMS TO BE IMPLICATED IN THE PATHOPHYSIOLOGY OF TREMOR IN PATIENTS WITH PHENYLKENURIA

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Background and objectives: Phenylketonuric patients under dietary treatment may have coenzyme Q10 deficiency and, consequently, increased oxidative stress and neuronal damage. This study aimed to investigate the relationship between tremor and coenzyme Q10 deficiency in phenylketonuric patients. **Methods**: In 41 phenylketonuric patients with a good metabolic control, tremor was evaluated applying the WHIGET Tremor Rating Scale and by accelerometer and surface electromyography recording. Plasma coenzyme Q10 concentrations were measured by chromatographic methods. To further evaluate the implication of coenzyme Q10 deficiency in the pathophysiology of tremor, four patients with tremor and coenzyme Q10 deficiency received supplementation with coenzyme Q10 at 200 mg per day for three months. **Results**: Ten out of 41 patients showed a high frequency (7–12 Hz) low amplitude postural and kinetic tremor. Student's t-test showed decreased coenzyme Q10 values in phenylketonuric patients with tremor when compared to those without tremor (p = 0.012). After coenzyme Q10 supplementation in four patients, plasma coenzyme Q10 concentrations increased (mean 1.27 μmol/L; range 0.62–2.10) when compared to basal levels (mean 0.21 μmol/L; range 0.12–0.27). Neurological examination revealed no improvement in two late-treated phenylketonuric patients; however, in two early-treated patients, tremor was neither detected on clinical examination nor on the neurophysiological recording. **Conclusions**: Coenzyme Q10 deficiency seems to be involved in the pathogenesis of tremor in phenylketonuria. Preliminary data suggest that early-treated phenylketonuric patients with tremor may benefit from coenzyme Q10 supplementation.

P-2-27

PSYCHIATRIC SYMPTOMS OF MOTHERS OF CHILDREN WITH PHENYLKETONURIA

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Phenylketonuria (PKU) is one of the most common inborn errors of metabolism in Turkey due to high rates of consanguinity. Although metabolic and genetic features of PKU have been well defined and thoroughly studied emotional status of mothers of children with PKU have not been evaluated. Brief Symptom Inventory (BSI), a self-rating scale for symptoms including somatization, obsessivecompulsive, interpersonal sensitivity, depression, anxiety, angerhostility, phobic anxiety, paranoid ideation, and psychoticism, was used to measure the psychiatric symptoms. BSI scores above 1.0 shows that the psychiatric symptoms are at pathological level. The socio-economic status and information on PKU were also evaluated. A total of 16 questions including educational level, income, inheritence pattern of PKU, normal phenlyalanine blood values, diet were asked. Mothers with past psychological disorders were excluded from the study. 100 mothers were evaluated; 25% of them were graduated from high school or university while the others had a lesser degree of education. 79% of the mothers were housewives, 21% had a full-time job. 34% of mothers had psychiatric symptoms at a pathological level. This group of mothers had co-existing social/familial/financial problems, and had significantly lower information and awareness on PKU ($p = 0.03$). Educational levels, working status and maternal age did not differ significantly between the two groups of mothers.

P-2-28

IDENTIFICATION AND CHARACTERIZATION OF LARGE DELETIONS IN THE PHENYLALANINE HYDROXYLASE (PAH) GENE

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Phenylketonuria (PKU) is an autosomal recessive disorder caused by mutations in the gene for phenylalanine hydroxylase (PAH). More than 500 different mutations have been described at PAH locus up to date. By previous detailed scanning of the PAH gene from 521 unrelated Czech PKU patients (1042 alleles), a mutation detection rate of 93.6% was achieved. Mutation analysis using exon by exon screening may fail to detect the mutant allele in case of large intragenic deletions avoiding primer annealing in the deleted area. Multiplex ligation-dependent probe amplification (MLPA) is a new method that detects larger DNA alterations. MLPA analysis was performed in 59 unrelated PKU patients (67 unknown alleles). A total of 19 deletions of exon 5 and 12 deletions of exon 3 were detected. 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. Two types of rearrangements were revealed in region spanning from intron 4 to intron 5, denoted as EX5del4232ins268 and EX5del995, detected on 17 and 2 unrelated mutant alleles, respectively. Breakpoint analysis of the exon 3 deletion showed loss of 4765 bp including the entire exon 3, formally described as EX3de4765. This mutation was found in 12 unrelated PKU families. Our results extend the spectrum of PAH defects and indicate that MLPA may be a useful new tool for the molecular diagnosis of PKU.

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P-2-29

MATERNAL PHENYLKETONURIA: AN IRISH PERSPECTIVE

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Introduction: Maternal phenylalanine (phe) is teratogenic. The incidence of phenylketonuria in Ireland is 1:4500 births. Ideally all pregnancies should be planned with weekly blood phe levels between 150 and 250 μmol/L pre and post conception and throughout pregnancy. **Objectives**: To assess biochemical control and outcomes in all pregnancies from 1997 to 2005. **Methods**: Initial presenting phe and all subsequent levels were recorded along with birth measurements (head circumference, weight and length). Genotyping was recorded, when available. **Results**: 38 women had 62 pregnancies resulting in 51 live births. Data was available on 50 births. Of these 18 (36%) were planned and 32 (64%) unplanned. In the planned pregnancies the mean (SD) presenting phe level was 274 (138) μmol/L with mean levels throughout pregnancy of 248 (15) μmol/L. The birth head circumference (HC) was 34.8 (1.3) cm, birth weight (Bwt) 3.680 (0.501) kg and length (Lth) 51.0 (2.7) cm. In the unplanned pregnancies the presenting phe level was 615 (335) μmol/L with mean levels throughout pregnancy of 311 (96) μmol/L. The HC was 33.8 (2.0) cm, Bwt 3.274 (0.489) kg and Lth 49.7 (3.0) cm. Of note no women who planned their pregnancies had microcephalic infants compared with 7 in the unplanned group. Genotyping is available in 10 of 38 women. 7 different mutations were identified with R408W being predominant. **Conclusion**: We report very favourable outcomes in our patient cohort during this period who had planned pregnancies, however, intensive education and follow-up is still required for non-compliant females who have unplanned pregnancies.

P-2-31

HIGH PREVALENCE OF A VENEZUELAN PKU MUTATION

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Classical phenylketonuria (PKU) is a recessive metabolic disorder caused by a deficiency of hepatic enzyme phenylalanine hydroxylase (PAH). Up to date, around 500 mutations have been described in the PAH gene, associated to different ethnic and geographical groups. The ethnic composition of the Venezuelan people arises from a particular mixture of Caucasians, principally from Western Europe, Amerindians and Negroids from Africa. The aim of this study is to understand the molecular basis of PKU in Venezuela, which could improve diagnosis and treatment of this disorder. We are currently searching for mutations in Venezuelan patients using mutation scanning methods based on PCR/DGGE and subsequent direct sequencing. Until now, we have investigated 30 independent mutant chromosomes. Seventy seven percent of the alleles have been identified, and a total of 13 different mutations have been detected. Interestingly, we found a high prevalence Venezuelan PKU mutation (S349L). The frequency of this mutation is 22% from the total alleles genotyped. Also, the frequency of the most prevalent mutation in Spain (IVS10nt-11g>a) is 22%. The rest of the mutations have been detected in one or two alleles. Out of 13 mutations detected, two have been reported only in Venezuela (S349L and P314fsdelC) and the other mutations (I65S, R241H, R243O, R252W, R261Q, E280K, IVS10nt-11g>a, T380M, V388M, A403V and R408W) had been found in European countries as expected. These results highlight the genetic heterogeneity of PKU in Venezuela and provide information on the genetic background of this population.

P-2-32

DEPRESSION, EXECUTIVE FUNCTION AND ADHD IN CHILDREN WITH EARLY AND CONTINUOUSLY TREATED PHENYLKETONURIA

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Aim: The primary aim of this study is to establish prevalence of depression, executive function deficits and attention deficit hyperactivity disorder in children with early and continuously treated (ECT-PKU). **Methods**: 20 children (13 with PKU and 7 control siblings) responded to questionnaires mailed to the 29 children in the 8–17 year age bracket attending the PKU clinic in Brisbane. The study is a mixed design (between groups and repeated measures) with two waves of data collection. The Children's Depression Inventory (completed by the child) and Behavior Rating Inventory of Executive Function (completed by parent) are used to measure depression and EF/ADHD respectively. Results are compared to same day and historical phenylalanine (phe) and tyrosine (tyr) levels and phe/tyr ratios. **Results**: Comparisons between PKU and control group has shown a statistically significant reduction of executive function in the PKU group. Further investigations also reveal a subset of four PKU children who display a high degree of overlap in terms of their EF dysfunction and high number of depression symptoms, both related to high historical phe levels. Females are more susceptible to high depression score ratings. We hypothesise a disruption to the same underlying neurotransmitter system as likely mechanism for both EF dysfunction and depression. **Conclusion**: These findings have implications for treatment of children with ECT-PKU, and provide further insight into the possible mechanisms underlying the neuropsychological profile.

P-2-33

BONE CONDITION AT PHALANGEAL QUANTITATIVE ULTRASOUND IN PHENYLKETONURIC PATIENTS

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Impaired bone status has been described in phenylketonuric patients, as a consequence of poor dietary control or insufficient proteic intake, and fracture rate is estimated to be more than two-folds increased in children over 8 years.

In order to investigate bone status in phenylketonuric subjects, we enrolled 26 patients aged 2.4–40.1 years for the evaluation of bone condition by phalangeal quantitative ultrasound (QUS). This technique evaluate the speed of ultrasound wave (AD-SoS) and the time of transmission (BTT) through bone, which are directly connected to bone mineral density and cortical thickness. Comparison with sex and aged matched healthy controls provides SD for these parameters (Z-score). For each patient, plasma phenylalanine levels and dietary compliance were recorded during the year before QUS.

Mean Z-scores were not reduced in the whole study group. Subjects aged less than 8, however, had higher values than older subject for AD-SoS ($p < 0.05$). All subject aged more than 20 ($n = 7$) had negative Z-scores. AD-SoS and BTT Z-scores resulted inversely related with phenylalaninaemia ($p < 0.05$) and with age ($p < 0.05$).

Four fractures occurred within the study group, all in subjects with negative BTT Z-score. Their mean Z-scores were lower than that of non fractured patients, but significance was not reached.

In particular, an humeral fracture occurred for low-energy traumatism in a 3.6 years male, with good adherence to the phe-restricted diet but very poor compliance to the protein substitute; his BTT Z-score was –1.61, confirming bone impairment, possibly due to low protein intake.

P-2-34

A REPORT OF PHENYLKETONURIA IN 2 THAI INFANTS

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Phenylketonuria (OMIM 261600) is an inborn error of metabolism resulting from deficiency of phenylalanine hydroxylase enzyme and characterized by delayed development/mental retardation. Early diagnosis of PKU is important because it is treatable by dietary means. Classical PKU is inherited in autosomal recessive manner and is the result of mutations in the structural gene for phenylalanine hydroxylase. PAH locus is on chromosome 12q 24.1 (OMIM-VAMcKusick).

We herein report 2 cases of PKU. Case 1. One year-11 month-old boy from Khonkaen Province in Northeastern Thailand, referred to Siriraj Hospital with history of hypopigmentation since age 4 months; seizures and delayed development since age 9 months. He was diagnosed with PKU at age 9 months; Phe level was 100 ugm/dl (normal <4 ugm/dl), by newborn screening program of Ministry of Public Health. Microcephaly, uncontrolled seizures and DQ 39 were observed on admission. Case 2. Two-month-old boy with PKU detected by newborn screening program at Siriraj Hospital. He was found to have abnormal PKU screening since day 1 (Day 1: Phe 6.355, Day 2: Phe 8.132, Day 11: Phe 37.713 mg/dl). He was admitted (day 12) and confirmation by quantitative plasma amino acid analysis demonstrated Phe of 1388.85 mmol/ml and urine organic analysis found 2-OH-phenyllactate, phenyllactate and phenylpyruvate. He was treated with breast milk/phenyl-free/PFD by titration until Phe level is at satisfactory level of 443.20 mmol/ml. Growth and development are normal. This is the first case of PKU from newborn screening program at Siriraj Hospital (started on October 21, 2005).

P-2-35

EVALUATION BY PKU PATIENTS OF FIRST SAVOURY FLAVOURED PROTEIN SUBSTITUTE

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A study was carried out to investigate the liking and changes in liking of the first reported savoury flavoured protein substitute (Milupa PKU activa tomato) for patients with phenylketonuria (PKU). A strong decrease in liking during consumption of a single portion is a possible indication of problems with longer term acceptance and therefore relevant for compliance.

The product that was virtually phenylalanine-free (9 mg Phe per 10 g protein equivalent/portion), was consumed warm as a soup and evaluated by 106 PKU patients (mean age 15 years) in six countries in Europe, with the use of a questionnaire that was self-completed, in some cases with the assistance of a carer. The questionnaire consisted of a general information part that was answered before tasting, a part that was answered at the beginning of consumption and a part that was answered at the end of consuming one portion (200 ml). For all hedonic questions a symmetrical 9 pt Likert scale was used, for all other questions 5 pt symmetrical scales were used.

72% of all participants liked the taste (mean rating taste +SE: 6.5 +0.2; aftertaste: 5.7+0.2; overall liking 6.6+0.2), 51% found the product as good as or better than expected (mean rating 3.5+0.1 on 5 pt scale). The overall liking decreased slightly during consumption (mean rating from 6.6+0.2 to 6.0+0.2), but changes in liking of the taste and aftertaste, the degree to which the product fulfilled the patients' expectations were not significant. It was concluded that savoury flavoured PKU products are promising.

P-2-36

6-PYRUVOYL-TETRAHYDROPTERIN SYNTHASE DEFICIENCY: THE CLINICAL SPECTRUM IN 3 CHINESE PATIENTS

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Objectives: To describe the clinical characteristics of 3 patient with 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency. **Methods**: The medical notes of these patients were reviewed. The clinical and the biochemical features were described. **Results**: The first two patients presented at 5 to 6 months old with episodes of prolonged eye staring and stiffening of the 4 limbs which lasted for 30 min to 1 h. These could happen few times per week. They had global delay. The third patient, who was 19 years old, had an initial diagnosis of dyskinetic cerebral palsy, epilepsy and mental retardation. Physical examination revealed a fair skin complexion. There was intermittent dystonia and choreoathetosis involving all the 4 limbs. Blood for amino acid in these patients showed hyperphenylalanaemia. Tetrahydrobiopterin (BH_4) loading showed complete normalization of plasma phenylalanine. Urine pterins showed decreased biopterin with normal to high neopterin. Cerebrospinal fluid analysis was low for biogenic amines including 5-hydroxyindoleacetic acid and homovanillic acid. All of them were diagnosed to have PTPS deficiency. All of them were confirmed by genetic testing. The first 2 patients' clinical symptoms and signs responded well to replacement of BH_4, l-dopa and 5-hydroxytryptophan. The third patient was just started on treatment. **Conclusion**: In places without newborn screening for inborn error of metabolism like Hong Kong, early recognition of this potentially treatable neurological condition is crucial to improve the associated neurological morbidity.

P-2-37

EFFECTS OF IRREGULAR USE OF AMINO ACID MIXTURE IN A GROUP OF PKU PATIENTS

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The National Newborn Screening Program (NNSP), created in Brazil in 2001, was set at the Public Health System and included phenylketonuria (PKU) as one of the diseases detected. Amino acid mixtures (AA) required for the patients treatment is supported by NNSP. Sometimes, due to logistic problems of AA local distribution, patients can pass several days without the appropriate formula for their treatment.

In order to ascertain the effects of irregular dietary intervention, the charts of 25 PKU children seen in a NNSP/PKU reference center were reviewed. Patients were followed up with medical, psychological, and nutritional assessments and blood phenylalanine (PA) level should be kept below 10 mg/dl. Patient's PA were studied in 3 different subsequent periods among June/2004 and September/2005: (A) a six months period of regular distribution of formula (but not necessarily age-adequate formula); (B) a three months period of irregular distribution (some patients did not receive any formula by this time); (C) a six months period of regular distribution of age – adequate formula. All patients increased their PA levels during period B and during period C they decreased the PA levels, even to lower levels than seen in the period A.

P-2-38

BH_4 TREATMENT WITH NO DIET RESTRICTIONS

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Several studies have shown that patients with mild PKU respond to oral administration of BH_4, with and without diet restrictions.

To verify that the BH_4 responsiveness in patients with one mutation (Y414C) in the PAH gene, can be treated just as safe and effective as on the conventional PKU diet. We wanted to find a reliable method to test for BH_4 responsiveness. The aim of the study was to verify whether this certain group of our patients in future will benefit from treatment with lowest possible BH_4 dosage, without any dietary restrictions. Twelve patients diagnosed with PKU with 2 known mutations in the PAH gene, where off Y414C is known, age 8–29 years old, was included in the study.

Diet record was registered initially and throughout the test period. S-phenylalanine was measured and analysed several times during the test period. Amino acid supplement was omitted from the diet and the amount of natural protein increased to 1 g/kg/day. The patients were asked to take supplements of vitamins and minerals. An individual diet had to be followed, to ensure the patient got a sufficient intake of natural protein. All patients had white bread, meat and sandwich spread included in their diet. Eleven patients had normal pasta and rice. Food items like eggs, dairy products and fish contributed only with a small amount of protein, due to former eating habits Three patients reported that they missed their amino acid supplement and all expressed an easier way of living.

P-3-1

A MOLECULAR LESION IN A JAPANESE PATIENT WITH SEVERE PHENOTYPE OF 3-METHYLGLUTACONIC ACIDURIA TYPE I

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We identified a novel mutation of the AUH (AU-specific RNA-binding protein) gene in a Japanese patient with a severe phenotype of 3-methylglutaconic aciduria type I (MGA1, MIM 250950). Direct sequencing of the genomic DNA, extracted from peripheral white blood cells of the patient, showed homozygosity of A-to-G transition at the 3′ (acceptor) splice site of intron 1, designated IVS1-2A>G, causing complete skipping of exon 2. In recent reports, five mutations of the AUH gene were reported to be molecular lesions of this disease. Four of these mutations caused truncation of the AUH protein, induction of a nonsense codon, or exon skipping in AUH RNA, and all were expected to be severe genotypes of this disease. However, the patients with these four mutations all showed mild developmental delay or no abnormality. In comparison, our case showed more severe neurological deterioration and persistent decompensated metabolic acidosis. The clinical phenotype of this patient is indeed caused by a splicing mutation of the AUH gene leading to complete skipping of exon 2. Based on patient findings, there seems to be no correlation between the clinical phenotype and mutational genotype in this disease.

P-3-2

ORGANIC ACIDEMIAS IN KOREA: EIGHT YEARS EXPERIENCE OF ORGANIC ACID ANALYSIS

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Purpose: We have done this retrospective study to know the relative incidence and clinical manifestations of organic acidopathies in Korea during 8 years (from July 1997 to May 2005). **Methods**: The results of quantitative organic acid analysis of samples of 1788 patients, referred from major university hospitals all over the country with neurological dysfunctions, were analyzed retrospectively according to four age groups (–2 months, 3 months–2 years, 3 years–12 years, over 13 years) and major clinical manifestations. Quantification of 83 organic acids was done with gas chromatography and mass spectometry. **Results**: We diagnosed 470 patients with 27 diseases of organic acid metabolism during this study period. Diseases found more than 10 cases are cytosolic 3-ketothiolase deficiency, mitochondrial respiratory chain disorders, PDHC deficiency, mitochondrial 3-ketothiolase deficiency, glutaric aciduria type, biotinidase deficiency, methylmalonic aciduria and propionic aciduria. Other diseases were diagnosed in less than 10 cases. **Conclusion**: Though the incidence of individual organic acidemia is low, the overall incidence of organic acidemia as a whole seems to be relatively high in Korea with diversity.

P-3-3

CLINICAL AND MOLECULAR STUDY OF 13 JAPANESE CHILDREN WITH GLUTARIC ACIDEMIA TYPE 2

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Glutaric acidemia type 2 (GA2) is an inherited autosomal recessive disorder of the fatty and organic acid metabolism caused by a defect of the electron transfer protein (ETF) which consists of α and β-subunits (ETFα and ETFβ, respectively) or the ETF dehydrogenase (ETFDH). GA2 has two clinical forms: the neonatal onset type (severe form) and the late onset type (mild form). We investigated the clinical manifestations and gene mutations of 13 Japanese patients with GA2 (8 boys and 5 girls), including four patients previously reported. There were 4 patients of the severe form and 9 of the mild form. Of the 13 patients, 8 patients had family history of child death in childhood, 3 had a polycystic kidney, and one had congenital heart disease. In the patients with the neonatal onset type, all 4 patients showed dyspnea, hypoglycemia or hyperammonemia, and died before 5 days after birth. In the late onset type, the ages at onset ranged from 4 months to 1 year, and poor feeding, hypotonia or convulsion were shown. Four of the 9 patients died before 3 years of age. In the molecular study, mutations of ETFα gene were identified in 3 patients. ETFβ gene in four and ETFDH gene in 4. In this study, 10 novel mutations were identified in the *ETF/ETFDH* genes. It was suggested that clinical and mutational spectrum of Japanese patients with GA2 was heterogeneous.

P-3-4

ACUTE INTOXICATION PRESENTATION OF MALONIC ACIDURIA – A CASE REPORT

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We present a Chinese girl born to non-consanguineous parents, who presented with acute circulatory collapse on day 5 of life with hypoglycaemia, ketosis and acidosis. She was born full term of good birth weight with no neonatal problems and was breastfed. She had poor suck on day 5 and was tachypnoeic. She was resuscitated, and put on intravenous 10% dextrose drip. Septic workup was done and she was given intravenous antibiotics and bicarbonate. Urine ketones were markedly elevated. She was transferred to a tertiary hospital where intravenous carnitine/biotin/vitamin B_{12} were given.

Biochemical investigations done showed blood sugar level of 1.9 mmol/l, metabolic acidosis with arterial blood gas pH 6.942 and anion gap of 21, mild hyperammonaemia 194 μmol/l and lactate 8.1 mmol/l. Septic workup was negative. Tandem mass spectrometry screening on Guthrie card showed grossly elevated malonyl-carnitine C3D3, consistent with malonic aciduria. Urine organic acid profile showed grossly elevated malonic acid and succinic acid, elevated methylmalonic acid and presence of adipic acid and glutaric acid. A 2-D echocardiography showed small patent ductus arteriosus, and left to right atrial septal defect/patent foramen ovale flow with no evidence of cardiomyopathy.

She responded quickly to treatment and was able to tolerate oral feeding from day 7. She was maintained on oral carnitine with a low fat and high carbohydrate diet, and remained well.

P-3-5

CHARACTERIZATION OF E252del MUTANT PROTEIN IDENTIFIED A PATIENT WITH MITOCHONDRIAL ACETOACETYL-CoA THIOLASE (T2) DEFICIENCY

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T2 deficiency is an inborn error of metabolism that affects the catabolism of isoleucine and ketone body. An Italian Sardinian T2-deficient patient was revealed to be a homozygote of a novel E252del mutation. We herein characterized the E252del mutation in detail. Transient expression analysis of wild-type and mutant cDNAs was done at 40, 37 and 30°C. The mutant T2 was detected with relative amount and activity of 30% and 25% to those of wild-type, respectively, in 37°C expression. This mutant protein was more stable in 30°C expression than 37°C expression but was hardly detected in 40°C expression, indicating temperature-sensitive stability of the mutant T2. Specific activity of E252del mutant was estimated to be 80% of wild-type. The mutant also reduced heat stability of T2 activity. Kinetic analysis showed that Km values for CoA and acetoacetyl-CoA were two-fold higher in E252del mutant than wild-type while Vmax value of E252del mutant is almost the same as that of wild-type. The effect of E252del for T2 tertiary structure was also investigated. In conclusion, E252del mutant was relatively unstable with temperature-sensitive manner but retained T2 activity with specific activity ∼80% wild type. This mutant was also a Km mutant.

P-3-6

SINGLE BASE SUBSTITUTION AT THE LAST NUCLEOTIDE OF EXON 6 (c.671G >A) RESULTED IN SKIPPING OF EXON 6, AND EXONS 6, 7 IN HUMAN SCOT GENE

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Succinyl-CoA:3-ketoacid CoA transferase (SCOT) deficiency, a rare ketolytic defect causes permanent ketosis and episodes of ketoacidosis that may lead to death. We performed molecular analysis of SCOT deficiency in a Turkey patient (GS12) who presented with ketoacidosis at the second day of her life. Defective SCOT enzyme activity in GS12 fibroblasts confirmed the diagnosis. In molecular analysis, GS12 had two shorter SCOT cDNAs: major cDNA had exons 6 and 7 skipping, and minor cDNA had only exon 6 skipping. No SCOT cDNA with normal size was detected. At the genomic level, we searched mutations in exon 6 and exon 7 and their exon/intron boundaries. A G>A substitution was identified at the last nucleotide of exon 6 (c.671G>A) and no other substitution was found around exons 6 and 7. To understand why the c671G>A causes exons 6 and 7 skipping, nuclear RNA was separated from cytoplasmic RNA and they were analyzed by RT-PCR. In nuclear RNA, SCOT mRNA with exon 6 skipping was predominant and mRNA with exons 6 and 7 skipping was hardly detected, whereas the latter was a dominant mRNA in cytoplasmic RNA. This discrepancy was interpreted as follows: exon 6 skipping causes a frameshift and nonsense mediated RNA decay in cytosol, so mRNA with exon 6 skipping was unstable. On the other hand, SCOT mRNA with exons 6 and 7 is a minor transcript but it is inframe and stable in cytosol. Hence the latter mRNA became more evident than the former RNA in RT-PCR at the steady condition.

P-3-7

AMNIOTIC FLUID ACYLCARNITINE LEVELS IN 5 SUBJECTS AFFECTED WITH PROPIONIC ACIDEMIA AS COMPARED TO CONTROLS

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Introduction: Propionic acidemia (PA) is one of the most frequent inborn errors of metabolism caused by a deficiency of propionyl CoA carboxylase. Prenatal diagnosis is usually performed using metabolites measurement in amniotic fluid and enzymatic measurement of deficient enzyme in chorionic villus samples (CVS). Acylcarnitines profile were evaluated in the same amniotic fluids taken by amniocentesis between 12 to 15 weeks of amenorrhea. **Materials and methods**: Acylcarnitines profiles were studied in amniotic fluids taken from 5 subjects affected with propionic acidemia: all of them demonstrated highly elevated levels of methylcitric acid (3.3 to 34 µmol/l, controls range: 0.37–1.50) and reduced activity of propionyl CoA carboxylase in CVS (<0.01 µmol/min/mg protein, controls range: 0.12–0.55). High risk subjects from affected families (n = 5) were studied. Control group consisted in 5 amniotic fluids taken from 12–17 weeks of amenorrhea for suspicion of chromosome abnormalities. Assays were run using API 3000 (Applied BioSystem) tandem mass spectrometer with electrospray ionization (ESI) through FIA (flow injection analysis). Deuterated acylcarnitines were used as internal standards. Sample preparation consisted in methanol extraction of acylcarnitines without derivatization step. Quantification of different acylcarnitines was run using MRM mode. Precursor ion m/z 85 scan was performed to evaluate profile of the samples. **Results**: The propionylcarnitine concentration of the 5 samples from affected foetus was highly increased (5.4 to 13 µmol/l) as compared to control group values (<0.35 µmol/l). Concentration levels observed in high risk family ranges from 0.2 to 1.3 µmol/l. **Conclusion**: measurement of propionylcarnitine in amniotic fluids could provide a useful tool in the prenatal diagnosis of propionic acidemia.

P-3-8

SUCCESSFUL INTRAUTERINE TREATMENT OF A PATIENT WITH COBALAMINE C DEFICIENCY

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We report on the outcome of a patient treated with high intrauterine dosages of hydroxycobalamine during pregnancy from the 16th weeks of gestation. The index case was the second child of a non related German couple. The first child is normal. Clinical and biochemical details of the second child in whom cbl C deficiency was diagnosed are published elsewhere (Tomaske et al., JIMD. 2001;24:511–2). To summarize this sibling is severely affected showing typical phenotype with psychomotoric retardation, growth failure, vision impairment and severe horizontal nystagmus. Homocysteine in serum and methylmalonic acid in urine are highly elevated (details on *www.ramedis.de* ID 160). Prenatal investigation in amniotic fluid in the third pregnancy revealed high homocysteine and elevated methylmalonic acid so that cbl C deficiency in the fetus was highly reliable. The parents decided to continue the pregnancy after genetic counselling. Treatment with hydroxycobalamine of the mother (3 times/week with 5 mg s.c.) was initiated from the 16th weeks of gestation up to delivery at term. Pregnancy and birth were uneventful, birth parameters were normal. Biochemical investigation after birth confirmed elevated homocysteine and methylmalonic acid so that treatment with hydroxycobalamine, betaine and folic acid was initiated soon after birth. The patient, now 16 months of age shows normal psychomotoric development, has no nystagmus, no microcephaly and no growth failure. So far only single cases are reported where there is treatment started in later pregnancy with a favourable outcome.

P-3-9

A RETENTION TIME LOCKED METHOD FOR THE AUTOMATED PEAK ANNOTATION OF URINE ORGANIC ACID CHROMATOGRAMS

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Urine organic acid analysis by GC/MS involves hand-written annotation of chromatographic peaks, a process which is time-consuming and potentially error prone. We developed a peak identification and labeling system utilizing mass spectral data and retention times to accurately and quickly annotate chromatograms. We used an Agilent GC/MS, Chemstation G1701CA software, trimethylsilyl derivatives, and an HP-5MS column. A new mass spectral library was compiled from our existing library with all retention times expressed in seconds. The method was then retention time locked, a process which ensures that these retention times remained constant regardless of changes such as column length. A database relating these retention times to mass spectra was then developed. This database was searched during each analytical run, using a macro program within the Chemstation method which was modified to facilitate annotation of peaks only if mass spectra and expected retention time were matched. Compared with hand-labeled chromatograms, 83% of peaks were successfully annotated, which increased to 91% as library/database times were more accurately defined. Very broad peaks had displaced retention times and were occasionally unlabelled. Trace concentrations of compounds were annotated even if they co-eluted with another peak. Data analysis time was reduced by approximately 50% allowing operators to concentrate on manual peak verification and annotation of any unlabelled peaks. Transcription errors associated with hand written annotation were eliminated for the 91% of peaks identified. Together with careful verification of peak identities by experienced operators, this development has been used successfully in our laboratory for over three years.

P-3-10

MULTIDIMENSIONAL DISTANCES BETWEEN ORGANIC ACID PROFILES

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Organic acid profiles of urine samples give diagnostic information about metabolic diseases in young children. The samples are analyzed using gas chromatographymass spectrometry (GC-MS) and full spectra are collected by continuous scanning. We have written in MATLAB a number of programs to facilitate the interpretation of the profiles. The results from the chemometric calculations are then returned back to the instrument software to identify the spectra and to calculate the concentrations.

After reading the raw data we use the OSCAR algorithm to isolate the spectra of the components. A new raw data file is then written. The synthetic raw data file contains compounds that are fully separated. The spectrum searches against spectrum libraries are made using the normal software of the GC-MS instrument.

From one urine sample it is possible to routinely obtain a concentration profile with 200 organic compounds. We have calculated the distances from individual patients to the mean value of all patients. The distances from the central point are smaller for non-pathological samples than for the pathological samples. The pathological values are outliers in the multidimensional space. Multidimensional distances are a practical way to rapidly find samples that need more detailed interpretation.

P-3-11

GASTROSTOMY IN IMD: POST OPERATIVE COMPLICATIONS

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Gastrostomy is a common procedure in IMD children and we report our experience in 20 children who have had 23 gastrostomy procedures since 1999. Their diagnosis included: organic acidaemias (n = 8); long chain β fatty acid oxidation disorders (n = 6); and GSD type 1a and 3 (n = 4). They had a median age of 2 y (range 1–11 years). 18 were percutaneous endoscopic gastrostomies and 5 were inserted laparoscopically. Post operative data was available on 21 of 23 procedures. The hospital bed stay for the gastrostomy procedure was 3 days or less in 33% (n = 7); 4–5 days in 38% (n = 8) and over 6 days in 29% (n = 6). Complications were reported in 15 (71%) procedures (compared with 30% for the rest of the paediatric hospital) and occurred within 5 days in 10 cases. In 9 procedures, infections (principle organism Staphylococcus aureus) were documented by positive swab cultures. In 3 procedures, the PEG was misplaced or came out of the tract and in a further 2 cases the tube came out through either infection (n = 1) and child tampering (n = 1). Post operative wound dehiscence (n = 1), silver nitrate burn (n = 1) and gastrostomy leakage (n = 5) were reported. Metabolic complications were uncommon but included metabolic encephalopathy (n = 1); hypoglycaemia (n = 1) and amino acid depletion (n = 1). **Conclusions**: Short term post operative complications were high in this group of young IMD patients.

P-3-12

SPINAL CORD DEMYELINATION ASSOCIATED WITH BIOTINIDASE DEFICIENCY IN 3 CHINESE PATIENTS

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Biotinidase deficiency is a treatable cause of severe neurological disorders and skin problems. Spinal cord impairment is a rare complication of this disease and is commonly unrecognized. We encountered three Chinese patients (2 boys and 1 girl) with progressive spinal cord demyelination associated with biotinidase deficiency. **Case 1** exhibited fatigue, proximal muscular weakness and hypotonic paraplegia from the age of 7 years and 4 months. Demyelination of cervical and thoracic cord was evident on magnetic resonance imaging (MRI). **Case 2** developed visual impairment, blepharoconjunctivitis and optic nerve atrophy from 5 years of age and combined with progressive hypertonic paralysis, ataxia and alopecia from the age of 7 years. His spinal MRI T2-weighted sequence revealed an extensive hyperintense lesion involving the cervical spinal cord C_2 to C_4. Bilateral optic nerves were significantly thick. In **Case 3**, intercurrent wheezing, tachypnea, dyspnea and lethargy occurred from the age of 1 year. Medulla and upper cervical spine edema and demyelination were found on MRI. Markedly elevated urine organic acids and decreased blood biotinidase activities were observed in the three patients. Biotin supplementation led to a dramatic improvement of clinical symptoms in 3 patients. Our findings indicate that biotinidase deficiency should be considered in the differential diagnosis of unexplained spinal cord demyelination since prompt diagnosis and treatment with biotin may enable an excellent recovery.

P-3-13

COMBINED LIVER-KIDNEY TRANSPLANTATION FOR THE MANAGEMENT OF METHYLMALONIC ACIDURIA

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Methylmalonic aciduria (MMA) is an organic aciduria that presents in infancy with recurrent bouts of acidosis, failure to thrive and developmental delay. Medical management of this disease is complicated by medication administration, adherence and palatability. Liver, kidney and combined liver-kidney transplantation for inborn errors of metabolism is gene therapy on a grand scale by providing an organ with functional enzyme. Over 15 cases of liver transplant, kidney transplant and combined liver-kidney transplant have been reported for the treatment of MMA. These cases have met with varying success. Here, we describe a case of a five year old boy who underwent combined liver-kidney transplant (CLKT) for phenotypic mut0 disease. His history was notable for more than 30 hospitalizations for severe acidosis, gross developmental delay, progressive visual loss, metabolic strokes, liver disease, pancreatic disease, chronic renal insufficiency with interstitial nephritis, and decreased quality of life. Post CLKT, there was a marked reduction in serum (92%) and urine MMA levels (97%) as well as a cessation of metabolic decompensations. Liver transaminases returned to normal. However, his post operative course was notable for suspected tacrolimus toxicity involving recurrent fevers, seizure activity, and speech disturbances. A subsequent cerebellar infarction with ataxia and aphasia also occurred. This case report underscores the reported post-transplant sequelae and natural history of this complex disease. Although CLKT offers the potential for improved quality of life, the role of organ transplantation in MMA remains to be established.

P-3-14

LENTIVIRAL-MEDIATED CORRECTION OF METHYLMALONYL CoA MUTASE DEFICIENT MOUSE FIBROBLASTS

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Aim: To evaluate the lentiviral vector-mediated transfer of the methylmalonyl CoA mutase gene as a treatment for methylmalonyl aciduria resulting from mutase deficiency, using fibroblast cultures from a methylmalonyl CoA mutase knockout mouse as an *in vitro* disease model. **Method**: A self-inactivating lentiviral vector expressing murine methylmalonyl CoA mutase from the elongation factor 1α promoter was constructed and used to transduce cultures of MMA CoA mutase knockout mouse fibroblasts. C^{14} propionate incorporation into TCA precipitable material was measured *in vitro* by a modified version of the method of Peters et al. Vector copy number was measured by real time PCR using a transferrin gene sequence as a single copy (diploid) control. **Results**: MMA fibroblasts were efficiently transduced with the vector and this resulted in the correction of the metabolic deficiency, as indicated by propionate incorporation into TCA precipitable material. **Conclusion**: These results show that lentiviral mediated transfer of the methylmalonyl CoA mutase gene is able to correct the defect in methylmalonyl CoA mutase deficient MMA cells, suggesting that the use of lentiviral vectors expressing MMA CoA mutase may be a viable approach for the development of a 'metabolic sink' gene therapy strategy for this form of MMA.

P-3-17

CLINICAL HETEROGENEITY OF HMG-CoA LYASE DEFICIENCY

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3-Hydroxy-3-methylglutaric aciduria is a rare autosomal recessive disorder of leucine metabolism classically presenting as Reye-like illness in infancy. We have identified 3 patients with HMG CoA lyase deficiency with different presentation over past 6 years. All of them had a typical urine organic acid GC-MS profile revealing large peaks of 3-OH-3-methyl-glutaric acid, 3-methylglutaconic acid, 3-methyl-crotonylglycine and 3-OH-isovaleric acid as well as dicarboxylic acids C6>C8>C10. Serum amino acid revealed hyperglycinemia and high glutamine. The first patient was a 19 year-old girl diagnosed at the age of 13 when she presented with epileptic encephalopathy and left hemipareisis due to a right parietal infarct after a bout of diarrhoea. The other two patients are siblings from the same family with consanguineous parents. The elder sister was diagnosed at 6 months of age when she presented with cyclical vomiting and failure to thrive associated with metabolic acidosis and rasied anion gap. Mother noted abnormal odour in her urine during each attacks and she had an episode of hypoketotic hypoglycemia in the neonatal period which she recovered without further investigation. Her younger brother was diagnosed at day 3 of life with hypoketotic hypoglycemia, metabolic acidosis with increased anion gap and hyperammonemia of 900 μmol/L requring peritoneal dialysis and ventilation. Both of them had the classical presentation requiring carnitine and leucine restricted diet before they improved. The first patient with atypical presentation required no specific therapy except for avoidance of fasting particularly during intercurrent illnesses. All of them remained well to date without significant neurological deficits.

P-3-18

AMINOACID FOLLOW-UP DURING SUCCESSFUL PREGNANCY IN A WOMAN WITH METHYLMALONIC ACIDAEMIA

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There are few reports about pregnancy occurring in methylmalonic acidaemia (MMA). We report a new case of a 23 years old woman diagnosed for MMA in the neonatal period. The diagnosis was made biochemically and subsequent enzyme studies determined the complementation group to be mut^-. Metabolic control during childhood and adolescence was good and she achieved normal secondary school. Pregnancy was followed weekly from the fourth week of pregnancy. Feeding difficulties (vomiting) occurred during the first trimester and improved from the second trimester to the end of the pregnancy. The weight remained stable until the 16th week of gestation and then increased regularly. Treatment included low protein diet (20–30 g of natural protein + 80 g of protein substitute without Val, Ile, Met and Thre), carnitine, cobalamine, iron and folate supplementation, and metronidazole. She was treated in the third trimester for a moderate hypothyroidism. Urinary methylmalonic acid excretion decreased after the first trimester and remained roughly stable afterwards. Delivery occurred by caesarean section, with a normal newborn boy (weight: 3.42 kg, height: 49 cm, head circumference: 35 cm). We followed glycine as a marker of metabolic decompensation and the sum of branched chain aminoacids (BCAA) as a marker of adequate natural protein intake. Glycine increased during the first trimester (vomiting), and then completely normalised while BCAA decreased (foetal growth and liver maturation). We suggest that serum aminoacid determination (glycine and BCAA) is a reliable tools to follow the metabolic and nutritional status during pregnancy in MMA.

P-3-19

IDENTIFICATION OF MMACHC GENE MUTATIONS IN NORTHERN CHINESE cBLC PATIENTS WITH COMBINED METHYLMALONIC ACIDEMIA AND HOMOCYSTINURIA

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The *cblC* type methylmaonic acidemia (*cblC* MMA, OMIM 277400), which is caused by defects in the *MMACHC* gene, is the most frequent inborn error of vitamin B_{12} metabolism. The defect impairs the biosynthesis of adenosylcobalamin and methylcobalamin, leading to dysfunction of methylmalonyl CoA mutase and methionine syntase. Accordingly, patients carrying *MMACHC* mutations will present clinical symptoms of combined methylmalonic acidemia and homocystinuria. In this study, the ORF region of exon 1 to 4 of the *MMACHC* gene was PCR amplified and sequenced subsequently to identify molecular defects in 25 unrelated Northern Chinese *cblC* MMA families. Thirteen different alterations, which accounted for 80% of diseased alleles, were identified. These mutations included 8 point mutations (c.1A>G, c.315C>G, c.365A>T, c.394C>T, c.457C>T, c.482G>A, c.615C>A, and c.609G>A), 3 insertions (c.445_446insA, c.568_569insT, and c.626_627insT) and 2 deletions (c.658_660delAAG and c.277-3_c.303del30). Among which, c.394C>T, c.457C>T, c.482G>A, and c.609G>A had been reported in other populations, while the other mutations were identified firstly in *cblC* MMA patients. The c.609G>A was found to be the most frequent mutant allele in Chinese *cblC* MMA (32%). This mutation had been reported in East Asian previously. The c.658_660delAAG (10%) and c.482G>A (8%) were found to be the second and the third frequent mutations in Chinese patients. Mutation c.315C>G, c.394C>T, c.457C>T, c.615C>A, and c.609G>A are nonsense mutation. Insertion mutations might cause frame shift. Two deletions might cause a deletion of amino acids. Most of these alterations were predicted to cause the impaiments of protein function and therefore lead to the disease.

P-3-20

GLUTARIC ACIDURIA TYPE I: IDENTIFICATION OF AFFECTED MOTHERS THROUGH THE NEWBORN SCREENING

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Glutaryl-CoA dehydrogenase deficiency (GA I, MIM# 231670) is one of the metabolic disorders included in the expanded newborn screening. About 60 000 newborns were screened by tandem mass spectrometry (MS/MS) and two were found to be sons of mothers affected with GA I.

The first case was a boy whose mother was diagnosed with GA I at 8 years of age. She presented epilepsy and a mild developmental delay. During her pregnancy, she did not respect the indication for carnitine supplementation nor necessary follow-up, and refuted prenatal diagnosis. At the second day of life, the newborn presented increased levels of glutarylcarnitine (0.22 μM) and high ratios glutarylcarnitine/ palmitoilcarnitine and glutarylcarnitine/ octanoilcarnitine. Due extremely low level of free carnitine, carnitine supplementation was recommended. At 4 months, the infant presented normal growth with normal physical and neurological examinations, although CT scan revealed some degree of brain damage.

The second case was a boy who presented a low free carnitine value at day 5 (3.9 μM), with no other alterations. Since he had extremely low serum carnitine level, he was admitted in our metabolic unit. At admission, this boy presented normal physical and neurological examinations. The newborn's mother showed mild mental retardation and macrocephaly and acylcarnitine profile revealed a glutarylcarnitine level compatible with GA I. This diagnosis was confirmed in urine by GC/MS. Our data suggest that very low levels of free carnitine in newborns may result from the presence of GA I in the mother, as already observed in other organic acidurias.

P-3-21

GLUTARIC ACIDURIA TYPE I IN A THAI INFANT

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Glutaric aciduria Type I (OMIM 231670) is a rare neurodegenerative disorder with megalencephaly, acute encephalitic-like crises, spasticity, dystonia, choreoathetosis, ataxia, dyskinesia, seizures, increased signal on imaging of caudate and putamen; and frontotemporal atrophy. Glutaric aciduria and deficient activity of glutaryl CoA dehydrogenase usually confirm diagnosis. Deficiency of glutaryl CoA dehydrogenase interferes the catabolic pathway of lysine, hydroxylysine and tryptophan.

We herein report a 16-month-old boy from Thai-Kmer border who developed seizure and spasticity 5 days prior to referral to Siriraj Hospital with clinical impression of encephalitis. There was history of consanguinity. Hepatomegaly, hypertonicity of all extremities and bilateral optic atrophy were noted. Cranial tomography demonstrated typical frontotemporal lobe atrophy. Urine organic acid analysis showed increased excretion of glutaric-2 and 3-OH-glutarate. Treatment included riboflavin, L-carnitine, low-protein diet and genetic counseling. In summary, organic acid analysis in patients with dystonic cerebral palsy with megalencephaly should be considered. Early diagnosis leads to appropriate treatment and eventual improved outcome. Molecular analysis will be presented. This is the first reported case of GAI in Thailand.

P-3-22

MULTIPLE CAROXYLASE DEFICIENCY IN A THAI INFANT: FIRST REPORTED CASE

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Multiple carboxylase deficiency (MCD) (OMIM 253270) is a disorder of biotin metabolism resulting in deficiencies of at least 3 biotin-dependent mitochondrial carboxylases: propionyl – CoA carboxylase, 3 methyl crotonyl – CoA carboxylases and pyruvate carboxylase. There are 2 forms: a neonatal form and a juvenile form. Biotinidase deficiency, late-onset (juvenile) multiple carboxylase deficiency is disorder of biotin recycling. Biotinidase deficiency has been described in over 80 symptomatic children. Clinical features include seizures, hypotonia, ataxia, hearing loss, optic atrophy, developmental delay, skin rash and alopecia. Abnormalities in immunoregulation cause conjunctivitis and fungal infections. Clinical expression is highly variable. Age of onset of symptoms ranges from several weeks to several years of age. Most common symptoms are severe metabolic ketolactic acidosis and organic aciduria.

We herein report an 8 months old male infant who presented with severe metabolic acidosis, an erythematous periorificial dermatitis and alopecia since 5 months of age. Quantitative plasma amino acids analysis was within normal limits: however urine organic acids analysis by gas liquid chromatography and mass spectrometry demonstrated a series of abnormal compounds characteristic of multiple carboxylase deficiency. Therapy consist of 60–80 mg of biotin per day with dramatic response. Enzyme assay and molecular analysis will be presented. This is the first reported case of multiple carboxylase deficiency in Thailand.

P-3-23

ALKAPTONURIA IN A THAI INFANT: FIRST REPORTED CASE

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Alkaptonuria (OMIM 203500) is a rare metabolic disorder in which enzyme homogentisic acid oxidase is deficient; therefore, there is accumulation of homogentisic acid (HGA) and is excreted in the urine. The metabolic defect causes a characteristic triad of homogenitisic aciduria, ochronosis and arthritis. It is inherited as an autosomal recessive condition. Sir Archibald Garrod (1902) described alkaptonuria as 'the first disorder of the Inborn Errors of Metabolism' The recent advance of nitisinone for long-term therapy require further evaluation.

We herein report 1 year 9 months old female infant from Chiangrai province in Northern Thailand whose dark-stained diapers and dark urine on standing was observed since age 1 month. Washing diaper with soap tends to make stains more intense. Parental consanguinity was denied, however, they came from the same village. Her developmental milestones were normal (DQ = 104). Urine organic analysis demonstrated homogentisic acid. Treatment with vitamin C to enhance HGA degradation has not proved beneficial. However, nitisinone (Orfadin) has been proposed as potential therapy. Enzyme assay and mutation analysis and will be presented. This is second reported case of alkaptonuria in Thailand.

P-3-24

HEARING LOSS IN BIOTINIDASE DEFICIENCY: PRELIMINARY RESULTS INDICATE GENOTYPE–PHENOTYPE CORRELATION

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Biotinidase deficiency (BD) is an autosomal recessively inherited disorder that results in failure to recycle the vitamin biotin. The clinical features of BD include cutaneous and neurological features, including hearing loss. We studied 18 children with BD; 3 asymptomatic children who were diagnosed and treated immediately after birth because an older sibling was affected (all were homozygous with mutations resulting in no enzyme protein; null mutations), 9 symptomatic children homozygous with null mutations, and 6 symptomatic children homozygous with missense mutations. Audiologic, impedancemetric, auditory brainstem response and otoacoustic emissions measurements were performed on all patients. Hearing loss of varying degree occurred in all symptomatic children with null mutations, whereas all symptomatic children with missense mutations (possibly having some residual enzyme activity) had normal hearing. Interestingly, the three children with null mutations who were treated since birth did not develop hearing loss. These preliminary results indicate that symptomatic children with null mutations are likely at risk of developing hearing loss, whereas those with missense mutations may not develop hearing loss even if they are not diagnosed and treated for a prolonged period of time. In addition, once hearing loss occurs, it seems to be irreversible despite biotin treatment, although treatment may prevent progression of the hearing loss. Biotin treatment immediately after birth appears to prevent hearing loss in children with null mutations. Our study further indicates that BD should be considered in all children with sensorineural hearing loss and all newborns should be screened for this readily treatable disease.

P-3-25

PROPIONIC ACIDEMIA IN THAI INFANTS: A REPORT OF 2 CASES

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Propionic acidemia (OMIM 232000) is a disorder of branched-chain organic acidurias caused by an abnormality of specific enzymes involving the catabolism of branched-chain amino acids (BCAAs). They can present clinically as a severe neonatal onset form of metabolic distress; an acute, intermittent, late-onset form or chronic progressive form presenting as hypotonia, failure to thrive and developmental delay.

We herein report 2 cases of severe neonatal-onset form. **Case 1**: (S.B.) A 3-year 9-month-old boy (born in 2000) who developed tachypnea and respiratory failure required mechanical ventilation at 10 h of age. He was first seen at Siriraj Hospital at 1 year and 5 months after severe vomiting, diarrhea, dyspnea with severe growth failure. Urine organic acid analysis confirmed diagnosis. Improvement was noted after aggressive treatment provided, however moderately severe delay development was noted. **Case 2**: (N.P.) A 10-month-old female infant (born in 2002) who developed sepsis-like symptoms at 2 days old which was treated accordingly. She developed seizure at one month of age with ketonuria; CT brain revealed hypodensity of white matter. Subsequently, she was hospitalized ($\times 10$) with different diagnoses ranges from UTI, fever, drowsiness. Wide amino gap metabolic acidosis was noted at 4 months of age. However, IEM was suspected at 10 months of age when plasma amino acid analysis revealed markedly high glycine and urine organic acid analysis showed increased excretion of propionylyglycine, 3-OH propionate, methylcitrate and glycine in the urine. Treatment include L-carnitine, Biotin, Propimex-1 and restricted protein with marked improvement. Mutation analysis will be presented.

P-3-26

METHYLMALONIC ACIDEMIA IN THAI INFANTS: A REPORT OF 3 CASES

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Methylmalonic acidemia is a rare inborn error of metabolism of organic acid disorder. There are 4 forms complete methylmalonyl CoA mutase deficiency (mut^0), partial deficiency (mut^-), deficiency of a mitochondrial Cbl reductase (cblA) and mitochondrial Cob (I) alamin adenosyltranferase (cbIB) deficiency. Most common clinical presentation are lethargy, failure to thrive, recurrent vomiting, dehydration, respiratory distress and muscular hypotonia. We herein reported: **Case 1**: 18-month-old infant with neonatal onset of severe metabolic acidosis, pancytopenia, increased anion gap, ketonuria, hyperammonemia and coma on day 18. Diagnosis of MMA was made via TMS and urine GC/MS. Treatment consisted of correction of metabolic acidosis, hydration, removal of toxic metabolites vit B_{12} and L-carnitine. She subsequently developed multiple episodes of severe metabolic acidosis responsive to aforementioned therapy. **Case 2**: 7-month-old boy with history of parental consanguinity, developed sepsis on day 6, responded well to antibiotics; later became lethargic also rapid breathing, vomiting and feedings refusal. At 6 months he developed pneumonia, lethargy and became comatose; subsequently referred to Siriraj Hospital where diagnosis was made. Quantitative plasma amino acid revealed elevation of glycine. TMS demonstrated significant elevation of propionylcarnitine. Urine organic acid analysis demonstrated increased excretion of methylmalonic acid and methylcitric acid. Treatment consisted of I-Valex-I formula, vit B_{12}, Shohl's solution and L-carnitine. **Case 3**: 8-day-old female newborn with intractable seizure, severe metabolic acidosis and coma; clinical brain death was noted on arrival and expired on day 10. Urine organic acid analysis demonstrated increased excretion of lactate, 3-OH-propionate, 2-OH-isovalerate and methylmalonate.

P-3-27

ISOVALERIC ACIDEMIA IN THAI INFANTS: A REPORT OF 5 CASES

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Isovaleric acidemia is a rare inborn error of organic acid metabolism due to deficiency of isovaleryl CoA dehydrogenase, which catalyzes the conversion of isovaleric acid to 3-methylcrotonic aicd in the leucine degradative pathway. It is inherited as an autosomal recessive trait. There are 2 clinical forms: acute neonatal form and chronic intermittent form. We reported 5 cases of isovaleric acidemias: **Case 1: Acute neonatal form** 6-week-old boy developed poor feeding since day 8; on respiratory support; hyperammonemia, pancytopenia, hypotonia, and increased anion gap observed; characteristic sweaty-feet odor; dried blood spot (TMS) and urine GC/MS confirmed diagnosis of IVA. **Case 2: Chronic intermittent form** 1-year-3 month-old male with intractable neonatal seizures, hypoglycemia, delayed growth/development; persistent severe metabolic acidosis, pancytopenia, generalized severe brain atrophy, bilateral neurisensoral hearing loss, bilateral optic atrophy;dried blood spots (TMS) demonstrated elevation of isovalerylcarnitine. **Case 3: Chronic intermittent form** 1-year-9 month-old male with intractable seizures, microcephaly, hepatomegaly, marked spastic quadriplegia; status epilepticus; subsequently expired and dried blood spot (TMS) demonstrated elevation of isovaleryl-carnitine. **Case 4: Acute neonatal form** 11-month-old girl who developed lethargy and feedings refusal since day 10; doing well until age 9 months when delayed development; pancytopenia, increased anion gap, elevated plasma glycine and increased excretion of urine isovalerylglycine demonstrated. **Case 5: Acute neonatal form** 2-month-old boy with poor feeding, unusual odor, hypothermia and lethargy since day 3; later developed acrodermatitis enteropathica. Urine organic acid analysis demonstrated increased isovalerylglycine, 4-OH-phenyllactate and 4-OH phenylpyruvate.

P-3-28

METHYLMALONYL-CoA MUTASE GENE MUTATIONS IN JAPANESE PATIENTS WITH METHYLMALONIC ACIDEMIA

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Methylmalonic acidemia (MMA) is an autosomal recessive inherited inborn error of propionic acid metabolism, due to the impaired isomerization of L-methylmalonyl-CoA to succinyl-CoA, including heterogenous conditions, defect of the methylmalonyl-CoA mutase (*MCM* gene) and abnormal production of the cofactor, 5′-deoxyadenoxylcobalamin (adocbl). To characterize the molecular lesions in Japanese MMA patients, we analyzed the *MCM* gene in 4 Japanese patients with MMA. All 4 patients were enzymatically diagnosed with complete mutase deficiency (mut^0). Six mutations (c.167C>T, c.509G>A, c.758C>T, c.1352G>A, c.2174-2175delAT, c.2179C>T) were identified in the *MCM* gene. All seven mutations were novel or private, suggesting that common mutations are not responsible for Japanese MMA patients. The patient with a homozygous c.2179C>T (p.R727X) showed relatively mild presentation during infancy and diagnosed at 11 month of age. The c.2179 mutation causes the truncation of 24 amino acid residues of the C-terminus in MCM apoenzyme. The C-terminus of this enzyme has β/α flavodoxin-like domain responsible for interacting with the lower face of cobalamin and its dimethylbenzimidazole bottom ligand. In this C-terminus, the core is formed by five parallel β strands (IIβ1-IIβ5) that alternate with α helices (Iiα1-Iiα5). The c.2179 mutation causes the loss of last α helices (IIα5) in the MCM apoenzyme, leading to the mut^0 biochemical phenotype.

P-3-29

FUMARATE HYDRATASE DEFICIENCY – A RARE CAUSE OF DEVELOMENTAL DELAY AND SEIZURES

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Fumarate hydratase deficiency is a rare disorder, described in only 32 cases. The clinical presentation is a severe psychomotor retardation, facial dysmorphism and severe brain malformations. Our patient is a girl born after an uncomplicated pregnancy of unrelated parents. She started to have focal seizures with secondary generalisation at the age of 9 months. She sat at 10 months and crawled at 15months. At the age of 29 months physical and neurological examination shows a normocephalic child who does not speak and stands with aid. She has a moderate developmental delay and seizures are controlled with anti-epileptic drugs. An MRI of the brain did not reveal any abnormalities. Caryotype was normal. On metabolic screen an increased excretion of fumaric acid was found. Activity of fumarate hydratase in cultured fibroblasts was decreased to 10% of low border of reference range. Two mutations were founding in the fumarase hydratase gene, one 1078 C to T resulting in a change of a histidine into tyrosine, and one in intron 9 c (A to G) creating new 5′ exon recognition site. Organic acid analysis in children with developmental delay and seizures can lead to a rare inborn error of metabolism.

P-3-30

NEW TECHNOLOGY CHANGES THE SHORT RANGE OUTCOME AND GENETIC COUNSELING ADVICE PROVIDED TO FAMILIES OF NEWBORNS AFFECTED WITH INBORN ERRORS OF COBALAMIN

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Background: There are 10 different inherited defects in the cobalamin pathway which frequently go unrecognized. A high degree of suspicion is usually necessary to pursue correct investigations. **Objective**: We wish to compare clinical outcome of three infants diagnosed with cobalamin C deficiency. **Methods**: Our patients had full metabolic and hematologic work-ups. Biochemical, complementation and molecular genetic studies were used. **Results**: Infant 1 was diagnosed at 6 months and had a complicated prenatal and postnatal course. Diagnosis was confirmed through complementation studies on skin cultured fibroblasts. The only method of prenatal detection was biochemical analysis of amniotic fluid and complimentation studies of amniocytes. This family terminated two affected pregnancies and then delivered a healthy baby. Infant 2 was diagnosed in the hospital at 10 weeks. He had a stormy first year and spent much time in the hospital. This family has not pursued another pregnancy since amniocentesis was their only option and they wouldn't terminate an affected pregnancy. Infant 3 was detected through newborn screening. At 8 months of age he has never required rehospitalization. We were able to confirm this infants diagnosis not only with complementation studies but also with DNA mutation analysis. This meant that in addition to amniocentesis, this family would also have the option of preimplantation genetic diagnosis. **Conclusion**: Expanded newborn screening provided this infant with a stable first year of life. The newly identified gene mutations allowed this family an alternate option of prenatal diagnosis.

P-4-1

NOVEL MAPLE SYRUP URINE DISEASE MUTATIONS IN CYPRIOT FAMILIES

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Maple syrup urine disease (MSUD) is a severe inborn error in branched-chain amino acid metabolism caused by mutations in any of the four genes encoding the three catalytic components of mitochondrial branched-chain α-ketoacid dehydrogenase complex (BCKDC). In this study, we investigated mutations responsible for the first three cases of MSUD among Cypriots from two unrelated families. These patients presented with symptoms within the first two weeks of life consistent with the severe, classic phenotype of MSUD. Western blot analysis of cell lysates showed that the Elα subunit was lacking in the first affected child and present in reduced amount in his parents. This patient was found to be homozygous for a type IA MSUD mutation (MIM no. 248600), a G-to-C transversion at a 3′-splice acceptor site of the Elα gene (IVS5-1G-to-C), which resulted in the deletion of the entire exon 6. Since no tissue was available from the other two affected children of the second family, cell lines from the parents were studied. The mother was found to harbor a novel type IB mutation (MIM 248611), a two-base deletion in exon 6 of the Elβ gene (709_710delCC). The father carried a different novel type IB mutation (IVS3[+3]delA) in the E1β gene, which resulted in the skipping of exon 3. The identification of these MSUD mutations allowed us to carry out carrier testing and six prenatal diagnoses for the two Cypriot families.

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P-4-2

PSYCHOSOCIAL AND BEHAVIORAL ISSUES IN FAMILIES AFFECTED BY MAPLE SYRUP URINE DISEASE

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The purpose of our study was to identify, delineate, and assess the behavioral and psychosocial issues faced by families affected by maple syrup urine disease (MSUD). This study is of clinical importance, as parents and caregivers of children with MSUD have expressed concerns about psychosocial and behavioral issues that affect their chronically ill child and impact other family members. Participants were recruited from the MSUD Support Group (USA) and included 52 families with children ranging in age from 5 to 18 years. Assessment measures included a Family Survey (FS), the Pediatric Quality of Life Inventory (PedsQL) and the Behavior Assessment System for Children (BASC) Parent and Teacher Rating Scales. The main themes and areas of concern that emerged from parents' responses on the FS were: (1) finances and insurance; (2) coping with medical staff; (3) what it means to have a child with MSUD; and (4) the child's experiences in school. On the PedsQL, children reported higher quality of life than parents; however, mean totals of children and parents were both closer to an oncology sample than to a healthy sample. Most children fell within the average range on the clinical scales of the BASC parent rating scale; however, there were elevations in hyperactivity, aggression, and attention deficits. Teachers reported significantly more internalizing symptoms on the BASC as compared to parents. Our findings identify specific psychosocial and behavioral issues that must be addressed by health care professionals in any comprehensive treatment plan for MSUD.

P-4-3

A CASE OF LYSINURIC PROTEIN INTOLERANCE PRESENTING WITH HEPATOSPLENOMEGALY

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In children, hepatosplenomegaly is an uncommon clinical problem with a wide differential diagnosis; and inherited metabolic disorders account for a vast majority. We report a 18-year-old girl with hepatosplenomegaly noticed at birth and progressed thereafter. Lactase deficiency, congenital cataracta of right eye and osteoporosis were also observed. Episodes of drowsiness followed intake of high protein diet. Metabolic findings included slight hyperamonemia, high plasma Citr, Ala, Gly, Glu, Ser, Thr, Orn and citrulinuria, lysinuria, glutaminuria, alaninuria, argininuria, prolinuria, hydroxyprolinuria, ornithinuria, orotic aciduria. Aversion to high protein diet strongly suggested a possibility of disorder resulting in hyperammonemia, and diagnosis of citrulinemia was suspected. However, subsequently the diagnosis of lysinuric protein intolerance (LPI) was made on the basis of biochemical and clinical features. LPI is caused by mutations in the SLC7A7 gene. A homozygous A-to-G transition was detected in a splice acceptor sequence 6 (IVS6-2A>G) in the patient. Interestingly, the mutation is located in the same nucleotide position as the Finnish LPI founder mutation, a transversion of A-to-T (IVS6-2A>T). This supports the pathogenic role of the mutation in our patient, and makes the clinical diagnosis of LPI is highly likely. This would be the first reported LPI case in Lithuania.

P-4-4

MOLECULAR ANALYSIS OF JAPANESE PATIENTS WITH CYSTATHIONINE β-SYNTHASE DEFICIENCY

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Cystathionine beta-synthase (CBS) deficiency is the most common cause of homocystinuria. More than 130 pathogenic mutations have been described. We present a mutation analysis of thirteen Japanese patients including two siblings. Fourteen mutations were found. D35Q, W43X, G148R, S217F, H232D and R266G were all novel mutations. The most frequent mutation found in Caucasian patients, I278T, was detected in two unrelated patients. We concluded that the CBS mutations have a diverse spectrum and no predominant mutation is detected in Japanese CBS-deficient patients. Several mutant CBS enzymes were expressed in *Escherichia coli*. All of the mutants except K441X exhibited severely decreased activity, and the capability to form tetramers of most mutants was severely impaired. As previously hypothesized, the increased aggregation of mutant CBS subunits might be a common pathogenic mechanism in CBS deficiency.

P-4-5

THE OUTCOME OF MAPLE SYRUP URINE DISEASE: A 6-YEAR EXPERIENCE IN MALAYSIA

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Maple syrup urine disease (MSUD) is a disorder of branched chain amino acids (BCAA) metabolism. Over a period of 6 years (1999–2005), 42 patients were diagnosed as having MSUD in Malaysia with an estimated incidence of 1 in 70 000 live births. The diagnosis was confirmed by elevated BCAA and the detection of alloisoleucine in plasma amino acids, and urine organic acids showing elevated peaks of branched chain ketoacids. Consanguinity was seen in 16 patients (38%). Two third (27) were the classical neonatal form. Early diagnosis and crisis intervention were given to 19 (70%) patients and 17 (90%) survived. Dialysis was performed for 14 patients with 100% success rate. The other third presented later as recurrent encephalopathy, cyclical vomiting, mental retardation, spasticity, autism or episodic movement disorders. Three of these variant patients (20%) succumbed to crises due to delayed diagnosis. To date 27 surviving patients were under our follow-up. Their age ranged from 2 months to 22 years and follow-up period from 2 months to 6 years. Their neurological outcome varied from normal to severe retardation. Five (12%) had normal neurological status and 2 were the classical form. Eight patients (19%) had mild developmental delay. In early period of the service, 11 patients (26%) from distant hospitals passed away before diagnosis was made. This had improved with increased awareness among the doctors. Early diagnosis, aggressive crises intervention and long-term close BCAA monitoring had improved the outcome of these patients. Further improvement necessitates newborn screening for the population.

P-4-7

SIX CASES OF CITRIN DEFICIENCY AND NINE MAJOR MUTATION SCREENING IN NEWBORNS IN KOREA

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Citrin deficiency resulting from mutations of *SLC25A13* is associated with two major clinical phenotypes; neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) and adult-onset type 2 citrullinemia (CTLN2). In Korea, 6 cases of citrin deficiency have been diagnosed based on biochemical and molecular findings. Three NICCD cases were identified by newborn screening using MS/MS. They are all males, presenting with conjugated hyperbilirubinemia, elevated liver enzymes, hypoalbuminemia, mild hyperammonemia, elevated citrullin, methionine and threonine. All of them have been spontaneously recovered from hepatic manifestation by the age of 6–8 months. Mutation analysis has been performed using their genomic and cDNAs obtained from skin fibroblasts. They turned out to be compound heterozygotes carrying each of 851del4, IVS11+1G>A, and IVS13+1G>A. Three CTLN2 patients were identified. Two adult male patients presented with a sudden loss of consciousness, seizure, vomiting, hyperammonemia and citrullinemia in their twenties. They carried an IVS13+1G>A, 851del4, and IVS11+1G>A mutant alleles. The other CTLN2 patient was 52 year old female patient, manifesting lethargy, altered consciousness, irritability and hyperammonemia. Similar clinical symptoms had recurred at the delivery of first and second babies in her past medical history. She was managed by hemodialysis and survived with neurological sequellae. Also, we screened the presence of 9 common mutations in 500 Korean newborns using dried blood spot of filter papers. None of allele carried any of 9 mutations. In conclusion, the entire picture of citrin deficiency in Korea including incidence, genotype, clinical features and natural courses, is still vague at the present time.

P-4-8

VARIABLE RESPONSE OF PATIENTS WITH HEPATORENAL TYROSINEMIA TO NTBC

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NTBC has revolutionized the treatment of hepatorenal tyrosinemia. It has improved the survival rate and reduced the need for urgent liver transplantation. We have diagnosed 6 patients with hepatorenal tyrosinemia over the past 6 years and treated all of them with NTBC. All had a large peak of succinylacetone in the urine, moderate tyrosinemia, increased urinary δ-ALA, Fanconic tubulopathy and markedly elevated α-fetoprotein. Two patients presented acutely as fulminant liver failure in the neonatal period. They did not respond to 1 mg/kg/day of NTBC and succumbed to the disease. The other 4 patients presented in later infancy or childhood with hepato-splenomegaly, failure to thrive, renal tubular acidosis and rickets beside chronic liver impairment. Renal tubulopathy and rickets were normalized by NTBC treatment. All had catch-up growth and liver function greatly improved with normalization of laboratory parameters. One patient had dysplastic changes in liver histopathology. He improved dramatically with 1 m/kg/day of NTBC and remained well after 3 years. Two patients suffered from porphyric crises with temporary unavailability of NTBC and recovered with restarting of treatment. One of them had a liver transplant done 2 years ago and remained well thus far. Our limited experience suggested that patients with acute neonatal form responded poorly to NTBC but those with chronic diseases responded well. Liver transplantation offered an alternative to these patients.

P-4-9

FOLLOW UP IN 27 CHILEAN PATIENTS WITH CLASSICAL MAPLE SYRUP URINE DISEASE

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Maple syrup urine disease (MSUD) is produced by a defect in the dehydrogenase enzyme complex of ketoacids of valine, isoleucine and leucine (VIL). The accumulation of leucine and (-ketoisocaproic acid are neurotoxic. Nutritional therapy consists in a leucine restricted diet, with special formula without VIL, supplementations of L- valine, isoleucine and thiamine. **Aim**: to evaluate the growth and development in MSUD patients in follow up at INTA. **Patients and methods**: 31 children with classical MSUD were studied. Protein, VIL aminoacids intakes were quantified. Blood leucine, nutritional status and psychomotor development were also measured. **Results**: Median age at diagnosis was 12 days and leucine levels of 1714 ± 893 μM/l, with alloisoleucine were measured. Five children were diagnosed before 5 days of life and 26 through clinical symptoms. 55% of the cases had no metabolic derangements during follow up and this was associated with diagnosis before 10 days of life. With regards to cognitive development, 33% show normal developmental and 77% have mental retardation. Analyzing the diet, it was observed that 66% of protein come from the special formula without VIL and 34% from natural foods. Every child receives supplementation with L-valine, L-isoleucine and thiamine. 67% have normal nutritional status and 33% is in nutritional risk due to deficiency or excess. **Conclusions**: the growth and development in MSUD children is related with the age at diagnosis and the metabolic status through follow up during life.

P-4-10

TYROSINEMIA TYPE 1, FOLLOW UP OF 8 PATIENTS TREATED WITH PHENYLALANINE (Phe) AND TYROSINE (Tyr) RESTRICTED DIET AND NTBC

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Tyrosinemia Type 1 (Tyr 1) is autosomal recessive inherited and is due to the deficiency of the enzyme fumarylacetoacetate hydrolase (FAH). Symptoms include acute liver failure, cirrhosis, hepatocellular carcinoma, Fanconi Syndrome and attacks of peripheral neuropathy. Diagnosis is confirmed finding hypertyrosinemia and high succinylacetone (SA). The prognosis with dietary treatment with restriction of Phe and Tyr is poor. Liver transplantation cures the disease. At present the drug (2(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione) NTBC, improves the hepatic and renal functions. **Method**: Eight patients with tyrosinemia Type 1 and treated with Phe and Tyr restriction and NTBC are presented. Five patients presented with hepatic failure, 2 before 2 months of age, 2 at 7 months of age and 1 at 17 months of age. Three patients had a chronic symptoms with rickets and Fanconi Syndrome. The diagnosis was confirmed with average SA 442 mM/L (normal value <0.1) and Tyr 333 mM/L (normal value 12–108). Treatment consists in Phe and Tyr restricted diet (1057 mg/day), protein 1.8 gr/kg/day and NTBC 1–2 mg/kg/day. Follow up has been in average 38 months. Five patients have normal hepatic function, one patient with a chronic form developed hepatocellular carcinoma and died, 1 patient was transplanted and 1 died 1 month after starting treatment. **Conclusion**: Treatment with Phe and Tyr restriction and NTBC is effective specially when initiated before 2 months of age.

P-4-11

THE MEASUREMENT OF SUCCINYLACETONE FOR THE DIAGNOSIS OF TYROSINEMIA TYPE I BY FLOW INJECTION MASS SPECTROMETRY

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Succinylacetone (SA) is a diagnostic metabolite for hepatorenal tyrosinemia type 1 (HT1). This disease presents with either an acute phenotype (severe, early onset and premature death) or a chronic phenotype (later onset and less severe). Diagnosis of the chronic form is normally by measurement of increased SA in plasma or urine. A direct infusion electrospray tandem mass spectrometry (ESI-MS/MS) method for quantifying SA in urine has been developed.

This method involves selective solvent extraction of urine (250 µL), derivatization with Girard T reagent and isotope dilution quantitation using synthesized d5-SA as an internal standard. A calibration curve of SA in urine demonstrated linearity up to 50 µmol/L. Precision was determined at two levels of SA, low (0.17 µmol/L) and high (7.4 µmol/L). Intra-assay CV (n = 6): 11.6% (low) and 1.8% (high). Inter-assay CV (n = 6): 12.9% (low) and 6.6% (high). Urine samples from normal children (2 weeks to 16 years, n = 20) showed a SA range of 0.095 to 0.44 µmol/L and 0.013 to 0.269 µmol/mmol creatinine. SA levels in urine and dried urine samples on filter paper of HT1 children, both presenting and on treatment will be presented.

This method is simpler, faster and more sensitive than published LC-ESI-MS/MS and GC/MS methods. Further simplification, with the 96 well format, will allow for the screening of SA in dried blood spots.

P-4-12

LYSINURIC PROTEIN INTOLERANCE: CLINICAL AND BIOCHEMICAL PROFILE IN 4 TUNISIAN CHILDREN

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Lysinuric protein intolerance (LPI) is a rare recessive autosomal defect of cationic amino acids transport (lysine, ornithine and arginine) caused by mutations of the SLC7A7 gene. We present the clinical findings and the biochemical results as well as the therapeutic evolution in Tunisian children with LPI. The goal is a better understanding of this pathology and for establishing of precocious diagnosis to allow effective therapy. Four children (3 girls and 1 boy, mean age at diagnosis 20 ± 5.59 months), were born to two consanguineous families. All patients were investigated for ammonemia measure, plasmatic and urinary amino acids (Ion Exchange Chromatography amino acids analyser), urinary organic acids profile and orotic acid quantification by GC-MS. Patients clinical features were dominated by intestinal protein intolerance and failure to thrive. Hyperammonemia varied from 73 to 474 µmol/l. Urinary excretion of lysine, ornithine and arginine was massively increased, (by 1879, 107.64 and 101 µmol/l, respectively), whereas plasmatic concentration was subnormal. In addition, the urinary orotic acid was augmented (ranged from 22.1 to 920 µmol/mmol creatinine). Protein restricted and L-citrulline supplementation was immediately started for these children. It was permitted to avoid ammonemia coma, improve progressive encephalopathy however does not correct lysine deficiency. LPI is generally under diagnosed in Tunisia. A better understanding of its clinical aspects shall enable an early diagnosis and a more efficiency therapy.

P-4-13

A NEW PROTEIN SUBSTITUTE FOR TYROSINAEMIA

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There are few protein substitutes (PS) for older people with Tyrosinaemia Type 1, 2, and 3. A new PS (*Tyr Express*: Vitaflo International) is available containing per 100 g: 60 g protein equivalent; 15 g carbohydrate, and added vitamins and minerals. **Aim**: An open, observational trial to assess the acceptability and efficacy of *Tyr Express* in a group of older children with Tyrosinaemia. **Methods**: 6 children (2 male; 4 female), median age 10.3 years (range 8–13 years), with Tyrosinaemia type 1 (n = 2), type 2 (n = 1), and type 3 (n = 3) were transferred to *Tyr Express* for 8 weeks (previous PS Tyr *Gel* (42 g/100 g protein equivalent). They had weekly fasting plasma tyrosine (tyr) and phenylalanine (phe) estimations; anthropometry at the study start and end; and they rated their impression of the *Tyr Express.* **Results**: The daily volume of PS intake was decreased (median daily intake of *Tyr Express* was 75 g (range 50–100 g); *Tyr Gel* 100 g/daily (60–160 g). The mean plasma tyr improved (on *Tyr Express:* mean tyrosine 305 µmol/l and pre *Tyr Express:* 345 µmol/l) and the mean plasma phe was unchanged (on *Tyr Express:* 72 µmol/l; pre *Tyr Express:* 79 µmol/l). Growth was satisfactory. All subjects tolerated the PS and stated the lower volume and flavour modules helped with their compliance. Conclusion: The new PS was well tolerated, convenient, aided compliance and did not adversely affect metabolic control.

P-4-14

GENOMIC DELETION WITHIN *GLDC* IS A MAJOR CAUSE OF NONKETOTIC HYPERGLYCINEMIA: SCREENING OF 65 PATIENTS BY MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION

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Objective: Nonketotic hyperglycinemia (NKH) is an inborn error of metabolism characterized by accumulation of glycine in body fluids and various neurological symptoms. NKH is caused by deficiency of the glycine cleavage multi-enzyme system with three specific components encoded by *GLDC*, *AMT*, and *GCSH*. The majority of patients are deficient of enzymatic activity of glycine decarboxylase, which is encoded by *GLDC*. Our recent study has suggested that there are a considerable number of *GLDC* mutations, which are not identified by the standard exon-sequencing method (Kure et al., Hum Mutat 2006;27:343–52). To improve the mutation detection rate we have developed a screening system for *GLDC* deletions by multiplex ligation-dependent probe amplification (MLPA). **Methods**: Two distinct cohorts of patients with typical NKH were screened by this MLPA method: the first cohort consisted of 45 families with no identified *AMT* or *GCSH* mutations and the second cohort was comprised of 20 patients from the UK who were not prescreened for *AMT* mutations. **Results**: *GLDC* deletions were identified in 16 of 90 alleles (18%) in the first cohort and 9 of 40 alleles (22.5%) in the second cohort. Fourteen different types of deletions of various lengths were identified, including one allele where all 25 exons were missing. We determined flanking sequences of interstitial deletions in five patients, and *Alu*-mediated recombination was identified in 3 of the 5 patients. **Conclusion**: *GLDC* deletions are a significant cause of NKH and that MLPA analysis is a valuable first-line screen for NKH genetic testing.

P-4-15

HUMAN MITOCHONDRIAL BRANCHED-CHAIN AMINO ACID AMINOTRANSFERASE DEFICIENCY

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The first step of human branched-chain amino acid (BCAA) catabolism involves transamination to keto-acids (BCKA). This step is catalysed by pyridoxine-dependent cytosolic and mitochondrial branched-chain aminotransferases encoded by the genes BCAT1 and BCAT2, respectively. We describe a female with a defect in BCAT2 (EC 2.6.1.42). She was first investigated at 14 years of age for a 2-year history of visual and auditory hallucinations, difficulties in micturition, midline frontal alopecia, CNS white matter changes and abnormal EEG but no seizures. Plasma BCAA were significantly increased with valine ranging from 719 to 2280 μmol/L (normal <285), leucine: 318 to 797 μmol/L (<169) and isoleucine: 234 to 725 μmol/L (<93). Remarkably, *allo*-isoleucine levels were only marginally increased: 3 to 7 μmol/L (<5), while BCKA and 2-hydroxy acids were normal. This metabolite pattern sharply contrasted with that seen in maple syrup urine disease and strongly suggested a deficiency of BCAT. Consistent with this, decreased leucine transamination (6% of controls) and decarboxylation (17% of controls) were found in intact skin fibroblasts. BCAT2 activity, measured directly in cell free extracts, was 37 nmol/h/mg cell protein versus a mean of 242 in controls ($n = 6$). Exon-sequencing of the BCAT2 gene revealed two changes: Q100E (c.298C>G) and T186R (c.594C>G). The latter, however, was found to be a polymorphism present in 9 alleles of 18 investigated random genotypes. A short trial of pyridoxine did not result in any clinical or biochemical improvement. Taken together, our patient appears to be the first with a proven BCAT2 defect in humans.

P-4-16

HOMOCYSTINURIA IN 2 THAI SIBLINGS: FIRST REPORTED CASES

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Homocystinuria (OMIM236200) is a disorder of transfuration. It is characterized by increased plasma homocystine and methionine and decreased plasma cystine. The cardinal manifestations usually occur in ocular, skeletal, cardiovascular and central nervous systems. Mental retardation, seizures, strokes, thromboembolism and early atherosclerosis, dislocated lenses, skeletal deformity are common features. The treatment of homocystinuria which is not responsive to pyridoxine usually lead to vascular, ocular and skeletal complications. We herein report 2 siblings with homocystinuria. **Case 1**: Nine-year-old girl with lens dislocation of the right eye, tall stature, osteoporosis, mental retardation, Ig A nephropathy and minimal thickening of common carotid artery. Elevated urinary homocysteine and marked elevation of plasma methionine and homocysteine were decmonstraled. **Case 2**: Six-year-old girl; younger sister (with history of parental consanguinity); lens dislocation was observed. Elevated urinary homocysteine and marked elevation of plasma methionine and homocysteine were also demonstrated. Both were treated with B6 and Betaine with poor response. Mutation analysis will be presented.

P-4-17

RAPID DIAGNOSIS OF GLYCINE ENCEPHALOPATHY BY ^{13}C-GLYCINE BREATH TEST

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Glycine encephalopathy (GE), also known as non-ketotic hyperglycinemia, is a life-threatening disease caused by inherited deficiency of the glycine cleavage system (GCS). Confirmation of diagnosis is currently problematic, requiring either invasive liver biopsy for measurement of GCS activity or exhaustive mutational screening of three GCS genes. The purpose of this study is to develop a rapid and widely available diagnostic test for early diagnosis and appropriate management. The GCS generates CO_2 by decarboxylation of glycine, suggesting that GCS activity could be functionally evaluated *in vivo* by analyzing exhaled $^{13}CO_2$ after administration of ^{13}C-glycine (Kure et al., Rapid diagnosis of glycine encephalopathy by ^{13}C-glycine breath test. Ann Neurol, 2006 in press). [1-^{13}C]glycine breath test was performed in ten control subjects and five GE patients including one with mild GE, in all of whom the diagnosis had been confirmed by identification of GLDC mutations. All the patients showed significantly lower cumulative ^{13}C recovery at 5 h than the control subjects ($p<0.0001$). Not only typical GE but also atypical GE can be reliably diagnosed by the ^{13}C-glycine breath test. Because it is rapid, non-invasive, and requires little expertise, the breath test could be useful as a standard test for diagnosing GE.

P-4-18

LONG TERM FOLLOW UP OF EIGHT PATIENTS AFFECTED OF TYROSINEMIA TYPE I ENROLLED IN THE INTERNATIONAL NTBC TRIALS, TREATED WITH NTBC AND TYROSINE RESTRICTED DIET

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This paper presented a long term follow up data of 8 Iranian patients with tyrosinemia type 1 treated with NTBC and tyrosine restricted diet. Age at diagnosis ranged from 17 days to 3 years old. Age at initiating treatment ranged from 5.5 to 44 months old. Follow up periods ranged from 2 to 3 years. Family history of hepatic failure was recognized in five families with 11 death in siblings. Convulsion due to hypoglycemia, prolonged hyperbilirubinemia, psychomotor, clotting abnormality and respiratory distress were observed in 2, 3, 2, 5 and 6 cases as the first clinical symptoms, respectively. NTBC dosage ranged from 0.9 to 1.2 mg/kg. Succinylacetone in plasma and urine, and 5 delta aminolevulinic acid in urine became normal in all after treatment. Alpha fetoprotein elevation and echographic abnormality of liver were not found. No patients has shown hepatocarcinoma so far. NTBC treatment as well as tyrosine restricted diet has been well tolerated. All 8 patients showed the significant improvement with NTBC treatment without major side effects.

P-4-19

DIET THERAPY AND LONG-TERM OUTCOME OF SEVEN PATIENTS WITH MSUD

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The aim of diet treatment in maple syrup urine disease (MSUD) is to decrease branched chain amino acids and ketoacids concentration. Seven patients (in it 5 with classical phenotype) were evaluated for diet compliance and efficacy by assessment of food intake, biochemical scores and psycho-motor development in long-term observation (mean 13.5 years). Energy value was in recommended range, as well as protein amount, though too low from protein substitutes (mainly in the beginning and the end of infancy). In four children mean fat intake was below 90%, and in six – mean carbohydrates intake was over 90% of RDI. Diet records with adequate leucine content was 89–34%. In all children but one (at least in one period) serum leucine concentration was more than 700 μmol/L. The lowest leucine concentrations were noticed in the whole group at age 3–6 months of life. Patients' body weight was ± 2 SDS during observation time. Three children exhibited slower growing rate in infancy, but final growth in all of them was within ± 1 SDS. Psychological evaluation expressed by IQ measurement showed mental retardation in four patients (twos with marked and mild retardation). In conclusion: diet compliance for energy and nutrients was satisfactory in majority of patients, leucine restriction in the diet was insufficient, early diagnosis and systematic biochemical control with parallel diet evaluation are crucial for good prognosis at the patients with MSUD.

P-4-20

LONG TERM FOLLOW UP OF BETAINE THERAPY IN TWO JAPANESE HOMOCYSTINURIA SIBLINGS

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We present two Japanese siblings homocystinuria due to cystathionine β-synthase deficiency (CBS) who are treated with betaine for 13 more than years. They were pointed out hypermethioninemia by mass screening of newborns. They are compound heterozygote for H232D and G259S and classified as vitamin B_6 non-responsive type. **Case 1**: The patient is 18 years old girl. Her CBS activity in peripheral blood was 2.0% of control group. At the age of 3 months, dietary therapy with methionine free formula was started. At the age of 22 months, her serum methionine levels increased and we started betaine therapy. But after that her mother moderated methionine-restricted diet. Her serum total homocysteine levels increased to 192.6 μM without the dietary therapy. **Case 2**: The patient is 13 years old girl (the younger sister of case 1). She was also hypermethioninemia at birth and used methionine free formula immediately. At the age of 8 months, the betaine therapy was started. She was good response to the betaine therapy at the beginning. But her compliance with the dietary therapy was also poor. Her serum total homocysteine levels increased to 269.6 μM. During this state with only betaine therapy, clinical complications did not occur. Their IQ were remained on average until their school age, but their IQ decreases to 86 and 73 at the present time. We suspect that the betaine therapy made them to be off their guard for the dietary therapy. More strict patient education should be considered.

P-4-21

NONKETOTIC HYPERGLYCINEMIA IN THAI INFANTS: REPORT OF 5 CASES

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Nonketotic hyperglycinemia (NKH) (OMIM 605899) is a disorder of mitochondrial glycine cleavage system causing glycine encephalopathy (GCE). It is inherited as an autosomal recessive trait. Gene locus is mapped on 16q24, 9p22, 3p21.2-p21.1 Most patients presenting in neonatal period with lethargy, hypotonia and myoclonic jerks and progressing to apnea and often to death. In the infantile form, patients presents with seizures and mental retardation. Glycine is elevated in serum and urine. No effective treatment exists (OMIM-VAMcKusick).

We herein report 5 cases of NKH: **Case 1**: An 8-month-old girl (born in 1998) with delayed development since age 3 months, recurrent vomiting, mild metabolic acidosis, urine OA/TMS-normal, persistent elevation of urine glycine and elevated CSF/plasma glycine ratio noted. **Case 2**: A 15-day-old male infant (born in 2000) with intractable seizure since age 9 h, elevated CSF/plasma glycine ratio observed, not responsive to L-carnitine, sodium benzoate, anticonvulsants. **Case 3**: An 8 year-11 month-old girl (born in 1992), older sibling of Case 2, developed seizure since day 1, require ventilatory support, cerebral atrophy, elevated CSF/plasma glycine ratio. **Case 4**: A 6-day-old female (born in 2004) became inactive/poor feeding at 20 h, developed neonatal seizure on day 2, apnea, on ventilatory support, cerebral atrophy noted. **Case 5**: A 4-day-old female (born in 2004) with neonatal seizure, apnea, hypotonia, on ventilatory support, feeding refusal and stupor, diffuse brain edema, not responded to treatment. Enzyme assay and mutation analysis are not available in Thailand.

P-4-22

REPORT OF 11 CASES OF CLASSIC MAPLE SYRUP URINE DISEASE IN THAI INFANTS: CLINICAL AND BIOCHEMICAL DATA

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Maple syrup urine disease (MSUD) (OMIM 248600) or branched chain ketoaciduria is caused by a deficiency in activity of the branched-chain (α-keto acid dehydrogenase (BCKD) complex, resulting in the accumulation of the branched-chain amino acids (BCAAs) leucine, isoleucine and valine and the corresponding branched-chain (α-keto acids (BCKAs). There are 5 phenotypes : classic, intermediate, intermittent, thiamine-responsive and dihydrolipoyl dehydrogenase (E3)-deficient. Classic MSUD has a neonatal onset of encephalopathy and is the most severe and most common form (Chuang DT, Shih VE from Scriver, Beaudet, Valle et al., 2001).We herein report 11 cases of classic MSUD in Thai infants: age of onset varying from 3–12 days; age of referral/diagnosis varying from 11–173 days/13–175 days; clinical symptoms were lethargy, coma, hypotonia, convulsion, spasticity, apnea; high level of leucine varying from 1449.28 to 10 778 nmol/ml plasma and outcome was profound mental retardation, denial of treatment and death. Enzyme assay and mutation analysis are not available in Thailand.

P-4-23

NEONATAL INTRAHEPATIC CHOLESTASIS CAUSED BY CITRIN DEFICIENCY (NICCD) IN A EUROPEAN PATIENT

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Neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) is a rare but potentially progressive disorder which can lead to liver failure and neuropsychiatric illness in later life. We describe the first case with NICCD in Europe. A male infant of non-consanguineous Caucasian parents was born after a normal pregnancy at term. He failed to thrive with persistent conjugated hyperbilirubinaemia from 2 days and at the age of 8 weeks was investigated for causes of neonatal cholestasis. Routine investigations confirmed liver dysfunction. Abnormal plasma amino acids (threonine 692 μmol/l, citrulline 512 μmol/l, methionine 139 μmol/l, lysine 280 μmol/l, arginine 166 μmol/l), urine amino acids (citrulline 213 μmol/mmol creatinine) and galactosuria suggested the possibility of citrin deficiency. A liver biopsy showed micro vesicular fatty changes. Western analysis of fibroblasts revealed citrin protein at 16–30% of normal. DNA analysis revealed he was heterozygous for a novel C489R mutation in the *SLC25A13* gene. mRNA analysis suggested a large deletion was present on the second allele. Further work is in progress to search for the second mutation.

All clinical symptoms and biochemical abnormalities resolved spontaneously by 1 year of age. The child is well at $2\frac{1}{2}$ years but remains at risk of developing clinical symptoms of citrullinaemia type II later in life. NICCD is a new differential diagnosis for cholestatic infants in Europe and early diagnosis may prevent subsequent neuropsychiatric complications by elective liver transplantation.

P-4-24

SCHIZENCEPHALY IN A PATIENT WITH NONKETOTIC HYPERGLYCINEMA

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We describe a first child of consanguineous Turkish parents, who became increasingly hypotonic on the second day of life. On the third day progressive lethargy, myoclonic jerks, generalized convulsions and repiratory insufficiency developed. Aminoacid analysis showed highly elevated concentrations of glycine in plasma (1480 μmol/L), CSF (183 μmol/L) and urine (593 750 μmol/L); the CSF/plasma glycine ratio was markedly elevated (0.12; RV: 0.04–0.08). Organic acid analysis in urine and CSF showed no abnormalities. On EEG a burst-suppression pattern was seen. CT-scan and MRI revealed agenesis of the corpus callosum and schizencephaly. The diagnosis of nonketotic hyperglycinemia (NKH) was confirmed by measurement of glycine cleavage enzyme activity in liver tissue which showed no residual activity and by molecular genetic analysis: the patient was homozygous for a nonsense mutation (c.2963G>A;R988Q) in the GLDC gene. An attempt of treatment with sodium benzoate and N-methyl-D-aspartate receptor antagonists failed, probably partly due to the severe structural brain deformities.

Schizencephaly is a rare severe defect of cell migration causing a full-thickness cleft within the cerebral hemispheres. Gyral malformations are already described by several authors in patients with NKH but to our knowledge schizencephaly, a severe congenital brain malformation disorder, has never been reported before in this metabolic disease.

P-5-1

IDENTIFICATION OF NOVEL MUTATIONS OF THE *HADHA* AND *HADHB* GENES IN PATIENTS WITH MITOCHONDRIAL TRIFUNCTIONAL PROTEIN DEFICIENCY

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Patients with long-chain 3-hydroxyacyl coenzyme A dehydrogenase (LCHAD) deficiency manifest with hypoketotic hypoglycemia, hepatomegaly, hypotonia, lactic acidemia, acute renal failure, cardiomyopathy, and sudden death. We describe four novel mutations of the α- and β-subunits of the mitochondrial trifunctional protein (MTP) in four patients from three unrelated families. Their plasma acylcarnitine profiles suggested the presence of LCHAD deficiency by demonstrating highly elevated 3-hydroxyacyl carnitines by tandem mass spectrometry. Patients 1 and 2 had siblings who died of lactic acidemia during neonatal periods. Patient 3 had a family history of Reye-like syndrome. She presented with acute renal failure, rhabdomyolysis, pericardial effusion, and myopathy at the age of 12 years. DNA analysis of patients 1 and 2 revealed homozygosity for a c.1689 +2T>G mutation of the *HADHA* gene, resulting in the skipping of exon 16 with an in-frame 69-bp deletion. Patients 1 and 2 have no protein activity for either α- or β-subunits of the MTP in the enzyme analysis using skin fibroblasts. Patient 3 was a compound heterozygosity of the *HADHB* gene, N307D/N389D. Patient 4, 25-month-old baby, presented with recurrent episodes of lethargy, metabolic acidosis, elevated liver enzymes, and dark urine since 10 months of age. Mutation analysis of the *HADHB* gene of patient 4 identified compound heterozygosity of N114D/N307D.

P-5-2

LONG-CHAIN TRIGLYCERIDE TOLERANCE IN VLCAD DEFICIENCY

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Dietary therapy of VLCAD deficient patients is primarily aimed at the avoidance of extended fast. Secondly, fat restriction is advised to avoid accumulation of toxic long-chain acylcarnitines. Medium-chain triglycerides are used in the diet to provide enough energy. **Objective**: To study long-chain triglyceride (LCT) tolerance in a 3.5-year-old boy with early-onset VLCAD deficiency (presenting in the neonatal period with hypoketotic hypoglycemia, with no signs of cardiomyopathy; confirmed by enzyme assay and mutation analysis showing compound heterozygosity for 272C>A and 577G>C; and recovering well on a diet of frequent feedings and LCT restriction) in a home controlled setting. **Methods**: Following a weekly based step-plan, LCT intake was extended using a non-variable diet from 1 to 1.5 g/kg over a period of two weeks. The latter regimen was maintained over the subsequent two weeks. Week 5, dietary variation was introduced. Follow-up during 3 months was performed clinically, and by monitoring of creatine kinase (CK) levels in plasma and acylcarnitine profiles in dried blood spots. **Results**: The increased LCT intake was well tolerated clinically. Plasma CK levels remained between the reference values. Acylcarnitine profiles in dried blood spots did not change and were hardly indicative for VLCAD. **Conclusion**: LCT tolerance can be higher in VLCAD deficiency than currently assumed. Further steps will be undertaken in this patient to confirm these findings.

P-5-4

PLASMA ACYLCARNITINES PROFILES IN 12 PATIENTS AFFECTED WITH CPT II DEFECT

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Introduction: Carnitine palmitoyl transferase II (CPT II) deficiency is an autosomal recessive disorder of carnitine dependant intracellular transport of long-chain fatty acids. The purpose of this study was to determine whether plasma acylcarnitines (AcC) profile could be used for late detection of mild enzymatic defects leading to a benign muscular form. **Methods**: AcC profiles were studied in plasma from 12 patients affected with CPT II deficiency whose age ranged from 20 to 50 years. All of them have reduced activity in leucocytes (<0.11 nmol/min/mg proteins, controls: 0.66–1.00). Assays were run using API 3000 (Applied BioSystem) tandem mass spectrometer with electrospray ionization (ESI) through FIA (flow injection analysis). Deuterated acylcarnitines were used as internal standards. Sample preparation consisted in methanol extraction of AcC without derivatization step. MRM mode was run for quantification and precursor ion m/z 85 scan for AcC profiles identification of 45 different AcC species. **Results**: Concentration levels of long chain AcC as saturated and unsaturated species was greater as compared to normal values: C14: 012–0.67 μmol/l (N<0.10), C16: 0.6 to 4.4 μmol/l (N<0.27), C18: 0.19–0.90 μmol/l (N<0.010), C18:1: 0.65–6 μmol/l (N<0.42), C18:2: 0.25–3 μmol/l (N<0.27) and especially the high C16+C18/C8 ratio: 16–70 (reference range: 0.011–0.048) were suggestive of CPTII deficiency. Free carnitine ranged from 22 to 36 μmol/l. **Conclusion**: Diagnosis could be suggested by a plasma AcC profile that disclosed accumulation of long chain AcC especially C16 and C18:1 and could be used as a criteria for screening before enzymatic measurement.

P-5-5

SHORT CHAIN HYDROXYACYL CoA DEHYDROGENASE DEFICIENCY ASSOCIATED WITH HYPERINSULINISM IN A YOUNG CHILD

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Introduction: Short chain hydroxyacyl CoA dehydrogenase(SCHAD) is an autosomal recessive fatty acid oxidation disorder. Fatty acid oxidation plays a major role in energy production, especially during period of fasting. Therapy of fatty acid oxidation disorders includes avoidance of fasting and treatment of acute hypoglycemia with intravenous glucose. Hyperinsulinism presents with non-ketotic hypoglycemia. If it co-exists with fatty acid oxidation defect, hyperinsulinism makes the dietary management more difficult. **Case Report**: We report on a 3-year old female presented with developmental delay and seizure at the age of 9 month. She was born at 38 weeks gestation. She had no neonatal problem. Clinical examination was unremarkable. Urine for organic acid showed persistently elevated 3 hydroxyglutarate and 3 hydroxymethylgluconate. A fasting study showed inappropriately high insulin (37 Mu/l) and high C peptide (675 pmol/l) with hypoglycemia (blood glucose 1.75 mmol/l). Serum cortisol, growth hormone, free and total carnitine were normal. Hydroxybutyrate was 0.05 mM and acetoacetate was 0.1 mM. Acylcarnitine profile revealed persistent mildly raised hydroxybutyryl carnitine. Fibroblast enzyme assay confirmed low SCHAD level. DNA study indicated that the patient was heterozygous for the mutation H170R. The patient was managed on 10% carbohydrate and diazoxide 5 mg/kg/day to maintain blood glucose within normal limits. **Conclusion**: This case report is another evidence for the associatation of SCHAD and hyperinsulinism. The pathogenesis of hyperinsulinism is not well understood.

P-5-7

LONG-TERM CLINICAL OBSERVATIONS OF THREE PATIENTS WITH INFANTILE CARNITINE-PALMYOYL TRANSFERASE-II DEFICIENCY

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Long-term clinical observations of three patients with infantile carnitine palmitoyltransferase (CPT) II deficiency are reported. They presented the initial symptoms from age 6 to 9 months with Rye-like syndrome with hypoglycemia, lethargy, and hepatomegaly. Their clinical courses have been observed for durations of 11 to 19 years. All three patients have been non-compliant with carnitine supplementation after they recovered from the episodes of metabolic decompensation in their infancy. Physical and intellectual developments of the patients have been normal. Fasting precaution was given since their infancy, which successfully prevented further episodes of severe metabolic decompensation. The 19-year-old patient recently developed the initial episode of rhabdomyolysis, which was preceded by an extensive exercise for 6 h at school. Plasma acylcarnitine, plasma carnitine, and CPK as well as cardiac ultrasound studies have been monitored in all of the patients. Although plasma free carnitine levels have been chronically significantly low in all patients (7–17.3 μM, reference range 36–74 μM), their cardiac functions remain normal without cardiac hypertrophy. Carnitine supplementation has been recommended to many metabolic disorders including fatty acid oxidation defects. Supplementation of carnitine to patients with CPT-II also involves theoretical concerns that accumulation of long-chain cartinine esters can be arrhythmogenic, which have been raised in LCHAD. Our patients with CPT-II have not had arrhythmia regardless they were on carnitine supplementation or not. Chronic management including carnitine supplementation and exercise restriction, and long-term prognosis of CTP-II deficiency will be discussed.

P-5-8

IS BREASTFEEDING AN OPTION IN THE DIETARY MANAGEMENT OF LONG CHAIN FATTY ACID OXIDATION DISORDERS? OUR EXPERIENCE WITH SEVEN PATIENTS

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There is limited literature regarding the use of breast milk in conjunction with other diet therapies for patients with inborn errors of metabolism. Specifically, ad lib breastfeeding has been contraindicated in patients with fatty acid oxidation disorders (FAOD) including long-chain 3-hydroxyacyl CoA dehydrogenase deficiency (LCHAD) or very long chain acyl CoA dehydrogenase (VLCAD) because of the high fat content of breastmilk. Management of long chain FAOD patients involves ensuring adequate caloric intake, fasting precautions, long chain triglyceride (LCT) restriction, supplementation of medium chain triglycerides, and careful monitoring.

We studied our cohort of seven patients with long chain FAOD (VLCAD: n = 5, LCHAD: n = 2) diagnosed through the newborn screening program. Four of the seven infants were on breastmilk as part of their diet – two patients were given calculated amounts of breast milk in conjunction with a low LCT diet, one patient was exclusively breastfed for the first month, and one patient was first given calculated amounts of a low fat formula and then breastfed ad lib for the first three months. All formula/breast milk combinations were designed to provide approximately 40% of calories from fat, with 10–20% of calories from LCTs. The mean duration of using breast milk was two months (range 1–4 months). Breastmilk was well tolerated by the four infants, with good growth, metabolic control, neurodevelopment, and normal cardiac and ophthalmic status. From this review, we conclude that some ad lib breastfeeding may be possible in the treatment long chain FAOD patients.

P-5-9

NEWBORN SCREENING AND MILD MCAD DEFICIENCY: IMPLICATIONS FOR FOLLOW UP AND LONG TERM CARE

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With the advent of newborn screening by MS/MS, a greater number of infants with unreported variations in the ACADM gene have been identified. The clinical implications of these findings are unknown. We describe a family of 8 children in which 4 siblings were identified as compound heterozygous for two MCAD mutations, 127G>A, reported in a previous cases diagnosed by newborn screening and 433T>C, not described previously, following a positive newborn screen in the youngest child (C8 of 1.39 μmol/L, abnormal >0.7; C8/C10 ratio 1.3, abnormal >5). Plasma follow up sample demonstrated elevated concentrations of C6 (0.25 μmol/L; ref. range: <0.23), and C8 (0.72 μmol/L; ref. range: <44). Urine hexanoylglycine excretion at 3 months of age was only minimal elevated (2.62 μg/mg creatinine; ref. range: 0.2-1.9). The three older siblings, ages 6, 8, and 18 years, carry the same genotype. Their biochemical findings included: normal carnitine levels, acylcarnitine profile and urine acylglycines in the 6 year old, normal carnitine levels and urine acylglycines while the acylcarnitine profile showed elevated concentrations of C6 (0.27 μmol/L, ref. range: < 0.17), C8 (1.12 μmol/L, ref. range: <0.78), C10:1 (1.22 μmol/L, ref. range: <0.47), and C10 (1.41 μmol/L, ref. range: <0.88) in the 8 year old, and normal carnitine, acylcarnitine profile and urine acylglycines in the 18 year old. All children are healthy and asymptomatic. This sibship has a seemingly mild clinical and biochemical phenotype and raises issues about how to follow and treat individuals with non-985A>G mutations ascertained by newborn screening.

P-5-10

PREGNANCY CHOLESTASIS AND PREMATURITY IN A CASE OF LONG-CHAIN 3-HYDROXYACYL-COA DEHYDROGENASE DEFICIENCY

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The authors report the clinical case of a preterm baby with long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHAD). Pregnancy was complicated by cholestasis. A girl was delivered in the 27th week of gestation by caesarean section, with a birth weight of 1120 g. She was ventilated for hyaline membrane disease and maintained on nasal continuous positive airway pressure until D36 for apnoeas. *Ductus arteriosus* was closed with indomethacin. *St. epidermidis* sepsis was diagnosed at D6. Enteral feeding was initiated at D2, without evidence of hypoglycaemia. Echocardiography, cerebral ultrasonography and ophthalmoscopic examination were normal. Diagnosis of LCHAD was made in blood card by the acylcarnitine profile and confirmed by the finding of the common mutation G1528C. She begun low fat diet, supplemented with medium chain triglycerides and L-carnitine on D30 and apnoea resolved in a few days. Third trimester pregnancy complications affecting mother's liver have been increasingly described associated with foetal diseases of energy metabolism, as in this case. Carnitine deficiency has been shown to be associated with myopathy and apnoea with respiratory failure in infants and in adults. On the other hand, treatment of prematurity apnoea with carnitine as been advocated by several authors although no controlled trials are known. Pregnancy cholestasis was probably caused by toxic metabolites due to LCHAD in foetus and specific treatment has resolved prolonged apnoea in this child.

P-5-11

HYPOCALCEMIA IN MITOCHONDRIAL TRIFUNCTIONAL PROTEIN DEFICIENCY

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A boy presented afebrile convulsions with severe hypocalcemia (Ca 4.8 mg/dl), that was normalized by 1α-vitamin D_3 and calcium lactate, at the age of 2 months. At his age of 13 months, severe hypotonia, cardiac failure and respiratory distress followed an upper respiratory tract infection. Blood examination revealed increases in creatine kinase, aspartate aminotransferase, and lactate dehydrogenase. He recovered, but hypotonia and respiratory distress had frequently occurred recurred subsequentlysince then. He was diagnosed as with mitochondrial trifunctional protein deficiency with the based on analysis of the urinary organic acids and the enzyme assay of cultured fibroblasts at the age of 2 years. Although his serum calcium level had been controlled within normal limits on with administration of 1α-vitamin D_3, hypocalcemia was observed when the patient had severe rhabdomyolysis. Because of the spontaneous restoration of serum calcium level with the improvement of rhabdomyolysis, it was supposed that this transient hypocalcemia was related to an increased propensity for calcium deposition in injured muscles. Moreover, the a reduction of in the dose of 1α-vitamin D_3 caused persistent hypocalcemia (Ca 6.3 mg/dl). Serum phosphate level was high (IP 7.3 mg/dl), and intact PTH concentrations had have been in low normal level (iPTH 10–21 pg/ml) in spite of the hypocalcemia. These data suggest that he is complicated withalso has hypoparathyroidism. Hypoparathyroidism in trifunctional protein deficiency was reported previously, but the relationship of between these disorders was has not been clarified. These cases suggested the possibility that the dysfunction of mitochondrial energy metabolism due to a fatty acid metabolism defect may cause hyposecretion of PTH.

P-5-12

CONGENITAL HEART DISEASE (VSD II) ASSOCIATED WITH SUSPICION OF SHORT-CHAIN ACYL-CoA DEHYDROGENASE DEFICIENCY NUTRITIONAL PROBLEMS

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We describe a case of child with suspicion of short-chain acyl-CoA dehydrogenase (SCAD) deficiency associated with ventricular septal defect (VSD II). Clinical findings (hypotonia, development delay, vomiting, poor feeding, failure to thrive – 2740 g body weight at 11 months) and biochemical tests (ethylmalonic aciduria in GC/MS analysis, elevated C4-carnitine in the acylcarnitine profile in MS/MS analysis) indicated classic SCAD or variant SCAD. The diagnosis will be confirmed after DNA mutations in the SCAD gene and SCAD enzyme activity in fibroblast analysis. Congenital heart defect (VSD II) diagnosis was based on echocardiography ('Swiss cheese' type). Preoperative management of this child required nutritional status improvement. Infants with VSDs have substantially higher (40%) total energy expenditure (TEE) than healthy infants. Our child needs TEE about 140 kcal/kg bw/day, nutrition was extremely difficult because of the prohibition of lipids iv and medium-chain triglicerydes (MCT) intake by patients with SCAD deficiency. After 1.5 months (by 3300 g bw) surgical repair was performed. Postoperative treatment was complicated by DIC and sepsis. The patient does not show hemodynamical abnormalities and nutritional status. The psychomotor development caught up at the age of 15 months. **Conclusion**: In preoperative treatment of children with SCAD deficiency and congenital heart disease associated, the prohibition of lipids iv and MCT intake makes it difficult to maintain good nutritional status and avoidance of perioperative complications.

P-5-13

LONG-TERM EFFECT OF SINGLE CARNITINE ADMINISTRATION ON FASTED CARNITINE-DEFICIENT JVS MICE REGARDING THEIR LOCOMOTOR ACTIVITY AND ENERGY EXPENDITURE

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Juvenile visceral steatosis ($jvs^{-/-}$) mice, an animal model of systemic carnitine deficiency, suffer from fatty liver, hypoglycemia, hyperammonemia, growth retardation and cardiac hypertrophy. We recently found that the voluntary locomotor activity (LA) was reduced at night in the $jvs^{-/-}$ mice following food withdrawal at 8:00 in the morning, and that reduced LA as well as all above symptoms was ameliorated by carnitine treatment. In the present study, we describe the long-term effects of carnitine on the reduced LA and energy metabolism of fasted $jvs^{-/-}$ mice. We found that a single carnitine administration to 24h-fasted $jvs^{-/-}$ mice in the morning increased both the LA and oxygen consumption at night not only on the same day, but also on the next day, when the carnitine levels in the blood and tissues were already as low as at the original carnitine-deficient state. We also found that fat utilization for energy production significantly increased under fasting even in $jvs^{-/-}$ mice and was stimulated in the carnitine-administrated fasted $jvs^{-/-}$ mice at night, in comparison to that observed in the saline-administered $jvs^{-/-}$ mice, at least for 2 days even under the low plasma and tissue carnitine levels. These results suggest that the low tissue carnitine levels are therefore not the sole rate-limiting factor of general fatty acid oxidation in carnitine-deficient $jvs^{-/-}$ mice.

P-6-1

COMPARISON OF CSF LACTATE LEVELS AND MR SPECTROSCOPY FINDINGS IN PATIENTS WITH MITOCHONDRIAL DISORDERS

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We compared CSF lactate levels with brain proton magnetic resonance spectroscopy (MRS) findings through record review of patients with mitochondrial disorders who underwent both procedures (n = 21). CSF lactate levels were elevated in 15 patients (range 2.3–14 mM/L) and normal in 6 (normal range 0.6–2.2 mM/L). MRS showed a lactate peak in 9 of 21 patients; in these 9, CSF lactate ranged from 2.1 to 14 mM/L (one: normal, eight: elevated). Six patients with MRS lactate peaks had CSF lactate values over 4.7 mM/L; 2 had values of 2.3 and 3.6 mM/L. A single patient with MRS lactate peak had normal CSF lactate. Twelve patients had normal MRS, all with CSF lactate values less than 5.3 mM/L; values were elevated in 7 and normal in 5. The two procedures were concordant in 61.9% (13/21) of cases. In 7 of the 8 discordant cases, MRS failed to detect a lactate peak in patients with CSF lactic acidosis (range: 2.6–5.3 mM/L). Thus, in patients with elevated CSF lactate values less than approximately 5 mM/L, brain MRS may fail to detect a lactate peak. Detection of a lactate peak on MRS may correlate well with CSF lactate values over approximately 5 mM/L; however, further studies are needed to confirm this correlation. In conclusion, MRS can complement direct CSF lactate measurements via lumbar puncture but cannot always provide independent evidence of cerebral lactic acidosis in patients with mild to moderate CSF lactate elevations.

P-6-2

FUNCTIONAL CAPACITY OF MITOCHONDRIAL ENERGY: GENERATING SYSTEM IN PREMATURE NEONATES

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Biosynthesis of OXPHOS proteins and their proper assembly in inner mitochondrial membrane is under direct influence of numerous nuclear and mitochondrial genes. Inadequate capacity of mitochondrial energy production may play an important role in the neonatal morbidity, especially in premature neonates. **Methods**: The amount and activities of pyruvate dehydrogenase (PDH) and respiratory chain (RC) complexes were analyzed in isolated muscle mitochondria obtained at autopsy in 19 premature neonates using spectrophotometric and radioenzymatic methods and Western blotting and Blue-native electrophoresis. Two groups of children recommended to muscle biopsy at the age of 0.5–2 and 3–18 years served as controls. **Results**: In the premature neonates, the protein amount of PDH subunits and RC complexes I, III, IV and V were lower in comparison with older children. The activities of PDH and RC complexes III, IV were lower in premature neonates in comparison with older children. On the contrary, the activity of complex I was higher in premature neonates than in older children. No difference was observed for complex II. In addition, PDH activity was lower (3.9 ± 2.2 nmol/min/mg protein) in 10 neonates with severe hyperlactacidemia than in 9 neonates (6.1 ± 2.4, $p < 0.05$) with mildly increased lactate. **Conclusion**: Our study documents the age dependent differences in activities of PDH and respiratory chain complexes in childhood. Lower functional capacity of mitochondrial energy generating system in premature neonates may be explained by delayed maturation of respiratory chain complexes and increased protein degradation during stress conditions.

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P-6-3

COMPLEX V SUBCOMPLEXES SUGGESTIVE OF A DEFECTIVE INTRAMITOCHONDRIAL PROTEIN TRANSLATION

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Introduction: In previous studies, complex V subcomplexes were detected in the patients with mtDNA mutations. In the present study, we searched for complex V subcomplexes in a large series of patients and report the underlying molecular defect in these patients. **Methods**: Blue Native PAGE (BN-PAGE) was performed in tissue samples from the patients suspected of having an OXPHOS defect. BN-PAGE gels were incubated in a specific staining solution to test for ATPase activity in the gel. The holo-complex V (620kDa) is visualised by this staining as well as the subcomplexes containing the alpha and beta subunits of complex V (370–470 kDa). Patients with complex V subcomplexes were screened for abnormalities in mtDNA (depletion, deletion, tRNA mutations, ATP6 mutations). **Results**: More than 350 tissue samples originating from 320 patients (185 muscle samples, 135 fibroblast strains and 35 heart and liver samples) were investigated. Complex V subcomplexes were detected in 32 tissues originating from 26 patients. Sixteen of these patients (62%) were found to harbour a mtDNA defect (tRNA mutation in 7, mtDNA deletion in 1, mtDNA depletion in 6, ATP6 in 2). **Conclusion**: BN-PAGE combined with activity staining in the gel allows detection of complex V subcomplexes and is, therefore, an easy method for screening mtDNA alterations.

P-6-4

CLINICAL IMPROVEMENT AFTER ADMINISTRATION OF CARNITINE AND UBIQUINONE IN POSSIBLE RESPIRATORY CHAIN DISORDERS

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Background: Mitochondrial disorders may have various clinical manifestations and can present at any age. Diagnostic criteria and standardized therapy for mitochondrial disorders in children remain controversial. **Case**: We present two siblings with mitochondrial disorder from healthy unrelated parents. They are seven years and eight months old boy; and five years old girl. Their early development was normal. At around 2 years of age, they developed muscle weakness and exercise intolerance. The symptoms worsen after years. We found spastic paraparesis, increased muscle tone, deep tendon hypereflexes and pathologic reflexes. Laboratory findings revealed a normal blood count and serum electrolytes with elevated lactate and anion gap. Brain magnetic resonance imaging study revealed leukodystrophy. Hepatic and renal functions were normal. Electrocardiography and echocardiography were also normal. No evidence of mutations in mt-DNA. Because of our limitation, we could not perform respiratory chain enzyme, pyruvate dehydrogenase complex and electron transport chain examinations. According to modified diagnostic criteria proposed by Bernier, et al. (2002), our cases were possible to have respiratory chain disorders. We decided to administer carnitine 50–100 mg/kg/day and ubiquinone 4–5 mg/kg/day for both patients. After 1 month of therapy, clinical improvement with better exercise tolerance and physical fitness was observed. **Conclusion**: Although the aim of treatment in mitochondrial disorders is to stabilizing rather than reversing the disease, administration of carnitine and ubiquinone showed clinical improvement in our patient.

P-6-5

NMR INVESTIGATION OF METABOLISM IN CULTURED HUMAN FIBROBLASTS: EFFECTS OF PDC DEFICIENCY AND DCA ADMINISTRATION

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The pyruvate dehydrogenase complex (PDC) is integral to mitochondrial metabolism and energetics. Congenital PDC deficiency leads to lactic acidosis, neurological degeneration and early death. Dichloroacetate (DCA), an investigational compound for such defects, activates the PDC by inhibiting the reversible phosphorylation of the E1a subunit and decreases its turnover. Primary human fibroblast cultures from 5 controls and 6 patients with mutations in the PDC-E1 component were grown in media $\pm$ 5 mM DCA, exposed to media containing 15 mM ^{13}C-labeled glucose, with the aqueous phase of the cell extracts studied by nuclear magnetic resonance (NMR) spectroscopy. Computer modeling of NMR-derived ^{13}C-glutamate isotopomeric patterns estimated relative carbon flow through tricarboxylic acid cycle-associated pathways and characterized effects of PDC deficiency on glucose metabolism and energetics. With the exception of one patient cell-line expressing an unusual splicing mutation, PDC-deficient cells had significantly higher glucose consumption, lactate production, and label-derived acetyl-CoA, indicative of increased glycolysis vs. controls. In all cells, DCA caused a major shift (40% decrease) from anaplerotic pathways (e.g. pyruvate carboxylase) toward flux through PDC. Ignoring the mis-spliced patient, DCA decreased average glycolysis (29%) in patient cells, but had no effect in control cells, and did not change lactate production or the nucleoside triphosphate to diphosphate ratio (NTP/NDP) in either cell group. Maintenance of NTP despite reduced glycolysis indicates that DCA improved metabolic efficiency by increasing glucose oxidation. This study demonstrates that NMR spectroscopy provides novel insight into the biochemical consequences of PDC deficiency and the mechanism of putative therapeutic agents thereon.

P-6-6

SCREENING MITOCHONDRIAL 12SrRNA GENE A1555G MUTATION ASSOCIATED WITH AMINOGLYCOSIDE-INDUCED HEARING LOSS

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Objective: Screening prevalence of mitochondrial DNA 12SrRNA gene A1555G mutation in Tianjin and its surrounding area, through detecting mitochondrial DNA 1555 nucleotide sequence of newborn and little infant. **Methods**: 300 blood samples were obtained from the patients who lived in newborn nursery in Jan 2005–Mar 2005. And genome DNA was extracted from the isolated leukocyte, including mitochondrial DNA. The mitochondrial DNA fragments were amplified by PCR, A1555G mutation was detected by restriction endonuclease digestion and DNA sequencing. **Results**: One of 300 infants has A1555G mutation. The incidence was 1/300. The A1555G mutation carrier has no maternal inheritance family medical history. Her auditory acuity is normal. **Conclusions**: The prevalence of mitochondrial DNA A1555G mutation in Tianjin and its surrounding area is higher. We ought to take care of using aminoglycoside antibiotics. Screening A1555G mutation before using them, finding A1555G mutation carrier, forbidding them to use aminoglycoside antibiotics, could reduced the incidence rate of aminoglycoside antibiotics induced-hearing loss.

P-6-7

SEVERE RENAL TUBULOPATHY AND CHOLESTASIS DUE TO BCS1L GENE MUTATION OF COMPLEX III: EXPERIENCE WITH HIGH DOSE SODIUM BICARBONATE THERAPY

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Defects of oxidative phosphorylation are a heterogenous group of disorders with various clinical presentations. Isolated complex III deficiency is also a rare disorder in childhood. Recently patients with early liver failure, renal tubulopathy and encephalopathy due to the mutations in the BCS1L gene coding for a structural protein in complex III have been described.

A ten days old female newborn was referred to our hospital because of intractable lactic acidosis. Physical examination revealed prominent hypotonia, and hepatomegaly. In the laboratory examinations, besides lactic acidosis, a lactate to pyruvate ratio of 34, an increase in blood alanine, alanineaminotransferase and aspartateaminotransferase levels, and generalized aminoaciduria were found. The tubulary phosphate reabsorbtion was reduced. Because of multisystem involvement, mitochondrial disease was suspected and the mutational analysis of the BCS1L gene revealed homozygote P99L mutation. As the patient was unresponsive to bicarbonate deficit, dichloroacetate and peritoneal dialysis, continuous intravenous sodium bicarbonate therapy with a dose up to 1.25 mEq/Kg was started. The patient got on well until the age of 7 months when she died of sepsis.

It was stressed that high dose intravenous continuous sodium bicarbonate therapy could be an alternative treatment option in patients with severe acidosis resistant to dichloroacetate and peritoneal dialysis.

P-6-8

ACUTE NEUROLOGICAL DETERIORATION IN A NEONATE WITH LEIGH SYNDROME AND CARDIOMYOPATHY CAUSED BY A COMBINED DEFICIENCY OF RESPIRATORY CHAIN COMPLEX I AND IV

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We report a 4-week-old boy who was hospitalized due to progressive failure to thrive and jaundice. He was born at term by Cesarean section because of fetal cardiac problems. Birth weight was 3038 g, length 47 cm. His mother and father have mild mental retardation of unknown origin. The examination revealed dysmorphic face (large eyes, upturned nose, large tongue), lethargy, muscular hypotonia and weakness, and hepatomegaly. Cardiac ultrasound showed hypertrophic cardiomyopathy. Laboratory investigations showed increased lactate in the blood (in range 0.9–4.2 mmol/l) and in the CSF (3.8 mmol/l), and mildly increased excretion of pyruvate, fumarate, malate, and citrate by urinary organic acid GC/MS analysis. Since 7 weeks of age he had progressive breathing difficulties and therefore needed artificial assisted ventilation. Additionally he had swallowing difficulties and therefore tube feeding was started. There was truncal hypotonia with limb hypertonia and brisk tendon reflexes. Brain MRT showed symmetric T2 hyperintensity of basal ganglia. Leigh syndrome was clinically diagnosed. He was treated with coenzyme Q10 and carnitine. Still, his clinical condition rapidly deteriorated and finally he died at the age of 3 months.

In suspicion of mitochondrial defects muscle biopsy was preformed. Histochemistry showed complete lack of cytochrome c oxidase activity and mild changes of mitochondria's ultrastructure; there were no ragged red fibres. Biochemical investigations of the muscle biopsy showed a reduced activity of respiratory chain (RC) complex I, and a reduced activity of RC complex IV, compatible with the histochemical observations. This confirms the diagnosis mitochondrial disorder due to a combined complex I/complex IV deficiency.

P-6-9

A SIX-YEAR CLINICAL COURSE IN A PATIENT WITH THIAMIN RESPONSIVE PDHC DEFICIENCY

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We present a patient with thiamin responsive pyruvate dehydrogenase complex (PDHC) deficiency who showed a remarkable response to thiamin during a 6-year follow up. The patient is a 10-year-old boy who developed a regression associated with a febrile illness at the age of 1 year and 10 months. He became unable to stand or raise his arms. Dichloroacetate-activated PDHC activity in the cultured blood lymphocytes with the presence of 1×10^{-4} mM thiamin pyrophosphate (TPP) was 28% of normal control. The activity with 0.4 mM TPP was 45% of normal, indicating thiamin responsive PDHC deficiency. The molecular analysis revealed R88C in the PDHC E1α subunit gene. By the time the definite diagnosis was made at the age of 4 years, he could walk but less than 500 m. He could not squat and he needed to hold his knees to stand up. With 100 mg of thiamin per day, he became able to walk 1 km without a fatigue. Squatting became possible and he could stand up without holding the knees. Currently, he is on 400 mg of thiamin. During the past 6 years, he did not develop regression or weakness except for occasional muscle pain or cramp. Thus, the PDHC deficiency with R88C showed a stable and good response to thiamin therapy.

P-6-10

TWO COMPONENTS IN PATHOGENIC MECHANISM OF MITOCHONDRIAL ATP SYNTHASE DEFICIENCY: ENERGY DEPRIVATION AND ROS PRODUCTION

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Isolated defects of mitochondrial F_1F_0-ATP synthase (ATPase) due to diminished biosynthesis of the enzyme represent new class of severe mitochondrial diseases of nuclear origin. The primary cause of decreased cellular content of ATPase appears to be a problem in assembly of the F_1-catalytic part of the enzyme. The clinical presentation shows early neonatal onset, lactic acidosis, hypertrophic cardiomyopathy and psychomotor retardation, in most cases with 3-methylglutaconic aciduria. Patients can die within the first weeks of life or show psychomotor and mental retardation to various degrees. With the aim to elucidate how the low ATPase content affects mitochondrial energy provision and production of reactive oxygen radicals (ROS), we have investigated fibroblasts from 4 patients with 70–90% decrease of ATPase content. Measurements of cellular respiration showed pronounced decrease in ATPase capacity for basal respiration, mitochondrial ATP synthesis was decreased to 26–33%. Cytofluorometric analysis using TMRM revealed altered discharge of mitochondrial membrane potential ($\Delta\Psi_m$) in patient cells, which was 20 mV increased at state 3-ADP compared to controls. Analysis of ROS production by CM-H_2DCFDA demonstrated a 2-fold increase in ROS levels in patient cells compared to controls. ROS production rate was sensitive to uncoupler (FCCP) and thus apparently related to increased $\Delta\Psi_m$. Our studies clearly demonstrate that low ATPase content and decreased mitochondrial ATP production lead to high values of $\Delta\Psi_m$ that are associated with activation of ROS generation by the mitochondrial respiratory chain. In conclusion, both the energetic deprivation and increased oxidative stress are important components of the pathogenic mechanism of ATPase disorders.

P-6-11

STRUCTURAL AND FUNCTIONAL CHANGES OF MITOCHONDRIAL ATP SYNTHASE CAUSED BY mtDNA 9205delTA MUTATION IN ATP GENE

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We have analyzed a new case with mtDNA 9205delTA mutation that cancels STOP codon of *ATP6* gene and changes cleavage site between *ATP6* and *COXIII* RNAs for subunit a of mitochondrial ATP synthase (ATPase) and subunit III of cytochrome *c* oxidase. (COX). Homoplasmic mutation was found in fibroblasts, blood cells and muscle in the affected boy with fatal encephalopathy, severe lactic acidosis and psychomotor delay. We found impaired processing of the primary *ATP8-ATP6-COXIII* transcript using NorthernBlot and RT-PCR. We observed a normal aurovertin-sensitive ATPase hydrolytic activity and 70% decrease of ATP production. Subunit a content was 10-fold decreased and [35S]methionine labeling showed pronounced decrease in subunit a *de novo* biosynthesis. The content of COX subunits (COXI, COXIV and COXVIc) was 30–60% decreased. Analysis of OXPHOS complexes by BlueNative and 2-dimensional electrophoresis revealed instability of the ATPase complexes and altered pattern of COX assembly. The results indicate that the 9205delTA mutation prevents the synthesis of the key component of ATPase proton channel subunit a and causes the formation of an incomplete, subunit a-lacking ATPase complexes that are capable of ATP hydrolysis but not of ATP synthesis. Mutation also affects biogenesis of COX, which is present in a decreased amount in patient cells. Interestingly, unchanged RNA processing and only some accumulation of F_1-intermediate were found in the only other known case of 9205delTA with much milder clinical presentation.

P-6-12

STUDY OF CLINICAL PHENOTYPE AND GENOTYPE IN JAPANESE PATIENTS WITH VERY-LONG CHAIN ACYL-CoA DEHYDROGENASE

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Objective: To clarify the characteristics of clinical phenotype and genotype in Japanese patients with very-long chain acyl-CoA dehydrogenase deficiency (VLCADD). **Methods**: We studied 28 patients diagnosed with the high level of C14:1 acylcarnitine in blood and the low enzyme activity. Clinical forms of VLCADD were defined according to the clinical and biochemical findings. Mutational analysis were performed and compared with the clinical phenotype. **Results**: Clinical forms of 28 Japanese patients with VLCADD were as follows: one (3.5%) showed the severe form; 3 (11%), the intermediate; 23 (82%), the myopathic form. In addition, one asymptomatic case (3.5%) was detected in newborn screening. Mutations of VLCAD gene were identified in 54 alleles. K264E was most common mutaion (14 alleles) and detected in the myopathic form. K382Q (9 alleles), 997 insT (6) and R450H (5) were followed. **Discussion**: In contrast to the previous reports published in Europe or USA, the myopathic form was prevalent in Japanese patients. Furthermore, several common mutations of VLCAD gene, such as K264E or K382Q, were idetified in the myopathic form of Japanese. There may be ethic difference in the clinical and genetic findings. Further study on other populations, such as Asian countries, will make the genetic background of VLCADD more clearly.

P-6-13

COMPLEX 1 RESPIRATORY CHAIN DEFICIENCY PRESENTING WITH SEVERE DILATED CARDIOMYOPATHY AND SEVERE RENAL TUBULAR ACIDOSIS IN A SAUDI BOY

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Introduction: Mitochondrial disorders, arising from deficiency of the electron transport chain give rise to a wide clinical presentation and are often progressive in nature. Hypertrophic cardiomyopathy with early onset and rapidly progressive course is often observed in mitochondrial disorder, dilated cardiomyopathy is exceptional especially in the neonatal period. **Case Report**: 18-month-old boy born at term to a consanguineous Saudi parent. Pregnancy and delivery were uneventful. He presented in the neonatal period with recurrent chest infection. Chest X ray showed cardiomegaly. Echocardiography confirmed severely dilated left ventricle and left atrium, poor left ventricular function, and mild mitral regurgitation. Despite adequate treatment of heart failure with diuretics, digoxin, and captobril, his weight, height, and head circumference remained below 3rd centile. Neurodevelopment was below average for his age. Examination was unremarkable with no dysmorphic features, pigmented retinopathy, or organomegaly. Laboratory results showed normal serum amino acids, acylcarnitine, and liver function test. Serum lactate was high. Urine showed moderate lactic aciduria and gross aminoaciduria. Blood gas revealed severe metabolic acidosis in many occasions with Ph <7.1, low bicarbonate, normal anion gap and hyperchloremia. Muscle biopsy confirmed low NADH Q Oxidoreductase (complex 1) at 17.0 nmol/min/mg protein (normal 30-150). Complex 2, 3, and 4 were normal. The patient was treated with oral sodium bicarbonate, carnitine, coenzyme Q and high fat diet. **Conclusion**: We reported on an infant with complex 1 respiratory chain deficiency presented with severe dilated cardiomyopathy, which is unusual as this condition is often associated with hypertophic rather than dilated cardiomyopathy.

P-6-14

MISDIAGNOSIS OF MITOCHONDRIAL NEUROGASTROINTESTINAL ENCEPHALOPATHY (MNGIE)

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MNGIE arises from deficiency of the mitochondrial enzyme thymidine phosphorylase (EC-2.4.2.4), which encoded by a nuclear gene (ECGF1, OMIM-603041). It is a severe metabolic disorder, usually presenting during the second decade but is described as early as 5 months. Early features may include borborygmi, but external ophthalmoplegia, ptosis, peripheral neuropathy, leukencephalopathy and myopathy ensue, with progressive gastrointestinal dysmotility typically leading to death in the early 30's. MNGIE can be definitively diagnosed by the presence of urinary or plasma thymidine and deoxyuridine, accompanied by raised thymine and uracil.

We present a 30 year old Iraqi male, who presented with a very mild phenotype: blurred vision and muscle fatigue but no gastrointestinal symptoms. On examination he had bilateral ptosis, external ophthalmoplegia and a myopathic facies. A urine specimen (transported interstate) exhibited grossly raised thymine and uracil (respectively: 72, 136 mmol/mol creatinine, normal <0.5, <15); dihydropyrimidines were not detected; thymidine and deoxyuridine were also absent. A provisional diagnosis of thymine-uraciluria was made. However, a repeat urine exhibited thymidine and deoxyuridine (35, 64 mmol/mol) with lower – but above normal – levels of thymine and uracil (23, 53 mmol/mol).

Thymine-uraciluria occurs in deficiencies of both dihydropyrimidinase and dihydropyrimidine dehydrogenase. Urinary uracil alone is a useful marker of urea cycle defects, but it can also be raised falsely by bacterial degradation of pseudouridine. We propose that in the first MNGIE urine sample the labile thymidine and deoxyuridine nucleosides were degraded during transport to their thymine and uracil bases. We suggest metabolic laboratories should be aware of potential pitfalls for diagnosis of MNGIE.

P-6-15

SEVERE PERIPHERAL NEUROPATHY IN A PATIENT WITH 3-METHYLGLUTACONIC ACIDURIA AND F_1F_0-ATP SYNTHASE DEFICIENCY OF NUCLEAR GENETIC ORIGIN

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Peripheral neuropathy (PN) is a quite common but often underdiagnosed finding in mitochondrial diseases in childhood. 3-Methylglutaconic aciduria (type 4) was described in various mitochondrial diseases. We report on a 18 years old girl with severe progressive PN with loss of deep tendon reflexes, ataxia, progressive muscular weakness, a reduced walking distance and horizontal nystagmus. She became symptomatic already on the second day of life with respiratory symptoms, lactic acidosis and 3-methylglutaconic aciduria. The course of the disease was characterized by repeated metabolic crises with elevated lactate and ammonia levels, especially during infections. 3-Methylglutaconyl-CoA hydratase was normal. In fibroblasts the oligomycin sensitive ATP synthase was diminished (3.7 mUnits/mg protein; normal: 36–167). In Blue Native electrophoresis the ATP synthase protein complex was more than 90% reduced. Sequence of the mitochondrial *ATP6/8* genes was normal. Motor nerve conduction velocity of the peroneal nerve was severely reduced (6 m/s, normal >50 m/s) with a significantly diminished amplitude (0.2 mV, normal >8 mV) proofing mixed axonal and demyelinating neuropathy. In conclusion, progressive PN in combination with lactic acidosis is highly suspicious for a mitochondrial disease. F_1F_0-ATP synthase deficiency has to be included in the differential diagnosis especially when 3-methylglutaconic aciduria is found.

P-6-16

SEARCHING FOR MUTATIONS ON PYRUVATE DEHYDROGENASE E1 ALPHA SUBUNIT GENE IN MAINLAND-CHINESE PATIENTS WITH LEIGH SYNDROME

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Leigh syndrome is a genetically heterogeneous disease caused by defects in enzymes involved in aerobic energy metabolism and the Krebs' cycle. Based on the reported data, deficiency of pyruvate dehydrogenase complex E1 alpha subunit (PDHA1) is one of the common causes of Leigh syndrome. To investigate the prevalence of PDHA1 gene mutations in Chinese patients with Leigh syndrome, we performed PDHA1 gene screening in 80 patients with Leigh syndrome who were hospitalized from 1998 to 2005. Patients were from 21 provinces or cities of Mainland China. Only one case of PDHA1 deficiency was found. An R72C mutation in exon 3 of the PDHA1 gene was detected. The patient was a boy with normal mental development, retarded motor development, general weakness, hypotonia and areflexia. Muscle histopathological findings suggested axonal peripheral neuropathy. Brain magnetic resonance imaging at 5 years of age revealed bilateral putamina lesions and periventricular white matter demyelination, supporting the diagnosis of Leigh syndrome. Two known SNPs have been found in other 13 patients. Our results suggest that PDHA1 gene mutations might be uncommon in Chinese Leigh syndrome patients.

P-6-17

MITOCHONDRIAL MOSAICS IN THE LIVER OF PATIENTS WITH PEARSON AND ALPERS-HUTTENLOCHER SYNDROMES

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Mitochondrial heteroplasmy has previously been visualized by cytochrome oxidase staining (COX) in muscle, but not in liver. We studied 2 pts, a girl 2.5 year old with Pearson syndrome (PS) (pancytopenia and exocrine pancreatic failure), and a boy aged 14 months with Alpers-Huttenlocher syndrome (APS) (myoclonal epilepsy, onset at 11 months, and hepatic disease). Both patients showed mitochondria with weak or absent COX activity in many liver parenchymal cells that otherwise looked viable; and strong mitochondrial staining in adjacent hepatocytes. COX activity was localized by DAB with and without cytochrome c after glutaraldehyde fixation, for light and electron microscopy. Mitochondrial staining was positive in bile duct epithelium, endothelium, Kupffer cells, vascular smooth muscle and neutrophils. In the Alpers pt mtDNA of skeletal muscle was depleted for 3/4 as seen by real time PCR. In blood of the girl with PS a deletion of 4091 bp located between positions 5969 and 10060 was found in 50% of the mtDNA. In her liver decreased protein and activity of complex IV, and subcomplexes of complex V were detected by blue native PAGE. Both pts had increased plasma lactate, but no ragged red fibers in muscle. OXPHOS activities were normal in muscle, fibroblasts and blood cells. Brain MRI of the PS pt demonstrated in the white matter of both frontal lobes a large cavitation with absence of the lactate MR spectrum. MRI of the AHS pt initially showed only slight diffuse cortical atrophy, which has become more obvious while convulsions persisted.

These results demonstrate the merit of liver biopsies and COX staining for fast diagnosis of oxidative phosphorylation defects, and as a clue to their phenotypic variation.

P-6-18

TWO NOVEL MUTATIONS CAUSING LEIGH SYNDROME

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Leigh disease is a devastating neurodegenerative disorder with extensive genetic heterogeneity in its causation. Mutations have been identified in many nuclear and mitochondrial-encoded genes involved in oxidative phosphorylation. We have diagnosed 12 patients with Leigh syndrome out of 265 patients with probable mitochondrial cytopathy. We report here 2 novel mutations found in 2 patients with clinical diagnosis of Leigh syndrome. First patient was a 5 year-old girl presented at 8 months with myoclonic epilepsy and recurrent encephalopathy associated with regression of milestones. She also had attention deficits. MRI revealed multiple T2 hypodensities in the brain. Glucose challenge revealed elevation of lactate. She had improved with coenzyme and vitamin therapy. The second patient was a 15-months girl presented with recurrent epileptic encephalopathy associated with lactic acidosis and rapidly progressive regression and finally succumbed to the illness. MRI changes were characteristic of Leigh syndrome. Mutation detection was 1st carried out on known mutation hotspots of Leigh syndrome for all patients and our subsequent strategy involved bi-directional sequencing of whole mitochondrial genome followed by nuclear gene analysis. We found a novel pathogenic mutation at ND5 of the mitochondrial genome A13276G resulting in amino acid substitution M314V for the first patient. This mutation predicted an abnormal complex 1 of the oxidative phosphorylation engine. The second case had multiple deletions of the mtDNA with the deletion at 8621-8850 of the ATP6 gene being novel. There were also large deletions involving both ND6 and tRNA-Val. Identification of pathogenic mutations enable future reproductive planning for the parents.

P-6-19

THREE NOVEL MUTATIONS IN POLG1 GENE

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The mitochondrial respiratory chain is the result of the interplay of two physically and functionally separated genomes, nuclear DNA (nDNA) and mitochondrial DNA (mtDNA). Therefore, in most cases, mutations in any of these genomes will be responsible for mitochondrial disorders, as progressive external ophtalmoplegia (PEO), a multiple deletions associated disorder. Multiple deletions are caused by mutations in nuclear genes encoding factors responsible for mtDNA maintenance and replication, like ANT1, Twinkle and *POLG1*, which encode DNA polymerase gamma. To study the relevance of *POLG1* gene in the pathogenesis of multiple deletions associated disorders, we screened 14 patients for mutations in this gene. The patients were chosen according to their clinic phenotype, such as PEO and myopathy and presence of multiple deletions and RRFs (red ragged fibers). The nDNA was isolated from skeletal muscle and the identification of multiple deletions was done using Southern Blotting method. The screening of mutations was performed by PCR, followed by cycle sequencing.

In POLG1 gene we identified three novel mutations, P648R, R807C and W918R and two other mutations already described, T252I and P587L. We also found two variants (c.1080G>T and c.3480G>A) and various already described polymorphisms.

Our results allowed the molecular characterization of four patients, one with SANDO syndrome, and the others with arPEO/adPEO, relating *POLG1* mutation with these disorders. Implementation of the molecular analyses in mitochondrial disorders of intergenomic communication in Portugal will open the possibility of more precise molecular characterization of patients, as well as familiar studies.

P-6-20

MITOCHONDRIAL ACETOACETYL–CoA THIOLASE (MAT) DEFICIENCY IN A THAI BOY: FIRST REPORTED CASE

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Mitochondrial acetoacetyl-COA thiolose (MAT) or 3-ketothiolase deficiency (OMIM 203750) is an inherited metabolic disorder of organic acids in which characteristic organic acids such as 2-methyl-3-hydroxybutyric acid, 2-methylacetoacetic acid, and tiglylglycine are present in the urine. The deficient enzyme is the K^+-dependent short chain mitochondrial thiolase, which plays a major role in ketone body metabolism.

We herein report a 10-year-old Thai-Chinese boy (born in 1991) who presented (at age 9 months) with fever, vomiting, hyperpnea and severe metabolic acidosis. Blood and urine samples were collected and tested in the USA since we were unable to perform plasma amino acid and urine organic acid analysis in Thailand in 1991, and initial diagnosis given was MSUD, intermittent type. In 2001 urine organic acid analysis by GC/MS donated by JICA demonstrated marked increased excretion of tiglylglycine and 2-methyl-3-hydroxybutyrate, characteristic of β-ketothiolase deficiency. Mutation analysis will be presented. This is the first reported case of MAT deficiency in Thailand.

P-6-21

CARNITINE PALMITOYLTRANSFERASE TYPE I (CPT I) DEFICIENCY IN A THAI INFANT: FIRST REPORTED CASE

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Carnitine palmitoyltransferase type I (CPT I) deficiency (OMIM 255120) is a rare autosomal recessive metabolic disorder of long-chain fatty acid oxidation characterized by severe episodes of hypoketotic hypoglycemia usually occurring after fasting or illness. Onset is in infancy or early childhood. There are two tissue specific isoforms, 'hepatic' and 'muscle' which are encoded by two separate genes. Carnitine palmitoyltransferse type I is a key enzyme of the carnitine shuttle and its deficiency results in a decreased rate of fatty acid beta-oxidation.

We herein report an 8-month-old baby girl (born in 2003) who developed lethargy, hypoketotic hypoglycemia after minor intercurrent illness. Metabolic acidosis, hepatomegaly and moderate degree of liver steatosis were observed. Urine organic acid analysis demonstrated increased excretion of adipate and suberate. Acylcarnitine profile showed substantial elevation of free carnitine to palmitoylcarnitine (FC/C16). Treatment consist of L-carnitine, low fat diet and special formula with marked improvement. Enzyme assay and mutation analysis will be presented. This is first reported case of CPT I in Thailand.

P-6-22

GLUCOSE PRODUCTION, LIPOLYSIS AND ENERGY EXPENDITURE IN AN INFANT WITH PYRUVATE DEHYDROGENASE COMPLEX DEFICIENCY

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Pyruvate dehydrogenase complex (PDHC) deficiency is an inherited disorder of carbohydrate metabolism, often resulting in severe lactic acidosis. We hypothesized that the metabolic disturbance and/or the treatment with ketogenic diet influence energy substrate production.

The aim of this study was to determine glucose production rate (GPR), rate of lipolysis and resting energy expenditure (REE) in a two month old boy with neonatal onset PDHC deficiency, treated with a high fat, low carbohydrate diet. Rates of GPR and lipolysis were analysed following constant rate infusion of [6,6-$^2H_2$]-glucose and [1,1,2,3,3-$^2H_5$]-glycerol for 6 h. Isotopic enrichments obtained during the last 40 min of the infusion after a 2.5 h fast, were used for calculation. REE was estimated by indirect calorimetry at the end of the investigation period. The rate of glycerol production was 13.7 µ1 mol kg^{-1} min^{-1}, GPR averaged 6.8 mg kg^{-1} min^{-1}, REE was 277 kcal and RQ was 0.97. Plasma glucose and lactate levels displayed low variation throughout the investigation. The levels of serum insulin were low. Lipolysis and GPR were in the upper range of those found in term neonates. The rather high energy substrate production and the low insulin levels probably reflects the ketogenic diet. REE was only 60% of estimated value for age and body size despite an adequate energy supply. Surprisingly the RQ was high indicating glucose oxidation, a fact that may reflect a sufficient rest activity of the PDHC during rest. These findings imply that ketogenic diet may not be the preference treatment for this disorder.

P-6-23

FATAL MITOCHONDRIAL DNA DEPLETION MYOPATHY DUE TO NOVEL MUTATIONS IN THE *TK2* GENE

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Mitochondrial DNA (mtDNA) depletion syndromes are autosomal recessive disorders of infancy or childhood, characterised by a marked decrease in mtDNA copy number in affected organs. Patients with a myopathic form of mtDNA depletion syndrome harbour mutations in the thymidine kinase 2 (*TK2*) gene, important for the maintenance of intramitochondrial nucleotide pools. Here we describe the first British family with mutations in this gene.

The proband is the 4th child born to non-consanguineous parents. They have two daughters aged 7 and 10 years who are well but their first child had increasing muscle weakness from 8 months of age and died from respiratory failure aged 14 months. The proband presented at the age of 14 months with a 1 month history of poor head control, reduced feeding and increasing difficulty with crawling and sitting. Examination revealed normal eye movements, but he had marked head lag and generalised muscle weakness. Muscle biopsy demonstrated $>50\%$ cytochrome *c* oxidase-deficient fibres and a severe decrease in the activities of respiratory chain complexes I, III and IV. Ultrastructural studies showed fibres with abnormally shaped mitochondria and focal increase in lipid. There was a progressive worsening of his myopathy and he died at the age of 18 months. Quantitative real-time PCR demonstrated a marked depletion of mtDNA copy number in muscle whilst sequencing of the entire coding region of the *TK2* gene revealed two novel, heterozygous changes – 259C > T in exon 2 and 299A > G in exon 3 predicting nonsense (Q87X) and missense (N100S) mutations respectively.

P-6-24

MITOCHONDRIAL RESPIRATORY CHAIN DEFECT AS A POSSIBLE CAUSE OF UEXPLAINED NEUROLOGIC DISORDERS

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Objective: To characterize clinical and laboratory features in children with neurological problems diagnosed as mitochondrial respiratory chain (MRC) defect and to provide more precise diagnosis and effective treatments. **Methods**: We retrospectively reviewed clinical and laboratory features of 28 children confirmed as MRC defect from muscle tissue using spectrophotometric assays. **Results**: (1) Mean age was 6.67 ± 4.44 years and sex ratio was 1.15:1. (2) Eighteen (64.3%) were MRC I deficiency, 8 (28.5%) of VI deficiency, 1 (3.5%) of II deficiency, and 1 case of combined deficiency of I and IV. (3) Eight cases (28.5%) were classified as Leigh syndrome, 1 case (3.5%) each as MELAS, Kearns-Sayre syndrome, and Alpers disease, but 17 cases (60.7%) were not clinically categorized for specific disease criteria. (4) Epilepsy was seen in 21 (75.0%) patients, developmental delay in 27 of 28 (96.4%). (5) Clinical symptoms most commonly started below 1 year of age, in 15 (53.6%) patients. (6) Brain MRI showed diffuse cortical atrophy in 18 (64.3%) and basal ganglia signal changes in 12 cases (42.9%). (7) Positive rate for laboratory studies were: 92.9% in increased plasma lactate/private ratio over 20, 66.7% in increased beta-hydroxybutyric acid/acetic acid ratio over 2, and 57.9% in MR spectroscopy. (8) Ketogenic diet showed clinical improvements in 75% of patients and mitochondrial cocktail with coenzyme Q_{10} and multi-vitamin in 75% of based on subjective judgments of the caretakers. **Conclusions**: MRC defect can be the cause of many unexplained neurological disorders including epilepsy. Treatments with coenzyme, multi-vitamine and/or ketogenic diet have shown considerable benefits.

P-6-25

METABOLIC ACIDOSIS AND FAILURE TO THRIVE

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Metabolic acidosis is one of the symptoms of inborn error of metabolism. We present a case about a 14-month-old boy who was admitted with 7 months history of feeding difficulties, failure to thrive and muscle weakness. He was just able to sit with support. He was mentally alert, both his sight and hearing was normal. There was slight dyspnea and marked muscle hypotonia. Blood gas analysis and serum electrolyte examination showed a metabolic acidosis, increased plasma anion gap and high blood lactate (8 mmol/L). Urine electrolyte examination revealed the diagnosis of distal renal tubular acidosis. No calcification was seen in his cranial CT-scan. High blood lactate support the suspicion of mitochondrial disorder. The patient was treated with enteral feeding, sodium bicarbonate, co-enzyme Q and L-carnitin. After a week his weight increased 1 kg and he could stand up. Finally he could walk without support within a month. This significant improvement support the diagnosis of co-enzyme Q deficiency, one of the mitochondriopathies. From this case we can conclude that mitochondriopathies should be suspected in metabolic acidosis with high blood lactate. Muscle biopsy with enzyme studies will establish the diagnosis. Recommended treatment in mitochondriopathy are co-enzyme Q, L-carnitin, riboflavine, ascorbate and suitable diet.

P-6-26

CLINICAL SPECTRUM OF POLYMERASE-GAMMA MUTATIONS IN 9 PEDIATRIC PATIENTS

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Alpers-Huttenlocher syndrome (AHS) is a progressive neurologic disorder characterized by the clinical triad of psychomotor regression, refractory seizures and liver disease after a normal development over the first weeks to years of life. Recently it has been shown that autosomal recessive mutations of mitochondrial (mt) polymerase gamma (POLG) result in mtDNA depletion and cause AHS. We present clinical data of 9 patients (male/female: 8/1) with *POLG1* mutations (2 homozygous, 7 compound heterozygous). Age at onset was between 5 months and 8 years. First symptoms were seizures ($n = 6$), failure to thrive ($n = 1$), gait disturbance ($n = 1$) and myopathy ($n = 1$). 4 patients had no signs of liver disease at onset. 3 patients with early onset died after a progressive course within 11 months, 6 patients are still alive. All patients developed a psychomotor regression. During the course of the disease 8 patients developed seizures, 7 with recurrent epileptic states. Liver disease was found in 8 patients, 2 of them died of liver failure. Finally 2 patients do not fulfill diagnostic criteria for AHS. One had a predominantly hepatopathic course with psychomotor regression but no seizures. The other presented with myopathy, progressive ataxia and seizures but without signs of liver disease (follow up 1 year). We conclude, that in addition to the complete AHS phenotype screening for *POLG1* mutations should be considered in patients with progressive neurodegenerative disorders of episodic course $\pm$ myopathy and liver disease to a various extent. Seizures are a clinical hallmark, but may be absent in early fatal hepatopathic cases.

P-6-27

SEVERE CARDIAC INVOLVEMENT IN CHILDREN CARRYING THE A3243G mtDNA MUTATION

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The phenotypic spectrum of the mitochondrial A3243G DNA mutation is highly variable, particularly when occurring in childhood. In contrast to the classical presentation in adulthood (MELAS syndrome; mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes) children show a different pattern of symptoms, often without the typical encephalopathy or psychomotor regression. We present six children carrying the A3243G mtDNA mutation with a heteroplasmy above 50% in muscle tissue. The age of the diagnosis ranged from 2 weeks up to 14.5 years (mean 5.6 years). Mitochondrial dysfunction was confirmed by fresh muscle biopsy in all cases. The clinical presentation was rather non-specific including muscle weakness, developmental delay, epilepsy and lactic acidemia. In contrast to the less frequent occurrence of cardiac involvement in the adult population with MELAS syndrome we discovered significant cardiac pathology in four out of six children already at an early stage of disease, including cardiomyopathy and biventriculair hypertrophy with rhythm disturbances. Based on the observation of arrhythmias, a partial heart block and a long QT syndrome without the presence of obvious clinical symptoms in our patients, we suggest performing ECG in children with a suspected mitochondrial disorder regardless of the presence of clinical symptoms of cardiac pathology.

P-7-1

ATYPICAL VARIANTS OF POMPE DISEASE: A REPORT OF 5 CASES

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A spectrum of clinical phenotypes has been described for Pompe disease. The well recognized classic form manifests as rapidly progressive cardiomyopathy and death within the first year. Disease variants present in later childhood or adulthood predominantly with skeletal myopathy. We had identified the disease variants in 5 patients (4 females and 1 male, all Southern Chinese) over past 3 years. All had characteristic histology in muscle. Diagnosis was confirmed by markedly reduced acid α-glucosidase in dry blood spots. Two sisters with absent acid α-glucosidase activity had infantile onset of hypotonia, motor delay and growth retardation but of different severity. One sister presented with symptomatic hypertrophic cardiomyopathy at 18 months and the other was diagnosed at 5 years with much attenuated symptoms. In another family, both the affected siblings with residual enzyme activity (0.1 μmol/h/L) were healthy during early childhood but had mild motor delay. They presented at 7 and 8 years old respectively with respiratory insufficiency following chest infection. They were still ambulatory and needed intermittent non-invasive ventilation at home via tracheostomy. The 5th patient was diagnosed at the age of 27 years after she developed respiratory failure though she presented earlier with weakness and gait problems in teenage. Phenotypic variation of Pompe disease may be due to epigenetic or environmental factors beside genetic heterogeneity with different residual enzyme activity. We need to be aware of these atypical presentations to avoid unnecessary delay in diagnosis as effective enzyme therapy is available.

P-7-2

DISTAL MYOPATHY WITH RIMMED VACUOLES: IMPAIRED *O*-GLYCAN FORMATION IN MUSCULAR GLYCOPROTEINS

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Distal myopathy with rimmed vacuoles (DMRV), is an autosomal recessive disorder with early adult-onset, displays distal dominant muscular involvement and is characterized by the presence of numerous rimmed vacuoles in the affected muscle fibers. The pathophysiology of DMRV has not been clarified yet, although the responsible gene was identified as that encoding UDP-N-acetylglucosamine 2-epimerase/*N*-acetylmannosamine kinase involved in the biosynthesis of sialic acids. To identify defective carbohydrate moieties of muscular glycoproteins from DMRV patients, frozen skeletal muscle sections from seven patients with DMRV, as well as normal and pathological controls, were treated with or without sialidase or *N*-glycosidase F, followed by lectin staining and lectin blotting analysis. The sialic acid contents of the *O*-glycans in the skeletal muscle specimens from the DMRV patients were also measured. We found that *Arachis hypogaea* agglutinin (PNA) lectin reacted strongly with sarcolemmal glycoproteins in the DMRV patients, but not with those in control subjects. α-Dystroglycan from the DMRV patients strongly associated with PNA lectin, although that from controls did not. The sialic acid level of the *O*-glycans in the DMRV muscular glycoproteins with molecular weights of 30 to 200 kD was reduced to 60 to 80% of the control level. The results show that impaired sialyl *O*-glycan formation in muscular glycoproteins including α-dystroglycan occurs in DMRV.

P-7-3

FIVE NOVEL MUTATIONS OF *AGL* GENE IN THREE KOREAN PATIENTS WITH GLYCOGEN STORAGE DISEASE TYPE III

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Glycogen storage disease type III (GSD-III), is a rare autosomal recessive disorder of glycogen metabolism. The affected enzyme is amylo-1,6-glucosidase, 4-alpha-glucanotransferase (*AGL*, glycogen debranching enzyme), which is responsible for the debranching of the glycogen molecule during catabolism. The disease has been demonstrated to show clinical and biochemical heterogeneity, reflecting the genotype-phenotype heterogeneity among different patients. In this study, we analyzed mutations of *AGL* gene in three unrelated Korean GSD-III patients and discussed their clinical and laboratory implications. Analysis of 34 exons and part exon-intron boundaries of the *AGL* gene in patients were carried out by direct DNA sequencing method using patients' peripheral leukocytes. The clinical features included hepatomegaly (in all patients), seizures (in patient 3), growth failure (in patients 1 and 2), hyperlipidemia (in patients 1 and 2), raised transaminases and creatine kinase concentrations (in all patients) and mild cardiomyopathy (in patients 3). Liver transplantation was performed in patient 3 due to progressive hepatic fibrosis. Administration of raw-corn-starch could maintain normoglycemia and improve the condition. Mutation analysis revealed that all three patients were compound heterozygotes. Patient 1 was a compound heterozygote of c.1282 G>A (R428K) and c.1306del (S603PfsX6, patient 2 with c.1160 G>A (R387Q) and c.1510_1511insT (Y504LfsX10), and patient 3 with c.3416 T>C (L1139P) and c.1735+1 G>T (Y538_R578delfsX4) mutations. Except R428K mutation, 5 other mutations of 3 patients were novel. In conclusion, GSD-III patients have variable phenotypic characteristics. The molecular defects in the *AGL* gene of Korean GSD-III patients were genetically heterogeneous.

P-7-4

GLYCOGEN STORAGE DISEASE TYPE II IN GERMANY: SPECTRUM OF MUTATIONS, BIOCHEMICAL AND MORPHOLOGICAL ASPECTS

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Mutations at the locus of the lysosomal alpha-glucosidase gene (GAA; OMIM: 606800) result in glycogen storage disease type II (GSDII, Pompe disease; OMIM: 232300). 42 unrelated patients were investigated in the past 7 years. Their age at the time of diagnosis ranged from 3 weeks to 77 years. The diagnosis was confirmed by reduced acid alpha-glucosidase acivity, elevated glycogen content and molecular genetic analysis of the GAA gene. We defined the molecular defect in 94% of the GAA chromosomes. By analysis of 66 GAA mutant alleles from 33 German patients, the allele frequency of mutations were determined: IVS1-13T>G (27%), c.525delT (14%) and delExon18 (9%). Fifteen mutations were novel: c.719C>T, c.877G>A, c.1564G>A, c.1703A>T, c. 1802C>T, c.1829C>T, c.1859G>A, c.1912G>A, c.2214G>A, c.1050-1051delG, c.1127-1130delG, c.1364-1369delG, IVS3+2T>C, IVS6-2A>G, and IVS18-2A>G.

The investigation of 9 Turkish patients living in Germany revealed 18 mutant alleles: 2741insC2743insG was the most common (39%), 3 were novel: c.896T>C, 1064T>C and IVS11-2A>G. Concerning the IVS1-13T>G mutation, two patients with compound heterozygosity IVS1-13T>G/c.1050-1051delG and IVS1-13T>G/c.2214G>A respectively, with elevated CK, ASAT ALAT and LDH but with no clinical presentation were diagnosed at infancy. The homozygosity for IVS1-13T>G was causative for GSDII in one adult patient.

Skeletal muscle biopsies from some patients with late onset form displayed globular 'reducing body'-like inclusions, autophagic vacuoles and aggregation of several proteins e.g. chaperone proteins αB-crystallin and heat-shock proteins. We show a wide variety of phenotypes which can be used to optimize the diagnosis of GSDII.

P-7-5

INFANTILE-ONSET GLYCOGEN STORAGE DISEASE TYPE II: REPORT OF A CASE PRESENTED W0TH LUNG HYPOLASIA

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Glycogen storage disease type II, also known as Pompe disease, is a rare autosomial recessive disease due to deficiency of lysosomal acid alpha-glucosidase. The infantile-onset form is the most severe, and most patients present with hypotonia and cardiomyopathy in early infancy. We reported a case of Pompe disease in a patient who died at 8 months of age due to cardiorespiratory failure. A seven-month-old-girl was referred due to respiratory failure with preliminary diagnosis of left lung hypoplasia. She had a history of developmental delay and did not reach major motor milestones, such as sitting and standing. Moreover, she was unable to hold up the head or move normally, resulting in floppiness and head lag. Moderate hepatomegaly was observed on her physical examination. Further laboratory investigation revealed cardiomegaly and markedly elevated plasma creatine kinase levels. Bronchoscopy was performed and left lung hypoplasia was ruled out. The lung position was secondary to the pressure of the hypertrophic heart and pericardial effusion. The diagnosis of infantile Pompe disease was confirmed by the almost total absence of cellular acid a-glucosidase activity in her fibroblasts culture after her death. So, presentation of this patient aimed to emphasize that infantile-onset of Pompe disease, which is treatable nowadays, should be kept in mind in infantile patients with cardiac failure and neurodevelopmental delay.

P-7-6

CONGENITAL DISORDER OF GLYCOSYLATION TYPE 1A PRESENTING WITH DILATED CARDIOMYOPATHY IN A SAUDI BOY

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Introduction: Congenital disorder of glycosylation type 1a (CDG1a) is an autosomal recessive condition caused by deficiency of the enzyme phosphomannomutase. CDG1a is a multisystem disease with broad clinical spectrum of dysmorphy and organ involvement. **Case Report**: We report on 6-year old Saudi boy. Parents are first- degree cousins. Pregnancy and delivery were uneventful. He has global developmental delay, with developmental age of around 3 years. He has partial epilepsy well controlled by carbmizabine. Clinical examination revealed bilateral exotropia, brachycephaly, short philtrum, widely spaced inverted nipples, central hypotonia, absent reflexes, and intact cranial nerves. No evidence of pigmented retinopathy or lipodystrophy. Cardiovascular examination showed ejection systolic murmur at apex with normal heart sounds. Echocardiography confirmed dilated cardiomyopathy with moderate performance of the left ventricle. MRI showed increased CSF spaces with evidence of brain atrophy. Serum amino acids, liver function test, coagulation profile, lactate, acylcarnitine profile and urine organic acids were normal. Studies of serum transferring isoelectric focusing were consistent with CDG1. **Conclusion**: The majority of reported cases with CDG1a were of Caucasian origin, here we report a case of CDG1a presenting with developmental delay, dysmorhic features and dilated cardiomyopathy, with Arabian descent.

P-7-7

GLUCOSE LEVELS CONTROLLED WITH AN INSULIN PUMP IN A PATIENT WITH COMBINED GLYCOGEN STORAGE DISEASE TYPE 1A AND TYPE 1 DIABETES MELLITUS

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Glycogen storage disease type 1a (GSD Ia) results from a deficiency in glucose-6-phosphatase (G6P) and is clinically characterized by hepatomegaly and hypoglycemia with fasting.

Our patient initially presented with hepatomegaly at 12 months of age. Clinical diagnosis of GSD Ia was confirmed by measuring low G6P on liver biopsy (8% of control activity) and DNA analysis showing compound heterozygosity for G188R and Q347X mutations. Management included overnight infusions of polycose by gastrostomy tube and cornstarch during the day. He had an unremarkable course until age 3, then developed muscle pain with a persistently elevated creatine kinase (CK) at 3 to 4 times the upper limit of normal. An echocardiogram showed increased left ventricular and atrial dimension. He complained of myofacial pain, but skeletal muscle biopsy was completely normal.

At age 7.5 years he presented with polyuria, polydipsia, and severe hyperglycemia. He was diagnosed with Type 1 diabetes and started on insulin therapy. Interestingly, shortly after starting insulin therapy, his muscle pain and elevated CK resolved. Initial basal-bolus insulin therapy failed to control his blood sugars, but successful glycemic control was achieved with an insulin pump. **Conclusion**: The combination of GSD Ia and T1DM puts patients at extreme risk of hypoglycemia. Insulin infusion pumps can allow tighter control of blood sugar than other methods of insulin injection. These patients would be prime candidates for continuous glucose monitoring as it becomes available.

P-7-8

D-GLYCERIC ACIDURIA IN THAI INFANT WITH VESICAL STONE: FIRST REPORTED CASE

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D-Glyceric aciduria (OMIM 220120) is a rare inborn error of metabolism with a very heterogenous group of symptoms, with D-glyceric acid excretion as the chief common characteristic. D-Glyceric acid is an intermediate metabolite of serine and fructose metabolism. Clinical symptoms observed in D-glyceric aciduria are severe metabolic acidosis from birth and failure to thrive, intermittent vomiting and abdominal distension, progressive neurologic disease (hypotonia, opistotonus, myoclonic jerks), delayed motor development, axial hypotonia and apastic paraparesis although there are also asymptomatic patients with only D-glyceric acid excretion in urine.

We herein report one-year-4month-old boy (born in 2001) who developed acute renal failure and vesical stone. There was history of parental consanguinity (Hmong hilltribe from Northern Thailand). Hyperglycinemia was not observed. Quantitative plasma amino acid analysis was normal; however urine organic analysis demonstrated increased excretion of glycine and glycerate. Diagnosis of D-glyceric academia was made. He underwent resolution of vesical stone and improved clinically. Mutation analysis will be presented.

P-7-9

CLINICAL AND BIOCHEMICAL FEATURES OF GALACTOKINASE DEFICIENCY

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Objective: Galactokinase deficiency (GALK-D), an inborn error of galactose metabolism, results in juvenile bilateral cataracts in untreated patients. **Methods**: We retrospectively collected genetic, biochemical and clinical data on 23 patients (12 males, 11 females), median age 10.1 years (range 0.5–30.2), with GALK-D (residual GALK activity: 02.5 μmol/h/g Hb). **Results**: 16 patients were diagnosed by neonatal screening, 7 by family or selective screening. *GALK1* analysis revealed the Romani founder mutation P28T homozygous in 11/17 families, 10/11 originating from former Yugoslavia. Before treatment, median urinary galactitol was 14.8 mmol/mmol crea (7.4–21.8; norm <0.01), RBC galactitol >1 mmol/L (norm <0.002), urinary galactose 51.2 mmol/mmol crea (37.9–106.5; norm <0.01), and blood galactose 9.9 mmol/L (4.5–14.9; norm <0.05). On diet, median urinary galactitol decreased to 0.2 mmol/mmol crea (0.03–0.5), RBC galactitol to 0.01 mmol/L (0.005–0.15), urinary galactose to 0.05 mmol/mmol crea (0.0010.66), and blood galactose to 0.03 mmol/L (0–0.74). Long-term follow-up revealed neonatal cataract (5/15), juvenile cataract (9/23), mental retardation (4/21), hypoglycemia (4/21), microcephaly (4/21), and seizures (1/21). **Conclusion**: Apart from juvenile cataracts, GALK-D may result in neurological manifestation. Pathomechanisms are, however, still obscure. Dietary treatment of GALK-D leads to a significant decrease of galactose metabolites, thus resulting in prevention or regression of cataracts.

P-7-10

FRUCTOSE-1,6-BISPHOSPHATASE DEFICIENCY (FBP_1) IN THAI INFANT: FIRST REPORTED CASE

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Hereditary fructose 1,6-bisphosphatase deficiency (OMIM 229700) is characterized by episodic spells of hyperventilation, apnea, hypoglycemia, ketosis and lactic acidosis; with a precipitous and often lethal course in the newborn infant. Later episodes are often triggered by fasting and febrile illness. Gluconeogenesis is severely impaired.

We herein report a 2 year-3 month-old boy referring to us with chief complaint of severe vomiting, lethargy $\times 4$ days prior to admission. History of recurrent vomiting required at least 10 previous hospitalizations which usually improved upon receiving intravenous fluids and correction of severe metabolic acidosis ($HCO_3^- <10$) with bicarbonate. Semicomatose condition and marked hepatomegaly, but no hypoglycemia were observed upon admission to the intensive care unit. Quantitative plasma amino acids analysis was normal; however, urine organic acid analysis via gas-liquid chromatography and mass spectrometry demonstrated increased excretion of lactate, 3-hydroxybutyrate (3HB), glycerol, glycerol-3-phosphate (G3P), marker compounds of fructose-1,6-diphosphatase deficiency (FDPD) were found in the urine.

Enzyme assay and molecular analysis will be presented. This is the first reported case of FBP1 in Thailand.

P-7-11

3 SUCCESSFUL SPONTANEOUS PREGNANCIES IN 2 WOMEN WITH CLASSICAL GALACTOSAEMIA

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Introduction: Classical galactosaemia in women is associated with primary and secondary hypergonadotrophic hypogonadism. We report 3 successful spontaneous pregnancies in 2 women with classical galactosaemia who were not compliant with diet. **Case 1**: Currently aged 22 years, was diagnosed by newborn screening and commenced on treatment. Genotype Q188R/Q188R. No menarche by 16 years when FSH was 98.0 μ/L (n: 2.0–10) and LH was 55.0 μ/L (n: 2–12). Commenced on oestrogen patches 25 μg daily. Preconception FSH and LH levels decreased to 3.9 and 6.0 μ/l prior to the occurrence of a spontaneous conception, whilst on HRT, and delivery of a healthy infant. **Case 2**: Currently aged 31 years. She was diagnosed at 5 weeks with severe near-fatal multi-system complications after false negative newborn screening. GALT activity <0.5 μmol substrate/hr/gHb, genotype Q188R/F194L. She had menarche at 16 years followed by irregular periods. FSH was 54 μ/l; LH 30 μ/l at 17.5 years. She had a spontaneous conception and delivery of a healthy baby at 19 years with a second spontaneous conception and successful outcome at 23 years. **Conclusion**: A number of hypotheses have been proposed to explain gonadal dysfunction in females with classical galactosaemia, including abnormalities of FSH glycosylation and receptor action (resistant ovary syndrome) and premature depletion of follicles from a direct toxic effect of Gal-l-P or other metabolites. The more liberal exposure of dietary galactose in pre-adolescence, and during the reproductive years, in these females may favourably protect glycosylation of FSH and its functions.

P-7-12

CLINICAL AND MUTATION ANALYSIS OF GLYCOGEN STORAGE DISEASE TYPE III

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Type III glycogen storage disease (GSD III) is caused by a deficiency of amyloglucosidase (AGL). In this study we studied the clinical and laboratory findings and performed mutation analysis of AGL gene in 22 patients with GSD III. The diagnosis was carried out by the clinical manifestations and epinephrine loading test. The major clinical symptoms were recurrent hypoglycemia (100%), hepatomegaly (100%), splenomegaly (95%), growth failure (72%) and muscle weakness (50%). The major abnormal laboratory findings were elevation of transaminase (100%), acidosis (68%), hypoglycemia (68%), hyperlipidemia (59%) and elevation of creatinine kinase (70%). There was no common mutation. Three novel mutations (E1450X, 1944del8, Y429X) were identified. These data suggested that patients with GSD III showed variable phenotypic expression and heterogeneity of gebe mutations.

P-7-13

MOYAMOYA DISEASE IN A CHILD WITH GLYCOGEN STORAGE DISEASE TYPE 1A

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Glycogen storage disease (GSD) is known as a metabolic disease which is related to the occurrence of moyamoya disease. Only a few cases of moyamoya disease in patients with GSD have been reported before. Here, we report a 16-year-old girl with continuous hyperlipidemia and metabolic acidosis, who had received surgery for moyamoya disease 4 years ago. At the age of 12, she came to the hospital due to progressive headache and the diagnosis of moyamoya disease was confirmed by four-vessel cerebral angiography. At that time hyperlipidemia and fatty liver was detected, but she received no treatment. After four years, she was hospitalized for dizziness and general weakness. Her height, weight and sexual development were in normal range. The cheeks of her face were chubby and in both sides telangiectasia was noted. An enlarged liver was palpable but the spleen was not enlarged. Laboratory data revealed metabolic acidosis and elevated levels of lactic acid, total cholesterol, LDL-cholesterol, triglyceride, and uric acid. The diagnosis of GSD type 1a was considered and found Gly122Asp missense mutation in the partial genomic DNA sequence of the glucose-6-phosphatase catalytic (G6PC) gene. After the patient's diagnosis, her younger sister, who had no symptoms but hyperlipidemia, was also diagnosed with GSD type 1a. The present case suggests that in children with moyamoya disease the analysis of its pathogenesis should include extensive metabolic studies on glycogenosis.

P-7-14

FANCONI-BICKEL SYNDROME IN AN AFGHANISTAN REFUGEE

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Fanconi-Bickel syndrome is a rare type of glycogen storage disease caused by mutations in the glucose transporter GLUT2. The characteristic clinical features include hepatomegaly, fasting hypoglycaemia, rickets and marked growth failure. The renal disease (Fanconi syndrome) is characterised by proximal tubular acidosis, glycosuria, phosphaturia and aminoaciduria.

A female Afghanistan refugee, aged approximately 4 years presented with hepatomegaly, severe abnormalities of the femoral head and severe short stature, height z-score –7.96. Early medical details were sketchy but elevated ALP (450 U/L), glycosuria and proteinuria had been noted. She had been treated with vitamin D by injection together with oral calcium.

Our initial investigations showed hypophosphataemia (PO_4^{2-} 0.56 mmol/L), elevated ALP 670 U/L (80–350), 25-OH vitamin D 508 µmol/L (50–150), aminoaciduria, proteinuria (0.4 g/24 h), glycosuria and phosphate excretion of 8 mmol/L per day. Urine N-telopeptide was 518 nmol BCE/mmol creatinine (14–74). A skeletal survey showed evidence of severe rickets and osteopenia. She was treated with phosphate supplements (5 mmol/kg per day). Although the rickets healed, her growth in height remained poor. She became hypoglycaemic with moderate to severe ketosis after a 12 h fast. A liver biopsy was consistent with glycogen storage disease. The diagnosis of GLUT2 deficiency was suspected and is awaiting confirmation by molecular genetic studies.

Uncooked cornstarch (UCCS) was started as two doses of 0.5 g/kg per dose overnight and later increased to 1.0 g/kg per dose. After 12 months, she is doing well with no documented hypoglycaemia. She remains acidotic and bicarbonate supplements commenced (2 mmol/kg per day). Her height has increased 8 cm in 12 months, z-score –6.40.

P-7-15

PREGNANCY AND LACTATION BY A PATIENT WITH CLASSICAL GALACTOSEMIA DUE TO THE Q188R/R333W MUTATIONS

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A 31-year old patient, with the galactose-1-phosphate uridyltransferase (GALT) mutations Q188R and R333W, delivered two healthy boys after uneventful spontaneous pregnancies. She had been identified in the neonatal screening in her second week and was then symptomatic with severe liver disease. No GALT activity could be detected in erythrocytes by long-term incubation. She has been on dietary treatment and had a normal development. The patient chose to breast-feed her first baby and her galactose metabolites in blood and urine were monitored closely. A temporary increase in her galactose-1-phosphate levels with a maximum of 0.30 mmol/L day 2 after delivery was observed and normalized day 10. Liver transaminases stayed normal. She stopped breast-feeding at 8 months after delivery and became pregnant again 2 months later. The second baby was also breast-fed with an increase in galactose-1-phosphate for 3 weeks with a maximum level of 0.25 mmol/L on day 7. Only minor changes in her urine galactitol values were noted during the study period but the values stayed in the range of treated galactosemia patients. It is thus possible that patients with classical galactosemia can breast-feed without harmful influence on their health.

P-7-16

CONGENITAL HYPERINSULINISM – THE NEED TO COMPLETE THE WORK-UP IN THE PROBAND

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Congenital hyperinsulinism (CHI) is the most frequent cause of infantile hyperinsulinemic hypoglycemia. Mutations in any one of five genes have been associated with CHI. Diffuse and focal forms of the disease have been described. Treatment can either be conservative medical management or require surgery. Focal disease requires a limited pancreatectomy while diffuse disease requires a near-total pancreatectomy. We describe a male infant who delivered at 32 weeks following a pregnancy complicated by polyhydramnios. Birth weight was 7 lb. By day #4, infant was noted to have severe hyperinsulinemic hypoglycemia. He failed to respond to medical management. At 8 weeks of age he underwent a subtotal pancreatectomy followed by total pancreatectomy resulting in diabetes mellitus. Echocardiogram revealed a cardiomyopathy that improved with treatment. Brain imaging studies are abnormal and he is neurologically impaired. Molecular analysis revealed a mutation in the ABCC8 gene which codes for the sulfonylurea receptor which may predict a focal form of hyperinsulinism. Future siblings may benefit from prompt treatment and studies aimed at localizing potential focal lesions via pancreatic venous sampling, PET scanning or intraoperative ultrasound. Management of patients with CHI still mainly depends on clinical parameters. Results from mutational analysis are usually not available for the index case when clinical decisions regarding type of treatment or extent of surgery have to be made. This child will not benefit from this information but future siblings will and may avoid the risk of neurological damage from hypoglycemia and diabetes mellitus which is so frequently associated with this disorder.

P-7-17

DO TUMOR NECROSIS FACTOR (TNFα) POLYMORPHISMS PREDICT BRONCHOPULMONARY DYSPLASIA?

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Background: Twin studies reveal a strong genetic foundation for the 'new' BPD, which is characterized by hypoalveolarization. *In utero* inflammation may disrupt lung development through genes such as TNFα. **Hypothesis**: SNPs of the pro-inflammatory TNFα gene place preterm infants at increased risk for BPD. **Methods**: DNA was analyzed from 105 infants <1 kg for the TNFα SNPs: –1031, –863, –857, –308, and –238. PHASE (v2.1) was used to reconstruct haplotypes and estimate their frequencies within the study population. LD between pairs of SNPs were quantified using D' statistic. **Results**: Differences in antenatal steroid administration, in birthweight and maturity (all $p<0.001$), but not in race were found. LD between all pairs of loci indicated 2 SNP blocks at loci (–1031, –863, –857) and (–308, –238). Haplotype-specific analysis revealed no significant association between BPD severity and any of the 5-marker common haplotypes with 10 or more copies in this study population. Additionally, no significant association was observed in any 3-SNP haplotypes at (–1031, –863, –857) and 2-SNP haplotypes at (–308, –238). **Conclusions**: No association between BPD severity and the 5 TNFα SNPs was found. Joint analysis with other candidate genes as well as the use of family data may improve the power in association analysis between BPD severity and genetic foundations from the TNFα gene.

P-7-18

DIETARY CONSIDERATIONS FOR PATIENTS WITH FOOD ALLERGY AND INBORN ERRORS OF METABOLISM

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Background: The incidence of food allergy is estimated at 10–20% of the paediatric population. It may be assumed that children with inborn errors of metabolism (IEM) will have an equal risk of food allergy. Since many of these children are managed by dietary manipulation of macronutrients including protein, and since the most common food allergens are proteins from cow's milk, soy, egg, wheat, nuts and fish, the additional exclusion of these foods increases the risk of nutritional inadequacies and poor growth in these children. **Patients**: During the past year we have managed 8 children (aged 7 months 9 years) with food allergy and IEM of carbohydrate metabolism (n = 2) protein metabolism (n = 3) and fatty acid oxidation (n = 3). **Interventions**: Individualised nutritional management was tailored for each patient, dependent on the dietary prescription necessary to manage both the food allergy and IEM. This included the use of alternative infant formulas, modified weaning advice and additional parental education on food choices to avoid allergens and include appropriate alternative food sources or supplements. Ongoing nutritional assessment was required to ensure adequate energy, nutrients and food variety given the limitations of the diet. **Recommendations**: (1) The combined dietary restrictions and modifications required for the management of food allergy and IEM requires careful consideration. (2) Patients and their carers may require intensive education and support to understand and construct such complex diets, including alternative nutrient sources, methods of maintaining dietary variety and extensive skills in label reading. (3) Regular dietary assessment to monitor growth and nutrient intake is needed.

P-8-1

HEPATOCELLULAR CARCINOMA ASSOCIATED WITH CITRIN DEFICIENCY: CASE REPORT AND REVIEW OF THE LITERATURE

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We report two patients with hepatocellular carcinoma (HCC) associated with citrin deficiency (51 year-old male and 40 year-old female). The male patient had never shown any neurological symptoms as seen in adult-onset type II citrullinemia (CTLN2) patients before partial hepatectomy. He was incidentally detected a liver tumor at age 51. After the operation he temporally suffered from liver failure with hyperammonemia. The female patient started to have occasional disturbance of consciousness due to hyperammonemia at age of 39 years. She was admitted to our hospital and HCC was detected at age 40. DNA analysis of *SLC25A13* gene revealed a homozygosity for IVS11+1G>A mutation in both patients and a diagnosis of citrin deficiency was made. So far, except for our patients, 13 HCC patients with citrin deficiency have been reported. The mean age at detection of liver tumor was 37.1 ± 12.3 years. There was no CTLN2/HCC patient with hepatic viral infection except for one patient. In addition to the clinical data of the 13 patients reported previously, the findings in our patients suggest that citrin deficiency can be one of the key disorders causing HCC especially at younger ages and HCC associated with citrin deficiency can develop even before the onset of CTLN2.

P-8-2

DEVELOPMENT OF AN ACCURATE, PRECISE AND RAPID MASS SPECTROMETRIC METHOD FOR THE QUANTITATION OF ARGININE AND CITRULLINE IN PLASMA

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Objective: Traditional methods for the determination of the urea cycle metabolites, arginine and citrulline, are either time consuming (ion exchange chromatography) or inaccurate (e.g. thin layer chromatography). Therefore, we wanted to develop an accurate and precise measurement for arginine and citrulline in plasma. **Method/Results**: Plasma (5 µl) was added to 100 µl methanol containing the internal isotope standards (Cambridge Isotope Laboratories, USA) in a 96-well filter plate (Millipore, USA; 0.22 µm). The mixture was centrifuged into polypropylene microtiter plates, fully evaporated at 55°C under a stream of air. Subsequently, the amino acids were derivatized with butanolic acid to yield the corresponding butyl esters. After another evaporation step the residue was resolved in acetonitrile: water and injected into the tandem mass spectrometer (Quattro micro, Waters, USA). No chromatography was required. To increase accuracy of the results an external calibration curve was measured for each batch. Using this method we obtained an average intraday precision of 5.8% and an average interday precision of 6.8%. Run-time per analysis was 2.2 min. Furthermore we measured a recovery of 101%, a limit of detection of 0.5 µM, a limit of quantitation of 2 µM and a linear range between 2 and 250 µM. **Conclusion**: We have demonstrated that mass spectrometry provides a rapid and accurate platform for the analysis of urea cycle metabolites. Especially, this method seems to be well-suited for the follow-up of patients with urea cycle defects.

P-8-3

ORNITHINE TRANSCARBAMYLASE DEFICIENCY WITH ORGANIC ACID PATTERN MIMICKING LONG-CHAIN 3-HYDROXYACYL-CoA DEHYDROGENASE DEFICIENCY

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Objective: Both ornithine transcarbamylase deficiency (OTCD, OMIM 311250) and long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD, OMIM 600890) may be accompanied by liver failure with hyperammonaemia. We present a newborn male with OTCD in whom urinary organic acids pattern mimicked LCHADD. **Case report**: Tachypnoea, fever and coma developed on the 3rd day of life. Biochemical testing revealed liver failure with hyperammonaemia. Acetaminophen (paracetamol) was included in complex therapy. The patient died at the age of 10 days. **Biochemical findings**: Hyperammonaemia (1355 μmol/l), elevation of transaminases, coagulopathy, hyperlactacidaemia and normoglycaemia were detected. Hyperaminoacidaemia with normal arginine concentration and citrulline under limit of detection was found. Analysis of urine showed massive 3-hydroxydicarboxylic and dicarboxylic aciduria, mild ketonuria, mild orotic aciduria and normal uraciluria. Plasma total and acylcarnitine concentration were elevated. Tandem mass spectrometric analysis revealed hyperaminoacidaemia and normal acylcarnitines profile. The diagnosis of OTCD was confirmed by enzyme assay of OTC in liver tissue and on molecular genetic level. **Discussion**: Association of acute liver failure in OTCD and organic acids pattern mimicking LCHADD has not been reported yet. Exposition to acetaminophen was probably the cause of secondary abnormalities in long-chain fatty acid oxidation function in our patient.

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P-8-4

MANAGEMENT OF UREA CYCLE DEFECTS IN THAI PATIENTS: PHRAMONGKUTKLAO HOSPITAL EXPERIENCE

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The urea cycle disorders (UCD) is uncommon inborn error metabolism in Thailand. Clinicians usually delay diagnosis due to unawareness and no available laboratory support. The first case of our report was 4-day-old newborn initially appeared normal but rapidly developed anorexia, hyperventilation hypothermia seizure and coma. She was referred from primary care hospital in the rural to tertiary care hospital at 8-day-old. The patient already had brain edema and sign of brain herniation. Laboratory investigations revealed hyperammonemia (821 mmol/L), high plasma glutamic acid low plasma citrulline and low level of urinary orotic acid. This case was suspected to be CPSI or NAGS deficiency. She was treated by peritoneal dialysis, mannitol infusion, high dose sodium benzoate, sodium phenylacetate and arginine. Her clinical condition couldn't be improved and expired at 36-day-old.The second case was milder (or partial) urea cycle enzyme deficiencies. This patient was referred to our hospital at 5-year-old. She had history of recurrent vomiting, lethargy and coma triggered by illness or protein ingestion at almost any time of life. Her sister had lethargy and coma, and died at 1.5 years old. She had mild hyperammonemia (105 mmol/L), low plasma citrulline, normal plasma ornithine and high level of urinary orotic acid. She was suspected to be OTC deficiency. She was advised low protein diet and admission in hospital whenever her illness. Genetic counseling is provided to both families.

P-8-6

CLINICAL MANIFESTATIONS OF JAPANESE LYSINURIC PROTEIN INTOLERANCE SUBJECTS

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Objective: Lysinuric protein intolerance (LPI; MIM# 222700) is a rare autosomal recessive disease. Several mutations in the human SLC7A7 (solute carrier family 7, member 7, MIM# 603593) gene were proven to be responsible for LPI. In this study, to evaluate the genotype-phenotype correlation in Japanese patients with LPI, we investigated the clinical aspects of Japanese affected subjects by questionnaire. **Methods**: Subjects are 27 Japanese patients (24 families) with LPI, in whom mutations in SLC7A7 gene detected in our laboratory. (Informed consent for genetic analysis was obtained.) A questionnaire about clinical aspects was designed and mailed to each physician. **Results**: A total of 21 (77%) physicians replied to this survey. Mean age at onset was 7.9 (range 0.8～38) years, while that of diagnosis was 11.9 (range 0.5～40) years. Most of patients showed protein aversion (The frequency was 95%), short stature (77%), Hepatomegaly (60%), mental retardation (55%), leucopenia (71%), and elevated serum LDH (90%). The frequencies of hyperammonemic encephalopathy (52%), bone fracture (17%), pulmonary involvement (5%) were relatively low. Immunological complication was associated with the S238F mutation more closely than with the R410X mutation. **Conclusion**: Japanese patients with LPI also showed clinical heterogeneity. But the genetic information of SLC7A7 gene did not seem enough to explain the clinical heterogeneity.

P-8-7

TREATMENT OF EARLY-ONSET ARGININOSUCCINATE LYASE DEFICIENCY – OPTIMIZING THE ARGININE DOSE

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Argininosuccinate lyase deficiency (ASLD) is a rare disorder of urea cycle, due to mutation in the argininosuccinate lyase gene. Arginine supplementation is a mainstay of the medical therapy. The recommended doses are quite high (up to 0.6 g/kg/day) and associated with supranormal blood levels of arginine, leading to increased accumulation of argininosuccinate (ASA), urea cycle intermediate potentially toxic for brain and liver. We studied the effect of different arginine doses on metabolic balance of five Finnish ASLD patients (three males, two females) followed up at our clinic. In order to reduce their ASA levels, the arginine doses were substantially reduced with simultaneous introduction of sodium phenylbutyrate (3 patients) or sodium benzoate (2 patients) to remove ammonia via alternate routes. All patients have also protein restricted diet, and multivitamin and calcium supplementations since diagnosis, and three patients have antiepileptic medication.

We compared plasma amino acid profiles, ammonium levels and aminotransferases of our ASLD patients at the time of their high and low arginine doses. The mean of the high dose of arginine was 0.81 g/kg/day, and the mean of the low dose 0.10 g/kg/day. The respective mean plasma concentrations of ASA were 719 and 232, arginine 210 and 76, citrulline 987 and 159, and glutamine 529 and 802 μmol/l; of NH4 45 and 37 μmol/l, and of alanine aminotransferase (ALT) 176 and 35 U/l.

The lower arginine dose was thus associated with lower levels of potentially toxic ASA and of ALT, indicating milder liver damage. Glutamine levels were slightly higher, but ammonium levels were comparable, and hyperammonemic episodes were not more common during the low dose of arginine.

P-8-8

IDENTIFICATION OF ONE MICROINSERTION, TWO NOVEL MISSENSE MUTATIONS AND ONE SPLICE-SITE MUTATION IN THE *OTC* GENE IN CHINESE PATIENTS WITH ORNITHINE TRANSCARBAMYLASE DEFICIENCY

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Ornithine transcarbamylase deficiency (OTCD) is the most common urea cycle disorder characterized by hyperammonaemia. It is caused by mutations in the *OTC* gene located on the X chromosome. Up to date, more than 278 mutations of *OTC* have been identified. In this study, we performed mutation analysis of the *OTC* gene in 12 unrelated Chinese patients diagnosed with OTC deficiency by direct DNA sequencing of all the exons and the flanking introns. We identified 4 novel mutations (c102-103insCTT, Asn198Ile, Glu239Gly and IVS5+1G>T) and 6 previously reported mutations (Arg26Gln, Glu122Gly, Arg129His, Lys144Term, Thr178Met and IVS7+1G>T) in 10 patients. The Arg129His mutation was found in 2 unrelated families with the late-onset phenotype. In one of the patients, no mutation was detected in the screened regions of the gene. Interestingly, a previously reported polymorphism Leu111Pro, was found. This polymorphism was only reported once in the literature in an OTCD patient. The rediscovery of Leu111Pro in another OTCD patient suggested that Leu111Pro might be a disease-causing mutation rather than a polymorphism. Mutation analysis of the *OTC* gene serves as a direct, efficient, and reliable way to diagnose OTCD, as well as to detect female carrier status and to perform prenatal diagnosis in families with an OTCD history.

P-8-9

LABORATORY FINDINGS IN CITRIN DEFICIENCY

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Citrin deficiency is an autosomal recessive disease discovered in Japan which shows at least two phenotypes: adult-onset type II citrullinemia (CTLN2) and neonatal intrahepatic cholestasis (NICCD). CTLN2 patients have been diagnosed on the basis of well-established criteria, including their symptoms and laboratory findings such as high blood ammonia, increased citrulline, arginine, ratio of threonine to serine and pancreatic secretory trypsin inhibitor levels, and decreased hepatic argininosuccinate synthetase activity/protein levels. NICCD patients show various and transient symptoms and laboratory findings, such as increased plasma citrulline, arginine, threonine/serine ratio, methionine, tyrosine, galactose, total and direct bilirubin, total bile acids, and/or α-fetoprotein with little or no increased blood ammonia [see Kobayashi and Saheki, GeneReviews (2005) www.genetests.org]. By the age of 1 year, most NICCD patients become apparently healthy probably due to some sort of metabolic change, adaptation or compensation. But one or more decades later, some of them suffer from CTLN2. It is difficult to diagnose NICCD without SLC25A13 mutation analysis. In order to understand citrin deficiency in greater detail, we report here a comparison of laboratory findings between NICCD (220 cases) and CTLN2 (155 cases) diagnosed by DNA analysis at Kagoshima University. A follow-up survey of NICCD patients will be needed in order to clarify symptoms following the improvement of NICCD and before the onset of CTLN2, to find early signs of CTLN2 onset, and to know the long-term prognosis of citrin deficiency.

P-8-10

CITRULLINEMIA: CLINICAL AND BIOCHEMICAL PROFILE OF INDIAN PATIENTS

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Objective: To determine clinical biochemical profile of Indian children with citrullinemia. **Subjects and method**: We selected 316 critically ill babies admitted in NICUs with clinical suspicion of IMD. There were 7 babies with citrullinemia (2.21%). **Results**: Of these 7 babies, 4 were males and 3 females. Two children were born out of 3rd degree consanguineous marriage (28.57%). The earliest presentation was 2nd day of life and latest by 6th day. Of these 6 babies expired (85.71%). The mean values for biochemical parameters were: ammonia 424 μmol/L, orotic acid 763 μmol/mmol Cr., Pl. citrulline 1014 μmol/L, Pl. arginine 107.4 μmol/L, Pl. glutamine 958.3 μmol/L and Pl. ornithine 74.83 μmol/L. All of them had normal carnitine/acyl carnitine profile and urine GC-MS for organic acids. High Ur orotic acid picked up all these babies in the very early stage. All except one were treated with sodium benzoate [SB] and one child received peritoneal dialysis in addition to SB. The only surviving child received arginine, SB and special diet UrC1 from ComidaMed (Germany). She has excellent weight gain and is thriving well. Motor and mental milestones are normal. Though her citrulline is always above 500 μmol/L, her ammonia is rarely above 60 μmol/L and Ur. orotic acid is always <11 μmol/mmol Cr. **Conclusion**: All sick newborns should be screened for citrullinemia as early treatment with SB, arginine and proper diet may lead to good recovery. Urine orotic acid screening is very helpful in early diagnosis. All the families had lost at least one child with similar disorder.

P-8-11

HUMAN CITRIN DEFICIENCY MODEL MOUSE

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A deficiency of citrin, the liver-type mitochondrial aspartate/glutamate carrier, results in adult-onset type II citrullinemia (CTLN2), characterized by hyperammonemia and citrullinemia, and neonatal intrahepatic cholestasis with multiple aminoacidemia and galactosemia (NICCD) in humans. A variety of the symptoms comes from loss of essential roles of citrin in supplying aspartate to the cytosol from mitochondria and transport of cytosolic NADH reducing equivalent into mitochondria as a member of malate/aspartate shuttle. Gene knockout (KO) of mouse Slc25a13, encoding citrin, did not generate any significant symptoms of CTLN2 and NICCD, although the perfused liver of citrin-KO mice revealed severely disturbed ureogenesis from ammonium chloride and gluconeogenesis from lactate. To test the hypothesis that the presence of glycerophosphate shuttle, another NADH shuttle, could circumvent the deficiency of citrin, we generated citrin and mitochondrial glycerophosphate dehydrogenase (mGPDH) double KO (double-KO) mice by mating citrin-KO and mGPDH-KO mice. Here we show that the double-KO mice present with hyperammonemia, citrullinemia and hypoglycemia. Interestingly, the double-KO mice become more severely hyperammonemic following oral sucrose administration, which confirms toxicity of sugar catabolism which generates cytosolic NADH in the liver in citrin deficiency.

P-8-12

OUTCOMES AMONG MALE OTC PATIENTS TREATED WITH AMMONAPS®

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A post approval study was conducted from 1999-2001 in eight European countries for urea cycle patients treated with Ammonaps®. One hundred eighty-seven patients were enrolled of which 34 were ornithine transcarbamylase deficiency males (OTC). Eighteen patients were >28 days of age, 12 <28 days, one was diagnosed antenatally, and data on three were missing. Patients received a median Ammonaps dose of 273 mg/kg/day (IQR 146–328). The long-term prognosis of UCD patients' has shown that their IQ's decrease in inverse proportion to the degree and frequency of hyperammonemic episodes they experience. Previous studies have shown a mean of three to four episodes of hyperammonemia per patient with poor neurological and cognitive outcomes. Seventy percent of the patients in this study experienced no hyperammonemic episodes. Normal cognitive function was reported in 47% of patients and normal neurological function was reported in 53% of the patients in this study. The median ammonia level was 56 µmol/L (IQR 40–64) among patients experiencing no episodes of hyperammonemic decompensation. Among patients experiencing hyperammonemic episodes the mean ammonia level was >2× the upper limit of normal. Daily treatment with Ammonaps resulted in fewer hyperammonemic episodes and improved neurological and cognitive outcomes than previously reported.

P-8-13

GENETIC ANALYSIS IN SEVEN JAPANESE PATIENTS WITH OTC DEFICIENCY

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Objective: Genetic analysis is an important tool for the early diagnosis with OTC deficiency. **Aim**: To detect the OTC gene mutations in seven Japanese patients. **Patients and methods**: We performed a retrospective study constituted of seven Japanese patients (M/F 5/2, average age of onset; 19 months, range; 4 days to 6 years) from five families with OTC deficiency between 1970 and 2005 in Kitasato university hospital. The diagnostic criteria of OTC deficiency were including hyperammonemia, characteristic amino acid profiles (hyperglutaminemia, hypocitrullinemia), orotic aciduria and OTC gene mutations and/or decreased hepatic OTC activities. All ten exons and intron-exon boundaries of the OTC gene were analyzed by PCR direct sequence of genomic cDNA obtained from peripheral blood lymphocytes in seven patients and their family members. **Results**: Genetic analysis revealed three previously reported mutations in four patients from three families and two novel mutations in three patients from two families. One novel mutation detected was a T to C substitution in codon 193 in unrelated patient and the other was a A to G substitution in intron 4+9 in two related patients. One novel mutation Trp193Arg detected was estimated to be a 'de novo' because the same mutation was not detected in her parents.The other A to G substitution in intron 4+9 detected was a maternal transmitted mutation.All three previously reported point mutations detected (Arg129Leu, Arg277Gln, Trp193Ter) were transmitted maternally. **Conclusion**: All two novel and two of the three known mutations detected in our study occurred a hot spot with CpG dinucleotides. Diagnostic usefulness of the OTC gene analysis will be discussed.

P-9-1

SCREENING FOR DEFECTS IN PURINE DE NOVO BIOSYNTHESIS BY TLC

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Two inherited enzyme deficiencies have been described in a ten-step purine de novo biosynthetic pathway (PDNS) – adenylosuccinate lyase deficiency and AICAribosiduria. We present here how thin layer chromatography (TLC) can be used for screening defects in second part of the PDNS. We synthesized dephosphorylated intermediates of the pathway and analysed it by TLC. One dimensional TLC was performed using cellulose plates using butanol:acetic acid:H_2O (4:1:1) as a solvent. Two dimensional TLC was performed using cellulose plates using isopropanol:NH_3 (4:1) and butanol:acetic acid:H_2O (4:1:1) as solvents. Detection was performed by diazoted sulfanilic acid (Pauly's reagent) after mild acidic hydrolysis of developed plates in vapours of hydrochloric acid (30 min). Potential usefulness of the method was demonstrated on samples from humans and Chinese hamster ovary cells defective in enzyme of PDNS. Detection with Pauly's reagent is very selective for the compounds of interest and no interferences were observed in 300 healthy urines analysed. One dimensional TLC allowed simple screening of the species of interest and two dimensional TLC confirms identity of the diagnostic compounds.

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P-9-2

ANALYSIS OF AMINOIMIDAZOLE RIBOSIDES BY CAPILLARY ELECTROPHORESIS – DIAGNOSING INBORN ERRORS OF SECOND PART OF PURINE DE NOVO SYNTHESIS

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Capillary electrophoresis (CE) is a powerful technique in diagnosing purine and pyrimidine inherited metabolic defects. Two inherited deficiencies have been described in purine de novo synthesis (PDNS). We present here a CE method for diagnosing defects in the second half of PDNS (from sixth to tenth enzymatic conversion) based on analysis of aminoimidazole ribosides – dephosphorylated intermediates – in urine. Separation were performed in uncoated fused silica capillary using two electrophoretic systems: 60 mmol/L borate – 2-amino-2-methyl-1-propanol – 80 mmol/L sodium dodecylsulfate (pH 9.6) and 200 mmol/L phosphate – sodium (pH 1.8). Conditions reported allowed separation of all the metabolites from major urinary constituents with analysis time less than 10 min and separation efficiency of 220 and 350 thousands theoretical plates per meter for borate and phosphate system, respectively. The intra- and interday imprecisions were less than 4.4% and 9.9% CV. Potential usefulness of the method was demonstrated on samples from patient with adenylosuccinate lyase deficiency and Chinese hamster ovary cell lines defective in PDNS. CE is useful and effective tool in analysis of aminoimidazole ribosides which enables diagnosis of known as well as not so far identified inherited defects of PDNS pathway.

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P-9-3

LESCH-NYHAN SYNDROME IN A THAI INFANT: FIRST REPORTED CASE

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Lesch-Nyhan syndrome (OMIM 300322) is a rare metabolic disorder of purine metabolism, causes by virtually complete deficiency of the hypoxanthine-guanine phosphoribosyltransferase (HPRT) enzyme. Gene map locus is on Xq26-q27.2. The disorder is characterized by hyperuricemia, choreoathetosis, spasticity, mental retardation and compulsive self-mutilation. Usually clinically normal at birth and developmental delay is evident by 6 months of age. Self-mutilation is present in most patients and may begin as early as age 6 months. Administration of allopurinol, an inhibitor of xanthine oxidase, reduces serum uric acid levels and prevents most of the symptoms associated with hyperuricemia.

We herein report 1-year-9-month-old male infant (born in 1995) with hyperuricemia, choreoathetosis, spasticity, mental retardation, compulsive self-destructive behavior and self-mutilation. He was referred to us because of delayed gross motor development (at age 5 months) and urine was noted to be turbid and contain yellow crystals. Orange crystals in diaper (sand or gravel like) or uric acid crystalluria was observed. Urine organic acid analysis was normal. Allopurinol treatment reduced uric acid concentrations; however he lost to follow up. HPRT assay was not yet done since it is not available in Thailand. Mutation analysis will be presented.

P-9-4

DIAGNOSTIC APPROACH TO UNEXPLAINED HYPOURICEMIA

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Hypouricemia is defined as a serum urate levels less than 2 mg/dL (119 μmol/L). Primary hypouricemia is related to several purine metabolic disorders. Recent identification of the urate transporter in the kidney (URAT1) led to the molecular elucidation of idiopathic renal hypouricemia (MIM 220150). Secondary hypouricemia is linked with the use of uricosuric drugs etc. Hypouricemia is sometimes overlooked. Therefore we have set up the diagnostic guideline. Uric acid was quantified by enzymic method and red cell enzyme, urinary nucleosides were measured by methods adapted to HPLC. Results: A proposed scheme for the investigation is as follows. Estimation of: (1) excretion fraction of uric acid, (2) urinary xanthine, S-sulphocysteine, thiosulfate, (3) measurement of (deoxy)guanosine, (deoxy) inosine in urine, in positive case performing assay of purine nucleoside phosphorylase in erythrocytes, (4) analysis of URAT 1 gene. The evaluation of clinical history with attention to renal failure, urolithiasis, seizures and immunodeficiency is important. These tests allow to differentiate (a) idiopathic renal hypouricemia, (b) hereditary xanthinuria, (c) molybdenum cofactor deficiency, (d) purine nucleoside phosphorylase deficiency. Also primary hyperuricemia can be excluded. We have detected four new families with hereditary xanthinuria, one case of hereditary renal hypouricaemia and one patient with molybdenum cofactor deficiency from 3300 blood and urine samples received during last three years. In conclusion – the exclusion of primary hypouricemia allows to search for the other causes. Available guideline will help for early diagnosis of purine disorders associated with hypouricemia.

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P-10-1

MR IMAGING AND PROTON MR SPECTROSCOPY IN JAPANESE AUTOSOMAL DOMINANT LEUKODYSTROPHY

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Autosomal dominant leukodystrophy (ADLD) is an extremely rare disease. The etiology has been undetermined. We recently encountered 5 cases of a Japanese family with dominantly inherited leukodystrophy in three generations. We used localized proton magnetic resonance spectroscopy (MRS) to assess metabolic abnormalities in the frontal and occipital lobe, and the cerebellum in a Japanese family with ADLD. In two progressive cases, MRS showed significant decrease of N-acetylasparatate (NAA)/Cceatine (Cr) ratio, and slight increase of choline (Cho)/creatine (Cr) ratio in the frontal and occipital lobes, and the cerebellum. NAA is neuroaxonal marker and a decrease of that compound is observed in the disease process with neuronal loss and damage. Cho is the hallmark of a cell membrane and that elevation of Cho indicates active demyelination. It is thought that a subsequent arrest of this process may lead to a Cho/Cr ratio in an already demyelinated region.

P-10-2

SUBSTRATE REDUCTION THERAPY IN SANDHOFF DISEASE: EVIDENCE FOR IMPROVEMENT IN NERVOUS FUNCTION IN PATIENTS TREATED WITH MIGLUSTAT

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Substrate reduction therapy with miglustat offers the first specific therapy for patients with neurological glycosphingolipid storage disorders. We describe 3 female Sandhoff patients from an extended Pakastani kindred who were treated with miglustat 100 mg bd or tds, according to tolerability. The patients were assessed at baseline and after 6 months of therapy. Plasma levels of miglustat at 6 months ranged from 0.98 to 1.95 mcg/ml and CSF levels were between 14% and 22% of plasma levels. The major adverse effects were diarrhoea and weight loss, which ranged from 5.6% to 9.8% of body weight. Brainstem auditory evoked responses at baseline showed delayed onset latencies in all 3 patients. In 2 patients who had repeat measurements at six months, latencies were within normal limits. In 2 patients, horizontal saccadic latencies were measured using a saccadometer; median latencies from 100 separate saccades were calculated. Median saccadic latencies at baseline were markedly prolonged at 710 ms and 410 ms (mean control value 160 ms). After 6 months there was improvement in both patients to 395 ms ($p = 0.02$) and 293 ms ($p = 0.002$) respectively. MRI with diffusion tensor imaging was performed on all patients. Consistent increases in fractional anisotropy were observed in a range of brain regions after 6 months therapy. Though it is difficult to monitor clinical progression of a chronic neurodegenerative disease over such a short interval, and validated surrogate markers are not available, these data suggest that treatment with miglustat can improve nervous function in Sandhoff disease.

P-10-3

CAN FABRY DISEASE BE MISDIAGNOSED AS SYSTEMIC SCLEROSIS?

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Fabry disease is an X-linked inherited disorder due to mutations in the gene encoding alpha-galactosidase A, a lysosomal enzyme. The enzymatic defect leads to the systemic accumulation of incompletely metabolized glycosphingolipids, primarily the substrate globotriaosylceramide (GL 3), in several tissues. The skin, kidney, heart and cerebrovascular systems can be affected. We report a case of Fabry disease with Raynaud's phenomenon, telangiectasias and swelling of the fingers referred to our institution as systemic sclerosis. The patient, a 33-year-old, male, had telangiectasias, Raynaud's phenomenon and recurrent chest pain. Physical examination showed Raynaud's phenomenon and swelling of the fingers, telangiectasias on face and trunk, cutaneous thickening and angiokeratomas on the shoulder, periumbilical and penis. Laboratory tests included erytrocyte sedimentation ratio (8 mm/1 st h), absent of antinuclear antibodies, urea = 70, creatinine = 2.99 mg/dl and proteinuria of 1.57 g/l. Electrocardiography was normal. Doppler echocardiography disclosed a left ventricule hypertrophy, moderate aortic enlargement, diastolic dysfunction. Skin biopsy showed epidermis with hyperkeratosis and mild acanthosis on dermis. Kidney biopsy showed moderate inflammatory reaction, intersticial fibrosis and tubular atrophy. Leucocyte alpha galactosidase activity ratio was decreased to 0.15 μmol/L/h (normal 1.37–7.99 μmol/L/h). Agalsidase beta and analgesics were given and caused the reduction of angiokeratomas, maintenance of renal function and improvement of his life quality. Fabry disease could be misdiagnosed as Systemic Sclerosis. A careful patient history and physical examination are fundamental tool to diagnosis.

P-10-4

NEUROTROPHIC FACTORS AND THEIR EFFECTS ON NEURONAL SURVIVAL IN THE BRAIN OF A MOUSE MODEL OF GAUCHER DISEASE

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Gaucher disease is a glycosphingolipid storage disease caused by a deficiency of glucocerebrosidase, resulting in the accumulation of glucosylceramide in lysosomes. The neuronopathic forms of this disease are associated with neuronal loss and neurodegeneration. However, the pathophysiological mechanisms leading to prenatal and neonatal death remain uncharacterized. To investigate brain dysfunction in Gaucher disease, we studied the effects of neurotrophic factors during development in a mouse model of Gaucher disease. The expression of brain-derived neurotrophic factor and nerve growth factor was reduced in the cerebral cortex, brainstem, and cerebellum of Gaucher mice, compared with that in wild-type mice. ERK 1/2 expression was downregulated in neurons from Gaucher mice and correlated with a decreased number of neurons. These results suggest that a reduction in neurotrophic factors could be involved in neuronal loss in Gaucher disease.

P-10-5

ANALYSIS OF THE EFFECT OF CHEMICAL CHAPERONE ON HUMAN MUTANT β-GALACTOSIDASE EXPRESSING MOUSE CELLS

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G_{M1}-gangliosidosis is a lysosomal storage disorder caused by deficient lysosomal β-galactosidase. A marked decrease in enzyme activity results in progressive accumulation of substrate (G_{M1}-ganglioside) in the cell, particularly in neurons, leading to severe neuronal dysfunctions. A chemical chaperone compound, *N*-octyl4-epi-β-valienamine (NOEV) have been shown to stabilize various mutant human β-galactosidase proteins against misfolding and increase the residual enzyme activities, both in cultured human patients' fibroblasts and enzyme deficient knockout mouse fibroblast expressing human mutant proteins. In this study, we examined 40 novel mutations of β-galactosidase for NOEV treatment by expressing in mouse fibroblasts and found that 8 mutations showed significant increase of enzyme activities. Immunofluorescence analysis using flag-tagged human β-galactosidase gene expression constructs showed restoration of lysosomal accumulation of G_{M1}-ganglioside by NOEV in R201C-expressing cells. We also examined NOEV effect on neuronal cells and revealed that treatment with NOEV on the primary astrocyte from G_{M1}-gangliosidosis mouse model increased enzyme activity and resulted in the reduction of G_{M1} accumulation. These results suggest that NOEV has a potent effect on human mutant β-galactosidase proteins expressing in mouse cells.

P-10-6

N-OCTYL-β-VALIENAMINE WORKS AS A CHEMICAL CHAPERONE FOR MUTATED β-GLUCOCEREBROSIDASE

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Gaucher disease (GD) is a most common lysosomal glycolipid storage disorder, due to mutations in the β-glucocerebrosidase (GBA) gene. It was demonstrated that N-octyl-β-valienamine (NOV), an inhibitor of human GBA, worked as a chemical chaperone to accelerate transport and maturation of F213I mutant GBA in human fibroblasts. In this study, we screened for GD fibroblasts with positive response to NOV and tested the effects of NOV on recombinant GBA. We found out GD fibroblasts with mutations N188S/G193W were also the target of NOV, in which NOV caused a 2.5-fold increase in cellular enzyme activity. The effects were not observed in other fibroblasts with GBA mutations D409H and nt 1447 del 20 ins TG. Using site-directed mutagenesis, we constructed plasmids containing wild-type and several mutations in flag-tagged GBA gene. The plasmids were transiently expressed in COS-7 cells and NOV was applied in culture medium for 24 h. In anti-flag immunoprecipitation products, the addition of NOV to COS-7 cell medium leads to 2.5-, 2.6-, 2.1-, and 2.8-fold increase in the activity of N188S, G202R, F213I and N370S respectively. However, no significant changes were observed in the activity of the wild-type, G193W and L444P mutated proteins. The immunofluorescent staining and endoglycosidase-H treatment revealed the recombinant flag-tagged GBA variants were retained in the endoplasmic reticulum despite of wild-type and mutant variants. It suggested that NOV stabilized N188S, G202R, F213I and N370S mutant GBA in the endoplasmic reticulum.

P-10-7

MEASUREMENT OF GLOBOTRIAOSYLCERAMIDE IN PLASMA AND URINE FOR DIAGNOSIS AND THERAPY MONITORING IN FABRY DISEASE

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The assay for measuring globotriaosylceramide (Gb_3Cer) levels in plasma and urine of patients with Fabry disease (FD) by tandem mass spectrometry has been established. Male reference ranges in urine were 1.7–119 μg Gb_3Cer/mmol creatinine (n = 38), in plasma 4.3–11 μg Gb_3Cer/ml (n = 28). The ranges for FD patients were in urine 440.9–1585.7 μg Gb_3Cer/mmol creatinine (n = 31), in plasma 13.2–50.1 μg Gb_3Cer/ml (n = 25). In plasma significant decrease of Gb_3Cer was found in all 14 patients receiving enzyme replacement therapy (ERT), whereas in urine the decrease was observed only in nine men. Significant shift in composition of Gb_3Cer isoforms towards long chain fatty acids (C24) in FD patients was found. ERT initiated positive tendency to normalize isoform pattern. Compared to formerly used techniques, this method, based on Boscaro et al. (2002), requires a small amount of a crude sample and a single dilution step without lipid extraction. Because of its specificity, sensitivity, and high-throughput, the assay provides useful diagnostic mean in Fabry disease and represents a model of detection strategy for other lysosomal disorders.

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P-10-8

RENAL INVOLVEMENT AS A RARE COMPLICATION OF DORFMAN-CHANARIN SYNDROME: A CASE REPORT

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Dorfman-Chanarin syndrome is a rare, autosomal recessive inherited lipid storage disease with congenital ichtiotic erythroderma due to an acylglycerol recycling defect. It is characterized by accumulation of neutral lipids in different tissues. Liver, muscle, ear, eye and central nervous system are generally involved, so we presented a patient with severe ichtiosis, lipid vacuoles in neutropils from bone marrow aspiration and a rare renal involvement.

A seven-month-old girl was presented with frequent respiratory infection, congenital ichtiotic erithroderma and suspicion for immune deficiency. On her physical examination hepatomegaly, developmental delay, palmar and plantar hypercheraposis and increased deep tendon's reflexes with clonus and high tonus were found. Laboratory investigations revealed elevation at tranaminases levels, hypo-albuminemia, hypergammaglobulinemia, presence of autoantibodies and eosinophilia. Vacuolization in leukocytes obtained by bone marrow aspiration confirmed Dormann-Chanarin syndrome, whereas no mutation at RAG1-2 and ARTEMIS genes ruled-out immune deficient status of the patient. At the age of eight months the patient died from severe edema, hepatic and respiratory failure. Her necropsies demonstrated microvesicular lipid accumulation not only at the liver but also at the renal species.

The variability of involvement of different system in Dorfman-Chanarin syndrome is well described, however the renal findings has not been reported previously at the literature.

P-10-9

AGE ADJUSTED SEVERITY SCORING FOR FABRY DISEASE

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Fabry disease is an X-linked LSD which affects both males and females and often presents in childhood. In the absence of a biomarker of disease burden or response to therapy, scoring systems, such as the MSSI have been developed. Such global scores are confounded by age-related progression, making comparisons across age groups invalid. **Aim**: To define an age and gender adjusted severity scoring system for FD. **Methods**: The MSSI scores, as adapted for data collected in the (Fabry Outcome Survey) FOS database, were calculated for 344 Mmales and 369 females with confirmed FD. Using linear regression an equation based on age and gender was derived from data where patients from UK were excluded. The initially excluded patients were used for validation. Studentized residuals for patients with specimen nonsense (R227X, n = 23) and missense (N215S, n = 33) mutations were compared. **Results**: The age adjusted severity score of a UK cohort of adult and paediatric patients was found to follow the model estimated by the European cohort thereby validating the methodology. The progression with age was estimated to be 4.5 and 2.2 units per decade among males and females respectively. The residuals of patients with nonsense mutation R227X were higher compared to those for patients with missense mutation N215S ($p < 0.001$). **Conclusion**: An age and gender adjusted scoring system for FD has been defined and validated in independent cohorts of patients. This scoring system allows the comparison of disease severity in different subgroups such as genotypes without age or sex as confounding factors.

P-10-10

IMPAIRMENT OF SURVIVAL OF DORSAL ROOT GANGLION NEURONS AND RETINAL NEURITE OUTGROWTH IN CULTURE FROM A MOUSE MODEL OF SANDHOFF DISEASE

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Sandhoff disease (SD) is a heritable neurodegenerative disease resulting from impaired degradation of GM2 ganglioside and related substrates. A mouse model of SD created by gene targeting displays progressive neurologic manifestations, and mimics pathological and biochemical features of patients with that disease. In the present study, we examined histopathological characteristics and survival/neurite outgrowth capability of dorsal root ganglion (DRG) neurons and retinal explants in SD mice. Immunocytochemical studies revealed an accumulation of GM2 ganglioside in the cytoplasm of DRG neurons and retinal ganglion cells (RGC) of SD mice in a time-dependent manner. The survival ratios of DRG neurons after 1, 2, 4 and 6 days in culture were significantly lower in 1-month-old SD mice than in the age-matched wild-type (WT) mice. Also, the number of neurites from the retinal explants after 7 and 10 days in culture were significantly lower in 2- and 4-month-old SD mice than in the age-matched WT mice. The application of brain-derived neurotrophic factor (BDNF) to culture medium (100 ng/ml) significantly improved neurite outgrowth from the retina in both SD and WT mice at 2 months of age. At 4 months of age, BDNF was much less effective at stimulating neurite outgrowth in the retina of SD mice than in retina of WT mice. These results suggest that lysosomal storage of GM2 ganglioside impairs the survival of DRG neurons and neurite outgrowth of retinal neurons in culture, and that BDNF is effective at diminishing the impaired neurite outgrowth during the early stage of disease.

P-10-11

ESTABLISHMENT OF IMMORTALIZED SCHWANN CELLS FROM SANDHOFF MICE AND CORRECTIVE EFFECT OF RECOMBINANT HUMAN BETA-HEXOSAMINIDASE A ON THE ACCUMULATED GM2 GANGLIOSIDE

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Beta-hexosaminidase (Hex) is a lysosomal glycosyl hydrolase that catalyzes the hydrolysis of beta-1, 4-linked N-acethyl hexosamine residues at the nonreducing ends of glycocojugates. Sandhoff disease is resulted from simultaneous deficiencies of both Hex A and Hex B, which results in storage of GM2 ganglioside in the nervous system, involving progressive neurological disorders. To develop the enzyme replacement therapy for Sandhoff disease accompanied by excessive accumulation of GM2 ganglioside and neurological manifestations, we have established spontaneously immortalized Schwann cell lines from dorsal root ganglia and peripheral nerves of Sandhoff mice. One of the cell lines exhibited genetically and biochemically distinct features of Sandhoff Schwann cells. The enzyme activities toward 4-methylumbelliferyl N-acetyl-beta-D-glucosamine (Hex A, Hex B, and Hex S) and 4-methylumbelliferyl N-acetyl-beta-D-glucosamine-6-sulfate (Hex A and Hex S) were decreased, and GM2 ganglioside accumulated in lysosomes of the cells. Incorporation of recombinant human Hex isozymes expressed in Chinese hamster ovary cells into the cultured Sandhoff Schwann cells via cation-independent mannose 6-phosphate receptors were found, and the incorporated Hex A degraded the accumulated GM2 ganglioside in lysosomes of the cells. In conclusion, we established immortalized Schwann cell line from Sandhoff mice. This cell line is useful for investigation and development of therapies for Sandhoff disease.

P-10-12

CARDIAC AND RENAL MANIFESTATIONS OF FABRY DISEASE IN CHILDREN AND ADOLESCENTS

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Fabry disease (FD) is a rare, X-linked disorder caused by a deficiency of the lysosomal enzyme α-galactosidase A. The progressive accumulation of the enzyme substrate, globotriasylceramide, leads to renal dysfunction and hypertrophic cardiomyopathy, which become apparent in the third decade of life. This study was performed to determine if signs of these pathologies are apparent in younger patients. Eight boys and 12 girls $\leqslant$18 years old with confirmed FD were studied with a 12-lead electrocardiogram (ECG), 2-h Holter ECG, and a 2-dimensional echocardiogram. Protein excretion was determined from 24-h urine samples and glomerular filtration rate (eGFR) was estimated from serum creatinine levels. At baseline, left ventricular hypertrophy (LVH) was evident in 2 of 8 boys and in 5 of 12 girls. Nearly all subjects without LVH had left ventricular mass indexed to height to the 2.7 power above the 75th percentile for age- and sex-matched normal children. Although eGFR was normal in all children, proteinuria ($>$200 mg/24 h) was evident in 4 of 8 boys and in 5 of 12 girls. During a mean 2-year follow-up in 14 children, left ventricular mass increased in 12 subjects and proteinuria was seen in 4 more children. Boys, but not girls, had evidence of reduced heart rate variability. These results indicate that cardiac and renal involvement of FD is frequent and progressive in children. Early intervention with enzyme replacement therapy may be warranted to slow or prevent the progression of irreversible organ damage.

P-10-13

THE SAFETY AND CLINICAL BENEFIT OF AGALSIDASE ALFA IN CHILDREN WITH FABRY DISEASE

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Fabry disease (FD) is an X-linked lysosomal storage disease whose pathologies begin in childhood. This open-label study was performed to evaluate safety and to explore efficacy of agalsidase alfa (0.2 mg/kg IV, every other week) in 19 boys and 5 girls (6 to 18 years old) with FD. Agalsidase alfa was well tolerated. Six boys and 1 girl experienced 1 or more infusion reactions (typically rigors, flushing, or fever), but only 1 infusion was stopped prematurely. One boy tested positive for IgG anti-agalsidase alfa antibodies at week 9, but was IgG-negative thereafter. No IgE antibodies developed during the study. After 26 weeks of treatment, mean plasma globotriaosylceramide (Gb_3) levels decreased 43% in boys ($p<.001$). Girls had normal Gb_3 levels at baseline that remained unchanged with therapy. Boys had a significant reduction in urine Gb_3 on treatment. Renal function and cardiac morphology were normal and did not change over 26 weeks. Heart rate variability was reduced in boys compared with girls and was improved with agalsidase alfa. Improvements in neuropathic pain, diarrhea, abdominal pain, and sweating were seen. These results show that agalsidase alfa, using the same weight-adjusted dose as in adults, is safe in children with FD and appears to be effective.

P-10-14

CLINICAL FEATURES AFTER ENZYME REPLACEMENT THERAPY IN HEMODIALYSIS PATIENTS WITH FABRY DISEASE

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Background: Recent reports have emphasized the usefulness of enzyme replacement therapy for Fabry disease. However, there are only a few reports on enzyme replacement therapy for Fabry disease with end-stage renal failure (ESRF). Here we present the clinical features of two hemodialysis (HD) patients with Fabry disease before and after enzyme replacement therapy. **Case Report**: Case 1 was a 60-year-old male who had been diagnosed with chronic glomerulonephritis since 1990. In 2003, he started HD for ESRF. However, Fabry disease was diagnosed after screening for plasma α-galactosidase A activity and since then he received enzyme replacement therapy. Before therapy, the patient had left ventricular hypertrophy (LVH) but no angiokeratoma, corneal opacities, acroparesthesia or hypohydrosis, which are typical manifestations of Fabry disease. Enzyme replacement therapy resulted in slow and progressive deterioration of LVH and appearance of premature atrial fibrillation in 2004. The patient died in January 2005 after acute heart arrest. Case 2 was a 26-year-old male who was diagnosed with the classic form of Fabry disease in 2002. Enzyme replacement therapy commenced soon after the diagnosis. However, in 2003, he was placed on HD for ESRF. Before enzyme replacement therapy, he had angiokeratoma, corneal opacities, acroparesthesia and hypohydrosis but no LVH. After enzyme replacement therapy, CTR by chest X-p was improved, but the development of LVH was confirmed by echocardiography in 2004. **Conclusion**: Enzyme replacement therapy for HD patients with Fabry disease might cause slow and progressive LVH. Evaluation of the stage of LVH in HD patients with Fabry disease requires echocardiography.

P-10-15

2 NOVEL SPLICE SITE MUTATIONS IN THAI SIBLINGS WITH NEURONOPATHIC GAUCHER DISEASE

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The objective was to identify mutations in Thai individuals with Type 3 Gaucher disease. Written informed consent was provided by patients' parents. Leukocyte glucocerebrosidase activity was measured by fluorometric assay. Genomic DNA and RNA were isolated from peripheral blood using standard protocols. Patient 1 was a 3-year, 10-month-old male with symptoms and signs consistent with type 3 Gaucher disease. Patient 2 is his asymptomatic 6-month old sister. Glucocerebrosidase activity was 0.57 nmol/h/mg protein (control 3.51). Both his mother and father had normal activity, 4.6 and 4.16. His sister was found to have reduced activity at 0.54; she developed symptoms at one year of age. Both were heterozygous for two novel splice site mutations. The paternal mutation was a G-to-C mutation at position –1 of intron 6, resulting in exon 7 skipping and leading to a truncated peptide with 255 amino acids. The maternal mutation, IVS9-3 C->G, results in activation of two cryptic acceptor splice sites residing in exon 10, leading to deletion of the first 4bp and the first 20bp of exon 10. This yields two distinct types of truncated peptide containing 486 aa (K464fs487X) and 479 aa (S463fs480X). In conclusion, we report 2 novel splice site mutations in patients with Gaucher disease type 3 allowing for additional genotype-phenotype information.

P-10-16

IMPROVING THE DETECTION OF *GBA* MUTATIONS IN GAUCHER DISEASE BY USE OF THE REAL/PSEUDO GENE-COMBINATION PCR

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Gaucher disease (GD) is an autosomal recessive, lysosomal disorder caused by mutations in the gene for the β-glucocerebrosidase (GBA) enzyme. Understanding the genotype and phenotype correlations in GD patients is very helpful for a treatment strategy and genetic counseling. Mutation analysis in the *GBA* gene has been emphasized and carried out worldwide. To date, 196 different mutations have been identified. In the Jewish patients with GD, more than 95% of the mutant alleles have been identified, while only 70–80% in the Asian populations. Complex rearrangement mutations resulted by homologous recombination between *GBA* and pseudo *GBA* genes or *MTX1* (metaxin 1) and pseudo *MTX1* genes have been found in approximately 3–10% of the Jewish and non-Jewish Caucasian GD populations. Southern blotting analysis has been mainly used for a detection of the recombinant alleles, but this method is not highly effective because of some demerits, a requirement of a large amount of DNA, a lower sensitivity, and difficulty for an interpretation of the result. In this study, in order to improve the detection efficiency and accuracy of the rearrangement mutations, we have developed a new method, the real/pseudo gene-combination PCR which is skillfully devised for convenient use. Using this method together with PCR-directed sequencing, we screened 31 unrelated Korean GD patients for mutations. We have found the entire 62 *GBA* mutant alleles of 31 patients (100%), including 53 single-nucleotide substitutions alleles, 5 single-nucleotide deletion alleles, 3 recombinant alleles, and 1 splicing error allele. This study will contribute to improving the genotyping efficiency in GD patients.

P-10-17

DIRECT COMPARISON OF ENZYME MEASUREMENTS FROM DRIED BLOOD AND LEUKOCYTES OF MALE AND FEMALE FABRY DISEASE PATIENTS – DIAGNOSTIC VALUE

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Anderson-Fabry disease is an X-linked disorder that is caused by deficiency of the lysosomal enzyme alpha-galactosidase A. Symptoms include chronic progressive painful small-fibre neuropathy, cornea verticellata, renal failure and heart disease. Interestingly, female heterozygous patients may also show severe symptoms. After clinical suspicion, the determination of alpha-galactosidase activity in leukocytes is usually requested first. Alternatively, an enzymatic assay using dried blood specimens has been described. Dried blood samples require less blood and are substantially more stable (several months at room temperature) than whole-blood specimens. Therefore, we compared enzyme activities of alpha-galactosidase, beta-galactosidase and beta-glucuronidase from 78 known Fabry patients (31 males, 47 females). All male patients were clearly identified by both methods. In leukocytes, 16 female patients would have been missed, while only 7 female patients showed activities above the cut-off threshold for dried blood (>0.15 nmol/spot, range of these patient 0.18–0.36 nmol/spot). In addition, the alpha-galactosidase to beta-glucuronidase ratio proved useful for another 7 female patients with low to normal alpha-galactosidase activities (0.11–0.15 nmol/spot alpha-galactosidase; JIMD. 2005:803–5). Furthermore, the direct comparison showed divergent behavior of enzyme activities between leukocytes and dried blood in some of the samples. It seems that plasma enzyme activities are not directly related to leukocyte enzyme activities. In blood spots it can be expected that the measured activity will reflect a sum of both sources. In summary, dried blood proved a reliable method for the diagnosis of male and a certain percentage of female Fabry patients.

P-10-18

CLINICAL AND LABORATORIAL OUTCOME OF BRAZILIAN FABRY PATIENTS ON ENZYME REPLACEMENT THERAPY (ERT): DATA FROM BRAZILIAN FABRY REGISTRY

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Introduction: The Fabry Registry is an ongoing, observational database that tracks natural history and outcomes of patients with Fabry disease. Fabry disease. **Results**: Nowadays there are 63 Brazilian patients enrolled in Fabry Registry and 27 (20M/7F) are on ERT with Algasidase beta (Fabrazyme®) standard dose of 1 mg/kg each other week for 6months to 46 months (average time of treatment of 28 months) and 9 of them are genotyped and the mutations presented by them are: L131P, R342Q, C94Y, IVS5-2^{A-G}. The female patients have an average of age of 51 years and average of age of first ERT infusion of 42 years. All female patients presented cornea verticillata, one of them has the cardiac variant and only 2 women presented proteinuria prior to ERT. In 3 patients there were a markedly improvement of cornea verticillata, and proteinuria markedly decreased after ERT. The male patients have an average of age of 39 years and average of age at start of ERT of 37 years. Prior to ERT one patient had already done a renal transplantation and another one were on hemodialysis, the average of creatinine clearance was 92.6 ml/min and the average of proteinuira was 1.665 mg/24 h (ranging from negative to 8.829 mg/24 h), 4 male patients started ERT with proteinuria higher than 2.000 mg/24 h. Renal transplantation was performed in 2 patients after average of 25 months of ERT. The average proteinuria after long term ERT are 551, 3 mg/24 h. **Conclusion**: The early diagnosis is important to prevent irreversible renal damage.

P-10-19

TANDEM MASS SPECTROMETRY OF SPHINGOLIPIDS AND LOADING TESTS ARE RELIABLE TOOLS FOR DIAGNOSIS OF SAP B DEFICIENCY

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The sphingolipid activator proteins (saposins A, B, C, D; SAPs) are important cofactors for the lysosomal degradation of sphingolipids with short hydrophilic head groups. Deficiencies of individual SAPs or their precursor prosaposin result in the blockade of catabolism of corresponding sphingolipids. In sap-B deficiency, major accumulated lipids are Gb3Cer and sulphatide, in prosaposin deficiency it is the whole array of sphingolipids. In contrast with classical enzymopathies caused by defects of enzyme protein, routine *in vitro* enzymology cannot give relevant answer.

We propose diagnostic approach based on the proof of lipid storage by tandem mass spectrometry (MS) in the first step with subsequent evaluation of loading tests with radiolabeled glycolipids. This is demonstrated on the case of sap-B deficiency.

Quantification by tandem MS revealed high urinary excretion of sulphatides and Gb3Cer (10 fold and 3 fold of normal, resp.). These findings perfectly correlated with loading experiments with tritiated substrates in fibroblast cultures. Degradation of sulphatides was completely blocked albeit *in vitro* arylsulphatase A activity was normal. Gb3Cer catabolism was impaired with only minute product formation. Catabolism of the other less polar glycosphingolipids and ceramide was found normal. We conclude that combination of tandem MS analysis of urinary sphingolipids with function experiments in cultured cells is reliable tool of the first hand diagnosis when SAPs deficiencies are suspected.

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P-10-20

PILOT STUDY OF FABRY DISEASE SCREENING BY MEASURING GL-3 IN PLASMA USING TANDEM MASS SPECTROMETRY IN KOREA

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Fabry disease (FD) is an X-linked inborn error of glycoshpingolipid metabolism resulting from mutation in the enzyme α-galactosidase A gene. 11 Korean Fabry patients were intermittently reported. To date, no data was collected on FD screening of more than 1000 patients in Korea. We screened 1100 outpatients from 8 hospitals to assess the incidence of FD among patients with renal dysfuction. We measured plasma GL-3 level in the patients who are under hemodialysis due to renal dysfunction or with proteinuria. Clinical manifestations were assessed, if positive for the plasma GL-3 measurement, and the patients were tested for α-Gal A enzyme and assayed for confirmative mutation analysis. We diagnosed 3 FD patients including 2 male and 1 female with incidence of 0.27 % among renal dysfunction patients. This report supports that the measurement of GL-3 in plasma is an effective tool for 1st line FD screening among renal dysfunction patients.

P-10-21

MUTATIONS OF THE NPC1 GENE IN JAPANESE PATIENTS WITH NIEMANN-PICK C DISEASE

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Niemann-Pick C disease (NPC) is an autosomal recessive lipidosis showing wide varieties of phenotype with hepatosplenomegaly and progress neurological symptoms. This disease results from mutations in the *NPC1* or *NPC2* gene, leading to a defect in intracellular trafficking of exogenous cholesterol that causes the lysosomal accumulation of unesterified cholesterol. To characterize the molecular lesions in Japanese NPC patients, we analyzed the *NPC1* and *NPC2* genes in 5 Japanese patients with NPC. Eight mutations (c.581592delinsG, c.1891A>G, c.2000C>T, c.2240delT, c.2800C>T, c.2974G>T, c.3418G>A, c.3615delA) were identified in the *NPC1* gene. Seven mutations were novel and one (c.2974G>T) was a mutation known to cause a Nova Scotia variant of NPC. This is the first case of NPC due to a homozygous c.2974G>T mutation found in another ethnic group, unrelated to Nova Scotia. This case showed a typical clinical course of the Nova Scotia variant, suggesting that ethnic background does not apparently influence the genotype-phenotype correlations in NPC. The c.2240delT causes a frameshift at codon 747 and a premature stop codon at codon 748. The transcripts containing this mutation were not detected by RT-PCR in the patient fibroblasts, suggesting that the nonsense-mediated decay pathway degraded the transcripts harboring premature stop codon.

P-10-22

GAUCHER DISEASE: RESULT OF A MULTICENTRIC SURVEY IN TUNISIA

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Gaucher disease (GD) is the most frequent among sphingolipidosis, it is caracterised by panethnic distribution and a large variability in phenotype.

Aim: describe epidemiological and clinico-biological features of GD in Tunisia through a multicentric study. We performed a retrospective multicentric survey covering a period of 22 years (1983–2005). A total of 53 cases of GD were collected among 45 families, 41 cases were diagnosed during childhood and 11 in the adulthood, 2/3 of cases were observed during the last 5 years. Thirty seven patients were diagnosed with type 1, 9 and 3 cases with type 3 and 2, respectively and one family with a prenatal form with ichtyosis. Mean age at diagnosis was 4.5 and 34 years in child and adult patients, respectively. Hematological abnormalities were more severe in adults, liver dysfunction was observed in the 2 groups, a cirrhosis was authentified in 3 children. Heart involvement was noted in 3 cases, bone disease was better documented in children and more severe in splenectomised patients. Seizures and tonus abnormalities were noted in all neurological forms. Enzymatic essay of β-Glucocerebosidase was performed in 70% of patients and molecular study in 6 families. Five patients deceased because a severe liver disease and 3 pediatric patients are under replacement therapy with favorable outcome. **Conclusion**: Gaucher disease was underdiagnosed in the adult population, the phenotype was mostly severe in this survey, inaccessibility to replacement therapy and bone marrow transplantation limited optimal management of Gaucher disease patients.

P-10-23

INVESTIGATION ON QOL OF PATIENTS WITH POMPE DISEASE IN JAPAN

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Introduction: Pompe disease is an inherited metabolic disease caused by the accumulation of glycogen due to the deficit of lysosomal enzyme, acid alpha-glucosidase. As clinical symptoms, it includes muscle weakness, cardiomegaly, respiratory failure or others. In Japan, approximately 40 patients have been confirmed as Pompe disease. We recently surveyed the clinical symptoms, Health Related-QOL and medical cares of the patients with Pompe disease in Japan. **Material and Method**: Among the patients who visited our hospital or belonged to 'Pompe disease Association', we surveyed 23 patients who gave us the informed consent. We used SF-36 as a measurement for health status in general. Also we created P-QOL as a disease specific measurement. **Results**: In the results of SF-36 questionnaire, the scores of all eight scales were lower than the national average. The significantly low score of physical health is probably due to their muscle weakness and respiratory failure. Many patients were concerned about not only disease itself, treatment, marriage and inheritance, but medical care system in the future as well. **Discussion**: The morbidity and mortality have been important in the epidemiologic study because they have been respected by its universality, clearness of definition and importance for both of the individual and the society. Currently, however, it is a trend to see the subjective evaluation by the patients themselves as the important markers so that HR-QOL which is now taking a role as an important indicator for 'medical outcomes' is considered as one of the medical evaluation from the standpoint of patients.

P-10-24

CLINICAL AND LABORATORIAL OUTCOME OF ONE PATIENT WITH FABRY DISEASE (FD) TREATED WITH ALGASIDASE BETA: CASE REPORT OF 39 MONTHS OF EXPERIENCE

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Introduction: We report the one of the first patient of Rio de Janeiro State with FD and has been treated with algasidase beta (1 mg/kg each other week) since 2003. **Case Report**: Male, 46 years old, at 7 years he presented onset of symptoms with diffuse and intermittent pain in both hands and acroparesthesias, at teenage he presented angiokeratomas and hypohidrosis. At 20 years old he presented fatigue, lymphedema in both legs, diminished peripheral sensibility and worsen of acroparesthesia. Along almost 30 years he looked for symptoms treatment with several physicians and was diagnosed as having arthritis and peripheral neuropathy. At 43 years he performed the alpha-Gal activity assay in dried blood spot on filter paper and was diagnosed as having FD. At the moment of diagnosis, he also had cornea verticillata, systemic arterial hypertension, diarrhea, right bundle branch block, mitral valve dysfunction, diffuse myocardial wall motion abnormality, reduced glomerular filtration rate (creatinine clearance: 39.06 ml/min), BUN and serum creatinine were normal and also normal value of 24 h proteinuria. After 6 months of ERT the diarrhea disappeared. Nowadays he is receiving ERT for 39 months, and he presented progressive improvement of sweating, fatigue and diminution of angiokeratomas. The BUN, serum creatinine and 24 h proteinuria remained stable. The creatinine clearance presented a slight improvement. **Conclusion**: Although FD presents characteristics symptoms, this patient had a delay of more than 30 years until the definite diagnosis. Nowadays with ERT, the sooner we diagnose and start treatment, the better we will be able to prevent irreversible damage.

P-10-26

IN VIVO EVIDENCE OF MORPHOLOGICAL IMPROVEMENT OF TWITCHER OLIGODENDROCYTES BY GALC TRANSDUCTION

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Globoid cell leukodystrophy (GLD) is a severe demyelinating disease caused by a genetic defect of β-galactocerebrosidase (GALC). To date treatment is limited to hematopoietic stem cell transplantation. Experiments on twitcher mouse, an authentic murine model of human GLD showed the potential usefulness of gene therapy in this disease. To clarify whether the transduction of GALC gene could provide beneficial effects on oligodendrocytes which are the primarily affected cells in GLD, we transduced twitcher oligodendrocytes by stereotactically injecting recombinant retrovirus encoding human GALC cDNA into the forebrain subventricular zone of neonatal twitcher. *In vivo* effects of exogenous GALC on twitcher oligodendrocytes were evaluated histologically at around 40 days of age. Results showed that GALC transduction led to dramatic morphological improvement of the twitcher oligodendrocytes comparing with those in untreated twitcher controls. This study provided direct *in vivo* evidence that GALC transduction could prevent or correct aberrant morphology of oligodendrocytes in GLD which may be closely related to the dysfunction and/or degeneration of oligodendrocytes and the demyelination in this disease.

P-10-27

THE EFFECTIVENESS OF ENZYME REPLACEMENT THERAPY FOR FABRY DISEASE: SHORT TERM CARDIAC EFFECT

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Objective: To determine the effectiveness of enzyme replacement therapy for cardiac lesion in Japanese Fabry Disease. **Methods**: We evaluated the effectiveness of enzyme replacement therapy for cardiac involvement of 21 patients have received enzyme more than 20 weeks. The evaluation of cardiac function was performed by electrocardiogram and echocardiography. **Results**: The cardiac symptom 3 out of 21 patients were improved by enzyme replacement therapy. Atrial fibrillation, atrioventricular block and complete atrioventricular block were found in 3, 1 and 1 patients respectively at baseline and were not resolved by enzyme replacement therapy. Left ventricular hypertrophy judged by electrocardiogram was found in 11 patients at baseline and improved in 2 patients. The ST-T change was found in 12 patients and was regressed in 2 patients. In 1 patient who needed renal renal dialysis during treatment and 1 patient who's compliance was poor, the ST-T change of electrocardiogram progressed. Myocardial hypertrophy, mitral regurgitation and aortic regurgitation judged by echo-cardiogram at baseline were found in 10, 18, and 5 patients respectively. Mitral regurgitation were improved in 2. However, in poor compliance case, the case who presented allergic reaction and elderly case, these echocardiographic findings were not improved. **Conclusions**: Enzyme replacement therapy can relief or prevent the cardiac symptoms of Fabry disease including left ventricular hypertrophy, the ST-T change, mitral regurgitation and aortic regurgitaton. However, poor compliance, elder age, and severe renal dysfunction and allergic reaction seem to be a risk factor for progression of cardiac involvement even though receiving enzyme replacement therapy.

P-10-28

LENTIVIRUS MEDIATED GENE THERAPY FOR KRABBE DISEASE

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Globoid cell leukodystrophy (GLD, Krabbe disease) is a severe demyelinating disease caused by a genetic defect of beta-galactocerebrosidase (GALC). To develop an efficient gene therapy, transduction of GALC cDNA into various tissues including CNS is very important issue. For this purpose, we constructed the recombinant lentivirus expressing GALC and eGFP (an enhanced green fluorescent protein). Both normal human GALC cDNA and eGFP reporter gene was placed downstream of the GALC cDNA with the ECMV internal ribosome entry site (IRES) preceding the eGFP gene. The titer of the virus was determined by counting of eGFP positive cells using flow cytometry after infection of this virus to 293 cell. We infected this recombinant virus to the fibroblast from twitcher mouse, which is authentic mouse model of GLD. The GALC was increased by the virus dose dependent manner when we transduced these cells at MOI = 8 and MOI = 80, the GALC activities increased from 2.97 nmol/mg protein/h (Mock) to 10.5 nmol/mg protein (MOI = 8) and 29.17 nmol/mg protein/h (MOI = 80) respectively, By adding chondroitin sulfate (CS) and polybrene into viral supernatant before infection, we detected more efficient transduction *in vitro* (4 times higher than that without CS). We administered this recombinant virus into the newborn twitcher mice intravenously, GALC activity in the liver was increased up to 10% of normal level 7 days after the infection. This recombinant lentiviral vector might be useful for the gene therapy of GLD.

P-10-29

CLINICAL MANIFESTATIONS OF JAPANESE HETEROZYGOTES WITH FABRY DISEASE

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Objective: Fabry disease is an X-linked lysosomal disorder resulting from mutations of the α-galactosidase A (α-GalA) gene. The disease manifestations of female heterozygote have been considered to be mild. However, several cases of female heterozygote with severe clinical course have been reported. The aim of this study is to clarify the heterogeneity of the clinical manifestations of Japanese female heterozygotes with Fabry disease. **Material and Method**: The study patients were 34 Japanese heterozygotes with Fabry disease from 27 families (4–67 years, average: 39 years). All of them were diagnosed on the basis of the mutation analysis of α-galactosidase A gene, the pathological findings of renal biopsy or abnormal accumulation of urinary globotriaosylceramide. We investigated the incidence of acroparesthesias, angiokeratoma, corneal opacities, proteinuria and left ventricular hypertrophy. **Results**: Twenty-nine out of 34 patients (85%) were symptomatic heterozygotes and most common symptom was corneal opacities (16 out of 34 patients, 47%). Less than 10 years old heterozygotes were asymptomatic. In 10–29 years old patients, most common symptom was acroparesthesias (8 out of 9 patients, 89%). In more than 30 years patients, left ventricular hypertrophy was most common symptom (15 out of 23 patients, 65%) and more prevalent in older patients. **Conclusion**: The ophthalmological examinations and ultrasound cardiography are useful to make a diagnosis of symptomatic heterozygote. Physicians should be cautious of the possibility that severe complications such as left ventricular hypertrophy occur in female heterozygotes as well as hemizygote patients.

P-10-30

AN OBSERVATIONAL PROSPECTIVE STUDY OF NEUROLOGICAL MANIFESTATIONS, AND OTHER COMORBIDITIES IN ADULT TYPE 1 GAUCHER DISEASE

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Gaucher disease (GD) is the most common of the glycosphingolipid storage diseases and has autosomal recessive inheritance. Clinical manifestations result from accumulation of glucosylceramide in various tissues. GD is a complex disease and comprehensive information on its natural history is relatively limited. The traditional classification of GD in three discrete subtypes has been recently questioned and several case reports have raised the possibility that neurological manifestations may be present even in GD1. To clarify this question, a multinational (5 countries, 6 centres) prospective study has been set up. It will recruit up to 100 patients with GD1 either untreated or treated by ERT. The primary endpoint of this study is to establish the prevalence of peripheral neuropathy at baseline. Evaluation and diagnosis of cases of peripheral neuropathy will be done by an independent central assessor. Secondary endpoints will include incidence of peripheral neuropathies over two years, incidence over two years and changes from baseline of a number of clinical and biological parameters such as neurological and neuropsychological status, skeletal symptoms, plasma electrophoresis and quality of life. As of March 2006, seventy-four patients have been enrolled in this study. Recruitment should be completed at the end of June 2006; therefore data on the prevalence of peripheral neuropathies and on key clinical manifestations of patients with GD1 untreated or treated with ERT should be available at that time. We expect the study to provide a first set of comprehensive information on GD1, leading to a better understanding and management of this disease.

P-11-1

PRENATAL MOLECULAR DIAGNOSIS OF X-LINKED ADRENOLEUKODYSTROPHY

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Objective: To carry out prenatal diagnosis on the first fetus of a 28-year-old woman at risk of being a carrier for X-linked adrenoleukodystrophy (X-ALD). **Methods**: Her father had been affected with adrenomyeloneuropathy and had been left in a vegetable-like state for more than 10 years. Amniocentesis was performed at 15 weeks' gestation. RNA and genomic DNA were isolated from cultured amniotic cells and leukocytes of both a client and her father. Four overlapping RT-PCR products that cover the entire open reading frame (ORF) of the cDNA were directly sequenced. Genomic DNA sequencing of the candidate exon-intron boundary was followed. **Results**: The amniotic fluid cells karyotype was found to be male. Systematic analysis of ORF of the ALD gene (ABCD1) in the propositus revealed 34 bp deletion which corresponded to the head of the 7th exon. A novel splice acceptor–site defect (IVS6-1g>a) was identified by the genomic DNA sequencing. Sequencing of the mother's RNA and DNA revealed the heterozygous pattern of this splice acceptorsite defect. This mutation was excluded in the fetus. **Conclusion**: The fetus had no IVS6-1g>a mutation on his ABCD1 gene and he was a normal hemizygote. Prenatal diagnosis with enough genetic counseling is a key to disease prevention for X-ALD.

P-11-2

A SECOND CASE OF PEX14 DEFICIENCY WITH PROLONGED SURVIVAL AND RENAL TUBULAR DYSFUNCTION

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Mutations of *PEX* genes cause peroxisome biogenesis disorders (PBD). *PEX14* is the 13th gene responsible for PBD, and only one patient with PEX14 deficiency has been described who died at 10 days of age. Here we report the second case with PEX14 deficiency. A male patient was born after uneventful delivery, but the abnormality of ABR was pointed out at the age of 6 days. He showed severe hypotonia from early infancy, failure to thrive, psychomotor retardation, dysmorphic features, hepatosplenomegaly, mild cataract, and severe sensorineural hearing impairment. Liver function has not been severely damaged. Brain MRI findings were within normal level and there has been no episode of seizure. At the age of 5 months, hyperaminoaciduria and hypophosphatemic rickets caused by renal tubular dysfunction were recognized. GCMS analysis of urine showed increased excretion of epoxydes and dicarboxylic acids. Serum levels of very long chain fatty acids and phytanic acid were also elevated. From these data, the patient was suspected as having PBD. Mutational analysis revealed that he is homozygous for a nonsense mutation of *PEX14*, c.538 C>T (Gln 180 X). He is now 15 months old and still shows severe hypotonia. In general, patients with PBD rarely survive over 1 year due to the severe hypotonia, feeding difficulty, liver involvement and apnea. In our case, the clinical phenotype is atypical for PBD in the point of the prolonged survival, mild liver dysfunction and existence of renal tubular dysfunction.

P-11-3

PREIMPLANTATION DIAGNOSIS IN A FAMILY AFFECTED WITH ZELLWEGER SYNDROME

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Background: Zellweger syndrome is the prototype and the most severe of Zellweger Syndrome spectrum (ZSS). ZSS is a group of clinically and genetically heterogeneous disorders, caused by defects in at least 12 PEX genes involved in normal peroxisome assembly. Patients affected with this syndrome have severe neurological dysfunction including hypotonia, seizures, severe mental retardation and characteristic craniofacial dysmorphism. Severe growth failure is the rule and death occurs in most patients within the first year of life. ZS is commonly observed in Saudi Arabia among other autosomal recessive diseases partially due to extensive consanguinity. Given the lethality of the condition and considering psychosocial and cultural issues, we opted for a preventive approach in a Saudi family affected with ZS by screening for mutations in PEX genes followed by preimplantation diagnosis (PGD). **Methods**: Index case in the family underwent mutation screening for PEX genes. This was followed by PGD using whole genomic amplification PCR and sequencing. **Results**: A new mutation in PEX 26 gene was identified in this family. Following PGD, a singleton pregnancy ensued after transfer of two normal embryos. A healthy baby girl was born and postnatal DNA testing revealed a normal homozygous genotype. **Conclusions**: We report successful prevention of ZS in a Saudi family by PGD following identification of a new mutation in PEX 26 gene.

P-11-4

EARLY STAGE OF CHILDHOOD X-LINKED ADRENOLEUKODYSTROPHY: A QUESTIONNAIRE SURVEY

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X-linked adrenoleukodystrophy (ALD) is an inborn error of peroxisomal metabolism, and is treatable by hematopoietic stem cell transplantation if patients are diagnosed early enough. To clarify the early stage of childhood X-linked ALD, we performed a questionnaire survey of 30 families with X-linked ALD who belong to the family association of ALD in Japan. Various early symptoms were described by the families as follows: learning disorder, psychological problem, visual disturbance, walking disturbance, and awkwardness. They often noticed subtle changes in behavior or response of their sons before an evident symptom appeared, however they thought it might be due to some psychological or emotional reaction. In one third of the patients, first symptom was recognized by a teacher or by a friend. About half of the parents visited Pediatric clinic first, however, others conferred with teachers or counselors, or visited eye or orthopedic or ENT clinics. Some of the families visited several departments or hospitals before the correct diagnosis was made. This indicates the importance of educational activity to schools and doctors not only in Pediatrics but also in other departments. Many families also described problems in inheritance. They hope sufficient and adequate genetic counseling.

P-11-5

LOVASTATIN INDUCTION OF THE REDUNDANT GENE: IMPLICATIONS FOR THERAPY OF X-LINKED ADRENOLEUKODYSTROPHY

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X-linked adrenoleukodystrophy (X-ALD) is an inherited demyelinating disorder associated with elevated level of saturated very long chain fatty acid (VLCFAs) in plasma and tissues. The purpose of this study is to elucidate the effects and mechanisms of lovastatin on transcriptional regulation of $ABCD_2$ gene and VLCFAs accumulation, so as to figure out if it can be a candidate for X-ALD therapy. **Methods**: skin fibroblasts of 10 X-ALD patients were cultured and then treated by 5 μM lovastatin for different time periods within 15 days. The content of VLCFAs and the level of $ABCD_2$ expression in X-ALD fibroblasts were determined by GC/MS and the half-quantitative reverse transcript PCR analysis. **Results**: Treatment with lovastatin at any timepoint, neither induced up-regulation of the expression of $ABCD_2$ gene nor corrected the accumulation of VLCFAs in the fibroblasts of X-ALD patients. **Conclusion**: Lovastatin has no effect on the VLCFAs level and $ABCD_2$ gene expression in fibroblasts of X-ALD patients, which cultured in usually medium. Whether lovastatin is effective in fibroblasts cultured in low lipid medium should be further studies. More experiments are needed to test if Lovastatin is benefit to X-ALD patients before it is used clinically.

P-11-6

RHIZOMELIC CHONDRODYSPLASIA PUNCTATA (RCDP) TYPE I IN 2 THAI INFANTS: FIRST REPORTED CASE

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Rhizomelic chondrodysplasia punctata (RCDP) Type I (OMIM 215100) is a rare autosomal recessive peroxisomal disorder characterized by the presence of stippled epiphyses, coronal vertebral clefting, dwarfing, joint contractures, rhizomelia, congenital cataracts, ichthyosis and severe mental retardation. Biochemically, RCDP patients have submormal levels of red cells plasmalogens and progressive accumulation of phytanic acid starting from normal at birth and increasing to levels more than 10 times normal by age 1 year. Gene locus is mapped at 6q22-q24. It is caused by mutations in the PEX7 gene, which encodes the peroxisomal type 2 targeting signal (PTS2) receptor (OMIM-VA McKusick).

We herein report 2 cases of RCDP, first reported cases from Thailand. **Case 1**: One year and 3 month old boy (born in 1993) with history of bilateral congenital cataracts, delayed development, elevated plasma very long chain fatty acid; phytanic acid level was 25 times higher than normal (Kennedy Krieger Institute). Contractures and shortening of the proximal limbs; stippled epiphyses were observed. Peroxisomal plasmalogen synthesis enzymes were deficient. **Case 2**: Four-month-old girl (born in 1993) with bilateral congenital cataracts, delayed development, contracture and shortening of the proximal limbs, stippled epiphyses. Phytanic acid was 3 times higher than normal (Kennedy Krieger Institute). Both patients died before 2 years of age. Enzyme assay and mutation analysis are not available in Thailand.

P-12-1

A WHOLE YOUNG ALuYa5a2 INSERTION MUTATION CAUSES MENKES DISEASE IN A JAPANESE BOY

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Menkes disease (MNK) is a multi-systemic lethal disorder of copper metabolism dominated by neurodegenerative symptoms and connective tissue disturbances. The disorder is inherited as an X-linked recessive trait and the responsible gene, *ATP7A*, is located on Xq13.3. MNK results from mutations in the *ATP7A* gene. To date, chromosome mutations including translocation, gross deletions and point mutations have been reported. We present the first patient with MNK causing by an alu insertion. A whole young AluYa5a2 element, which was 382-bp long, was identified within exon 9 of the *ATP7A* gene, and all of exon 9 was aberrantly skipped in the cDNA, predicting severely truncated proteins. Using an exonic splicing enhencer finder the Alu element created two new high-score exonic splicing enhancer sequences in mutant nearby the site of insertion. Exon 9 is necessary for the normal function of ATP7A protein, because it encodes the first and the second transmembrane domains. Here, we present the first report of an Alu element insertion mutation causing Menkes disease by an Alu element's interfering with splicing regulatory elements.

P-12-2

IDENTIFICATION OF NOVEL MUTATIONS OF THE *ATP7A* GENE AND PRENATAL DIAGNOSIS OF MENKES DISEASE BY MUTATION ANALYSIS

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Menkes disease is an X-linkedrecessively inherited disorder caused by the mutation of the *ATP7A* gene encoding copper-transporting P-type ATPase. Mutation analysis has been carried out in 5 unrelated Korean Menkes patients using cDNA from cultured skin fibroblasts or genomic DNA from peripheral leukocytes. They presented with depigmented wool-like hair, progressive neurologic deterioration, and hypotonia in infancy. Serum copper and ceruloplasmin levels were decreased. Brain magnetic resonance imaging revealed tortuous intracranial vessels. One patient with deletion of exon 8 and 9 of the *ATP7A* gene died at the age of 4 years. Three novel mutations have been identified from three different families; c.3511+1G>A (p.E1099_N1171delinsMfsX18), c.4005+5 G>A (p.V1268_R1335del), and c.1870_2172del (p.S624_Q724del). The rest of the two mutations (c.3352 G>A (p.G1118S), and c.1933 C>T (p.V1268_R1335del))were previously reported. Prenatal diagnosis was performed in two cases using chorionic villi samples. One was diagnosed as normal, while the other turned out to be a female heterozygotewith p.S624_Q724del mutation of the *ATP7A* gene. Prenatal diagnosis in families at risk is critical in order to choose preventive options including an early treatment with copper-histidine therapy or therapeutic termination. In conclusion, most mutations of the *ATP7A* gene were frame-shift mutations and prenatal diagnosis has been successfully carried out.

P-12-3

COPPER AND ZINC CONCENTRATIONS IN THE BREAST MILK OF MOTHERS WITH WILSON DISEASE AND EFFECTS ON INFANTS

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This is only a model abstract. Female patients with Wılson disease who are being treated with a chelating agent or zinc can become pregnant. Most of the mothers want to breastfeed their infants while continuing their treatment for Wılson disease. However, the copper concentration has not been investigated in the breast milk of mothers with Wılson disease. We report here copper and zinc concentrations in the breast milk of mothers with Wılson disease and the effect of the milk on their infants. **Materials and Methods**: Using atomic absorption spectrometry, the copper and zinc concentrations in the breast milk from three patients with Wılson disease were analyzed at several times. A patient was sequentially treated with zinc and trientine. In addition, the infant's serum levels of copper and zinc were analyzed. **Results and Discussion**: Although the serum copper levels of the patients were significant lower than that of control subjects, the copper and zinc concentrations in the breast milk from the patients while they were taking zinc or trientine were normal levels. The serum copper and zinc levels were also normal in the infants receiving the breast milk from the patient taking zinc and trientine. However, the copper and zinc concentrations in the breast milk from the patients taking penicillamin were lower than those of controls. These results indicate that mothers with Wılson disease receiving zinc or trientine may safely breastfeed their infants.

P-12-4

COMPARATIVE PROTEOME ANALYSIS OF SERUM OF THE PATIENTS WITH WILSON DISEASE

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Wilson disease (WD) is an autosomal recessive metabolic disorder characterized by the toxic accumulation of copper in the body, primarily in the liver, kidney, and brain. To date, ceruloplasmin is a key biomarker for the diagnosis of WD. Efforts have been made to correlate the serum protein profiles of 5–6 year old, three (two male and a female) asymptomatic WD patients with two (a male and a female) normal age and sex matched controls by comparative proteome analysis in order to identify diagnostic biomarkers for WD. Fractionated serum proteins were displayed on two-dimensional electrophoresis gel using multiple affinity removal columns (MARC) to removes six high-abundant proteins. Analyses of these gels allowed us to identify two differentially expressed proteins that were remarkably reduced in WD patient, compared with the healthy controls. These reduced spots were analyzed by MALDI-TOF/TOF. MS/MS analysis and database searching revealed that the reduced proteins were complement component C3c (C3C), carbonic anhydrase (CA1), thiol specific antioxidant (PRDX2), dihydropyrimidinase-like 1 variant (DPYSL2), and complement factor B (BF). It indicated that enhanced oxidative stress caused by excessive copper accumulation might down-regulate the expression of PRDX2, C3C, and BF, which were known to be associated with oxidative stress. CA1, DPYSL2, and metalloprotein were also decreased by excessive copper accumulation. Our strategy using comparative analysis of parallel protein quantitation on 2-DE gels will provide us with cues to accelerate the discovery of novel serum protein biomarkers for the diagnosis of Wilson disease.

P-12-5

IDENTIFICATION OF *ATP7A* MUTATION ASSOCIATED WITH MENKES DISEASE

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Menkes disease (MD) is an X-linked recessive disorder of copper metabolism characterized by progressive neurological degeneration and connective tissue abnormalities. The disease is caused by mutation in the *ATP7A* gene, which encodes a copper-transporting P-type ATPase. *ATP7A* is a large gene, and consists of 23 exons spanning a genomic region of about 150 kbp. We examined mutations in the *ATP7A* gene in 36 unrelated Japanese patients with MD. Further, carrier detection was performed in 18 mothers of the patients, and prenatal or neonatal diagnosis was performed in 7 male siblings. Genomic DNA was prepared from peripheral blood lymphocytes, cultured fibroblast or amniocytes, and amplified by PCR. The direct sequencing of exons was performed with a 3700 DNA analyzer. Thirty three different mutations were identifeid in the 36 patients; 9 nonsense mutations, 6 missense mutation, 9 splice-site mutations, and 12 insertion/deletion mutations. Most of *ATP7A* mutations in the patients were identified in important functional/structural domains of protein products such as copper biding domains, transmembrane domains, CPC motif, and ATP binding domains. Thirteen of 18 mothers (72.2%) were carriers of Menkes disease. Five of 7 siblings suffered from Menkes disease. These results indicate that gene analysis is useful for the early diagnosis of MD and for quality of life of patients and families.

P-12-6

DETECTION AND ANALYSIS OF MUTATIONS IN PATIENTS WITH WILSON DISEASE

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Objective: To determine mutation characters and distribution of ATP7B gene in Chinese and explore importance and methods of gene diagnosis for Wilson's disease (WD) patient. The genotype and phenotype correlation in patients with WD were also studied. **Method**: 35 individuals, 13 patients (9 male and 4 female) from 12 no kinship WD families were enrolled in this study. The age of onset ranged from 5–13 years. Genomic DNA from patients was subjected to polymerase chain reactions (PCR) for exons 5, 8 and 12 of ATP7B gene. PCR products were analyzed by single strand configuration polymorphism (SSCP), denaturing high-performance liquid chromatography (DHPLC) and by direct sequencing. **Results**: Eleven of 13 patients had present with hepatic manifestation, 9 of them had only hepatic manifestation, one had hepatic and neurological manifestation at the same time and other one had hepatic and other symptom. One patient had no symptom. Six mutations were identified by DNA sequencing, including three missense mutations (R778L, D765N, R952K), one deletion (2790del3) and two polymorphisms (2310C→G,2850G→C). R778L occurred in 6 patients and they were all heterozygous. **Conclusion**: Gene diagnosis is a sensitive and specific method for identification of patients and carriers in WD families. DHPLC and SSCP are dependable tool for screening ATP7B gene mutations. The 2790del3 are novel mutations found in patients with WD. The R778L mutation is common mutation of ATP7B gene in Chinese, with frequency of 6/26 in this study, and may not be a mild mutation.

P-12-7

MENKES DISEASE IN A THAI INFANT: FIRST REPORTED CASE

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Classic Menkes disease (OMIM 309400) is an X-linked recessive disorder of copper metabolism caused by abnormalities in a gene that encodes a copper-transporting ATPase. Gene map hocus is on Xq12-q13. Clinical features result from loss of function of specific cuproenzymes, include abnormal hair and pigmentation, laxity of skin, metaphyseal dysplasia, cerebellar degeneration and failure to thrive.

We herein report a 4-month-old boy (born in 2001) who developed intractable seizures after minor intercurrent infection. He had skin hypopigmentation and scanty, whitish lackluster, kinky hair since birth. Low serum copper and ceruloplasmin were noted. Hair electron microscopic examination revealed characteristic twisting (pili torti) and fractures of the shaft at regular intervals. Brain MRI demonstrated generalized cerebellar atrophy. He did not respond to copper sulfate treatment; subsequently severe neurologic impairment developed and progressed rapidly to death within one year. Mutation analysis will be presented.

P-12-8

DETECTION OF *IN VITRO* AND *IN VIVO* FERRITIN AND TRANSFERRIN OXIDATIVE MODIFICATIONS BY Q-TOF LC-MS/MS: HEREDITARY HEMOCHROMATOSIS AS A MODEL

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Hereditary hemochromatosis (HH) is an inherited, recessive autosomal disorder characterized by accumulation of excess iron. When iron binding proteins become saturated, concentrations of free, or non-transferrin-bound iron (NTBI) rise, a condition thought to be responsible for the adverse effects associated with HH. To test the hypothesis that free radical injury plays a role in disrupting iron homeostasis in HH, protein carbonyls (markers of oxidative damage in proteins) were measured in Ferritin and Transferrin isolated from normal patients and from patients with HH. Protein carbonyls were 2–16 times higher in patients with HH than in controls, with the greatest increases being observed in untreated HH patients with high ferritin and >90% transferrin saturation with iron. *In-vitro* oxidation of transferrin and ferritin standards with hydrogen peroxide and excess free iron, followed by immobilized trypsin digestion (Poroszyme), high-resolution LC/MS/MS analysis (Q-TOF Ultima, Waters), and MS/MS data processing (PEAKS, Bioinformatics Solutions), identified several tryptic peptides containing oxidized methionine, histidine and tryptophan residues. Mapping of the oxidized ferritin residues showed them to be located on the inner face of each sub-unit, the face directed toward the ferritin core where iron is normally stored. Using the same methodology, oxidized residues were subsequently detected in ferritin and transferrin isolated from patients severely affected patients with HH. These data show that elevated NTBI may be involved in oxidative modification of the iron binding proteins ferritin and transferrin, and that such modifications may play a significant role in the pathophysiology of HH.

P-12-9

STRATEGY OF MOLECULAR DIAGNOSIS FOR THE JAPANESE PATIENTS WITH WILSON DISEASE

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Introduction: Wilson disease is an autosomal recessive disorder resulting from defective function of copper transport P-type ATPase (ATP7B). Copper is accumulated primarily in the liver, brain, cornea, kidney and other organs. Clinical features of this disease are liver cirrhosis, extra pyramidal signs and Kayser-Fleischer ring. More than 200 disease-specific mutations of ATP7B gene have been reported. The mutation spectrum of ATP7B showed a population-dependent distribution. Thus, the strategy for molecular analysis and diagnosis for this disease should be established in each population. This study reports the results of mutation analysis performed on Japanese patients with Wilson disease and investigates effective strategy of molecular diagnosis for this disease. **Material and methods**: A total of unrelated 55 Japanese patients with Wilson disease were examined in this study. Gene analysis was performed under written informed consent. Genomic DNA was isolated from the peripheral blood leukocytes of the subjects. Twenty-six sets of oligonucleotide primers were prepared to amplify all exons of ATP7B gene in genomic polymerase chain reaction (PCR). The PCR-amplified DNA fragments were analyzed by direct sequencing. **Results and discussion**: Twenty-one mutations were detected, including one base insertion, one or two bases deletions, exon skipping and missense mutations in the coding region. More than 80% mutations will be detected to analyze exons 8, 11, 13 and 18. These four exons should first be analyzed. We will next analyze exons 5, 10, 12, 14, 16 and 17. This is effective protocol for the molecular diagnosis for Japanese patients with Wilson disease.

P-12-10

MOLECULAR DIAGNOSIS FOR ATYPICAL PATIENTS AND CARRIERS WITH WILSON DISEASE

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Wilson disease is a genetic disorder of copper metabolism characterized by hepatic and/or neurological manifestations. This disease is caused by mutations in the gene of copper transporting ATPase (ATP7B). Wilson disease is a treatable disorder. Treatment involves the removal of excess copper by chelating agents (D-penicillamine or trienthine 2HCl), and/or blocking intestinal copper absorption by oral administration of zinc salt. Early diagnosis is very important to improve the prognosis of this disease. However, biochemical studies are not sufficiently effective for the definitive diagnosis of young patients, atypical cases and carriers. This study presents the molecular diagnosis of patients and carriers of Wilson disease. Genomic DNA was isolated from peripheral blood leukocytes of patients and their family. All exons of the ATP7B gene were amplified by genomic polymerase chain reaction (PCR), and then analyzed by direct sequencing. Three patients, one infant, one carrier and one atypical case were diagnosed by ATP7B gene analysis. We conclude that the molecular diagnosis of Wilson disease is very useful for the identification of young patients, atypical cases and familial analysis.

P-13-1

NOVEL MUTATIONS OF ZMPSTE24 IN A JAPANESE FAMILY WITH MANDIBULOACRAL DYSPLASIA

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Mandibuloacral dysplasia (MAD) is a rare autosomal recessive phenotypically heterogeneous disorder characterized by craniofacial anomalies including mandibular hypoplasia, dental overcrowding, bird-like face with prominent eyes and thin beaked nose, and short distal phalanges with acroosteolysis, delayed closure of the cranial sutures, lipodystrophy, skin atorophy with mottled hypopigmentation, stiff joints and growth disturbance. We report Japanese female siblings with severe form of MAD, who presented at birth with characteristic face, atrophic skin and represented severe expressions of MAD in early childhood. Skin atrophy and joint rigidity were more severe in elder sister. Their lipodystrophy pattern was partial. And their subctutaneous fat of cheeks, thighs and arms were spared. The genomic analysis revealed both of them had a novel compound heterozygous mutation in an endprotease, the zinc metalloprotease (ZMPSTE24) gene involving in posttranslational processing of prelamin A to mature lamin A. Clinical phenotypes of our family were similar to the MAD patient with a ZMPSTE24 gene mutation, nevertheless mutation point and subcutaneous fat distribution was quite different. We conclude that MAD is a genetically and phenotypically heterogeous disorder.

P-13-2

CORTICAL NEURONAL MORPHOLOGY AND MR SPECTROSCOPY IN WALKER-WARBURG SYNDROME CAUSED BY NOVEL POMT1 MUTATION

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Walker-Warburg syndrome (WWS) is an autosomal recessive disease, belonging to the group of congenital muscular dystrophies. Clinically, main characteristics are congenital brain and ocular abnormalities and muscular dystrophy. The syndrome is genetically heterogeneous, with mutations found so far in five different genes (POMT 1, POMT 2, POMGnT1, fukutin and FKRP gene). Although all these genes encode known or putative glycosyltransferases involved in glycosylation of alpha-dystroglycan, the variations of clinical, radiological and neuropathological features are significant and only partly understood. We report new neuropathological and neuroradiological changes in an infant with WWS and novel genotype. The patient was born after uncontrolled pregnancy in a travelers family. Body measures were about lower normal range. Since birth the boy was very adynamic and hypotonic with no psychomotor development. Ocular changes included dense opacities of anterior parts, glaucoma and buphtalmus. Creatine kinase was up to 20× normal. Both kidneys had cysts. Brain imaging revealed completely disturbed architecture, thick lissencephalic cortex, hydrocephalus with fused frontal horns, basal ganglia atrophy, small third chamber, hypoplasia of corpus callosum, hypoplasia of cerebellar hemispheres and vermis aplasia. Spectroscopy showed atypical, huge increase of peaks between 1.3 to 1.4 ppm. He died at age 2.5 months. Golgi staining revealed novel abnormalities of pyramidal neurons: (a) frequent abnormal (oblique, horizontal or inverted) orientation; (b) unusual bending of apical dendrites, and (c) heterochronic decoupling of dendritic arbor development within the same subsets of pyramidal neurons. The boy was homozygote for the IVS4+1G>T mutation of POMT1 gene.

P-14-1

SEASONALITY VARIATION IN INCIDENCE OF PRIMARY CONGENITAL HYPOTHYROIDISM IN JAPAN

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In Japan, a nationwide neonatal screening program for congenital hypothyroidism (CH) has been ongoing since 1979. An analysis of the accumulated data between 1994 and 2003 obtained from a long-term follow-up system performed by Aiiku Maternal and Child Health Center revealed seasonality variation in both suspected and confirmed cases of CH. A total of 4666 suspected cases and 1465 confirmed cases were detected through the primary thyroid-stimulating hormone screening method and the follow-up study. December was the month with the largest numbers of both suspected and confirmed cases, and June was the month with the fewest cases. Nationwide live births peaked in the period April to October. Using Roger's test and the chi-squared test of goodness-of-fit, *p*-value was less than 0.001, indicating statistical significance. This result was similar to that found in the Niigata area of Japan (in 2005) and England (in 1999). The sex ratios (female/male) of nationwide live births, suspected cases, and confirmed cases were 0.95, 0.94, and 1.24, respectively. Therefore, the seasonal variation in primary CH suggests that environmental factors may be important in the development of primary CH.

P-14-2

ACTIVATION OF LUTEINIZING HORMONE/ HUMAN CHORIOGODANOTROPIN RECEPTOR LEADS TO NEURONAL DIFFERENTIATION OF PC12 CELLS

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Familial male-limited precocious puberty (FMPP) occurs in boys with genetic activating mutations in luteinizing hormone/ human choriogodanotropin receptor (LHR). They are often shown to have behavioral problems which may be related to the dysfunction of brain cells caused by the aberrant expression of the mutated LHR. LHR is a member of G-protein-coupled receptor and has been shown to express both in the intact central nervous system and primary neuronal and astroglial cultures. The non-gonadal expression of LHR suggests LHR may have non-gonadal functions in the neural cells which are yet unknown. In this study by activating LHR through human choriogodanotropin (hCG) stimulation on PC12 cells which stably express wild type LHR, we show the differentiation of these cells toward neuronal cell type. These cells show neurite outgrowth and expression of an early neuronal marker, β-tubulin III. This effect is mainly through receptor activation other than ligand itself since transfecting naœve PC12 with a constitutive activated mutant LHR (D578H) without hCG stimulation is sufficient to achieve this effect. The neuronal differentiation of PC12 cells may involves mitogen-activated kinase (MAP kinase) pathway through a sustained activation of ERK1/2 which has been shown to be the main signal transduction pathway for P12 differentiation by NGF or other G-protein couple receptors. The results indicate that LHR activation possibly through its ligands, LH or hCG, may participate in the fate determination of neural cells. Further study is to investigate the neural function of LHR *in vivo* and possible relationship to behavioral abnormalities of FMPP patients.

P-14-3

LEPRECHAUNISM SYNDROME: AN EXAGGERATED HYPERGLYCEMIC RESPONSE TO ACTH CHALLENGE

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Leprechaunism is an autosomal recessive condition characterized by hyperinsulinism due to a possible defect in the insulin receptors. Good control of blood sugar homeostasis is often difficult to achieve. **Aim**: To describe the glucose and cortisol response to ACTH challenge in a neonate with Leprechaunism. ACTH test was performed as part of the assessment of adrenal function in view of the high 17 hydroxy-progestrone on newborn screening. **Case Report**: A full term baby girl born to non-consanguineous Jordanian parents. Pregnancy and delivery were uneventful. Birth weight was 2 kg. Clinical features include microcephaly, large low set ears, depressed nasal bridge, gingival hyperplasia, lipodystrophy, prominent nipples, hypertrichosis, umbilical hernia, clitoral enlargement, wrinkled loose skin and bilateral ovarian cysts confirmed on MRI of the abdomen. The baby was feeding well in the first two weeks of life with tendency towards hypoglycemia. Chromosomes were 46 XX. Insulin was inappropriately high (1626.1 mU/ml, normal 3–17 Mu/ml) when glucose was relatively low (3.2 mmol/l). Newborn screening test showed high level of 17 hydroxyprogestrone (30.1 ng/ml, normal <2.5 ng/ml). Accordingly, standard short ACTHstimulation test was performed to assess the adrenal function and to rule out congenital adrenal hyperplasia. 62.5 μg of ACTH was administered intramuscularly. The baseline glucose was 3.2 mmol/l and cortisol was 6.5 mg/l (normal 4–34 mg/l). One hour later, glucose went up to 19.6 mmol/l and cortisol was 53.6 mg/l. The blood sugar remained above 17.1 mmol/l for 24 h. 17-Hydroxyprogesterone remained within normal levels. Gluconegenic effects of cortisol and possible insulin suppression may explain this exaggerated hyperglycemic response to ACTH challenge. ACTH or cortisol may be worth trying in patients with Leprechaunism with severe persistent hypoglycemia unresponsive to usual measures.

P-14-4

CAT EYE SYNDROME ASSOCIATED WITH SEVERE GROWTH FAILURE CAUSED BY ISOLATED GROWTH HORMONE DEFICIENCY IN A SAUDI MALE

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Introduction: Cat eye syndrome is a chromosomal abnormality resulted usually from an extra chromosome derived from two identical segments of chromosome 22. Anal atresia and colobomata of the iris, initially considered the hallmarks of the disorder, are present in combination in only a minority of the patients reported. The majority of the cases showed normal growth. **Case Report**: We report on a 16-year-old Saudi boy with consanguineous parents. Pregnancy and delivery were uneventful. Birth weight was 2.7 kg. Neonatal examination revealed bilateral multiple ear tags, hypertelorism, and bilateral undescended testicles. At one year of age, he presented chronic constipation. Examination revealed multiple anal fissures, anal stenosis with fibrous ring, and redundant rectum. He was treated with anal dilatation and anorectoplasty. In view of the anorectal anomalies, chromosomal analysis were requested and showed 47 XY +del 22q11, which confirmed the diagnosis of cat eye syndrome. At 15 year of age, he was referred to the endocrine clinic with extreme short stature, with height of 129 cm well below 3rd centile and weight below 3rd centile. He was prepubertal and had no colobomata. IQ was normal. Bone age was significantly delayed consistent with 9 years of age. Brain MRI showed normal pituitary. Pituitary function test showed isolated growth hormone deficiency with normal ACTH-cortisol axis, normal thyroid and sex hormones. Growth hormone peaked at 1.1 Mu/L (normal >20) with insulin induced hypoglycemia test (glucose <1.7 mmol/l). Growth hormone therapy was commenced. Conclusion: We reported on an unusual case of Cat eye syndrome presented few dysmorphic features, anorectal anomalies and severe growth failure due to isolated growth hormone deficiency.

P-14-5

OESTOPETROSIS PRESENTING HYPOCALCEMIC SEIZURE EARLY IN THE NEONATAL PERIOD

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Introduction: Oestopetrosis is a genetic disease resulted from defective bone resorption. The basic defect is thought to involve osteoclast differentiation. Most of the manifestations are due to failure to remodel growing bones. **Case Report**: A full term baby girl was born to a Saudi consangeounous parents. Pregnancy and delivery were uneventful. She presented on day 8 of life generalized tonic clonic convulsions. Clinical examination was unremarkable. Investigations revealed serum calcuim of 4.2 mg/dl (normal 8.4–10.2 mg/dl). Serum phospherus, alkaline phosphatase, parathyroid hormone, and vitamin D were normal. Seizure responded to calcuim infusion. At one month of age she presented poor feeding and pallor. Examination revealed hepatomegaly of 6 cm and splenomegaly of 4 cm. Complete blood count showed Hb of 6 g/dl (normal 11–14 g/dl) and platelet of 47 000 (normal 120 000-400 000). Skeletal survey demonstrated markedly dense bones with effaced medullary spaces consistent with oestopetrosis. The patient was maintained on regular transfusion, oral calcuim, and oral vitamin D supplement. The ultimate treatment is bone marrow transplantation. **Conclusion**: This case report demonstrated that oestopetrosis can present significant hypocalcemia early in the neonatal period. The possibility of oestopetrosis may be considered in neonates with unexplained hypocalcemia.

P-14-6

GROWTH HORMONE TREATMENT IN RESPIRATORY CHAIN DEFICIENCY

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Genetic defects of oxidative phosphorylation (OXPHOS) are known to account for a variety of neuromuscular and non-neuromuscular symptoms in childhood, including growth hormone (GH) deficiency. However, it is not clear if GH treatment can be administered to OXPHOS deficient patients without any risk of acute worsening of the disease. Indeed GH is a mitosis-stimulator which may increase energy demand for cell proliferation. Noticeably GH administration leads to liver damage in animal models. Here, we report the observation of three unrelated children with OXPHOS enzyme deficiency and growth retardation who required GH therapy. The condition of one patient quickly deteriorated under GH administration and GH therapy was then stopped and subsequent clinical improvement was noted. However, neurological deterioration occurred over the next few years following the cessation of GH treatment, due to natural course of the disease. Two other patients had no acute or dramatic consequences following GH administration but various organ system failures progressively appeared during the several years under GH administration. In all patients, no dramatic positive growth response was obtained but length was maintained in the same level of SD. Interestingly, the patient who dramatically worsened after GH administration had no GH deficiency while the two others had low GH response to test stimulations. An excessive GH level in the first patient leading to ATP failure is questionable. These observations question the use of GH as a treatment of growth retardation for patients with OXPHOS deficiency.

P-14-7

CONGENITAL LIPODYSTROPHY ABOUT A NOVEL CASE

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Generalised lipodystrophy(GLD) of the Berardinelli-Seip type is a rare autosomal recessive disorder caused by the absence of functional adipocytes leading to an aberrant storage of lipids, severe insulinoresistance and ultimately to a diabetes mellitus. Intellectual impairment is also described. Authors report a novel case of GLD with a central nervous system involvement. Acromegaloid feature was noted in the neonatal period in a girl originated from Lybia, she was referred to clinical investigation at 5 months: lipoatrophy, muscular hypertrophy, with pseudoathletical morphotype and hepatomegaly were noted on physical exam, we also noted hyperactivity, quadripyramidal syndrom without intellectual impairment. Hypertrophic and hyperkinetic cardiomyopathy was objectivated on echocardiography. Hypertriglyceridemia, high level of muscular enzymes and hyperinsulinemia oriented the diagnosis. Hypointensities of periventricular white matter and basal ganglia were visualised on cerebral MRI.after dietary therapy reducing caloric intake,fast carbohydrates and saturated lipids, we assisted to reversibility of hypertrophic cardiomyopathy and normalisation of hyperlipemia.

P-15-1

VARIABLE MANIFESTATION AND THERAPEUTIC RESPONSE OF AROMATIC L-AMINO ACID DECARBOXYLASE DEFICIENCY

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Aromatic L-amino acid decarboxylase deficiency (ALAADD) is a rare autosomal recessive pediatric neurotransmitter disorder of catecholamine and serotonin synthesis. There is clinical heterogeneity. Females are generally more severely affected with poorer outcome. Three female cases of ALAADD were diagnosed and treated for the past 3 years in Malaysia, giving an estimated incidence of at least 1 in 500 000 live births. All had a peak of vanillactic acid in their urine organic acid. Diagnosis was confirmed by absent or extremely low 5HIAA and HVA in the CSF with markedly elevated substrates for ALAAD: 3OMD, 5OH-tryptophan and L-Dopa. Two of them presented with dystonic crises in infancy associated with irritability, abnormal sweating and temperature instability. One also had recurrent encephalopathy with hypoglycemia. The other one mimic Prader Willi syndrome with central hypotonia and obesity before dystonia was noted. All showed positive response to dopamine receptors agonist bromocriptine 0.5 mg/kg/day in 2–3 divided doses. Two patients became more alert and active with total cessation of dystonic crises. Further increase in the dosage resulted in increased choreoathetoid movements. All were also given MAOI inhibitors tranylcypromine (0.2–0.4 mg/kg/d in 3 divided doses) or selegilline (1.25 mg bd) without side effects, folate 5 mg daily and pyridoxine 50 mg o.d. or b.d. Episodic insomnia was successfully controlled by either benzodiazepine or chloral hydrate. To date, all of them remained globally delayed and totally dependent.

P-15-2

TWO NEW MUTATIONS ON TYROSINE HYDROXYLASE GENE (TH) PRESENTING AS LATE ONSET DYSTONIC-DYSKINETIC SYNDROME

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TH (E.C. 1.14.16.2) deficiency is a rare disorder causing the recessive form of Dopa-responsive dystonia. We report a new Italian patient presenting with a mild phenotype who carried two new mutations on TH gene.

This 17-year-old boy was born after an uncomplicated pregnancy and labour. Psychomotor development was normal until the second year of life when toe-walking gait, frequent falls and delay in language development progressively became apparent. Starting from the age of 5 he suffered from generalized epilepsy requiring antiepileptic drugs. At the age of 11 he developed a dystonic-dyskinetic syndrome which worsened in the following months. At the first examination, at the age of 14, he suffered from generalized dystonia, choreoathetosis and myoclonic jerks of upper limbs, oculogyric crisis with vertical gaze paresis, severe dysarthria, and mild mental retardation. Brain MRI, blood prolactin and the Phe/Tyr ratio at conventional Phe loading test were all normal. A trial with L-Dopa resulted in a doubtful response, while CSF assessment revealed a defect of dopamine synthesis (HVA: 68 nmol/L, n.v. 148–434; 5HIAA 143 nmol/L, n.v. 68–115) with normal pterins (neopterin 2 μg/L, n.v. 2–4; biopterin 5.3 μg/L, n.v. 6-13). Molecular analysis of TH gene (mRNA variant 1, NM_000360) revealed two missense mutations, c.1179 G>A (exon 11; G383R) and c.1437 T>A (exon 13; L479Q), which (a) are both located in the catalytic domain of the enzyme, (b) affect two highly conserved amino acids in the eukaryotic species, and (c) were not found in any of 100 alleles of control subjects.

P-15-3

NOVEL SLC6A8 MUTATION IN A PATIENT WITH CREATINE TRASPORTER DEFECT RESPONSIVE TO ARGININE SUPPLEMENTATION

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Three inborn errors of creatine (Cr) metabolism are known, two affecting Cr biosynthesis (arginine-glycine amidinotransferase and guanidinoacetate methyltransferase deficiency) and one its transport (CT1). Clinical picture is heterogeneous including mental retardation, epilepsy, movement disorders, language delay and autistic-like behavior. While Cr supplementation is able to correct biosynthetic defects, it is completely ineffective in the transporter defect. We report a new patient affected by CT1 defect who responded positively to the supplementation with Arginine, a precursor of Cr synthesis. A 9 year-old boy was born at term after an uneventful pregnancy and presented mild respiratory distress at birth. The psychomotor development was delayed and from the age of 13 months he presented partial seizures not completely drug responsive. The clinical examination showed clumsiness, behavioural and attentional disorder, delay of language comprehension and severe impairment of verbal communication ('empty speech') due to a severe oral dyspraxia. Brain ^{1}H-MRS showed a decrease of Cr peak and GC/MS plasmatic and urinary Cr values demonstrated increased urinary Cr (12543.66 μmol/L; n.v. 200–5500) and an high ratio Cr/Crn (2.35; n.v. <1.8). The mutational analysis of SLC6A8 identified a novel *de novo* mutation (c.1006delAAC) confirming the diagnosis of X-linked CT1 defect suggested by biochemical and spectroscopy findings. Cr uptake in cultured fibroblast was absent. Lacking any alternative treatment, we decided to evaluate the effect of Arginine supplementation (300 mg/kg/day) on clinical status and brain Cr depletion. After five months of treatment a clinical improvement associated with a slight increase of ^{1}H-MRS Cr peak was observed.

P-15-4

A NOVEL TREATMENT FOR CANAVAN DISEASE

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Background: Canavan disease (CD) is a fatal hereditary neurodegenerative disorder. Currently, supportive treatment is the only available treatment modality. The disease is caused by mutations in the gene for the enzyme aspartoacylase (ASPA). ASPA acts to deacetylate N-acetylaspartate (NAA), thus generating brain free acetate, which is crucial to myelin lipids synthesis. Recently it was demonstrated that administrating oral glyceryl triacetate (GTA), to mice resulted in up to 17-fold increase in brain acetate concentration with no significant increase of NAA levels in brain, and no overt adverse effects. **Objective**: To determine whether oral supplementation of GTA improves the clinical prognosis of CD. **Design**: We have conducted a Phase I clinical trial after IRB approval by administrating oral GTA to two consenting CD patients One patient is a one year old Ashkenazi Jewish girl with a diagnosed homozygous c.854A to C mutation, and the second, is a seven months old Arab male with a diagnosed homozygous IVS4+1G>T mutation. The following outcome criteria were evaluated prior to initiation of treatment and will be reviewed at 4 months of age: neurological status, brain MRI and MRS, urine NAA levels and ophthalmologic examination. Adverse effect will be screened clinically and by blood work of CBC, SMAC and ABG. **Results**: After three months (the latter seven weeks at maximum dosage) of treatment no adverse effects were recorded. Neurological assessment revealed no deterioration. Further follow up is mandatory in order to conclude whether GTA is a new treatment modality for CD, decelerating patients' neurological deterioration.

P-15-5

INHERITED METABOLIC DISEASES AS A CAUSE OF ISOLATED PSYCHIATRIC DISORDER IN 56 PATIENTS FROM ARGENTINE

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Objective: To describe a group of patients with isolated psychiatric disorder as the only initial manifestation of an inherited error of metabolism. **Methods**: This study was conducted in the Laboratory of Neurochemistry Dr. Chamoles. Medical records were reviewed for all admissions over a 33-year period between 1970 and 2003 and a subset of 56 patients with psychiatric disorder as the only initial feature of an inherited error of the metabolism were identified. **Results**: All the patients were referred due to psychiatric symptoms starting between 1 and 37 years of age. According to DSM IV, the diagnosis were attention deficit disorder (38%), autism (16%), schizophrenia (16%), learning disability (13%), depression (5%), anorexia (4%), anguish disorder (4%), psychotic episode (2%), panic (2%).The diagnosis of the metabolic disorder was achieved between 1 to 20 years after the first manifestation and was: X-linked adrenoleukodystrophy (38%), phenylketonuria (21%), methacromatic leukodystrophy (11%), Wılson's disease (11%), muchopolisacharidosis III (4%), Krabbe's disease (2%), GM2 gangliosidosis (2%), Niemann-Pick C disease (2%), homocystinuria (5%), Pompe's disease (2%) and 4-hydroxibutiric aciduria (2%). MRI abnormalities were suspicious for metabolic disease in 63% of the cases. **Conclusions**: These results suggest that the diagnosis of inborn errors of metabolism in psychiatric disorders is probably established late. MRI abnormalities could in some cases help in the diagnostic approach. This aetiology should be considered to give the patient the possibility of a better support.

P-15-6

BASAL GANGLIA INVOLVEMENT IN CHILDHOOD: CREATINE DEFICIENCY SYNDROME CAUSED BY GUANIDINOACETATE METHYLTRANSFERASE (GAMT) DEFICIENCY

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Creatine deficiency syndromes are defined as inborn errors of creatine synthesis and transport including GAMT and AGAT deficiencies, and creatine transporter disorder. Clinical features are mental retardation, speech delay, intractable seizures, extrapyramidal movement disorder and behavioural changes. Magnetic resonance spectroscopy (MRS) shows almost complete depletion of cerebral creatine pool. A 17-month-old boy presented with motor and mental developmental delay and febrile seizures at the age of 11 months. He had peripheral spasticity, axial hypotonia and dystonic movements. There is a 22-year-old sister with mental retardation and seizures. MRI-MRS showed bilateral hyperintensity in the globus pallidi and decreased creatine and increased guanidinoacetate peak in cerebral deep gray matter. There was increased concentration of guanidinoacetate (868 mmol/mol cre; N: 11–284 mmol/mol cre) by GC-MS analysis in the urine. GAMT mutation analysis is pending. The patient is on 500 mg oral creatine supplementation daily for the last three months. Differential diagnosis of basal ganglia involvement in childhood mainly includes hypoxia, mitochondrial disorders and organic acidemias. Bilateral globus pallidus involvement along with MRS findings in our patient with a complicated neonatal period and family history lead to the diagnosis of a creatine biosynthesis defect, which is a treatable condition.To the best of our knowledge this is the first patient defined with GAMT deficiency from Turkey. We conlude that MRS should be included in the evaluation of patients with basal ganglia involvement to present the status of creatine pool.

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P-15-7

SIGNAL TRANSDUCTION DEFECTS (STD): NEWLY RECOGNISED CAUSES OF PSYCHOMOTOR RETARDATION

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Defects in signal transduction can lead to mental retardation. Platelets are a good tool to assess STD abnormalities. A $3\frac{1}{2}$ year old boy was seen because of developmental delay (sit unsupported at 11 months, walked at 17 months, said only a few words at age 3), and dysmorphia (sunken nose bridge, epicanthal folds, hypertelorism, protuberant ears, and brachydactyly of 4th and 5th digits in both hands). Neurological examination was normal. Metabolic and chromosomal studies were unremarkable but he had thrombocytosis [398×10^9/L (130–350)], decreased mean platelet volume (MPV) [7.4 fL (8.6–9.7)], and raised LDH [567 U/L (240–480)] with normal AP, TSH, CPK, and transaminases. Mild and variable disturbances of these parameters are compatible with a STD, thus platelet studies (bleeding time/aggregation studies) were performed. These were clearly abnormal (reversible aggregation for ADP, delayed collagen aggregation and an absent secondary response to epinephrine) in line with a STD. Investigations to pinpoint the precise defect are in progress. STD should be considered in mentally retarded (especially patients with brachydactyly). We suggest measuring thrombocytes, MPV, LDH, AP, TSH, CPK, and transaminases in these patients. If abnormal, platelet studies are indicated.

P-15-8

RECESSIVE CONGENITAL METHEMOGLOBINEMIA TYPE II: OVERLOOKING THE CYANOSIS LEADS TO DELAY IN DIAGNOSIS

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Introduction: Recessive congenital methemoglobinemia (RCM) is due to deficiency of cytochrome b_5 reductase (cb_5r). The enzyme plays a role in microsomal electron transport, fatty acid elongation and desaturation and cholesterol biosynthesis. In erythrocytes it catalyzes methemoglobin reduction. Methemoglobin has a chocolate brown color. It appears blue through the skin and gives a sallow greyish complexion. In RCM type I cyanosis is the only symptom (patients are 'more blue than sick'). In RCM type II cyanosis is associated to a severe encephalopathy which is prominent. Around 50 cases of RCM type II have been published. Definite diagnosis and knowledge of the gene mutation allow prenatal diagnosis. **Patients**: We report features in 4 new cases of RCM type II, aged 2 to 11. The patients had severe psychomotor retardation, generalized dystonia, microcephalia, strabismus and hypotrophy. One had West's syndrome. Diagnosis was delayed. In two cases, cyanosis was very mild and the diagnosis was delayed until the cyanosis became obvious during a bronchial infection (age $2\frac{1}{2}$ and 8). In two cases, although cyanosis was obvious, diagnosis was also delayed (age 1 and 4). The methemoglobinemia levels were 16 to 28 % ($n < 1\%$) and cb5r activity in erythrocyte and leucocytes 0 to 5.7% of controls. **Conclusion**: In children with unexplained encephalopathy associated with dystonia, cyanosis is the most valuable diagnostic clue in RCM type II. Cyanosis may become evident only during environmental stress (illness, cold weather, etc.). In these cases, a sallow or greyish complexion should alert. Methemoglobinemia measurement should be made, even though cyanosis is not obvious.

P-15-9

SAP-B DEFICIENCY IN SIX SAUDI CHILDREN

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Sphingolipid activator proteins (SAP) are cofactors for lysosomal degradation of sphingolipids. PSAP codes for the SAP precursor which is processed to SAP-A, SAP-B, SAP-C, and SAP-D. SAP-B stimulates the hydrolysis of sulfatide by arylsulfatase-A (ASA). PSAP mutations resulting in SAP-B deficiency are known to cause features similar to those of metachromatic leukodystrophy (MLD). Of the many leukodystrophy cases that we have evaluated in our tertiary care center (KFSH&RC), we found only one patient, in two years, with ASA-deficient MLD. During the same period, 6 patients from 3 unrelated Saudi families were found to have SAP-B deficiency. The presentation resembled juvenile MLD in four patients and late infantile form in one. Brain MRI findings were highly suggestive of MLD but the ASA assays were normal. Analysis of PSAP found that the three families segregate the same mutation despite they are from different tribes. The patients were homozygous for a g.722G>C transversion resulting in Cys241Ser change which was previously reported in an Arab patient with SAP-B deficiency [1]. We screened the patients' siblings and an asymptomatic 4-month-old girl was found to be homozygous for the same mutation. Her neurological exam, brain MRI, and nerve conduction velocity have been normal. This report suggests that, in our population, SAP-B deficiency is probably more common than ASA-deficient MLD. It also shows that the C241S is likely to be the most prevalent mutation in Arab patients with SAP-B deficiency.

[1] Holtschmidt et al. (1991) J Biol Chem. 266:7556–60

P-15-10

APPROACH TO GENETIC METABOLIC DISORDERS IN SAUDI ARABIA

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Middle Eastern cultures are tribal and heavily consanguineous. Marriage between cousins is mainly cultural, for a millennia leading to 'founder' effect and a large number of autosomal recessive diseases. In Saudi Arabia like other Middle East countries first cousin marriages account for 60–70% of all marriages, leading to uniquely common disorders which are either rare by Western standards or are unknown. The practicing physician must include these unusual disorders in his diagnostic considerations, since cybernetic trees described for European countries or USA may not be valid for the Middle East.

A review of the combined files of the Armed Forces Hospital and the King Faisal Specialist Hospital and Research Centre, Riyadh, over 10 years period, documented more than 150 varieties of neurodegenerative disease among 2000 children. Some autosomal recessive disorders are common e.g. sickle cell anaemia. Others are unique e.g. Sanjad Sakati syndrome. In these disorders the exact molecular defect is found. Therefore, prevention is possible by either pre-implantation genetics diagnosis or prenatal diagnosis according to the recommendation of our Islamic scholars.

These diseases are clinically recognizable through certain symptoms and signs. Their early recognition is important to initiate treatment and to prevent neurologic crippling. The treatment of storage diseases is experimental and is either through administration of purified enzymes (Ceredase-Gaucher) or bone marrow transplantation. A large number of these genetic metabolic disorders can be recognized in a clinical setting and have treatment modalities. However, treatment is either difficult or expensive or unavailable in most centers. Therefore, prevention is of utmost importance.

P-15-11

GENETIC METABOLIC DISORDERS: THE SAUDI EXPERIENCE

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Saudi Arabian cultures are tribal and heavily consanguineous. Marriage between cousins has been part of the culture for millennia leading to 'founder' effect and a large number of autosomal recessive diseases.

A review of the combined files of the Armed Forces Hospital and the King Faisal Specialist Hospital and Research Centre, Riyadh, documented more than 150 varieties of neurodegenerative diseases among 2000 children; 27 of which constitute more than half of these files.

The early recognition of these disorders is important to initiate early treatment especially in cases of organic acidurias and amino acidurias, urea cycle disorders, and lysosomal storage disorders to initiate enzyme treatment, to prevent neurologic crippling.

P-15-12

NOVEL MUTATIONS IN JAPANESE PATIENTS OF LEUKOENCEPHALOPATHY WITH VANISHING WHITE MATTER (eIF2B-RELATED LEUKODYSTROPHY)

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Leukoencephalopathy with vanishing white matter (VWM; MIN #603896) is a rare autosomal recessive leukodystrophy. Mutations in the five eIF2B subunits have been found in patients with VWM. We report two Japanese patients with novel mutations in eIF2B2 and 2B5. **Case 1**: She was born as the first child of healthy, non-consanguineous parent. At one year old of age, she showed lethargy with a high fever. Her cranial CT showed remarkable low density on the bilateral deep white matter. At many times she was suffered from the event of lethargy and deteriorations. At 17 months old of age she was bedridden. At 4 years old of age she died. We found mutations in eIF2B2, c.254T>A (V85E) in exon 2 and c.913–915 del in exon 8. **Case 2**: She was born uneventfully between healthy parents with consanguinity. In her childhood delayed speech and school difficulty were noted. At 9 years old of age, she fell down on the ground. After this event, mild spastic diplegia was remained. The findings of her cranial CT suggested that she was suffered from unknown leukodystrophy. At 29 years old of age, she was examined again because her symptoms had become gradually progressive. At this time she showed severe quadriplegia with spasticity, mental retardation. Her cranial MRI showed typical findings of VWM. We found homozygous mutation of c.395 G>C (G132A) in exon 3. We confirmed this diagnosis on the basis of molecular biological approach in Japanese patients with VWM.

P-15-13

CORD BLOOD STEM CELL TRANSPLANTATION IN NIEMANN-PICK DISEASE TYPE 1A

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Niemann-Pick type 1A (NPA; MIM 257200) is an autosomal recessive lysosomal storage disorder caused by deficiency of acid sphingomyelinase and accumulation of sphingomyelin and unesterified cholesterol. It is characterized by failure to thrive, hepatosplenomegaly, and a rapidly progressive neurodegenerative course culminating in death by 3 years of age. There is no known effective treatment. We report the case of a prenatally diagnosed girl who underwent cord blood stem cell transplantation (CBSCT) at 3 months of age. She is the youngest patient with this condition to have undergone any form of transplantation. An affected brother died of NPA at 23 months of age. Sphingomyelinase level on direct CVS was 0.55 nmoles/h/mg protein (controls: 11.0, 8.9). The diagnosis of NPA was confirmed at 9 days of age [leukocyte acid sphingomyelinase level of 0.1 (control: 0.6–1.8)]. Neurologically, she appeared intact. Hepatosplenomegaly was detected at 6 weeks of age. It regressed following CBSCT. Recovery was complicated by graft-versus-host disease. She suffered from recurrent respiratory infections, failure to thrive and feeding difficulties. She continues to show marked global developmental delay, generalized hypotonia, and signs of neurological regression, despite continued engraftment as shown by normal leukocyte acid sphingomyelinase levels and molecular genetic studies in blood. Bilateral cherry red spots were detected 7 months post-CBSCT. Although she is doing better than her affected brother, she shows little overall benefit from CBSCT. Although CBSCT seems to have slowed the progression of the disease in comparison to her affected sibling, it has not prevented the onset of progressive neurodegeneration.

P-16-1

COMPLEX GLYCEROL KINASE DEFICIENCY (GKD): A CASE REPORT

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Introduction: Glycerol kinase deficiency (GKD) is a rare X-linked recessive disorder. There are of two types: an isolated form and a complex form. The complex GKD is an Xp21 contiguous gene deletion involving the glycerol kinase locus together with the adrenal hypoplasia congenita (AHC) or Duchenne muscular dystrophy (DMD) loci or both, clinical features depending on the loci involved. Clinical characters are mainly caused by AHC, usually not by GKD. Here we report a case with AHC. **Case report**: a one-year and seven-month old boy was admitted to our department with complaint of progressing hyperpigmentation of gum and skin, recurrent convulsion after born for one week, accompanying with feeding difficulty, severe motor and mental retardation. Physical examination, he is in light coma, malnutrition, obvious pigmentation on gum and skin, with a straight nose and a triangular face, Adrenal insufficiency was considered. Further laboratory tests revealed hypoglycemia, hyperglycerolemia, serious hyponatremia and hyperkalemia, low serum cortisol and testosterone levels and high ACTH levels, a lot of urinary glycerol tested by gas chromatography mass spectrometry (GC/MS). After hydrocortisone and fludrocortisone replacement and a low fat diet therapy for one week, the boy improved quickly. But the parents gave up, and the boy died 15 days later. **Conclusion**: urinary levels of glycerol in patients of congenital adrenal hypoplasia should be analyzed by GC/MS to screen out complex GKD. Main cause of death in complex GKD is due to adrenal crisis, so prompt clinical recognition and management of adrenal function should be highly notable.

P-16-2

PLASMA SITOSTEROL AND CHOLESTEROL ABSORPTION IN SMITH-LEMLI-OPITZ SYNDROME

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Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive disorder caused by a defect in the final enzyme of cholesterol (CH) synthesis. Dietary CH is a potential therapy. Thus, the absorption (ABS) rate of dietary CH is important to monitor, but current methods of determining ABS are complex or involve radiation exposure, limiting their use in longitudinal studies. In adults, plasma plant sterols including sitosterol (SIT) corrected for CH (μg/mg) correlate with fractional CH ABS. As there are no data comparing plasma SIT to measured CH ABS in children, we determined if plasma ratio of SIT to CH (μg/mg) correlates with measured CH ABS in SLOS. (1) We measured plasma SIT and CH in 17 controls and 20 SLOS children by GC or GC/MS. (2) In 10 SLOS children we also measured dietary intake of CH and SIT. (3) We measured CH ABS (fecal dual isotope ratios: ^{3}H-sitostanol and ^{14}C-CH) in 7 SLOS children. Plasma SIT (mg/dL) was lower in SLOS (0.17 ± 0.09) than in controls (0.35 ± 0.19; $p < 0.001$), but the ratio of SIT to CH was similar: 1.75 vs. 1.66 μg/mg. Dietary CH and SIT intake (mg/kg/d) ranged from 1.9 to 50 and from 2.0 to 6.3, respectively. There was no effect of CH or SIT intake on plasma SIT or its ratio to CH. Fractional CH ABS in SLOS children was $35.7\% \pm 10.7$. We found no correlation between plasma SIT to CH ratio (μg/mg) and fractional CH ABS. Thus, plasma SIT may not be valid as an index for CH ABS in SLOS.

P-16-3

ROLES OF TOLL-LIKE RECEPTOR 4, PROINFLAMMATORY CYTOKINES AND STATS IN THE PATHOGENESIS OF NIEMANN-PICK DISEASE TYPE C

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Niemann-Pick disease type C (NPC) is an autosomal recessive lipid storage disorder and its brain pathology is characterized by lipid storage, neuronal loss and glial proliferation. We demonstrate constitutive secretion of proinflammatory cytokines and activation of transcription factors STATs (signal transducers and activators of transcription) in NPC cells and brains. Cultured human NPC fibroblasts secreted three cytokines, interferon-beta interleukin-6 and 8 (IL-6 and 8), and contained increased levels of STATs. These cells also contained increased levels of Toll-like receptor 4 (TLR4) that accumulated in cholesterol-enriched endosomes, and siRNA against TLR4 suppressed secretion of the cytokines. Immunohistochemistry of Balb/C *NPC1*$^{-/-}$ mouse brains revealed expression of TLR4 and IL-6 by glial cells and increased expression of STATs by both glial and neuronal cells. Genetic deletion of TLR4 in *NPC1*$^{-/-}$ mice reduced IL-6 secretion by primary-cultured fibroblasts but failed to affect brain pathology or life span. However, genetic deletion of IL-6 normalized STAT levels, reduced gliosis and caused a modest, but significant increase of their life span. These results suggest involvement of TLR4, proinflammatory cytokines and STATs in NPC pathology, providing a link between intracellular lipid transport and innate immune responses.

P-16-4
MOLECULAR ANALYSIS OF THE *DHCR7* GENE IN AUSTRALASIAN SMITH-LEMLI-OPITZ SYNDROME PATIENTS

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Background: The preferred means for the rapid and accurate prenatal diagnosis of Smith-Lemli-Opitz syndrome (SLOS) is the direct measurement of 7-dehydrocholesterol levels in chorionic villus (CV) tissue. However, the provision of small CV samples can make direct analysis impossible causing significant delays and potentially compromising the degree of accuracy. To minimise the potential for delays in prenatal diagnosis we have introduced molecular testing of the *DHCR7* gene. **Objective**: To assess the frequency of the reported common mutations in the *DHCR7* gene amongst Australasian SLOS patients. **Method**: DNA from Australasian SLOS patients was screened for six common mutations in the *DHCR7* gene (c.964-1G>C, p.T93M, p.V326L, p.W151X, p.R404C and p.R352W). These are reported to account for 71% of *DHCR7* alleles in patients of Caucasian origin (Witsch-Baumgartner *et al.,* Hum Mutat. 25(4):412, 2005). **Results**: Analysis indicates that c.964-1G>C accounts for 60% (27/45) of tested SLOS alleles in the Australasian cohort. The remaining 5 mutations constitute only a small proportion of SLOS alleles. We have used analysis of c.964-1G>C to provide rapid prenatal diagnosis of SLOS in a couple at a 1:4 risk for SLOS. Molecular testing of the *DHCR7* gene has also enabled confirmation of the diagnosis of SLOS in a terminated fetus by detection of the c.964-1G>C mutation in both parents. **Conclusion**: The c.964-1G>C mutation accounts for a high proportion of the SLOS alleles in the Australasian population. Molecular testing of the *DHCR7* gene strengthens our diagnostic ability for SLOS, particularly in the prenatal setting.

P-16-5
DISORDERS OF LIPID METABOLISM AND CHANGES OF THE LEFT VENTRICLE IN CHILDREN

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Background: Lipid disorders contribute to an individual cardiovascular risk that dramatically increases with the combination of risk factors. They start the process of the atherosclerosis early in childhood. AIM: We studied the relationship between various atherosclerosis risk factors and changes of the left ventricle geometry. **Methods**: Lipids analysis, 24 h blood pressure monitoring, anthropometric characteristics, echocardiographic assessment of the left ventricle mass (LVM), LVM index to height (LVMIV) and left ventricle geometry were performed in 90 children. **Results**: Obesity and overweight were associated with significantly higher LVM, LVMIV, blood pressure and combined hyperlipidaemia. Higher total cholesterol was associated with concentric remodelling, higher triacylglycerol with concentric hypertrophy of the left ventricle. LVM and LVMIV were significantly positively correlated with the body weight, height, BMI and blood pressure. Correlation between LVM, LVMIV and total and LDL cholesterol and apolipoprotein B was surprisingly significantly negative ($p < 0.01$, $r = -0.47$, $r = -0.37$, $r = -0.38$), and remained unchanged when adjusting to age and gender. The relationship between these lipids and the height and weight was similar. However, after elimination of the influence of the body height and weight the correlation had positive trend. **Conclusion**: Revealed interesting relationship between the body height, weight, lipids and left ventricle changes requires further study. Heart pathology caused by lipid disorders in combination with other atherosclerotic risk factors starts in childhood and therefore an active intervention is necessary.

P-17-1
LYSOSOMAL STORAGE DISEASES DIAGNOSED IN HONG KONG

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To identify the known lysosomal storage diseases (LSD) diagnosed in Hong Kong, we (1) reviewed the database of the Joint Metabolic Clinic at the Prince of Wales Hospital and (2) performed a literature search on publications of local LSD cases.

The Joint Metabolic Clinic at the Prince of Wales Hospital, one of the 2 university teaching hospitals in Hong Kong was established in January 1997. A complete patient registry was maintained and updated regularly. Up until January 2006, there are 22 LSD patients in the registry. They include 16 patients with mucopolyssacharidoses (3 MPS I, 4 MPS II, 5 MPS III, 1 MPS IV and 3 MPS VI), 3 patients with mucolipidosis II and 3 patients with Niemann-Pick type C disease. All these diagnoses were confirmed by enzymatic assays or cultured fibroblasts studies or molecular genetic studies.

Literature search through international as well as local journals identified the following LSD cases – Gaucher disease (1 case), neuronal ceroid lipofuscinosis (1 case), Fabry's disease (4 cases), glycogen storage disease Type II (3 cases of juvenile form), Niemann-Pick disease (4 cases) and fucosidosis (1 case).

In conclusion, many of the known LSDs have been identified among the local HK population. Patients are mainly of ethnic Chinese and few of Pakistani origin. Various types of MPS are the commonest LSDs seen. The set up of a local LSD patients registry will shed light towards local disease prevalence and pattern, the data of which will help towards services planning and resources distribution.

P-17-2
MOLECULAR MECHANISMS UNDERLYING JOINT AND BONE DISEASE IN THE MUCOPOLYSACCHARIDOSES (MPS)

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Animal models of the MPS have provided important insights into the causes of bone and joint pathology in these disorders. The hypothesis guiding this research is that a better understanding of these underlying mechanisms will translate into more effective therapies for human MPS patients, and may also lead to the identification of new biomarkers. At 6 months of age, an abnormal cellular and molecular profile is seen in bones and joints of MPS animals, with characteristic increases in cytokines, MMPs, and apoptotic cells. Further studies have identified the chemotactic and pro-inflammatory role of macrophage inflammatory proteins in the development of this pathology, and the formation of osteoclast-like, multinucleated cells. The production of tumor necrosis factor-alpha (TNF-α) in MPS animals up-regulates an essential osteoclast survival factor, ligand of receptor activator of NF-κB (RANKL), likely explaining the appearance of these osteoclast-like cells. The role of TNF-α and RANKL in the MPS animals is clinically supported by the occurrence of osteopenia. The results of our studies have shown that TNF-α promotes many different aspects of MPS disease. Therefore, in the future it will be important to evaluate treatment strategies aimed at mediating this effect.

P-17-3

SANFILIPPO SYNDROME TYPE D: CASE REPORT OF 2 SISTERS

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Mucopolysaccharidosis type IIID is a rare form of mucopolysaccharidosis. We present the clinical findings of 2 siblings with this condition. The older sister attained a level of learning unusual in Sanfilippo syndrome. There have been few published reports and the degree of mental retardation has been variable.

J.A. first presented at 4 years of age with coarse facial features, hirsuitism and mild developmental delay. Speech development started at 2 years old. She attended special school from 5 years of age and by 7 years, was able to write, count to 15 and had learnt sign language (AUSLAN). She was also able to read simple words. She was sociable and communicated well verbally. She was able to speak in sentences. At 12 years, her cognitive skills deteriorated and her behaviour became aggressive. By 14 years old, she had no verbal communication. She lost ability to eat and chew at 15 years old and has difficulty walking.

Her younger sister, AA, had normal development initially. She had early problems with speech and articulation, but now speaks well. She is now 11 years old and attending special school. She knows her letters, but is unable to read. She has attained less in comparison to her sister at the same age. She is sociable, talkative and able to assist with her sister's care. She has not shown signs of neurological deterioration.

Both girls exhibited the physical features typical of the condition. Deficiency of N-acetylglucosamine-6-sulphatase in peripheral blood leucocytes was demonstrated in both cases.

P-17-4

INTELLIGENCE IMPAIRMENT IN MORQUIO SYNDROME TYPE IVA: A CASE REPORT

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Background: Morquio syndrome IVA, also known as mucopolysaccharidosis IVA is characterized by short stature, severe bones disease and preservation of intelligence. We report a case of Morquio syndrome IVA with delayed speech and mild mental retardation. **Case presentation**: A 3-year-old boy was referred by pediatric psychiatrist due to growth abnormality and delayed speech. The clinical manifestation were flat nasal bridge, frontal bossing, small teeth, short barrel chest, disproportionately long arms, hyperextensible wrist and knock knees. We also found hearing impairment and delayed speech. Mild mental retardation was revealed based on psychiatrist observation. Perinatal history was unremarkable. The bone x-ray revealed J-shaped sella tursica, irregular vertebrae body and irregular cone shaped of metacarpal epiphyses. Odontoid hypoplasia was showed in cervical CT scan examination. We also found a mild cerebral atrophy and mild enlargement of bilateral ventricles from brain CT scan. The diagnosis was established by finding low level of N-acetyl-galactosamine-6-sulphate-sulphatase enzyme and elevated level of mucopolysaccharide in his urine. The patient was given supportive therapy including correction of hearing impairment by using hearing aid and speech therapy. He showed significant improvement after the therapy although his speech ability was still below normal age. **Conclusion**: Our Morquio syndrome type IVA had delayed speech and mild mental retardation. In this patient, mental retardation may be due to mild cerebral atrophy. We could not determine the cause of cerebral atrophy since his perinatal history was unremarkable.

P-17-5

A BATAKNESE FAMILY OF TWO SIBLINGS WITH SANFILIPPO SYNDROME: FIRST CASE REPORT IN CIPTOMANGUNKUSUMO HOSPITAL, JAKARTA, INDONESIA

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Introduction: Indonesia has many tribes with various customs and tradition. Bataknese is one of the tribe lives in Indonesia. In Bataknese tribe, there is some rule that prohibit marriage between relatives, so that inherited disorders are rare. **Case**: We report a 7 year-old boy and his younger brother, 4 year-old. The patients were referred by pediatrician with suspected MPS based on the radiological examination. The clinical manifestations were present at the age of 3 year-old as delayed development, delayed speech, hyperactivity, aggressive behavior, coarse face, flat nasal bridge, low set ear, frontal bossing, short stature, hepatomegaly and joint stiffness. Bone survey revealed MPS features. The diagnosis was established by clinical manifestations, radiological features and urine electrophoresis which showed abnormal accumulation of heparan sulfate without accumulation of dermatan sulfate or keratan sulfate. The patient's younger brother has the same symptom but milder. Genetic counseling should be done to inform the possibility of having child with Sanfilippo syndrome in the next pregnancy. **Conclusion**: We have to think about the possibility of genetic disorders in the Bataknese family although consanguinity is prohibited by the culture.

P-17-6

THE EFFECTS OF LARONIDASE TREATMENT IN A PATIENT WITH HURLER SYNDROME: RESULTS OF ONE YEAR THERAPY

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Mucopolysaccharidosis type I is an autosomal recessive lysosomal storage disease caused by the deficiency of L-iduronidase. The clinical spectrum in L-iduronidase deficiency varies from the severe Hurler to mild Scheie phenotype. Recombinant laronidase enzyme treatment has been progressed as an treatment opportunity in L-iduronidase deficiency.

A 4 years old female patient diagnosed to have Hurler Syndrome has been started L-iduronidase treatment in our department 12 months ago. Laronidase has been given intravenously with a dose of 100 IU/Kg dose every week. After 12 months of therapy besides the decrease in liver and spleen volumes and in total mucopolysaccharide excretion in the urine, improvement in cardiac functions, phenotypic appearance, growth parametres and physical performance were noticed.

It was stressed that laronidase treatment especially improves the physical performance and organomegaly in Hurler patients.

P-17-7

A COINCIDENCE OF MUCOPOLYSACCHARIDOSIS TYPE IIIA AND FAMILIAL HYPERCHOLESTEROLAEMIA TYPE II A: A CASE REPORT

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The Sanfilippo type A syndrome (mucopolysaccharidosis type IIIA; MPS IIIA) is one of the most frequent forms of mucopolysaccharidosis III, characterized by severe mental retardation, progressive neurological degeneration, and mild somatic changes. It is caused by a deficiency of the lysosomal enzyme heparan N-sulphatase. The disease is transmitted through an autosomal recessive mechanism, and more than 60 gene mutations have been identified.

A four year-old girl was presented with delayed speech, aggressive and extremely hyperkinetic behaviours. She had normal height, fewer skeletal deformities and progressive psychomotor retardation. The diagnosis of MPS III A was primarily suspected because of increased glycosaminoglycans in the urine and then confirmed by enzyme analysis in fibroblast (heparan N-sulphatase 0.4 nmol/h/mg). Moreover, the family history of hypercholesterolaemia and high levels of total cholesterol and LDL-cholesterol was reason for further investigation of patient's lipids status. So, her lipoprotein electrophoresis revealed a hyperlipidemia type II a. She was put on statin treatment.

Familial hypercholesterolaemia which is an autosomal dominant disorder of lipoprotein metabolism is caused by mutations in the gene for the low-density lipoprotein receptor, and to date more than 700 mutations have been reported worldwide. However, the coincidence of MPS and familial hyperlipidemia has not been described previously.

P-17-8

THE TWO CASES OF SALLA DISEASE IN TURKISH CHILDREN

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Sialic acid storage disorder known as Salla disease is a rare autosomal recessive lysosomal disorder produced by a defect of a proton-driven carrier that is responsible for the efflux of sialic acid from the lysosomal compartment. Two main categories of the disease have been classified: a conventional subtype and a severe subtype with more severe defects however, Salla disease had an extremely wide clinical variation.

A two- and four -year-old girls were referred to the metabolic unit due to the history of global developmental delay. Both patients were born from consanguineous marriage and had a health sibling. On their physical examination the height and weight were on the 25 centile for the first patient, whereas the second one had a short stature with height below the third centile; the muscle tonus was increased in both, with prominent trunkal ataxia and bilateral optic atrophies in the first and abnormal gait and without ophthalmological involvement at the second patient. The both patients had a delay at their major motor developmental milestones and linguistic skills. Their laboratory investigations revealed negative reducing substance in urine, normal blood and urine profile of aminoacids, normal glycosaminoglycans and extremely high sialic acid excretion in urine. Thereafter confirmatory fibroblast culture demonstrated accumulation of sialic acid. Magnetic resonance images revealed severe cerebral and cerebellar hypomyelination in both patients.

Regarding these clinical findings of patients with Salla disease might had a considerable variation this disorder should be put in the differential diagnosis in patients with neurodevelopmental delay born from consanguineous patients.

P-17-9

HUNTER SYNDROME IN A GIRL CAUSED BY A BALANCED TRANSLOCATION

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Introduction: Hunter syndrome is an x-linked recessive disorder. It is due to deficiency of the enzyme iduronate sulfatase. Clinical features include facial dysmorphysim, bone and joint dysplasia, hepatosplenomegaly, and neurological abnormalities. It is extremely rare for Hunter syndrome to occur in females. **Case Report**: We report on a 9-year-old girl with history of mild developmental delay. She attends mainstream school but also gets remedial and resource help on a regular basis. She had history of recurrent otitis media treated with grommet insertion. She wears hearing aids in left ear. She receives speech, language and occupational therapy. Examination showed no dysmorphic features or organomegaly. Weight and height were at 50th centile. Rest of examination was normal. Skeletal survey showed no stigmata of mucopolysachridosis. Urine revealed increase dermatan and heparan sulphate. Leukocyte enzyme assay confirmed low iduronate sulphatase. Karyotype showed 46,XX, t (X; 11) (q28; q13), balanced reciprocal translocation between X chromosome and long arm of chromosome 11. The break point appears to be Xq28 and 11q13. This translocation has occurred de novo, as cytogenetic analysis of both parents was normal. The gene for Hunter syndrome is located at Xq28 and explains the rare occurrence of Hunter syndrome in a female.

P-17-10

THALASSEMIA/SICKLE CELL SYNDROME, GLUCOSE 6 PHOSPHATE DEHYDROGENASE DEFICIENCY, AND MUCOPOLYSACHRIDOSIS TYPE 3 EXPRESSED IN ONE CHILD

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Introduction: Inbreeding between consanguineous partners is potentially harmful as it brings recessive alleles into the homozygous state in the offspring. In general, the rarer the disease and the greater the frequency of consaguinous marriage, the higher the proportion of recessive homozygotes produced by them. The risk of one person to harbour more than one genetic disease is small, however this risk is increased by consanguinety. **Case Report**: We report on 4 year old Saudi boy presented with hyperactivity and speech delay. Parents are first degree cousins with no family history of genetic disorder. Finland scale for social maturity showed that the patient had moderate low average intelligence. Clinical examination showed weight and height below 3rd centile, hepatosplenomegaly, and umblical hernia, with no evidence of dysmorphism. Complete blood count was normal. Peripheral blood film showed microcytosis and asisocytosis. Hb electrophoresis confirmed sickle cell/Thalassemia syndrome. Glucose 6 phosphate dehydrogenase (G6PD) was 9 mU/RBC (120–240 mU/RBC). Urinary glycosamino glycan (GAG) was 390.4 mg/l. GAG/creatine ratio was 87 (normal 2-15). GAG electrophoresis findings were cosistent with mucopolysachridosis type 3. **Conclusion**: This case report illustrates four genetic hits (Thalassemia/Sickle cell syndrome, G6PD, and mucopolysachridosis type 3) occurring in one child with consangeonous parents.

P-17-11

PHYSIOTHERAPEUTIC EVALUATION OF A 4 YEARS OLD HURLER PATIENT AFTER ENZYME REPLACEMENT THERAPY (ERT) WITH LARONIDASE: CASE REPORT OF 22 MONTHS OF EXPERIENCE

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We describe a male patient who has 4 years old, Hurler phenotype, with moderate mental impairment, mild mitral regurgitation, hepatosplenomegaly, hydrocephalus, moderate joint limitation and markedly neurodevelopmental delay. He was evaluated by physiotherapists specialized in children with mucopolysaccharidosis prior to ERT with laronidase (Aldurazyme®) with standard dose of 0.58 mg/kg each week, and during his treatment. He had several interruptions due to upper way respiratory tract infections and due to chronic urticaria (not related to ERT). Physiotherapeutic evaluation was performed by measuring the large articulations angles with an universal goniometer for large articulations (CARCI Indústria e Comércio de Aparelhos Cirúrgicos e Ortopédicos LTDA. São Paulo, Brazil) in lateral and dorsal lying position, and in sitting positions. Shoulder, elbow, forearm, wrist, hip, knee and ankle joints were analyzed. Goniometry procedures were based on Goniometry Manual from Prof. Dr. Amélia Pasqual Marques. During the ERT period, improvements in shoulder flexion and extension, elbow flexion, distal interphalangeal flexion, knee extension and residual flexion, cervical flexion and extension were observed. Also, a better joint mobility and an improvement of walking with help was detected, resulting in a better agility of the child, as reported by his mother.

P-17-12

OBSERVATION OF ORAL COMMUNICATION CHANGES AND ORAL MIOFUNCTIONAL CHANGES IN 9 BRAZILIAN MUCOPOLYSACCHARIDOSIS TYPE I (MPS I) PATIENTS ON ENZYME REPLACEMENT THERAPY (ERT)

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Introduction: The aim of this study was to observe possible language and oral measurements changes in nine MPS I patients who participated of a multicenter, multinational, randomized, dose-optimization study of the safety and pharmacodynamic response of Aldurazyme® (laronidase) in patients with mucopolysaccharidosis I and continued receiving laronidase at the dose 0.58 mg/kg each week after the end of the study. Nowadays they are on ERT for one year. The patients were four female and five male, from 4 to 20 years old. **Method**: We evaluated the patients with ABFW TEST (a Brazilian Language Test) to speech and language function and we used a paquimeter to oral measurements. **Results**: At the end of this period, many changes occured in oral communication: there was an improvement in vocabulary and grammatical structure in younger children who spoke few words or had no oral communication. Oral structures (lips and tongue) had a better aspect and tonus, and these findings improved the speech ability. Some of them could go to school due to better speech and acquirement of socialization. The oldest MPS I patients had a better comprehension and language use and one teenager began to change the voice.

P-17-14

MULTIDISCIPLINARY EVALUATION IN 9 MPS I PATIENTS PRIOR TO AND AFTER A CLINICAL STUDY OF DOSE OPTIMIZATION WITH ENZYME REPLACEMENT THERAPY (ERT) WITH LARONIDASE

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Nine patients, 5M/4F, 4–19 years, were evaluated prior and after a clinical trial of dose optimization study with Laronidase by multidisciplinary professionals. Prior to ERT, they presented facial dysmorphisms, corneal clouding, cardiac murmur, hepatosplenomegaly, umbilical and inguinal hernia, skeletal deformities, joints stiffness, macrocephaly, hypotonia of limbs, piramidal signs with increased tendon reflexes, Babinski sign, hypoactive tendon reflexes and behavior disturbances. The joint limitations were measured by physiotherapist. All patients had aortic and mitral valve involvement with augmented leaflet thickness; profuse rhinorrea, hypertrophic turbinates and adenoids, macroglossia and sleep apnea was present, most of them severe. After the end of study, all except one patient were evaluated again by echocardiogram but no significant changes were found; all patients presented an improvement in oral communication evaluation, in hepatosplenomegaly, in neurological examination and in reduction of impairment to daily life activities. Some patients presented improvement of corneal clouding. Although there were not significant changes in echocardiographic findings in these 12 months period it is necessary a long term follow up to correctly evaluate the changes the ERT might cause.

P-17-15

MUCOPOLYSACCHARIDOSIS TYPE III (MPS III): CLINICAL AND BIOCHEMICAL FINDINGS IN 48 PATIENTS FROM ARGENTINA

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MPS III is characterized by a disproportionate involvement of the CNS with mild somatic disease; this feature is unique among the MPSs. To achieve a better delineation of the clinical picture of MPS III, findings of 48 Argentinean patients were analyzed. This study was conducted in the Laboratory of Neurochemistry Dr. Chamoles. Medical records were reviewed for all admissions over a period between 1974 and 2004 and a subset of 48 patients with MPS III diagnosis were identified. Mean age at clinical onset was 3 years and at diagnosis was 5 years. For the whole sample, developmental delay accompanied with speech delay, and hyperactivity were found at admission in 70% and 30% of the cases, respectively.

The most frequent abnormalities reported on clinical exam were: slightly coarse face, hepatomegaly, gait disturbances and hernias. The referring physician's speciality was in order of frequency, paediatric neurology, paediatrics, genetics and endocrinology. The urinary MPS screening was strongly positive in 37.5% of the patients and mild positive in the rest. MPS urinary chromatography revealed abnormal amounts of heparan sulphate in all patients. The enzymatic assay confirmed the diagnosis. No correlation between the age of diagnosis, biochemical findings and clinical severity were found. Although the urinary MPS screening was effective, these results suggest that the diagnosis of MPS III is probably established late. We think that is necessary to continue with the educational programs for health carers and improving the availability of biochemical diagnostic tests to give the patient the opportunity of a better support.

P-17-16

A CHILD WITH SUSPECTED MORQUIO SYNDROME: A CHALLENGE TO ESTABLISHED DIAGNOSIS

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A three years old girl was consulted to division nutrition and metabolic disease with unproportion short stature and suspected to have inborn error of metabolism. She was born as a normal baby but her parent noticed that she did not gain height normally after one year of age. Physical examination showed mild coarsening of facial with broad mouth, short anteverted nose, prominent glabella, wide fontanel, and low set ears, she also had a prominent umbilical. Head circumference was normal. Skeletal manifestations were short neck and trunk, kyposcoliosis, flaring of rib, pigeon chest, genu valgus, short stubby hands and bradydactili. Nutritional status is under nutrition with low caloric and protein intake. No history of loss developmental milestones or mental deficiency and no consanguinity. Radiology findings showed kyphoscoliosis, pectus carinatum and illeal hypoplasia. There were widened metaphyses and epiphyses, widened metacarpals with pinched proximal metacarpals 2–5 and short of the phalang and long pedicles. Other radiological feature such as sella, cervical spine and odontoid were normal. Bone age showed a result as average girl. Clinical features presented in this case were matched with disorder of lysosomal metabolism with high suspicion of muchopolysaccharidosis type IV or Morquio syndrome. To establish the diagnosis we plan to investigate urine spot tests to screen for mucopolysaccharides (GAG), spectrophotometric assays with dimethylene blue and blood enzyme tests. Unfortunately we do not have those specific laboratory investigations due to financial and distance (300 km out from Jakarta). However, some laboratories that could perform examination from dry urine and blood spot with affordable trice are needed.

P-17-17

CLINICAL AND LABORATORIAL ALTERATIONS IN 3 MPS I PATIENTS RECEIVING ENZYME REPLACEMENT THERAPY (ERT) WITH LARONIDASE: A 22 MONTHS EXPERIENCE

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We have analysed the clinical manifestation and glycosaminoglycans (GAGs) urinary excretion of 3 patients with MPS I using Aldurazyme®, 0.58 mg/kg each week prior to the ERT and during the last 22 months of treatment. **Case 1**: female, 10 years, Hurler Scheie phenotype, mild mental impairment, hepatosplenomegaly, corneal opacity, severe obstructive sleep apnea, concentric hypertrophic cardiomyopathy and mild pulmonary stenosis, normal-pressure hydrocephalus. The urinary GAGs excretion prior to ERT was 119.6 mg/L of urine, predominantly of dermatan sulfate (DS). After 22 months treatment, urinary GAGs presented a decrease of 75% of baseline value, also a decrease in DS and an increase in condroitin sulfate (CS) excretion. There was clinical improvement and hepatosplenomegaly reduction with improvement in echocardiogram findings. **Case 2**: male, 10 years, Hurler phenotype, corneal opacity, moderate mental impairment, severe hydrocephalus, hepatosplenomegaly, concentric hypertrophic cardiomyopathy, mitral valve thickness and normal polysomnography exam. The urinary GAGs excretion after 22 months decreased 96% of baseline value, nowadays it is normal and predominantly of CS. The hepatosplenomegaly normalized and there was resolution of hypertrophic cardiomyopathy. **Case 3**: male, 4 years, Hurler phenotype, corneal opacity, moderate mental impairment, mild mitral regurgitation, hepatosplenomegaly. He had several interruptions due to recurrent upper airway tract infections not related to Laronidase, and a 2 months period without ERT due to governmental reimbursement problems. The urinary GAGs excretion prior to ERT was 164.6 mg/L urine of DS, there was a 37% reduction of baseline value. He had clinical improvement and hepatosplenomegaly reduction.

P-17-18

ADL IN PATIENTS WITH HUNTER DISEASE

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To clarify the activities of daily life (ADL) of Hunter disease, type II mucopolysaccharidosis, we investigated 29 Japanese patients with Hunter disease with the use of modified scoring system of the Functional Independence Measure (FIM). Total scores of motor and cognitive function in patients with the severe phenotype were highest between 5 and 7 years old, then the score progressively decreased, and reached to the minimum score at around 10 years old. Cognition scores decreased more rapidly than motor scores, generally reaching a minimum level at around 7 years old. In children with the attenuated phenotype, total scores increased progressively with age, which is similar to the pattern of healthy children, and some of the boys reached to the maximum score. However, adult patients did not show maximum scores, and patients over 25 years old showed decreasing scores. Two children and 2 adults showed lower scores as compared with other mild patients, indicating that these 4 moderately impaired patients in FIM scores might represent intermediate forms. This study helped elucidating the precise ADL of patients with Hunter disease. FIM scoring system should help accurately evaluate the severity of the disease and effectiveness of therapeutic approaches in MPS.

P-17-19

ENZYME REPLACEMENT THERAPY FOR INFANTS OF HURLER SYNDROME IN JAPAN

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Mucopolysaccharidosis I (MPS I) is a storage disorder caused by the deficiency of α-L-iduronidase (IDUA). MPS I has historically been classified into three clinical symptoms; Hurler, Hurler-Scheie, and Scheie. After multi-national world-wide clinical trial, recombinant human IDUA (laronidase) has been approved to use in more than twenty countries. Since the patients with Hurler-Scheie syndrome at the age of more than 5 years old participated in the clinical trial, only limited information is available about the therapeutic efficacy in patients with Hurler syndrome less than 5 years old. We evaluated safety and efficacy of enzyme replacement therapy in two Japanese infants with Hurler syndrome; one-year-old boy and two-year-old girl. We obtained laronidase by international charitable access program, because it has not yet been approved in Japan. Both patients received laronidase, 100 units (0.58 mg)/kg BW every week. To minimize possible infusion associated reactions (IARs), they received an antipyretic and an antihistamine before infusion. Urinary glycosaminoglycans excretion reduced within 4 weeks; 430 to 94.4 and 551 to 96.5 mg/g creatinine, respectively. Liver and spleen volume is normalized after 24 weeks. General conditions were dramatically improved, and growth and development was facilitated significantly. One patient showed IARs such as rash and mild wheezing. There was no apparent correlation between laronidase IgG antibody titer and the incidence of IARs. We conclude that enzyme replacement therapy using laronidase is effective for developmental delay as well as physical symptoms in patients with Hurler syndrome, although the improvement in mental retardation might be indirect and transient.

P-17-20

MUCOPOLYSACCHARIDOSES IN TUNISIA: A MULTICENTRIC STUDY COVERING THE PERIOD 1970–2005

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Mucopolysaccharidoses (MPS) are heterogenous disorders affecting the catabolism of glycosaminoglycans. To better define the frequency of MPS in Tunisia and to evaluate laboratory diagnosis and management of patients, we performed a retrospective multicentric survey covering a period of 36 years (1970–2005). **Results**: A total of 132 patients were collected among 104 families. The global prevalence of MPS was estimated at 8.1 case/1 million habitants. A definitive diagnosis was obtained by urinary analysis and/or enzyme assay in 92 cases. MPS III was the most frequent type (27 cases), followed by type I (22 cases), type IV (18 cases), type VI (13 cases), and type II (6 cases). Seventy percent of the cases have had heart ultrasonography. Mitral valvular dysplasia and hypertrophic cardiomyopathy appeared as the most frequent cardiac abnormalities, found in 43 and 25% of patients, respectively. Carpal tunnel syndrome was looked for in only 25% of cases, brain and cervico-occipital imaging were performed in only 25 and 15% of patients respectively. Of all MPS, 80% were still alive 10 years after diagnosis, compared to 50% for type I. Symptomatic treatments have been proposed to a minority of patients. **Conclusion**: Our study confirms that MPS are relatively frequent in Tunisia and constitute a serious public health problem. The large number of multiaffected families reflect the deficiency of genetic conselling. We also conclude that the lesional investigations and symptomatic treatments are not optimal in the absence of a multidisciplinary approach.

P-17-21

INTERNATIONAL MORQUIO A REGISTRY

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To understand natural history of MPS IVA, we collected the data including present age, sex, statue, race background, clinical course, age of onset, symptoms, present status, intelligence, activity etc. questioning through the web site (*www.morquio.com*). In total, 300 respondents from 39 countries have been summarized for statistical study.

Features included as follows. Sex ratio of male to female is 55% to 45%. Resident country is 27% from USA, 12% Italy, 10% Germany, 7% Canada, 5% etc. The average of age of diagnosis is 4.8 years. The current age ranges between 1 and 76.8 years old (20.6 years on an average). The final height over 13 years old is –7.8 SD below the standard. The deduced final height averages 116.5 cm for female patients and 121.3 cm for male patients while the birth height is 51.8 cm, compatible with the standard height. Major initial symptoms are short statue, knocked knee, kyphoscoliosis, abnormal gate, and pigeon chest noticed in over 40% of the patients. Current symptoms are short statue and bone deformity appeared in around 90% of the patients. Bone deformities, laxity of joints, and abnormal gait occupy over 60%. Intelligence is kept normal or excellent in 90% of the patients. 61% patients walk less than 200 meters, 33% are wheel-chaired and 66% of patients are operated surgically.

These statistical data help define the natural history of MPS IVA and contribute to assessment of effectiveness of advanced treatments such as enzyme replacement treatment, and also in aid in timing of the operation.

P-17-22

DIFFERENCES IN METHYLATION PATTERNS IN THE METHYLATION BOUNDARY REGION OF IDS GENE IN HUNTER SYNDROME PATIENTS: IMPLICATIONS FOR CPG HOT SPOT MUTATIONS

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Hunter syndrome, an X-linked disorder, results from deficiency of iduronate-2-sulfatase (IDS). Around 40% of independent point mutations at IDS were found at CpG sites as transitional events. The 15 CpG sites in the coding sequences of exons 1 and 2, which are normally hypomethylated, account for very few of transitional mutations. By contrast, the CpG sites in the coding sequences of exon 3, though also normally hypomethylated, account for much higher fraction of transitional mutations. To better understand relationship between methylation status and CpG transitional mutations in this region, the methylation patterns of 11 Hunter patients with transitional mutations at CpG sites were investigated using bisulfite genomic sequencing. The patient cohort mutation spectrum is composed of one mutation in exon 1 (1 patient) and 3 different mutations in exon 3 (10 patients).

We confirmed that in normal males, cytosines at the CpG sites from the promoter region to portion of intron 3 were hypomethylated. However, specific CpG sites in this area were more highly methylated in patients. The patients with p.R8X (exon 1), p.P86L (exon 3), and p. R88H (exon 3) mutations had a hypermethylated condition in exon 2 to intron 3 but retained hypomethylation in exon 1. The same trend was found in four patients with p.A85T (exon 3), although the degree of hypermethylation was less.

These findings suggest methylation patterns in the beginning of IDS genomic region are polymorphic in humans and that hypermethylation in this region in some individuals predisposes them to CpG mutations resulting in Hunter syndrome.

P-17-23

EXPRESSION OF FUNCTIONALLY ACTIVE HUMAN IDURONATE-2-SULFATE SULFATASE IN *ESCHERICHIA COLI*

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Iduronate-2-sulfatase (IDS)-(EC 3.1.6.13), is a glycosilated lysosomal enzyme involved in the catabolism of heparan and dermatan sulfate. The enzyme deficiency is responsible for Hunter Syndrome. The aim of this study was to examine the feasibility of expressing an active human sulfatase in *E. coli*. IDS cDNA was introduced in bacteria containing the prepro-protein sequence using the pUC13 plasmid. The IDS expression was evaluated measuring enzyme activity by a flurometric assay and Western-blot, using an anti-hIDS monoclonal antibody. Activities obtained for this construct ranged from 1.1 to 6.681 nmol h^{-1} mg^{-1} of protein. No activity was found in non-transformed cells. Western Blot revealed bands of 97, 62, 52, 49 and around 40 kDa in supernatant and cell lysates. The bands correspond closely to those identified in the mammalian enzyme. A second construct using the plasmid pGEX-3X, gave activities from 29 to 32 nmol h^{-1} mg^{-1} of protein. Our reference values for leucocytes are between 4 and 8 nmol h^{-1} mg^{-1} of protein. We report for the first time activity of a human sulfatase expressed in *E. coli*. Transformation of cysteine into formylglycine is essential for sulfatase activity. Our findings suggest that this mechanism is active in *E. coli*. In spite of differences in glycosilation patterns between *E. coli* and humans, this and other recent results from other laboratories suggest that *E. coli* may be useful for the expression of post-transcriptionally modified proteins, capable of being used in enzyme replacement therapy.

P-17-24

A 10 Mb DELETION INVOLVING IDURONATE-2-SULFATASE GENE OF A PATIENT WITH HUNTER SYNDROME (MPSII)

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Mucopolysaccharidosis type II (Hunter syndrome) is an X-linked lysosomal storage disorder. A novel mutation is described in a MPS II patient in whom the disorder is caused by a 10 Mb deletion.

From birth this patient has generalized hypotonia and weakness. His facial appearance was coarse. When he was 10 month old, his tongue and enlargement of liver became progressively more evident.

The definitive diagnosis was made by enzyme analysis for iduronate sulfatase in white blood cells. His enzyme activity is 0.5 nmol/mg protein/4 h (normal control: 58.4–114), this confirms the diagnosis of Hunter syndrome. PCR analysis and direct sequencing revealed that the patient DNA has no exon of the iduronate-2-sulfatase (IDS) gene. Subsequent micro-satellites analysis with four microsatellites marker (DXS102, DXS984, DXS15, DXS1108) did not detect allelic loss in chromosomal X. But the array-based comparative genomic hybridization analysis showed partial deletion of the long arm of X chromosome. This deletion span is 10 Mega bases long.

This result demonstrates this patient has a 10 Mb deletion which contains the IDS region and the FMR-2. This patient has severe hypotonia which is atypical phenotype of Hunter syndrome. This phenotype maybe contribute by the observed large deletion.

P-17-25

REVIEW OF CLINICAL COURSE FOR 9 AUSTRALIAN CHILDREN WHO HAVE MPS 1 TREATED WITH BONE MARROW TRANSPLANTATION

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Nine children with Hurler Syndrome diagnosed in Australia have had a bone marrow transplant, between 1990–2003. One patient died three weeks after transplant. The 8 remaining children are between the ages of 3.5 and 17 years. A cluster of 7 children were transplanted at an average age of 16 months (range 14–27 months) with the 8th child transplanted at 5 years of age. Intelligence is stable but has deteriorated from pre-transplant scores in all. Most have required surgery for carpal tunnel syndrome, and all have short stature with heights ranging from 2nd percentile to –8.1 SD for the eldest. Corneal clouding decreased in all but is still present. All have cardiac complications with one requiring surgery for prolapsing mitral valve leaflets. One has required multiple surgeries for cervical spine instability and wears a halo brace. All others have spinal pathology ranging from a gibbus to kyphoscoliosis. One developed sequelae from meningitis at 4 years of age. Two children of the 8 had received enzyme replacement therapy prior to and for several weeks after transplant. Six out of the 8 families have expressed their satisfaction in their decision to transplant their children.

P-17-26

MUCOPOLYSACCHARIDOSIS TYPE I (HURLER SYNDROME) IN THAI CHILDREN: REPORT OF 4 CASES (MPS IH, IH/S, IS)

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Mucopolysaccharidosis Type IH (Hurler syndrome) (OMIM 607014) is caused by mutation in the gene encoding alpha-L-iduronidase (IDUA). Deficiency of alpha-L-iduronidase can result in wide range of phenotypic involvement with 3 major recognized clinical entities: Hurler (MPS IH), Scheie (MPS IS) and Hurler-Scheie (MPS IH/S) syndromes. Hurler and Scheie syndromes represent phenotypes at the severe and mild ends of the MPS I clinical spectrum, respectively and the Hurler-Scheie syndrome is intermediate in phenotypic expression (McKusick, 1972)). The clinical features of Hurler syndromes include coarse facies, corneal clouding, mental retardation, hernias, dysostosis multiplex and hepatosplenomegaly. Children with Hurler syndrome appear normal at birth and develop the characteristic appearance over the first years of life (Wraith et al., 1987).

We herein report 2 cases of Hurler syndrome (MPS IH), 1 case of MPS IH/S and 1 case of MPS IS. **Case 1**: Two year-11 month old girl (born in 1997) with coarse facies, cloudy cornea, hirsutism, dysostosis multiplex, claw-hand deformity, umbilical hernia, hepatosplenomegaly, hearing loss, mental retardation and positive urine turbidity. **Case 2**: Two-year-old girl (born in 1998) with coarse facies, hepato-splenomegaly, cloudy cornea, hearing loss and dysostosis multiplex. **Case 3**: Four year-5 month-old boy with short stature, macrocephaly, joint stiffness, claw-hand deformity, cloudy cornea and hepatosplenomegaly. **Case 4**: Five-year-old girl (born in 1986) with cloudy cornea referred by an ophthalmologist with normal intelligence; subsequently received corneal transplant ($\times 2$). Enzyme assay was done (Prof. EH Kolodny, NYU, USA). Urinary glycosaminoglycan (GAG) demonstrated dermatan and heparan sulfate in all 4 patients.

P-17-27

MUCOPOLYSACCHARIDOSIS TYPE II (HUNTER SYNDROME) IN THAI CHILDREN: REPORT OF 16 CASES

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Mucopolysaccharidosis Type II (OMIM 309900) caused by deficiency of iduronate sulfatase enzyme which results in tissue deposits of mucopolysaccharides and urinary excretion of large amounts of chondroitin sulfate B and heparan sulfate. It is inherited as X-linked recessive; usually less severe clinically and no cornea clouding observed. Clinical manifestations include short stature, coarse facies, hepatosplenomegaly, deafness and dysostosis multiplex. Gene locus is mapped on Xq 28. (OMIM-VA McKusick). We herein report 16 cases of Hunter syndrome in Thai Children: age of onset varying from 1–4 years; age of referral /diagnosis varying from 2–13 years/2–21 years; clinical manifestations were a short stature, coarse facies, hirsutism, clear cornea, DD/MR, hepatosplenomegaly, umbilical hernia; urine glycosaminoglycans (dermatan/heparan sulfate) were detected in all cases; outcome ranged from MR/lost to follow up/death. Enzyme assays were performed in some patients (6/16). Mutation analysis is not available in Thailand. These are first reported cases of Hunter syndrome from Thailand.

P-17-28

MUCOPOLYSACCHARIDOSIS TYPE III (SANFILIPPO SYNDROME) IN THAI CHILDREN: REPORT OF 4 CASES

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Mucopolysaccharidosis Type III (Sanfilippo syndrome) (OMIM 252900) is an autosomal recessive lysosomal storage disorder due to impaired degradation of heparan sulfate. There are 4 types, each due to the deficiency of a different enzyme: heparan N-sulfatase (type A); alpha-N-acetylglucosaminidase (type B); acetyl CoA: alpha-glucosaminide acetyl-transferase (type C) and N-acetylglucosamine 6-sulfatase (type D). It is characterized by severe central nervous system degeneration, but only mild somatic disease. Onset of clinical features usually occurs between 2 and 6 years; and death typically during second or third decade of life. Type A has been reported to be most severe, with earlier onset and rapid progression of symptoms and shorter survival. Gene locus is mapped on 17q 25.3 (OMIM-VA McKusick).

We herein report 4 cases of Sanfilippo syndrome in Thai patients. Enzyme assay and mutation analysis are not available in Thailand.

P-17-29

MUCOPOLYSACCHARIDOSIS TYPE IVA (MORQUIO SYNDROME) IN THAI CHILDREN: REPORT OF 3 CASES

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Morquio syndrome (MPS IVA) (OMIM 253000) is caused by defective degradation of keratan sulfate. Two enzyme deficiencies resulting in Morquio syndrome are recognized, each with a wide spectrum of clinical manifestations; a deficiency of N-acetylgalactosamine 6-sulfatase in MPS IVA and of β-galactosidase in MPS IVB. Both types of Morquio syndrome are characterized by short-trunk dwarfism, fine corneal deposits, a skeletal (spondyloepiphyseal) dysplasia distinct from that of other MPS, and normal intelligence. Gene locus is mapped on 16q24.3 (OMIM-VA McKusick).

We herein report 3 cases of Morquio IVA: **Case 1**: Six year-old female (born in 1989) with kyphoscoliosis, prominent wrists, hand-dropping and bowed-leg deformity at age 4; coarse facies, clear cornea, pectus deformity, normal intelligence. Cervical cord (C_1-C_2) compression developed at age 6; subsequently placed on ventilatory support $\times 6$ months and expired. **Case 2**: Two-year-2 month-old female (born in 1992) with mild coarse facies, clear cornea, hirsutism, urine metabolic screen-mild positive for MPS, urine turbidity-positive and dysostosis multiplex. **Case 3**: One year-11 month-old female (born in 1999) with history of weakness of right arm and leg after a fall, subsequently developed cervical cord (C_1-C_2) compression required surgery at age 2 years and 5 months. Coarse facies, pectus carinatum, mild corneal opacity, dysostosis multiplex, hypoplasia of odontoid process and normal intelligence were observed. Urinary glycosaminoglycan (GAG) in all 3 cases demonstrated keratan sulfate and chondroitin sulfate. Enzyme assay and mutation analysis are not available in Thailand.

P-17-30

MUCOPOLYSACCHARIDOSIS TYPE IVB (MORQUIO SYNDROME) IN THAI CHILDREN: REPORT OF 4 CASES

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Morquio syndrome (MPS IVB)(OMIM 253010) is caused by defective degradation of keratan sulfate. Two enzyme deficiencies resulting in Morquio syndrome are recognized, each with a wide spectrum of clinical manifestations; a deficiency of N-acetylgalactosamine 6-sulfatase in MPS IVA and of (β-galactosidase in MPS IVB. Both types of Morquio syndrome are characterized by short-trunk dwarfism, fine corneal deposits, a skeletal (spondyloepiphyseal) dysplasia distinct from that of other MPS and normal intelligence (OMIM-VA McKusick).

We herein report 4 cases of Morquio IVB: **Case 1**: Nineteen-year-old male (born in 1983) with history of pectus deformity and kyphoscoliosis since age one; developed cervical cord (C_1-C_2) compression after a fall without surgical correction due to parental refusal; subsequently unable to walk by 12 years of age. Short stature, pectus carinatum, joint contracture, clear cornea, kyphoscoliosis and dysostosis multiplex were observed. He died with pneumonia and respiratory failure. **Case 2**: Five-year-3 month-old girl (born in 1985) with pectus deformity, knocked-knee deformity, mild corneal clouding, initially diagnosed as SED congenita. Positive urine turbidity and dysostosis multiplex were observed. **Case 3**: Six-year-10 month girl (born in 1987) with short stature, clear cornea, skeletal abnormality, pectus deformity, positive urine turbidity, dysostosis multiplex and moderate mental retardation. **Case 4**: Two-year-old male with short stature initially suspect SED congenita, knocked-knee deformity and positive urine turbidity. Urinary glycosaminoglycan (GAG) demonstrated keratan sulfate in all 4 cases. Enzyme assay and mutation analysis are not available in Thailand.

P-17-31

MUCOPOLYSACCHARIDOSIS TYPE VI (MAROTEAUX LAMY) IN THAI PATIENTS: FIRST REPORT OF 4 CASES

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Maroteaux-Lamy (OMIM 253200) is recognized as Hurler-like syndrome with normal in- telligence and excretion of dermatan sulfate, caused by deficiency of N-acetyl galactosamine 4-sulfatase (arylsulfatase B). Clinical manifestations are normal intelligence, impressively short stature, coarse faces, hirsutism, short trunk, protuberant abdomen and lumbar lordosis, progressive limited joints, claw-hand deformity, inguinal hernias, corneal opacities, hepatosplenomegaly and dysostosis multiplex. Gene locus is mapped to 5q 13-14 (OMIM-VA McKusick).

We herein report 4 cases of Maroteaux-Lamy. **Case 1**: Four-year-old female (born in 1988) with umbilical hernia, short stature, hirsutism, cloudy cornea, claw-hand deformity and joint stiffness. **Case 2**: Eleven-year-old girl (born in 1998) with growth retardation, coarse faces, cloudy cornea, hirsutism, unbilical hernia, hepatosplenomegaly, joint stiffness, dysostosis multiplex. **Case 3**: Thirteen-year-old boy (born in 1992) with short stature, cloudy cornea with secondary glaucoma, hepatosplenomegaly, inguinal hernia, claw-hand deformity, thickening of mitral and aortic valves. **Case 4**: Eight-year-old boy (born in 1996), sibling of case 3, with short stature, coarse faces, cloudy cornea, hepatosplenomegaly, claw-hand deformity. Urinary glycosaminoglycan (GAG) demonstrated dermatan sulfate in all 4 cases. Enzyme assay and mutation analysis are not available in Thailand.

P-17-32

MUCOPOLYSACCHARIDOSIS TYPE VII (SLY SYNDROME) IN A THAI BOY: FIRST REPORTED CASE

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Sly syndrome (MPS VII) (OMIM 253220) is a mucopolysaccharidosis with short stature, unusual faces, protruding sternum, hepatosplenomegaly, umbilical hernia, thoracolumbar gibbus, marked vertebral deformities and moderate mental retardation, caused by deficient β-glucoronidase enzyme. Fine corneal opacities is noted at later age and dysostosis multiplex are moderately severe. Hepatomegaly, inguinal/umbilical hernias, corneal clouding have wide spectrum of severity. Urinary glycosaminoglycan demonstrated dermatan sulfate, heparan sulfate, chondroitin 4,6-sulfates. Gene locus is mapped at 7q21.11 (OMIM-VA McKusick).

We herein report a first case of Sly syndrome in Thailand. Ten-year-old boy was referred to us with learning difficulty, mental retardation, coarse faces, claw-hand deformities, pectus deformities, lordosis, knocked-knees, mild cloudy cornea, hearing loss, short stature and dysostosis multiplex. Urinary glycosaminoglycan (GAG) findings were consistent with MPSVII. Enzyme assay sent to New York University (Professor EH Kolodny) for confirmation of diagnosis of Sly syndrome. Enzyme assay and mutation analysis are not available in Thailand.

P-17-33

GAUCHER DISEASE: REPORT OF 5 CASES IN THAI CHILDREN

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Gaucher disease (GD) is an inborn error of glycosphingolipid metabolism and inherited as an autosomal recessive trait. GD is the most common of the lysosomal storage diseases resulting from deficiency in the lysosomal acid B-glucosidase enzyme that leads to accumulation of glucocerebroside (glycosylceramide). There are 3 types: Type I (OMIM 230800) – nonneuronopathic (splenomegaly, pancytopenia, hepatomegaly, bony pain, fractures, avascular necrosis), Type II (OMIM 230900) – infantile cerebral type or acute neuronopathic (hepatosplenomegaly, retroflexion of head, splenomegaly, hypertonicity, rapid neurologic deterioration and death within 2 years), Type III (OMIM 231000) subacute neuronopathic (hepatosplenomegaly usually precedes neurologic abnormality, supranuclear gaze palsies and death in childhood).

We herein report 5 cases of Gaucher disease: 3 cases of Gaucher Type I with age of diagnosis ranged from 6–12 years (by bone marrow aspiration), enzyme deficiency was identified in one case, all 3 cases are alive, one case receive ERT; one case of Gaucher Type II with age of diagnosis at 6 month (post mortem); one case of Gaucher Type III with age of diagnosis at 10 months (by bone marrow aspiration) with enzyme deficiency and receive ERT. Enzyme assay and mutation analysis are not available in Thailand.

P-17-34

FIRST CHILEAN CASE OF MPS 1 ON ENZYME REPLACEMENT THERAPY IN AN EARLY TREATED PKU IN CHILE

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MPS-1 is due to deficient lysosomal alpha-L-iduronidase and is characterized by the deposit of mucopolysaccharides in multiple organs. The clinical phenotype ranges from severe form (Hurler) to adult Scheie variant. DGC is a male patient, 5 years 4 months old, first son of healthy, nonconsanguineous parents. He started treatment for an early diagnosed classical PKU (PHE 1.308 μM/L and TYR 66 μM/L) at 24 days of life. At 12 months short stature was noted and later mild developmental delay. At age 3 years coarse facial features were evident, corneal clouding appeared at 3 years and 6 months. Alpha-L-iduronidase was 0 mU/mg. The boy developed thick skin, large liver and spleen, dysosotosis multiplex and later ventilatory difficulties. He continued on a Phe restricted diet and started enzyme replacement therapy with alpha-L-iduronidase (Aldyrazyme®), dosis 0.58 mg/kg/week at 5 years 1 month of age. He has experienced decreased ventilatory disturbances; more articular mobility and less hepatosplenomegaly alter 13 weeks enzyme replacement. To conclude we emphasize to investigate early treated PKU patients with abnormal development to initiate the treatment of other conditions like MPS 1 as early as possible.

P-17-35

PSYCHOSOCIAL OUTCOMES OF BONE MARROW TRANSPLANT FOR INDIVIDUALS AFFECTED BY MPS I HURLER DISEASE: PATIENT SELF-REPORT OF PERSONTALITY AND PERSONAL ADJUSTMENT

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Aims: To explore the composite scores, and clinical and adaptive scales of the Behaviour Assessment System for Children: Self Report of Personality (BASC-SR) (Reynolds and Kamphaus, 1992) for individuals affected by mucopolysaccharidosis I Hurler Disease (MPS IH) post-BMT. Particular attention was given to the Personal Adjustment composite and to its contributors. **Participants and Method:** Eighteen patients participated, along with their mothers. Patients' ages ranged from 8 to 25 years (M = 14.5 years). Semi-structured interviews with patients' mothers were utilised, and patients were administered tests of cognitive function and the BASC-SR. **Results:** Hierarchical multiple regression on the Personal Adjustment composite of the BASC-SR demonstrated that 95% of the variance could be explained ($F = 18.741_{3,2}$, $p = .051$) by aspects of the MPS condition in terms of adaptive skills, and physical and learning disability; and by parental and family factors, particularly maternal coping and family environment. Regarding the clinical and adaptive scales, no overt behavioural difficulties were observed. However, possible trends emerged, which highlighted adjustment difficulties with school and feelings of inadequacy for the 8–11 year age group; and a tendency towards inhibition and withdrawal for the 12 years and over age group. **Conclusion:** The findings illustrate how aspects of the MPS condition contribute to individuals' personal adjustment. They also implicate parenting and family factors. These issues therefore require attention when providing support to patients and their families. Appropriate classroom support for this patient group also requires attention, as does the question of whether psychosocial support should be considered within the school environment.

P-17-36

LIVING WITH MPS I HURLER DISEASE POST-BMT: PATIENT COGNITIVE FUNCTION AND EDUCATION

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Aims: To explore possible determinants of cognitive function of individuals affected by mucopolysaccharidosis I Hurler Disease (MPS IH) following bone marrow transplant (BMT); and to explore education and cognitive function according to patient age. **Participants and Method:** Forty-four patients participated. Their ages ranged from 16 months to 25 years (M = 9.7). Semi-structured interviews with patients' mothers were utilised, and patients were administered tests of cognitive function. **Results:** Hierarchical multiple regression on patient cognitive function as measured by the WISC-III UK (Wechsler, 1991), WAIS-III UK (Wechsler, 1997) or Griffiths Mental Development Scales (Griffiths, 1971) demonstrated that 65% of the variance could be explained ($F = 5.733_{3,22}$, $p = .005$) by aspects of the child's physical health and care needs, by the mother's past or present job classification, and by mothers' expectations of the child. No significant differences were found between the mean scores of cognitive function between patients aged under 12 years and patients aged 12 years and over. **Conclusion:** The findings illustrate potential links between child cognitive function post-BMT and a number of medical and organic factors associated with MPS IH. They also implicate aspects of the mother, particularly her expectations of the child and the seniority of her present or previous job in child cognitive development. Such information requires attention when providing support to patients and their families. The study also illustrates child attendance at mainstream and special needs schools, and presents parents' ratings of satisfaction with their children's education.

P-17-37

PSYCHOSOCIAL OUTCOMES OF BONE MARROW TRANSPLANT FOR INDIVIDUALS AFFECTED BY MPS I HURLER DISEASE: PATIENT WITHDRAWAL, AND ADAPTIVE AND SOCIAL SKILLS

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Aims: To explore patient withdrawal, and adaptive and social skills as measured by the Behaviour Assessment System for Children (BASC) (Reynolds and Kamphaus, 1998). **Participants and Method:** Thirty-nine families with children affected by MPS IH post-BMT participated. Patients' ages ranged from 2.5 to 25 years (M = 10.4). Semi-structured interviews with mothers were utilised. Fathers were administered questionnaires by telephone, and patients were administered tests of cognitive function. **Results:** When comparing scale scores for patients aged 2.5–5 years, 6–11 years, and 12+ years, a pattern emerged, which indicated greater withdrawal and less well-developed adaptive and social skills for the younger and older age groups, but scores within the normal range for the 6–11 age group. Hierarchical multiple regression on adaptive skills demonstrated that 78% of the variance could be explained ($F = 3.332_{4,10}$, $p = .056$) by patient self-esteem, maternal stress and coping, maternal anxiety about child welfare, and aspects of the family environment. For social skills (86%, $F = 8.481_{4,10}$, $p = .003$) the most significant contributors were patient cognitive function, self-esteem, and internalising problems, plus parenting and family environment variables. Regarding patient withdrawal (77%, $F = 3.270_{4,10}$, $p = .059$), adaptive skills, maternal coping, and family cohesion contributed significantly to the variance. **Conclusion:** The findings illustrate how patient adaptive skills and social competency can be impacted upon by disabling aspects of the condition. They also show how internal resources such as self-esteem, and external influences, such as parenting and family-related factors can play a role in the development of such skills.

P-18-1

INDUCTION DEATH IN CORTICAL NEURONS CELLS BY DNA DISORDER

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DNA damage, as an important initiator of neuronal cell death, has been implicated in numerous neurodegenerative conditions. In this experimental model, primary embryonic cortical neurons were prepared from E14-16 rat embryos. A density of 1.2×10^5 cells/well plated on the wells, pre-coated with poly-D-lysine. Camptothecin (10^{-5} M) was added to neuronal culture after 24 h. After 4, 6, 24 and 48 h, expression of the P38 and ATF-2 was studied using primary antibody in the immunocyto-chemistry technique, and number of healthy and death nuclei were counted by cell lysis buffer. Then percentage of the healthy, death and expression of the cells was analyzed by one way ANOVA followed by Tukey's post test. Percentage of the expression of P38 was 4%, 20%, 40% and 55%, and percentage of the survival was 95%, 85%, 64% and 50% for 4, 6, 24 and 48 h, respectively.The expression of ATF-2 was also 3%, 20%, 30%, 45% and percentage of the survival was 97%, 85%, 64% and 50%, respectively. Percentage of the expression and survival of the P38 neurons for 24 h were 40% and 64% and it was for ATF-2, 30 and 64% respectively which these results compared to control were increased significantly ($p < 0.05$). This study revealed that expression of the P38 and ATF-2 was increased simultaneously.Thus, in this model, Camptothecin induces neuronal death by stimulation P38-ATF-2 pathway.

P-18-2

CITRIN DEFICIENCY (NICCD) IN SOUTH CHINA: A REPORT OF 2 CASES

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Two patients in Guangdong, China,were reported with citrin deficiency (NICCD). Patient 1, male, 6 months, was referred because of jaundice nearly 6 months. Physical examination showed obvious jaundice and hepatomegaly. Laboratory tests revealed elevated GGT, ALP, AST, ALT, TBA, conjugated bilirubin, ammonia, lactate, cholesterol, triglyceride, AFP, free fatty acids, and Tyr, Met, Cit and Thr as well, with decreased total protein/albumin and fibrinogen in the blood,and large quantity of galactose and galactitol in the urine. SLC25A13 gene mutation analysis revealed a compound heterozygote of mutation 851del4 and 1638ins23. After treatment with Arg, multiple fat-soluble vitamins,and a formula free of lactose and enriched with medium-chain fatty acids, jaundice disappeared rapidly, laboratory findings recovered gradually,and hepatomegaly retracted gradually. Patient 2, female, was suspected to have galactosemia or PKU by neonatal screening. She was also once diagnosed putatively as a case of succinylacetone-negative tyrosinemia type I, based on the abnormal liver function indices, increased serum level of AFP, and blood levels of Met, Tyr, Arg and Cit, and increased levels of 4-hydroxyphenyllactate,4-hydroxy-phenylpyruvate, galactose,and galactitol in urine. However, mutation 851del4 in one allele of the gene SLC25A13 was found at her age of 1 year, and galactosemia was rule out by GALT and GALE analysis, together with galactose and galactose-1-phosphate detection. These findings lead to the gradual reduction of the lactose and Phe/Tyr-free formula. She is 15 months presently, and develops well. Due to the laboratory findings and her benign prognosis, she was clinically suspected to have NICCD. There are also NICCD cases in south China, and the right diagnosis and treatment in time will benefit such patients quite well.

P-18-3

CONGENITAL DISORDER OF GLYCOSYLATION TYPE 1a IN MALAYSIA: CASE REPORT AND OUTCOME

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Reports of congenital disorder of glycosylation (CDG) are uncommon in Asian patients. A one-day-old Malay baby girl was referred for dysmorphic features. The parents were second cousins with no significant family or antenatal history. She was delivered at term with a birthweight of 3.6 kg. During the postnatal examination, she was noted to have bilateral inverted nipples, abnormal fat distribution over her thighs, buttocks and suprapubic regions. She has a flat nasal bridge and prominent nares. The rest of the examination was normal.

Investigations showed cerebellar hypoplasia on the cranial MRI. An echocardiogram showed pericardial effusion. The serum transferrin isoform pattern analysis showed elevated levels of disilo-transferrin isoform and trace asialo-transferrin isoform of CDG type 1. Peripheral leucocytes showed decreased phosphomannomutase: 0.6 nmol/min/mg protein (normal 3.6–9.0) and normal level of phosphomannose isomerase confirming CDG type 1a. Further investigations showed hypothyroidism, persistent hypoalbuminemia and impaired coagulation. The patient was treated with anti-cardiac failure therapy, L-thyroxine and intravenous albumin infusions. She developed recurrent acute respiratory distress from the severe pericardial effusion. She was transfused with fresh frozen plasma before pericardiocentesis was performed. She succumbed to her illness at the age of 7 months.

To the best of our knowledge, this is the first case report of CDG type 1a in Malaysia. A high index of suspicion is needed to achieve an early diagnosis and to allow treatment of the major complications related to CDG type 1a. Despite her early demise, the diagnosis brought comfort to the couple as prenatal diagnosis will be possible for their future pregnancies.

P-18-4

ROLE OF CYSTILAC IN THE NUTRITION OF CHILDREN WITH CYSTIC FIBROSIS

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Cystic fibrosis (CF) is an autosomal recessive disease affecting 1 in 2500 newborns among Caucasians. The major clinical characteristics are progressive lung disease and pancreatic insufficiency Problems with digestion lead to malnutrition. Milupa Cystilac – CF formula – is a hypercaloric formula based on the specific nutritional needs of children with CF in their first years of life. Cystilac is higher in energy, fat, protein, vitamins, minerals and trace elements than a formula for healthy children. The aim of our study was to assess effectiveness of Cystilac in nutrition for children with CF. Thirty-three children aged from 3 to 15 months were enrolled in this 6 month duration study. Twenty-eighy children finished the observation. Each had their height, weight and head circumference measured every 3 months. Blood tests were performed at the beginning and at the end of the study (prealbumins, total protein, sodium, vitamins A and E, RBC, WBC, haemoglobin). Every month acceptance and tolerance of Cystilac were checked. After 2 and 5 months the parents prepared detailed nutritional protocols. Statistical analysis of the studied children and a control group was based on the T-Student test. There was no statistical difference in somatic development between the groups. The parameters of vitamin A, E, protein and prealbumins were however significantly higher in the Cystilac group, as were energy, protein, and carbohydrate dietary intake. Cystilac is a practical, effective and convenient diet for CF children. It is well tolerated and accepted by CF infants and children.

P-18-5

IDENTIFICATION OF NOVEL MUTATIONS AND THE COMMON MUTATION IN THE HUMAN *NOTCH3* GENE OF KOREAN PATIENTS WITH CADASIL

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Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a small vessel disease of the brain, which is characterized by recurrent subcortical ischemic attacks, stepwise or progressive cognitive decline, and white-matter abnormalities on brain magnetic resonance images (MRI). The disease is caused by mutations in the *NOTCH3* gene, which encodes a transmembrane receptor expressed in vascular smooth muscle cells. It has been reported that the mutations of *NOTCH3* were mostly found in EGF-like domain 2–8 of NOTCH3 protein, which corresponded to exon 2 to 6 of the gene. To characterize molecular defects of the *NOTCH3* gene in Korea patients with clinical diagnosis of CADASIL, we screened 388 unrelated patients with stroke, cerebral infarction, and/or vascular leukoencephalopathy by PCR-directed sequence analysis of hot spots on *NOTCH3* gene using genomic DNA isolated from peripheral leukocytes. We identified 16 distinct nucleotide variations in 55 out of 388 patients tested, including seven novels; three non-cystein involved variations (R75P, R75Q, P167S) and four cystein involved mutations (C988Y, R587C, C606R, C971S). The R544C mutation was the most common mutation, which was identified in 26 out of 55 patients. In conclusion, we have identified 16 distinct mutations including 7 novels of *NOTCH3* gene. Genetic testing might be feasible as a confirmatory diagnostic test in clinically ambiguous cases.

P-18-6

A SIMPLE FLUOROMETRIC SPOT TEST FOR DIAGNOSING CHITOTRIOSIDASE DEFICIENCY

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Objective: Determination of plasma chitotriosidase activity is increasingly being used in diagnosis and monitoring the activity of Gaucher's disease and screening of lysosomal storage disorders. Its usefulness as a surrogate marker in these conditions is hampered by the relatively high rate($\sim$6%) of chitotriosidase deficiency. In this study, a simple fluorometric spot test for diagnosis and/or screening of chitotriosidase deficiency is presented. **Method**: The principle of present spot test largely rely on the the method developed by Hollak et al. (J Clin Invest. 1994;93:1288–92). Briefly, 25 μL of plasma was incubated with 100 μL of 22 μmol/L methylumbelliferryl-β-D-N-N′-N′-triacetyl chitotriosidase (Sigma M-5693) in McIlvain's phophate-citrate buffer; pH = 5.2, for 1 h at 37°C in darkness. The reaction was terminated by adding 120 μL 0.5 mol/L Na_2CO_3-$NaHCO_3$ buffer, pH = 10.7. Immediately following this step, 10 μL of the reaction mixture was spotted on Whatman No:1 filter paper and its fluorescence intensity was evaluated in a dark cabinet by naked eye under long wave-length UV light. **Results**: The samples those were predicted to be within the normal range of chitotriosidase activity (4–195 nmol/h/ml) yielded a light-blue fluorescence, while the samples with chitotriosidase deficiency (null-activity) just reflecting light-brown spots without any fluorescence. **Conclusion**: This simple fluorometric method unnecessitating sophisticated instrumentation may successfully be used in population screening studies and rapid discrimination of individuals with chitotriosidase deficiency; moreover, this simple method may assist in choosing the alternative tests for diagnosing and monitoring the affected individuals.

P-18-7

IDENTIFICATION OF A NOVEL MUTATION IN THE ASPARTYLGLUCOSAMINIDASE GENE IN A CONSANGUINEOUS MANDEAN FAMILY

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Aspartylglucosaminuria (AGU, MIM 208400) is a rare lysosomal storage disorder caused by a deficiency of the enzyme aspartylglucosaminidase. Although relatively common in the Finnish population due to a founder effect, cases in most other ethnic groups are rare. The Mandean community follow an ancient faith and now number less than 100 000, living mostly in southern Iraq and the neighbouring region of Iran.

We report 4 cases of aspartylglucosaminuria in two generations of a consanguineous Mandean family originating from southern Iran and now living in Australia. Three of 4 children born to first cousin parents showed signs of delayed development from around 18 months of age and mild coarsening of facial features. All 3 showed increased urinary aspartylglucosamine and the enzyme defect was confirmed in skin fibroblasts. A maternal uncle was also shown to be affected.

We devised primers covering the exon/intron boundaries for the 9 exons and sequenced the PCR products to identify the mutation. A homozygous sequence variation c.941G>A at the beginning of exon 9 resulting in a glycine to aspartate change in amino acid 314 was identified in the affected family members with the mother being heterozygous. The amino acid substitution lies in β sheet 6 of the mature protein and is highly conserved.

P-18-8

IMPROVED SERVICE DELIVERY FOR 'HIGH RISK' METABOLIC PATIENTS

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Background: Mortality and morbidity rates are high in some metabolic conditions during acute episodes of decompensation. Therefore, recognising signs of deterioration and prompt management are critical. The State of Victoria (land area >228 000 square kilometres; population 5 million) has a centralised metabolic service based at the Royal Children's Hospital, Melbourne. Patients from across the state would likely present at other medical centres in an emergency, where health professionals may lack knowledge in the recognition and treatment of rare metabolic conditions. Many patients/parents feel a medical alert bracelet is unsuitable because of age or stigma. **Objective**: To provide an alternative means of improving service delivery to an at risk' group of patients in an emergency situation. **Method**: A laminated, credit-card sized Emergency Management' card was developed (costing approximately A$1 per card). The card states the diagnosis, possible presenting symptoms, emergency treatment and contact details of the Metabolic Service. Conditions covered include defects in urea cycle, carbohydrate, amino acid, fatty acid and mitochondrial energy metabolism. **Results**: A preliminary survey of over fifty parents/patients who use the card has yielded positive feedback. The card is also unanimously endorsed and utilised by health professionals within hospitals and others in the community setting (family practitioner, community nurse, childcare workers etc). An ongoing evaluation process is under way. **Conclusion**: The usage of the card has resulted in improved service delivery during emergency situations.

P-18-9

ETHYLMALONIC ENCEPHALOPATHY DUE TO ETHE1 MUTATION

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Ethylmalonic encephalopathy is a progressive inborn error characterized by neurologic impairment, petechiae, acrocyanosis and high excretion of ethylmalonic acid in the urine. Recently Tıranti, Briem and co-workers have showed that the mutations in ETHE1 gene are the main cause of the disease. Four years old male patient admitted to our department with mild mental and motor retardation, intermittent diarrhea and acrocyanosis in the hands. The physical examination revealed increased deep tendon reflexes and positive Babinski sign. Bilateral hypodensity in the white matter was noticed in magnetic resonace imaging. As a very high excretion of ethylmalonic acid was noticed in the urine DNA sample was investigated for possible ETHE1 mutation in Pierfranco and Luisa Mariani Center, Italy. The investigation showed homozygous G→T mutation at nucleotide position 3 of the cDNA affecting the first methionine of the ETHE1 gene.

P-18-10

INCIDENCE OF OSTEOPOROSIS IN A METABOLIC UNIT

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Osteoporosis is a common disease characterized by reduced bone mass, with a consequent increase in bone fragility and susceptibility to fracture risk. Bone mineral density (BMD) measurement is a reliable test that is used to make the diagnosis of osteoporosis and to predict fracture risk. Dual Energy X-ray Absortiometry (DEXA) is the most popular and effective method utilized for osteoporosis screening. The aim of this study to determine the prevelance and types of inherited metabolic disease in osteoporotic patients followed-up in our metabolic unit. Data of 98 patients with osteoporosis followed-up for last 10 years were evaluated retrospectively and presenting symptoms, anthropometric data, BMD Z scores, comorbid diseases and therapy were noted. Mean diagnosis age of cases was 11.5±4.1. Female: male ratio was 32:66. Median BMD Z score was –2.96± 096. Of the cases 22 % had inherited metabolic diseases and median BMD Z score was –3.69 ±1.18. In this group, 11 osteoporosis patients caused by primer defect were included 8 of patients with osteogenesis imperfecta, 2 patients with homocystinuria, 1 patient with infantil hypophosphatasia. 10 osteoporosis patients caused by secondary defect were included 5 of patients with glycogen storage disease type 1, 3 patients with Gaucher disease, 1 patient with Fabry disease, 1 patient with propionic acidemia and 1 patient with phenylketonuria. Osteoporosis patients were treated with calcium-rich diet, vitamin D_3 and biphosphonates. In conclusion, in our patients inherited metabolic diseases were an important cause of osteoporosis and median BMD Z score was higher in this group. For this reason, patients with inherited metabolic disease should be evaluated carefully for osteoporosis.

P-18-11

DILATED CARDIOMYOPATHY AND HYPERTRANSAMINASEMIA IN A CONGENITAL DISORDER OF GLYCOSYLATION TYPE-Ix

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Congenital disorders of glycosylation (CDG) are a rapidly expanding family of genetic diseases characterized biochemically by abnormal glycosylation of serum and cellular glycoproteins. We report two male siblings whose parents are 1st degree cousins. The older brother was referred at age 1 year for evaluation of mild hypertransaminasemia discovered since age 5 month as part of evaluation of FTT with normal psychomotor development. Persistent hypertransaminasemia prompted a liver biopsy at age 13 months that showed mild portal inflammation and periportal fibrosis. Serum transferrin (Tf) isoelectric focusing (IEF) showed a type 1 profile suggesting a glycan assembly defect (CDG-I). However, analysis of the patient's fibroblasts eliminated CDG-Ia and CDG-Ib and lipid-linked oligosaccharide analysis did not show any abnormal accumulation of glycan intermediates.

At age 6 years, a finding of a new systolic murmur led to the diagnosis of dilated cardiomyopathy (DM). Screening of the family for cardiomyopathy revealed DM, hypertransaminasemia and abnormal serum Tf-IEF in his asymptomatic 5y old brother. **Conclusion**: these siblings represent a novel type of CDG-Ix, seemingly restricted to the liver and the heart. CDG should be considered in the differential diagnosis of children presenting with clinically asymptomatic hypertransaminasemia, and in patients presenting with cardiomyopathy.

P-18-12

PHARMACOKINETICS OF MIGLUSTAT IN JUVENILE GM2 GANGLIOSIDOSIS

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GM2 gangliosidosis (GM2) is an inherited neurodegenerative disorder caused by lysosomal β-hexosaminidase A deficiency. Substrate reduction therapy is currently one of the therapeutic options for GM2. **Objective**: To establish the pharmacokinetics (PK) of miglustat (Zavesca®) given as single and multiple doses in patients with juvenile GM2. **Methods:** Five patients received oral miglustat at the dose of 100–200 mg t.i.d. Patients underwent PK assessments at day 1 and after 3 months of treatment. Blood samples were drawn immediately before and up to 24 h after drug administration. **Results**: The mean AUC_τ after a single dose was less than after multiple doses, ($10\,871 \pm 2918$, and $21\,081 \pm 8200$, respectively), suggesting accumulation of about 2-fold. No differences were found between tmax, C_{max}, and $t_{\frac{1}{2}}$ for single and multiple doses. The major side effects observed among the 5 studied patients were diarrhea and weight loss. Both improved with dietary modification. Two patients who showed frequent and severe diarrhea showed the highest values of AUC_τ and C_{max}. **Conclusion**: The PK of miglustat was similar after single- and multiple-dose administration. Dose-dependent side effects of the drug were common but manageable.

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P-18-13

POTENTIAL PHARMACOLOGICAL CHAPERONES FOR JUVENILE AND LATE-ONSET GM2 GANGLIOSIDOSIS

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GM2 gangliosidosis is an inherited neurodegenerative disorder caused by lysosomal β-hexosaminidase A (Hex A) deficiency. **Objective**: To identify by high throughout screening (HTS) potential chemical compounds that enhance mutant enzyme activity *in vitro*. **Results**: Fibroblasts with diverse *HEXA* and *HEXB* mutations were cultured in media with two compounds, N-acetylglucosamine thiazoline (NGT) and one of the hits from HTS, H1. Treatment with H1 or NGT for 3 days produced 3-fold and 2.5-fold increases, respectively, in Hex A activities in TSV fibroblasts with a G269S/null allele genotype. H1 or NGT treatment of SV fibroblasts with C137Y/C137Y genotype, produced 3-fold and 2-fold increases, respectively, of Hex A activity. H1 or NGT treatment of fibroblasts with R505E/Dexon 13 genotype showed 2–4-fold increases in Hex A activities. Similar treatment of TSV fibroblasts with other juvenile mutations showed modest enhancement of Hex A activities. **Conclusion**: NGT and H1 function as pharmacological chaperones for specific *HEXA* and *HEXB* mutant gene products causing GM2 gangliosidosis. These chemical compounds may stabilize the conformation of the mutant enzyme, increasing the amount of Hex A reaching lysosomes. The compounds constitute a potential alternative therapeutic approach for late-onset forms of GM2.

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P-18-14

IDENTIFICATION OF A NOVEL MUTATION IN A FAMILY WITH HEREDITARY COPROPORPHYRIA (HCP)

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Hereditary coproporphyria (HCP) is an autosomal dominant disorder that results from defects in the enzyme coproporphyrinogen oxidase (CPOX) which is the sixth enzyme in the haem biosynthetic pathway. A major clinical feature is neurologic damage that leads to peripheral and autonomic neuropathies and psychiatric manifestations. Clinical expressions of acute porphyrias are usually linked to environmental factors such as nutritional status, drugs and hormones or their metabolites. These precipitating factors may cause acute episodes of a variety of neuropathic symptoms.Generally, the porphyrias have incomplete penetrance, making it more difficult to diagnose carriers by solely relying on biochemical methods. Mutation screening was carried out in a family with one member being symptomatic for HCP. Primers were designed for the exon/intron boundaries of the 7 exons of the CPOX gene located on chromosome 3. A novel mutation was identified in exon 5 at c.1064A>C causing a substitution in amino acid 355 from glutamine to proline (p.Q355P). Evolutionary analysis of other CPOX sequences shows that this amino acid is highly conserved across species. A DHPLC method has been developed to screen the extended family for this mutation. We intend to collect data through a clinical questionnaire to assess the penetrance of this mutation. Identification of carriers who may be prone to acute attacks will benefit from early preventative measures and avoiding high risk environmental factors.

P-18-15

ASYMPTOMATIC PRESENTATION OF MORBUS POMPE

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Morbus Pompe is a muscular glycogen storage disease. The early onset form is lethal, usualy within the first year of life. The late-onset form comprises a wide spectrum of age at presentation and disease progression. A case without symptoms or signs was diagnosed by chance because of persistently increased transaminases and creatine kinase at age 1.5 years. Muscle biopsy did not show clear abberations or signs of glycogen storage

The diagnosis M. Pompe was confirmed by a decreased acid alpha-glucosidase (GAA) acitivity in leukocytes (16 nmol/h/mg protein, ref range 58–228 nmol/h/mg protein using glycogen as a substrate) and fibroblasts (159 nmol/h/mg protein, ref range 679–1865 nmol/h/mg protein using glycogen and 2 nmol/h/mg protein, ref range 23–84 nmol/h/mg protein using 4-MU as a substrate). Two common Dutch mutations (IVS1-13T→G en del exon 18) were found in the gene encoding acid alpha-glucosidase.

Enzyme replacement therapy is considered a useful treatment strategy. The earlier treatment is started the more effective it seems to be. However, the treatment procedure can be considered to have a high impact on day to day life.

We conclude that we diagnosed a young case of M. Pompe, being asymptomatic and without specific changes in muscle biopsy. In this case it is hard to determine prognosis and to decide whether – and if yes when – enzyme replacement therapy should be started.

P-18-16

CONGENITAL DISORDERS OF GLYCOSILATION TYPE I A (CDG Ia) IN ARGENTINE: CLINICAL, BIOCHEMICAL AND MOLECULAR FINDINGS IN TWO PATIENTS

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CDG-Ia is the most common form of CDG and is caused by phosphomannomutase (PMM2) deficiency. The typical reported features are highly variable ranging from mild clinical manifestations to severe systemic disease. We report the findings in the two first cases detected in Argentine.

Patient 1: Eighteen years old female. In the neonatal period she presented with low APGAR score, hypotonia, feeding difficulties, low weight, strabismus and fat pads. After that she developed ataxic gait, lower extremities hypotrophy, stroke-like episodes, venous thrombosis, amenorrhea and hypothyroidism. Brain MRI showed a progressive cerebellar atrophy. **Patient 2**: Nine months old male. CDG was first suspected at 2 months because of failure to thrive, stroke like episodes, inverted nipples, moderate psychomotor retardation and fat pads. He also developed strabismus, hypoacusia, pericardial effusions, perianal chronic dermatitis, hepatomegaly, and a severe multiorganic involvement. Laboratory tests showed metabolic acidosis and low levels of free carnitine in serum. The MRI was consistent with cerebellar hypoplasia. In both cases, the screening test of CDG revealed a typical CDG I pattern and the deficiency of phosphomannomutase activity and the mutation analysis (mutations R141H and p.R141H, R141H and V231M respectively) confirmed the diagnosis of CDG-1a. To our knowledge this is the first report of CDG Ia patients from Argentine. A program is underway aiming a better dissemination of information on this pathology among health professionals and at a broader availability of screening tests in our country.

P-18-17

A NEW CASE OF A COMPLETE DELETION OF THE GLUT-1 GENE

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Aim: To report a new case of infantile epileptic encephalopathy and hypoglycorrhachia, a condition usually due to a mutation involving one allele of the glucose transporter 1 (GLUT-1) gene. **Case report**: This girl, first child of consanguineous maghrebian parents, was evaluated at the age of 4 years for epilepsy and psychomotor delay. She was born after an uneventful term pregnancy, with normal weight and head circumference. Developmental delay was present from soon after birth. At age one year, febrile convulsions appeared and valproate was initiated. At $4\frac{1}{2}$ years she was oriented to a special center for neurologically disabled. Occasional febrile or afebrile convulsions, frequent absences, and atonic seizures were still observed, especially in the morning. Physical examination showed global psychomotor retardation, axial hypotonia, and ataxia. Electroencephalogram showed diffuse slow dysrythmia. Atypical absences were recorded. CSF analysis showed low glucose (29 mg/dl) and low normal lactic acid (1.5 mEq/l), with normal glycemia 77 mg/dl). Lamotrigine add-on improved partially the epilepsy. A ketogenic diet was eventually initiated. From day to day patient became seizure free but she remained ataxic. DNA analysis evidenced a complete deletion of the GLUT-1 gene, confirmed by Multiplex Ligation dependent Probe Amplification (MLPA) technique. **Conclusion**: New case of GLUT-1 deficiency, characterized clinically by features belonging to the 'classic' phenotype, genetically by a complete deletion of the GLUT-1 gene. This case further confirms that ketogenic diet effectively controls the seizures, however additional treatment would be needed to treat the other symptoms.

P-18-18

MONOCYTE CHEMOATTRACTANT PROTEIN-1 POLYMORPHISM IN KOREAN PATIENTS WITH PULMONARY TUBERCULOSIS

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We analyzed single nucleotide polymorphisms (SNPs) in monocyte chemoattractant protein (MCP)-1) gene in 129 tuberculosis cases and 162 healthy controls. In control subjects, genotypes of MCP-1 gene were in Hardy-Weinberg (HW) equilibrium. The allele G of the MCP-1 gene was strongly associated with tuberculosis, compared to healthy controls, with a significant $\chi^2 = 32.279$ ($p = 0.0001$), and an odds ratio 0.381 (95% confidence interval 0.272–0.533). Carriers of MCP-1 genotypes AG and GG were significantly overrepresented among tuberculosis cases, compared to healthy controls. The odds ratio for heterozygous AG in tuberculosis cases versus healthy controls was 2.8 and strongly increased to 6.9 for the comparison of homozygous GG. These data suggest the polymorphism at the MCP-1 in individuals homozygous for the (–2518) G allele could contribute to their increased risk of developing tuberculosis in Korea.

P-18-19

CONGENITAL DEFECT OF GLYCOSYLATION (CDG) IN THAI INFANTS: REPORT OF 2 CASES

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Congenital defects of glycosylation (CDG) (OMIM 212065) is a group of hereditary multisystem disorders with major nervous system involvement; previously called carbohydrate-deficient glycoprotein syndromes (CDGs). They are caused by defects in mannose addition during N-linked oligosaccharide assembly. CDGs can be divided into 2 types, depending on whether they impair lipid-linked oligosaccharide (LLO) assembly and transfer (CDG I), or affect trimming of the protein-bound oligosaccharide or the addition of sugars to it (CDG II). Gene locus is mapped to 16p 13.3–13.2 (OMIM-VAMcKusick). Major phenotypic manifestations are failure to thrive, developmental delay, hypotonia, inverted nipples, esotropia and as unusual lipodystroply, pericardial effusions, hepatic dysfunction and pontocerebellar hypoplasia. Biochemical characteristic is presence of secretory glycoproteins which are deficient in their carbohydrate content. Terminal trisaccharides are characteristically missing (Jaeken J et al., 1987). Diagnosis is confirmed by demonstration of carbohydrate deficient transferrin in serum. No effective treatment has been reported.

We here in report 2 cases of CDG: **Case 1**: Six-year-old boy (born in 2000) with neonatal seizure, hypotonia, inverted nipples, thrombocytopenia, pericardial effusions ($\times 2$), olivopontocerebellar atrophy, diagnosed since age 1 year and 7 months. Consanguinity was denied. **Case 2**: Two-year-old boy (born in 2000) with severe delayed development, pericardial effusion at 4 months, seizures, thrombocytopenia, bilateral foveal hypoplasia, inverted nipples, olivopontocerebellar atrophy on CT brain, normal quantitative plasma amino acid and urine organic acid analysis. Enzyme assay and mutation analysis are not available in Thailand.

P-18-20

A CONCEPTUAL APPROACH TO INBORN ERRORS OF METABOLISM WITH DYSMORPHIC FEATURES

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Many distinctive malformations and dysmorphic syndromes have been found to have an underlying metabolic cause. Inborn errors affecting the metabolism of single molecules (intermediate metabolism) do not usually lead to dysmorphic features unless they secondary affects on macromolecules. Dysmorphic manifestations can be expected as a¥ result of disorders of large molecules, which form the structural framework of cells and extracellular matrix. We present two examples. Fırst one with affected synthesis of large molecules (disorders of cholesterol biosynthesis) and second with affected degradation0of large molecules (lysosomal storage diseases). With known morphological and biochemical defects and mutations, Smith-Lemli-Opitz syndrome became a paradigm of way from biochemical genetics to dysmorphology and creates new idea in biochemical syndromology. In lysosomal storage disorders distension of lysosomes with undigested macromolecules leads to secondary effects on the macromolecules, tissues and organs. Dysmorphic features, which are now important clues for diagnosis of IEM, require conceptual approach using Human Phenome Project (Freimer and Sabatini 2003, Scriver 2004). Phenomics approaches require collecting phenotypic information and then determining how these features can profitably be studied together. So we present list of the more distinctive dysmorphic findings (phenomic database) which are connected with IEM. In conclusion, inverse mapping shifts focus from the traditional search for shared genotypes to a search for shared dysmorphic features.

P-18-21

TOTAL LEUKOCYTE CYSTINE MEASUREMENT, EUROPEAN QUALITY ASSURANCE INITIATIVE

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Cystinosis is characterised by an intracellular accumulation of cystine. Measurement of total leukocyte cystine content is critical in establishing the diagnosis and optimising the use of the drug cysteamine. Results are expressed relative to the protein content nmol $\frac{1}{2}$ cystine per mg protein. Establishing quality assurance for the whole procedure is difficult because it necessitates large volumes of abnormal, fresh, whole blood. It was therefore proposed to circulate material that emulated the isolated leukocyte pellets. A base material of leukocyte supernatant was prepared. Aliquots were spiked with added cystine and distributed together with a freeze-dried pellet of bovine serum albumin. There have now been 28 supernatant samples distributed over 3 years. There are currently 26 participants using three principal assays; competitive binding protein, ion exchange chromatography and HPLC. There have been some seriously erroneous outliers in the returned results. Although it is likely that this is often a result of miscalculation there are also some clear analytical problems. In one of last year's distribution pairs the mean result was 6.94 nmol $\frac{1}{2}$ cystine per mg protein with a range of results between 2.83 and 29.7. Once outliers were excluded the mean recovery of added cystine was close to 100%, so there does not appear to be a problem with specificity.

We have shown that quality assurance of this assay is possible. The results of the scheme to date have demonstrated that there is considerable room for improvement in laboratory performance.

The scheme has been organised through the ERNDIM organisation.

P-18-22

CLINICAL AND GENETICAL ASPECTS OF AUTOSOMAL DOMINANTLY INHERITED OSTEOGENESIS IMPERFECTA TARDA

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Osteogenesis imperfecta (OI) dominant type is caused by mutations in the type I collagene genes, COL1A1 and COL1A2.

The main point of our haplotype analysis was to get information about the informativity of 8 polymorphic short tandem repeat (STR) markers in the analysis of 12 OI pedigrees. The molecular genetic analysis supported the linkage to COL1A1 locus in 5 OI type I families. The linkage to the COL1A2 locus was supported in 2 OI type I, 1 OI type III and 1 OI type IV families. The haplotype analysis was non conclusive in 3 families. As both genes consist of more than 50 exons the haplotype analysis is very important before direct mutation screening. To achieve the maximum theoretical LOD scores for haplotype analysis more STR markers are needed as in many cases our markers were non informative.

P-18-23

RAPID HPLC-MS/MS METHOD FOR THE DETERMINATION OF GUANIDINOACETATE, CREATINE, AND CREATININE – USING NATIVE AND DERIVATIZED SAMPLES

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Introduction: Determination of guanidinoacetate (GAA), creatine (CT) and creatinine (CTN) in biological fluids is essential for selective screening for inborn errors of creatine metabolism. Best results were acquired using a time consuming cation-exchange LC method (2 × 2 h/sample). A previously described tandem mass spectrometry (MS/MS) method without HPLC-separation detects only GAA and CT. Here we report a fast method for detecting GAA, CT, and CTN using HPLC-MS/MS. **Methods**: After normalization for CTN, urine specimens and internal standards were evaporated in a microtiter plate at 55°C under circulating air. Samples were prepared in duplicate with and without butylation (3N butanolic HCl, 65°C, 15 min). All data were acquired using an IONICS EP 10+ LC/MS/MS, a performance-enhanced Sciex API 365 (Sciex, CA) with a turbo spray ion source operated at 200°C. The orthogonal sampling interface is directly heated up to 300°C. A HPLC reversed phase C18 column was used for separation (2 × 4 min/sample). **Results**: Analysis of samples without butylation shows good precision and recovery for CT and CTN. Reliable GAA determination needs butylation. The combination of native and butylated measurements under identical HPLC-conditions showed linearity over large concentration ranges. Therefore it is equivalent to the cation-exchange LC method with the advantage of high throughput. **Conclusion**: The HPLC-MS/MS method using native and derivatized samples is applicable for diagnosis and therapy monitoring of guanidinoacetate methyltransferase, arginine:glycine amidinotransferase, and creatine transporter deficiency in urine.

P-18-24

IDENTIFICATION OF A NOVEL MUTATION IN A TAIWANESE PATIENT WITH CITRIN DEFICIENCY

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Citrin, a liver-type mitochondrial aspartate glutamate carrier, is encoded by SLC25A13 gene, and the citrin deficiency causes adult-onset type II citrullinemia (CTLN2) and a type of neonatal intrahepatic cholestasis (NICCD). So far, over twenty SLC25A13 mutations have been identified in the patients with citrin deficiency: 155 CTLN2 and 220 NICCD, and 14 of them have been published. However, the mutation in 4–5% of mutated alleles is still unknown. In the present study, we report a novel mutation found in a male Taiwanese NICCD patient (birth date: April 25, 2005). He presented with mild failure to thrive and jaundice at the age of one month. Workup showed a neonatal hepatitis picture with mixed hyperbilirubinemia and profound fat malabsorption with vitamin K deficiency. His expanded newborn screen was interpreted as consistent with citrullinemia type II, based on an elevated citrulline level (323 μmol/L). This was then followed by a complete set of plasma quantitative amino acids, which were also interpreted as consistent with NICCD. His liver biopsy showed cholestasis and pseudoacinar formation, consistent with metabolic disease. DNA diagnosis for known 18 SLC25A13 mutations revealed that the patient has 851del4 mutation in an allele. Western blot analysis using the cultured fibroblast cells revealed that the patient is a citrin deficiency. Then we performed RT-PCR/sequencing by using mRNA extracted from the cultured fibroblast cells and found a novel mutation C1189T (Q397X) in exon 12 of SLC25A13 gene. We detected the Q397X mutation in the father's allele and the 851del4 mutation in the mother's allele.

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DEVELOPMENT OF A WEB LOG (BLOG) FOR THE ONLINE METABOLIC AND MOLECULAR BASIS OF INHERITED DISORDERS: FACILITATING DISCUSSIONS ON INBORN ERRORS OF METABOLISM

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Web logs, or blogs, are the new trend in online communications. They are websites which allow all users to participate in discussions, post news or ideas which will be available to the online community. The Online Metabolic and Molecular Basis of Inherited disorders (OMMBID), is a widely used, exhaustive reference concerning inherited diseases. The OMMBID blog, available at www.ommbid.com, is a free resource which permits discussions on inherited disorders, the posting of news concerning recent discoveries, the promotion of conferences, and will ultimately stimulate research collaborations. Really Simple Syndication (RSS) feeds enable, for example, members of the inborn errors of metabolism scientific community to have instant access to blog entries from their web browsers. We believe this communication tool will ultimately benefit investigators, physicians and patients by facilitating discussions and education on inherited diseases.

Author Index