

9th ASIAN-EUROPEAN WORKSHOP OF INBORN ERRORS OF METABOLISM

Prenatal diagnosis of mucopolysaccharidoses (MPS): The first Egyptian experienceAhmed About Nasr¹, Ekram Fateen²¹Obstetrics and Gynecology Department, Faculty of Medicine, Cairo University and ²Biochemical Genetics Department, National Research Centre, Cairo, Egypt

Objective: Prenatal diagnosis of mucopolysaccharidoses (MPS) in pregnant females with previously affected child or more.

Design: Prospective clinical study.

Subjects: The present study included 14 pregnant females with previously affected child or more with one the MPS types. These were 5 type I (Hurler), 3 type II (Hunter), 3 type III (Sanfilippo), 1 type IV (Morquio) and 2 type VI (Maroteaux-Lamy). Eleven patients came from Cairo metropolitan area (Cairo, Giza and Kaluobia governorates). Consanguineous marriage was present in 11 (78.6 %) couples. Six families have no normal children and eight families have normal children, 5 have girls and 3 have boys. The gestational age at the of their first visit was 13 weeks or less in 9 cases and more than 13 weeks in 5 cases. All the pregnant

females were subjected to history taking, pedigree construction, clinical examination and ultrasound scan. Proper counseling was done and patients were scheduled for diagnosis. One case (type II) did not come in scheduled time.

Intervention: Amniocentesis was done at 15 weeks gestational age in 10 cases to withdraw 10 ml of amniotic fluid for the analysis of the glycosaminoglycans (GAG) by the two-dimensional electrophoresis (2-DEP). Chorionic villus sampling (CVS) was done at 11–12 weeks gestational age in 3 cases (type I, type III B and type IV A) to perform the specific enzyme assay fluorimetrically which was developed during the study.

Results: 10 cases were proved to be normal fetuses.

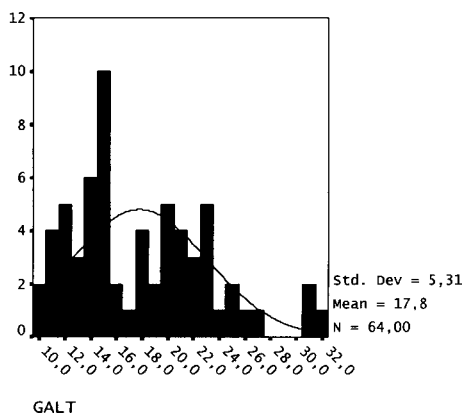
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Quantitative analysis and reference value determination of galactose-1-phosphate uridyl-transferase activity in healthy babiesGunes Ak Basol¹, Tugba Tuncel², Isin Yaprak², Tijen Tanyalcin¹¹Ege University Medical School and Hospital Department of Biochemistry, ²SSK Tepecik Training Hospital Child Health and Diseases Clinics, Izmir, Turkey,

Classical galactosemia, a life-threatening disorder with severe symptoms in the neonatal period, is caused by deficiency of the enzyme galactose-1-phosphate uridyl transferase (GALT; EC2.7.7.12). In this autosomal recessive inherited disorder, in-

gestion of milk causes accumulation of galactose in the blood and urine and leads to high intracellular concentrations of galactose-1-phosphate (gal-1-P). Neonates with classical galactosemia are prone to failure to thrive, jaundice, hepatic dysfunction, cat-

Statistics		
GALT		
	Valid	64
N		
Mean		17,7661
Median		16,5600
Mode		22,62
Std. Deviation		5,3062
Percentiles	2,5	10,2375
	50	16,5600
	97,5	31,1663



aracts and severe sepsis, due principally to *Escherichia coli*. Galactose-restricted diet reverses these acute complications immediately. However, because of suboptimal clinical awareness, diagnosis is often delayed or symptoms are misinterpreted as sepsis or isoimmunization, leading to death or sequelae because of late intervention.

In this study our aim was to determine the GALT activity in erythrocytes of healthy babies aged between 1 month–1 year and to observe the reference value determination of this enzyme among these babies. Whole blood collected into the EDTA tubes was used for enzyme activity and hemoglobin determination. Kinetic method based on uridine 5'-diphosphoglucose (UDPG) consumption was used to determine the activity of GALT. In the UDPG consumption assay based on the method of Beutler, the disappearance of UDPG from a mixture containing UDPG, gal-1-P and hemolysate was estimated by measuring residual

UDPG in the NAD-linked reaction catalysed by UDPG dehydrogenase. For every sample one sample blank was used to monitor the activity at 340 nm using Shimadzu UV-VIS spectrophotometer. The reaction was followed for about 45 minutes until there was no further change in optical density. On the basis of our data for current situation, we observed that the values show a non-parametric distribution. Asymptotic significance is near to 0,05. Therefore we evaluated nonparametrically. So the lower end of the GALT activity was 10.23 U/gHb (2.5 % percentile) and the higher end of it was 31.16 U/gHb (97.5 % percentile) giving a cut off value of 9.49 obtained by ROC curve analysis.

The confidence intervals were also calculated according to IFCC recommendation. We suggest to use this method as a confirmatory test for the babies who are diagnosed galactosemic with the other mass screening kits.

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Urea cycle disorders – pitfalls in diagnosis

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Introduction and aim: The urea cycle disorders (UCDs) represent metabolic diseases caused by defects in genes encoding enzymes and transport proteins involved in the urea cycle. As a group UCDs are considered the frequent and treatable inherited metabolic diseases (IMD). Patients with different UCDs were investigated in University Children's Hospital (capacity of 450

beds). The aim of our study was to analyse retrospectively initial clinical and biochemical findings in this series of patients and evaluate pitfalls in setting up an accurate diagnosis.

Patients and methods: Over the period of the last 10 years, ten UCD patients were investigated, including two cases with a severe neonatal form and eight patients with a late onset of the

Tab. 1. Clinical manifestation in a series of UCD patients

Patient No/Sex	Initial symptoms (diagnosis)	At the age	Definite diagnosis	At the age
1/M	Subdural haemorrhage	2 d	OTC deficiency	p.m.
2/F	Pneumonia	3 d	ASS deficiency	4 d
3/F	Viral hepatitis A	3 m	OTC deficiency	11 m
4/F	Hypotonia, hepatopathy	1 m	OTC deficiency	3 y
5/F	Psychosis, vomiting	5.5 y	OTC deficiency	6 y
6/F	Lethargy	childhood	OTC deficiency	28 y
7/F	Haedache, vegetarian	childhood	OTC deficiency 2	9 y
8/M	Somnolence, ataxia	20 m	OTC deficiency	24 m
9/M	Myopathy, hepatitis	10 m	HHH syndrome	23 m
10/M	Cerebral palsy	5.5 y	AG deficiency	6.5 y

OTC — ornithine transcarbamylase, ASS — argininosuccinic acid synthetase, AG — arginase, HHH — hyperornithinaemia-hyperammonaemia-homocitrullinuria, p.m. — post mortem

Tab. 2. Concentration of plasma ammonia in UCD patients during the initial investigation.

Patient	1	2	3	4	5	6	7	8	9	10
Deficiency	OTC	ASS	OTC	OTC	OTC	OTC	OTC	OTC	HHHsy	AG
Age	3 d	4 d	5 m	3 y	6.5 y	28 y	29 y	24 m	23 m	6.5 y
Sex	M	F	F	F	F	F	F	M	M	M
Ammonia (µmol/l)	1029	638	364	498	139	n	n	148	n	n

disease. Our interest was focused on the age at the first clinical symptoms, the initially established diagnosis and the age, when an UCD was proved. The concentration of ammonia was judged and all specialized biochemical investigations were evaluated in details.

Results: Diverse predominant clinical findings were observed in individual patients and the time of delay in diagnosis ranged from 4 months to many years (Table 1). Several surprising findings were disclosed during laboratory investigation. Hyperammonemia was absent in four patients when initial examination was carried out (Table 2). It was of interest that a slight isolated hyperornithinaemia was the unique initial biochemical finding in a patient with HHH syndrome, who showed only mild changes during his further testing. It was remarkable, that moderate

biochemical findings were detected in a boy with argininaemia despite the complete arginase deficiency in his erythrocytes.

Conclusion: UCDs can be difficult to recognise and diagnose. A careful stepwise approach to diagnostic testing and a high index of suspicion for a UCD might provide the best results. Authors emphasize that more frequent and repeated testing for ammonia in critically ill newborns and children and adults with unexplained hepatogastric, neurologic and psychiatric symptoms should be carried out to establish the diagnosis of UCD. Means of assaying ammonia should be available around the clock, even in small hospitals. Nevertheless, when hyperammonaemia is not detected, further investigations (amino acids, orotic acid, loading tests, enzyme and DNA testing) are necessary to be performed in patients with a strong clinical suspicion.

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A case of Rett syndrome from Ukraine – Clinical diagnosis confirmed by mutation analysis of the MECP2 gene

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Rett syndrome is an X-linked disorder caused by mutations in the methyl-CpG-binding protein 2 gene (MECP2). The incidence is 1:10,000-1:15,000 females worldwide. To date, the mutational spectrum of MECP2 in the Ukrainian population is not known. Here we present first Ukrainian girl with classic clinical signs of Rett syndrome, in whom clinical diagnosis of Rett syndrome was confirmed by mutation analysis of MECP2 gene. Total genomic DNA was extracted from a dry blood spot using the QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's protocol. Genomic DNA was used to amplify coding sequence and exon/intron borders of MECP2 gene. Products were examined by restriction analysis and automatic direct sequencing. The sequencing analysis of our patient revealed a small deletion of 4

bases AAAG at position 856–859 in exon 4 of MECP2 gene (856_859del4). This mutation leads to a frameshift (K286fs) and a premature stop codon. The creation of premature stop codon results in synthesis of truncated MeCP2 protein. Localization of the mutation into TRD probably affects the function of MeCP2 protein in the process of transcriptional repression. To our knowledge this is the first case from Ukraine, where mutation of MECP2 gene was detected. Mutation analysis of further patients are needed to establish the mutational spectrum of MECP2 in the Ukrainian population.

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Biochemical diagnosis of Niemann–Pick disease A, B and C: The important role of sphingomyelinase

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Niemann-/Pick disease (NPD) A and B is a lysosomal storage disorder which is characterized by acid sphingomyelinase deficiency. On the other hand, NPD-C, an autosomal-recessive disorder due to a unique error in the transport of cholesterol with a normal sphingomyelinase expression, has been nevertheless shown to accumulate sphingomyelin as NPD-A and B. The clinical manifestation of various types of NPD is quite different, however, the major symptom found in nearly all phenotypes is hepatosplenomegaly. The sphingomyelinase activity was determined by modified methods of our laboratory applying the spectrophotometric and the radioisotopic procedure. The normal value in leucocytes is generally much lower than in fibroblasts amounting about only 3–5 % (0.5–3.0 by the photometric method and 0.11–0.50 by the radioisotopic method). However, the leukocyte activity among NPD-A and B patients are easily distinguishable from normal values and therefore this technique can be used for the diagnosis of NPD-A and B. In two patients with NPD-A, the ac-

tivity measured by two methods ranged from 0.8–3.5 and 0–0.25 respectively in cultured fibroblasts. The sphingomyelinase activity in three patients with NPD-B was 1.5–34.5 and 0.2–3.6 respectively in fibroblasts showing a considerable amount of the residual activity. The patients with NPD-C are known to accumulate unestered cholesterol, which can be traced by Filipin dye. The sphingomyelinase activity in leukocytes of all 10 patients with NPD-C was found to be normal, but in fibroblasts five patients showed a somewhat decreased sphingomyelinase activity (see the table). Among almost all patients with NPD-C in this study, however, the sphingomyelinase activity in fibroblasts increased significantly when cultured in the lipoprotein (fetal bovine serum, FBS) containing medium in contrast with control cells as well as cells of NPD-A and B (see the table). These results indicate that the diagnosis of NPD-C can be supported by assay of sphingomyelinase in cells cultured in two different mediums.

Tab. 1. Sphingomyelinase activity in cultured fibroblasts (nmol/h/mg protein).

Patient	Spectrometr. met.	Radioisot. met.	Increase by FBS	Filipin dye
1	20.2	3.5	14.0	classic
2	25.4	3.6	19.0	variant
3	52.3	6.9	23.3	classic
4	20.8	3.7	43.8	variant
5	30.4	3.6	42.1	classic
6	256.0	n.d.	n.d.	variant
7	115.0	n.d.	n.d.	classic
8	217.0	„	23.2	variant
9	53.3	„	9.5	n.d.
10	17.7	„	14.9	n.d.
Normal range	40–230	5~36	0~50	negative

n.d. not determined

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Percutaneous endoscopic gastrostomy in patients with inborn errors of metabolismFabriciová K¹, Bzdúch V¹, Bibza J², Králik R², Klčová V¹¹First Department of Pediatrics and ²Department of Pediatric Surgery, Comenius University Children's Hospital, Bratislava, Slovakia

Percutaneous endoscopic gastrostomy (PEG) – method for long-term enteral feeding – is known for more than 20 years. It was initially developed for patients with swallowing difficulties (patients with onkological process in coli region or with absent of swallow reflex).

In a view of well-tolerated PEG procedure with relatively low complications, PEG is used not only in these common indications.

There were 5 indications of PEG in our Paediatric Department till now. Three of them were in patients with inborn errors of metabolism (IEM). Two of them suffer from organic acidurias (methylmalonic aciduria and maple syrup urine disease (MSUD)), one patient from disorder of carnitine cycle.

The idea to use PEG in patients with IEM is based on importance of high caloric intake in contrast with often present anorexia in these patients. We indicated PEG placement when serious anorexia appeared which lead to metabolic decompensations. PEG displaced need for parenteral feeding.

The mean age of PEG placement was 9.5 months (13, 10 and 6 months).

The oldest patient – 4.5 year old girl with MSUD – has used PEG more than 3 years without serious complications, the youngest patient has had PEG for only 3 months.

Children with PEG showed better metabolic compensation with decreased time spent in hospital. As a result there was progress of their psychomotor development.

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Diagnosis and management of galactosemia: An Egyptian experienceEkram Fateen¹, Sahar El Shafei², Hanaa El Karakasy³, Mona Mahmoud¹, Susan Roshdy², Samia El Temtamy⁴, Yoon Shin⁵¹Biochemical and ⁴Clinical Genetics Departments, ²National Research Centre Cairo, Medical Research Institute Alexandria University, ³Children Hospital Cairo University, Egypt and ⁵Children Hospital, Munich University, Germany

Objective: To screen normal and high risk Egyptian neonates for galactosemia.

Subjects and methods: The study included 2168 neonates classified into two groups. Group I included screening of 1794 normal newborns. Group II included 374 high risk neonates (jaundice, hepatomegaly and failure to thrive). Total galactose was determined by enzymatic colourimetric method in dried blood spot (Quantase). The enzymes activities (uridyltransferase and epimerase) were measured using C14.

Results: One case of galactosemia was found in the first group and 26 cases in the second group. 19 patients suffered from uridyltransferase deficiency, the parents of 16 (88.8 %) of this classic form were consanguineous and 5 (27.7 %) parents had history of

a previously affected child. Mean age of diagnosis was 3.8 month with a mean total gal value of 52.9 mg/dl. 10 (55.5 %) of them have cataract. The other 8 affected neonates were epimerase deficiency patients. 5 (62.5 %) of them born to consanguineous parents. Parents of the epimerase deficiency neonates have no previously affected children. Mean age of diagnosis was 7.2 month with a mean total gal of 17.5 mg/dl. All eight patients have cataract.

Conclusion: Mass screening program is not available yet in Egypt. Screening of the high risk neonates is a priority. Diagnosis of different galactosemia forms is mandatory to structure the management strategy accordingly.

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Four enzymes involved in mucopolysaccharidosis III (Sanfilippo syndrome): Expression in various tissue and the diagnostic possibility using leucocytes

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Mucopolysaccharidosis (MPS) III, also known as Sanfilippo syndrome, is an autosomal recessive lysosomal storage disease with somewhat less phenotypic variations than other MPSs. Biochemically, however, MPS III can be divided into four distinct subtypes, which involve 4 different enzymes degrading heparan sulfate: heparan N-sulfatase (type A), α -N-acetylglucosaminidase (type B), acetyl CoA: α -glucosaminide acetyltransferase (type C) and N-acetyl glucosamine 6-sulfatase (type D). Clinical symptoms of Sanfilippo syndrome characterize a severe, progressive central nervous system degeneration and manifest usually between the age of 2 and 6 years. Presenting features include aggressive hyperactivity, sleep disorder, delayed speech development, hearing loss and loss of social and adaptive skills. All four enzymes are expressed in fibroblasts, leucocytes and lymphocytes. The heparan sulfatase activity (MPS IIIA) was the highest in fibroblasts (23–152), followed by that in lymphocytes (3.2–22) and then in leucocytes (2.3–12). The normal ranges of three other enzymes are as follow: the activity of acetylglucosaminidase (MPS IIIB) in

fibroblasts 18–42 nmol/h/mg protein and in leucocytes 1.5–3.5, that of glucosaminide acetyltransferase (MPS IIIC) in fibroblasts 27–479 nmol/17 h/mg protein and in leucocytes 8.6–48, and that of N-acetyl glucosamine 6-sulfatase (MPS IIID) in fibroblasts 31–197 nmol/17 h/mg protein and in leucocytes between 8.5–30.1. In this study we report the first case of a 5-year old Turkish girl suffering from MPS IIIA whose diagnosis was established by enzyme assay of peripheral blood cells. The heparan sulfatase activity in leucocytes of the girl was 0 and of the mother lied below the normal range (1.4 nmol/17 h/mg protein) and of the father in the lower limit of the normal range (2.5 nmol/17 h/mg protein; normal range 2.3–12). As in the case of MPS IIIA, the respective enzyme activity among MPS B-D patients is very low not amounting more than 5 % of the normal values in fibroblasts as well as in leucocytes. The activity of four enzymes in leucocytes are much lower than in fibroblasts, however, the respective homozygotes and eventually the heterozygotes can be diagnosed by using 2–3 mL EDTA blood.

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Genotyping patients with fatty acid oxidation defects: Why, when and howGregersen N¹, Andresen BS²

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The genome structures of most of genes coding for enzyme proteins that are deficient in the fatty acid oxidation defects, have been elucidated during the last decade. This has enabled identification of a large number of gene sequence variations associated to the various disorders. Genotyping of patients with metabolic diseases, including the fatty acid oxidation defects, have aimed to: 1) Add an additional level of diagnostics to the clinical, metabolic and enzymatic levels. In this regard genotyping has been used as a confirmatory test and as a supplement to the enzymatic diagnosis; 2) Develop easy, rapid, specific and sensitive methods of genotyping. Not as a supplement to enzymatic tests, but as an alternative, that is applicable on tiny amounts of test material, such as blood-spots or biopsies, directly following indications obtained from blood and/or urine analyses. In addition

to genetic diagnosis of 'new' symptomatic patients and 'patients' identified by neonatal screening, genotyping has been used for prenatal diagnosis, giving very specific and clear answers. New single and multiplex genotyping methods has facilitated and will promote this development; 3) Perform genotype-phenotype studies in order to judge the severity of the disease and help in the management decisions. The experience from this has been that a genotype-phenotype correlation exists for certain defects in long-chain fatty acid oxidation, but not for medium- and short-chain fatty acid oxidation defects, notably MCAD and SCAD deficiencies; and 4) Elucidate the molecular pathogenesis and cellular pathophysiology of the respective gene variations in *in vitro* cell systems and *in vivo* in test animals.

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The experience of diagnosis of mitochondriopathies in Ukraine

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Mitochondriopathies are heterogeneous group of heritable diseases, which is characterized by pathology in system of mitochondrion's (violation of frame, function of mitochondria's). It results in organopathy, mainly of those organs, in which they are concentrated mostly. Mitochondriopathies have the special mode of inheritance – maternal (cytoplasmatic, nonmendelian), which is connected with presence of mitochondrion's in female gametes and absence of them in spermatozoon's. Mitochondria's mutations are localized in locuses of mitochondrial DNA, which completely was sequenced. The additional locuses in nuclear DNA are fixed.

Biochemically mitochondriopathies are the disorders of enzymes or their complexes immediately participating in formation of chemical energy with the help of oxidizing phosphorylation (a pyruvate dehydrogenase complex – PDH, respiratory chain and ATP-synthase). From the point of view of the clinical signs, pathophysiology and genetics, there is an appreciable overlap between different disorders, and it may be explained by the fact, that some proteins are coded by different enzyme complexes and the cumulative metabolites can inhibit other enzymes.

The pathology of mitochondrion's treats to serious disabling diseases.

During last years the pathological mutations mtDNA in each type of mitochondrial genes have been described and they are considered to underlie mitochondrial diseases.

Materials and methods: There were observed 55 patients with presumptive mitochondriopathies in the Kharkiv Center of Clinical Genetics and Prenatal Diagnostics. All cases were available for detailed clinical examination, biochemical investigation. Molecular genetic testing was performed in Laboratory of Molecular Anthropology, University of Pennsylvania, Philadelphia PA from native blood or blood dried blood spots.

Results and discussion: There were observed 55 patients, the average age of which was 10 years 6 months ± 2 years 8 months. 26 patients (47.3 %) are males, and 29 patients (52.7 %) are females.

There were registered the following clinical signs:

- The delay of psychomotor and physical development;
- Symptoms of myocardiopathy;
- Symptoms of epilepsy;
- Ophthalmologic symptoms;
- Symptoms of neurosensor deafness.

And laboratory sings:

- Increased level of LDH in serum;
- Increased level of ALA in serum;
- Increased level of CK in serum;
- Decreased level of glucose in serum;

– The level of ketoacids in the morning urine (qualitative tests).
In research of results with the help of factor analyses there were received the following clinical and laboratory criteria's of mitochondriopathies:

- 1) Factor "laboratory sings":
 - Increased level of LDH in serum, factor load – 0.8 (for 69.1 % patients);
 - Increased level of ALA in serum, factor load – 0.7 (for 69.1 % patients);
 - Inclination to hypoglycaemia, factor load – 0.6 (for 69.1 % patients).
- 2) Factor "the level of psychomotor and physical development":
 - The delay of psychomotor development, factor load – 0.8 (for 80.2 % patients);
 - The delay of physical development, factor load – 0.7 (for 73.7 % patients);
- 3) Factor "The pathology of hearing organs", factor load – 0.7 (for 57.2 % patients).
- 4) Factor "Symptoms of epilepsy, factor load – 0.9 (for 82.3 % patients).
- 5) Factor "Myopathy with of ophthalmological symptoms":
 - Symptoms of myopathy, factor load – 0.8 (for 70.6 % patients);
 - Ophthalmological symptoms, factor load – 0.7 (for 65.9 % patients).

Myocardiopathy was not characteristic for observed patient (factor load "–0.7", more than 60 % patients had not any sings of the defects of cardiac muscle), decrease ketoacid formation was not characteristics for our patient, factor load – 0.5 were absent in less than 50 % patients.

The blood samples were screened on the 8993T/C/G mutation, which was associated with the NARP/LFIGHS disease and the 13513 G/A mutations, which usually in investigated in the cases with MELAS syndrome and Ley syndrome.

The DNA of genome was extracted from the blood samples. The samples were screened on the general SNPs locuses of the mitochondrial DNA, which were associated with the different neurological and muscle decrease. The PC-RILP method was used. The mutation 8993 or 12513 were not detected. The samples will be screened on the 10–12 SNPs locuses.

The tRNA Leucine gene was sequenced in the mitochondrial DNA for the control of pathogen variants. The changes were not detected.

Now we are planning to provide the investigation of the sequenced gene sequences of the tRNA Lysine, the sequention of the nuclear OXPHOS-genes, which can be involved in the disease expression of these patients.

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Unexplained familial methylmalonic aciduria: a benign situation or an unidentified inborn error?

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Elevated urine methylmalonic acid concentrations in children could be seen due to various etiologies such as vitamin B12 deficiency, bacterial overgrowth in the intestines but also some inborn errors. A total of five patients aged 9 months–11 years (two siblings) have been being followed-up in the Department of Metabolism because of unexplained methylmalonic aciduria ranging from 68 to 1194 mmol/mol creatine (Normal: 0). All the children have mild mental retardation. There were no specific findings in tandem mass examinations, normal B12, folic acid and

homocysteine levels. No specific findings were seen with valine and isoleucine loading tests and neither with a short therapy of metranidasole. No specific excretion of any metabolite other than methylalonic acid was noticed with gas chromatography. Investigations of the cobalamine defects and methylmalonyl CoA mutase activities in three of them revealed normal results. The similarity of the clinical as well as the laboratory findings in all children suggest an unidentified genetic background. Further investigations are planned.

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Developments of neonatal screening in TaiwanKwang-Jen Hsiao^{1,2}, Szu-Hui Chiang², Tze-Tze Liu¹*¹Institute of Genetics and Genome Research Center, National Yang Ming University, University System of Taiwan, and ²Department of Medical Research and Education, Taipei Veterans General Hospital; Taipei, Taiwan 112*

Some of the congenital metabolic disorders have no specific clinical symptoms during neonatal period, if not treated early irreversible damages such as mental retardation will occur. The permanent damages can be avoided if these are able to be detected biochemically by neonatal screening in the early stage of life, and treated immediately with appropriate therapy and intervention. Method development pilot programs (including dried blood sample collecting, screening tests, confirmatory diagnostic procedures and treatments) were carried out in mental retarded children between 1982 and 1983 in Taiwan. Based on the methods developed, the nationwide project to set up neonatal screening for congenital hypothyroidism (CHT), phenylketonuria (PKU), maple syrup urine disease (MSUD), homocystinuria (HCU), and galactosemia (GAL) was started in January 1984. After the nationwide neonatal screening system was established in July 1985, the method for screening of glucose-6-phosphate dehydrogenase (G6PD) deficiency was developed. The incidence of G6PD deficiency was estimated to be about 2% (male 3%, female 0.9%) in Taiwan based on the screening program. Since no MSUD was found from 200,000 newborns screened, after a two year (1985.7–1987.6) pilot study on G6PD screening, MSUD was replaced by G6PD in the routine nationwide neonatal screen-

ing program. The screening coverage rate in Taiwan has reached 80% in 1990 and 99% since 1996. From 1984.1 to 2002.12, 4,462,600 newborns have been screened. The incidences of CHT, PKU, HCU, and GAL were reported to be about 1/1,800, 1/31,400, 1/263,000, and 1/744,000, respectively. Most of the affected cases were detected and treated accordingly within one month of birth and are developing normally at the present time.

Each of the three neonatal screening centers in Taiwan has started individual voluntary program paid by the parents for selective screening of congenital adrenal hyperplasia (CAH) and defects in other amino acids and acylcarnitines metabolisms, which were detected by tandem mass spectrometry (MS/MS), since 2000.3 and 2001.8, respectively. The incidence of CAH was estimated around 1/15,000 from 600,000 newborns screened between 2000.3 and 2003.10. Six cases of 3-methylcrotonyl-CoA carboxylase deficiency (3MCC), 4 cases of citrullinemia (CIT), 3 cases of MSUD, 2 cases of glutaric aciduria type I (GAI), 1 case of methylmalonic aciduria (MMA), and 1 case of nonketotic hyperglycinemia were detected from 216,000 newborns by the MS/MS screening between 2001.8 and 2003.10.

Recently, a technological assessment research supported by the Bureau of Health Promotion, Department of Health, Taiwan,

has reached a consent recommendation about the adjustment of the items for neonatal screening in Taiwan: 1) the 5 current routine items should be kept, 2) CAH, MSUD, MCAD, CIT, GAI, MMA, and isovaleric academia (IVA) should be included as routine items, 3) biotinidase deficiency, argininosuccinase deficiency, propionic academia, and C5OH-carnitine should be included as routine items for a pilot project, 4) any other disease which could be detected by MS/MS should be considered as a research

item only at the present time, 5) any disease incorporated into the routine services, including pilot project items, should have confirmatory diagnosis and follow up treatment system prepared in place before its screening program starts, 6) the positive results of CAH, G6PD, GAL, and MS/MS tests should be referred for follow-up no later than 7 days after birth, 7) the routine screening items should be available to all the newborns non-selectively, 8) the routine screening items should be reviewed periodically.

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Glutamine and glutamate in the differential diagnosis of hyperammonemias, organic acidurias, the hyperinsulinism/hyperammonemia (HIHA) syndrome and hyperglutamic acidemia

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Glutamine is an amino acid that is increased in all hyperammonemias, caused by defects of the urea cycle, together with alanine and lysine. In addition to ammonia, it does contribute to the clinical manifestations and brain disturbances, as has been shown in animal studies. Therefore, acute management of hyperammonemic attacks also consist of lowering the glutamine level by phenylbutyrate. In spite of the increased ammonia level, which may exceed a factor 10 above normal in cerebrospinal fluid, patients with the HIHA syndrome do not show the typical clinical characteristics of hyperammonemia, like drowsiness or ataxia. In plasma of these patients the glutamine level is even decreased. The hyperactivity of the glutamate dehydrogenase enzyme leads to a decreased synthesis of glutamine, thus pre-

venting its adverse effect. A typical patient will be discussed. In organic acidurias the hyperammonemia in an acute attack does not lead to an increased glutamine, as will be shown in a patient with propionic acidemia. Hyperglutamic acidemia with a normal glutamine level has been found in a number of patients in the Rotterdam Pediatric centre. These patients have in common, that they are newborns with lactic acidosis receiving Calcium-levulinic acid as medication, because of hypocalcemia. Withdrawal of this drug leads to normalization of the glutamic acid in plasma and of the metabolic acidosis. In the workup of acutely presenting patients with hyperammonemia the determination of plasma amino acids may give an important clue. Organic acid analysis in urine is also necessary.

9th ASIAN-EUROPEAN WORKSHOP OF INBORN ERRORS OF METABOLISM

Maternal hyperphenylalaninemias in healthy Czech population of pregnant women: 30 years experience with screening, prevention and treatment

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Introduction: The increased level of phenylalanine (Phe) in maternal blood – hyperphenylalaninemia (mHPA) has a detrimental effect on the early development of healthy foetus (1965). The toxic effect causes spontaneous abortion or retards intrauterine

growth, skeletal malformation, cardiac anomalies can appear. However the most frequent are microcephaly, mental retardation and hypotrophy.

Patients and methods: Simultaneously with the introduction

of obligatory “Newborn Screening Program” in CR also the facultative screening for mHPA was introduced („Maternal Hyperphenylalaninemia Preventive Screening Program“). Since 1975 till now 271 796 healthy pregnant women (16–47 yrs) from city Prague and its area (cca 2 mil. inh. have been screened for increased Phe in blood by Efron’s chromatographic screening test (1964); Phe cut off value: 240 $\mu\text{mol/l}$. Nonfasting venous blood has been taken in 2nd–3rd month of pregnancy during the first antenatal visit. All positive cases have been verified with quantitative Phe estimation on amino acid analyzer incl. pterines analysis in urine. For differentiation of detected mHPAs the Güttler’s scheme (1980) has been used. Mutations for Phe-hydroxylase gene analyzed by restriction enzyme digestion after Guldberg (1994).

Results: The average incidence of mHPA detected at the beginning of pregnancy was found 1:7,992. The major part (65.3 %) of all detected mHPA belongs to mild or moderate form of phenylketonuria (PKU) with most frequent PAH gene mutations R408W, Y414C, IVS11 nt8g-a, R158Q, IVS12ntlg-a and R261Q. 19.2 % corresponds to atypical or classical PKU with prevailing mutation R408W. Only in 15.3 % were detected non-PKU (persistent HPA) with mutations R408W, Y414C, IVS12ntlg-a, IV11nt8g-a and A403V. 28 offsprings born from

pregnancies on low-phenylalanine diet (LPD) introduced at least 2 months before the conception and during the whole pregnancy show normal psychomotoric development. In 7 offsprings without LPD or after delayed introducing or on PLD or badly monitored showed malformations (microcephaly and hypotrophy).

Discussion: Relatively high incidence of mHPA detected in healthy population of pregnant women of Prague area differs from findings of Buist (1989) or Levy (1994) from American pregnant women screened for mHPA from umbilical blood. We consider that screening performed at the beginning of pregnancy from nonfasting venous blood is more effective compared to umbilical blood from two reasons: the Phe level in maternal blood is increased during first trimester of pregnancy due to sucking effect of placenta in comparison to decreased Phe level at the end of labour. Umbilical blood for screening of mHPA is not quite suitable to detect the atypical or mild forms of Phe disturbances which prevailed in our slavonic population of pregnant women. The particular data about patients and their offsprings as well as atypical cases and family histories incl. reasons of not successfully treated and monitored pregnancies esp. in the last decade of Screening Program are discussed in details.

9th ASIAN-EUROPEAN WORKSHOP OF INBORN ERRORS OF METABOLISM

Ketogenic diet

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Ketogenic diet is a special high-fat, low carbohydrate diet that helps to control seizures in children with difficult-to-control epilepsy. It is not clear exactly how the ketogenic diet works. It involves a change in the body’s metabolism by replacing glucose with fats as a major source of energy. Recently, the rationale for therapeutic trials of ketogenic diet is deficiency of glucose transporter 1 (GLUT), which causes hypoglycorrhachia (low cerebrospinal fluid glucose) infantile seizures a developmental delay.

Authors present the successful treatment of a 3 years old child with frequent seizures (20 per day), which could not be controlled by medication. On the fourth day after commencing ketogenic diet seizures suddenly ceased and marked improvement of the child’s clinical state was observed. After two weeks of

treatment sporadic seizures again appeared and have been controlled by combination of ketogenic diet and antiepileptic drugs.

The kind of foods that provide fat for the ketogenic diet were butter, oil, cream, mayonnaise. Intake of calories should be approximately 75 % of the recommended calories for a child’s age and ideal weight. Because the diet does not provide all vitamins and minerals found in a balanced diet, vitamin and mineral supplements were recommended. Child begins by fasting (except for water) under close medical supervision (24 hours for infants). To ensure that the diet is safe and nutritionally adequate for a child, each child’s daily intake is individually calculated by an experienced dietician.

In conclusion, the ketogenic diet has recently been rediscovered and is achieving increasingly widespread use.

9th ASIAN-EUROPEAN WORKSHOP OF INBORN ERRORS OF METABOLISM

Population analysis in east asia of twelve SLC25A13 mutations found in Japanese patients with citrin deficiency (CTLN2 and NICCD)

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SLC25A13 gene encodes citrin, a liver-type mitochondrial aspartate glutamate carrier. Citrin plays a role not only in urea, protein and nucleotide syntheses by transporting aspartate formed in mitochondria to cytosol in exchange of glutamate and proton, but also in transport of NADH reducing equivalent from cytosol to mitochondria as a member of malate aspartate shuttle. Mutation of SLC25A13 gene causes idiopathic neonatal hepatitis associated with intrahepatic cholestasis (NICCD) in neonatal period and adult-onset type II citrullinemia (CTLN2) at adult age. NICCD, suffering from transient aminoacidemia involving citrullinemia, galactosemia, hypoproteinemia, hypoglycemia and cholestatic jaundice, is generally not severe and can be managed using nutritional manipulation; symptoms disappear within a year. CTLN2 is a severe disorder, characterized by episodes of neurological symptoms associated with hyperammonemia involving

disorientation, abnormal behaviors, seizure, coma and potentially death from brain edema. We have found a population analysis of SLC25A13 mutations that the frequency of heterozygote in Japanese population is approximately 1 in 69. On the other hand, since we have diagnosed Chinese (three CTLN2 and four NICCD) and Vietnamese (two NICCD) patients as carrying the same mutations as Japanese, we have started population analysis in East Asia. We have found many heterozygotes with SLC25A13 mutations; so far 25 in 2077 (China), 20 in 1369 (Taiwan), and 7 in 411 (Korea). We noticed some regional specificity in mutation type in East Asia and regional difference in mutation frequency in China. These results suggest that at least 50,000 East Asian are calculated to be homozygous in SLC25A13 mutations. It is now important to find out patients with CTLN2 and NICCD, to treat them, and to prevent onset of severe CTLN2.

9th ASIAN-EUROPEAN WORKSHOP OF INBORN ERRORS OF METABOLISM

Tetrahydrobiopterine (BH4) responsive phenylketonuria

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Background: Phenylketonuria (PKU) is an inherited metabolic disease that is due to phenylalanine hydroxylase deficiency (classical PKU) or defects of BH4 metabolism. We have diagnosed 110 patients during the last 25 years, 105 of them were diagnosed as classical PKU. Others were atypical type.

Method: BH4 deficiency is screened by performing analysis of pterins in urine and measurement of dihydropteridine reductase (DHPR) activity in blood. To explore the therapeutic efficacy of BH4, we performed BH4 loading test (a single oral dose of 20 mg/kg of BH4). And, we analyzed PAH gene in 80 patients with PKU.

Result: 14 patients of study group responded with a decrease in blood phenylalanine level after BH4 challenge; 5 of them were

confirmed with BH4 deficiency and the others were confirmed with phenylalanine hydroxylase deficiency. We also observed some mutation (R241C, A259T, R243Q AND R53H) are associated with BH4 responsiveness in classical PKU.

Conclusion: Since 1999 an increasing number of patients with PKU were reported to be able to decrease their plasma phenylalanine concentrations after BH4 challenge. The major finding of the current report is that BH4 treatment provides an improvement in phenylalanine tolerance. The advantages of such treatment include decreased stringency and easier management of diet. The data strongly emphasize the necessity of the BH4 loading test in patients detected in the newborn PKU screening.

9th ASIAN-EUROPEAN WORKSHOP OF INBORN ERRORS OF METABOLISM

Clinical, biochemical and molecular heterogeneity of branching enzyme deficiency (Andersen disease)Hyunkyung Lee¹, Teodor Podskarbi², Yoon S. Shin¹¹University Children's Hospital, Munich, Germany, ²Molecular Genetics and Metabolism Laboratory, Munich, Germany

Glycogen storage disease type IV known as Andersen disease or amylopectinosis, is a rare autosomal recessive disorder caused by deficiency of glycogen branching enzyme. The gene for this enzyme is located on chromosome 3q14. Although all forms of GSD type IV are due to aberration of this gene, but their clinical manifestation is extremely variable. The classic form, a severe liver form is characterized by hepatosplenomegaly and progressive hepatic fibrosis in the first 18 months of life, which usually leads to death before the age of 5 years. Patients with a rare non-progressive variant (a mild liver form) do not develop cirrhosis and survive until adulthood. Patient with the infantile neuromuscular form of the disease may present at birth with severe hypotonia, muscle atrophy, and neuronal involvement resulting in death during neonatal period. Furthermore, in addition to a severe multisystem neonatal form, there are the juvenile form with myopathy and/or cardiomyopathy, an adult neuromuscular form

of polyglucosan body disease (APBD) as well as an isolated skeletal muscular form with adult onset have also been reported. The branching enzyme activity is deficient in leukocytes, liver and cultured fibroblasts of the neonatal and infantile liver forms as well as of the cardiac form. About a 10% residual activity was found in leukocytes among patients with APBD and the mild liver forms. Isolated muscle forms of GSD IV on the other hand show the deficient activity in muscle only and a normal activity in other tissues such as leukocytes, fibroblasts and liver. The molecular study of a German female with APBD revealed a compound heterozygosity for two missense mutations in the branching enzyme gene (R515H, R524Q) and of a Polish boy with the mild liver form for H528R and IVS5+2t>c. Further biochemical and molecular studies of various forms are necessary to understand the marked heterogeneity of the disease.

9th ASIAN-EUROPEAN WORKSHOP OF INBORN ERRORS OF METABOLISM

Mitochondrial fatty acid oxidation deficiencies: an overviewLehnert W¹, Matern D²¹University Children's Hospital, Metabolic Unit, Freiburg, Germany, ²Division of Laboratory Genetics, Mayo Clinic, Rochester, MN, USA

During periods of prolonged fasting and once glycogen stores are depleted, mitochondrial fatty acid oxidation (FAO), closely associated with ketone body formation, becomes an important source of energy for the brain. Furthermore 60–70 % of the energy requirement of the heart muscle is provided by FAO under normal and even more under stressed conditions. Consequently defects in this important pathway result in severe clinical disturbances affecting mainly the brain, liver, heart and skeletal muscle.

Mitochondrial FAO is a complex process involving transport of activated fatty acids into the mitochondria and successive removal of acetyl-CoA units. These are in turn metabolised by the tricarboxylic acid cycle or converted in the liver into ketone bod-

ies. The resulting "reducing equivalents" are then used as fuel for the respiratory chain to produce ATP. Each step in FAO requires an enzyme or depends on a transport protein.

At least 22 different disease causing inborn errors of fatty acid β -oxidation have been identified so far. Diagnosis is not straightforward because clinical symptoms may be very similar in different diseases and in many cases pathognomonic urinary organic acid profiles may be absent during asymptomatic periods. Recently the recognition of FAO defects has been greatly facilitated by additional analysis of blood acylcarnitine patterns using tandem mass spectrometry. For further confirmation and characterisation biochemical and molecular genetic methods are applied.

9th ASIAN-EUROPEAN WORKSHOP OF INBORN ERRORS OF METABOLISM

Modern dietary therapy of maple syrup urine disease

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Human maple syrup urine disease (MSUD) obtained its name from the native Canadian maple tree because the smell of the patients' urine and body fluids is the same as that of the popular syrup. This peculiar smell of the body fluids is an important part of the diagnosis in case of inborn metabolic diseases.

The aim is a diet relatively low in proteins, leucine, isoleucine and valine but rich in alanine. The amount of protein intake is the same as expected at that age but the ratio is different. Two thirds of proteins are provided with a synthetic amino acid mixture that does not contain the above mentioned three amino acids. The remaining one third is obtained from natural proteins, mainly fruits, vegetables, low-protein milk substitutes and fruit

pulp. The following low-protein food types were used: low-protein pasta, bread powder, egg substitutes, cake powders, bread-crumbs, rice, vegetable cans, low-protein chips, cereals, chocolate and starch – as the base of this diet.

When setting the diet we had to face the following problems: Since this disease is not examined during infant screening the problem of these small patients is diagnosed late. Getting used to unfamiliar tastes that differ from the usual diet takes a long time for the babies, and they need a lot of attention and patience from the family and the staff of health centers since this diet is a lifelong program for them.

9th ASIAN-EUROPEAN WORKSHOP OF INBORN ERRORS OF METABOLISM

The heart and Fabry disease: Current knowledge

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Fabry disease is a rare lysosomal storage disease caused by the X-linked defect of the enzyme α -galactosidase A leading to the intracellular accumulation of glycosphingolipids in various organs and tissues. Cardiac involvement is frequent and, in individuals with some residual enzyme activity, may be the sole manifestation of the disease. Hemizygous men are generally more seriously affected than heterozygous women. Left ventricular (LV) hypertrophy, represents the dominant cardiac manifestation of the disease, which, in some patients, may mimic hypertrophic cardiomyopathy. LV systolic function is usually preserved by means of traditionally measured parameters. On the other hand mild to moderate LV diastolic dysfunction is regularly detected and is responsible for the symptoms of congestive heart failure.

Valvular abnormalities are frequently noted. However, hemodynamically significant lesions are rare. Conduction system involvement leads initially to the shortening of atrioventricular conduction. Nevertheless, with a progressive substrate accumulation, atrioventricular blocks and various forms of supraventricular and ventricular arrhythmias typically appear in later stages of the disease. Myocardial ischemia in Fabry disease has in most cases a functional origin due to endothelial dysfunction of coronary arteries and also due to the increase oxygen demand of hypertrophied myocardium. The results of so far performed studies with enzyme replacement therapy are promising in preventing further deterioration and even improving function of affected organs.

9th ASIAN-EUROPEAN WORKSHOP OF INBORN ERRORS OF METABOLISM

Identification of the PCCA and PCCB gene mutations in Chinese propionic acidemia patientsMei-Ying Liu¹, Yu-Ning Liu¹, Su-Jen Wu¹, Tze-Tze Liu³, Kwang-Jen Hsiao^{1,2,3}¹Institute of Genetics, National Yang-Ming University, ²Department of Medical Research and Education, Taipei Veterans General Hospital, ³Genome Research Center, National Yang-Ming University; Taipei, Taiwan

Propionyl carboxylase (EC 6.4.1.3), a biotin-dependent mitochondrial enzyme, catalyzes carboxylation of propionyl CoA to D-methylmalonyl CoA. PCC is composed of two subunits, PCCα and PCCβ subunit. Defects in either subunit result in deficient PCC activity and lead to propionic acidemia (PA, MIM 232000, 232050). In this study, both the exons of the PCCA gene and PCCB gene were analyzed by PCR-based sequencing for 6 Chinese PA patients from 5 unrelated families. One PA family was found to have nucleotide alteration in the PCCA gene, while the other 4 families were found to have nucleotide alterations in the PCCB gene.

One alteration c.1193C>T (P398L) in the PCCA gene, which had been found in a Japanese patient, was identified in one Chinese PA patient. This alteration in the PCCA gene could not be detected in 100 normal alleles in Chinese. Five novel mutations in the PCCB gene, namely c.491C>T (A164V), c.560_561delinsA (S187X), c.580T>C (S194P), c.601G>A (A201T) and c.1301C>T (A434V), were identified in four PA patients. None of these five alterations identified in the PCCB

gene were detected in 100 Chinese normal alleles. These data indicated that c.1193C>T in the PCCA gene, and c.491C>T, c.560_561delinsA, c.580T>C, c.601G>A and c.1301C>T in the PCCB gene might be disease-causing mutations in Chinese PA patients.

Two of these PA patients from non-consanguineous family were found to be homozygotes of c.491C>T and c.1301C>T mutations in the PCCB gene, respectively. A STR marker, D3S3528, was analyzed to study whether the transmission of c.491C>T and c.1301C>T of PCCB gene were in linkage disequilibrium. The homozygous c.491C>T mutation found in one PA family was linked to the same 272bp allele of D3S3528. Three c.1301C>T alleles identified in two PA families were linked to the same 270bp allele of D3S3528. The 270bp and 272bp allele of D3S3528 were found to be less frequent in normal Chinese population (11.0% and 6.1%, respectively). These data suggested that the c.491C>T and c.1301C>T mutation in Chinese PA patients might have founder effects.

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Methylmalonyl CoA mutase gene mutations in Chinese methylmalonic acidemiaTze-Tze Liu¹, Yu-Ting Teng², Shu-Fen Lee², Wa-Fong Lam¹, Mei-Ying Liu², Sheu-Jen Wu³, Kwang-Jen Hsiao^{1,2,3}¹Genome Research Center and ²Institute of Genetics, National Yang Ming University; ³Department of Medical Research and Education, Taipei Veterans General Hospital; Taipei, Taiwan 112

Mut type methylmalonic acidemia (mut MMA, MIM 251000) is an autosomal recessive disorder of organic acid metabolism caused by methylmalonyl CoA mutase (MCM, E.C.5.4.99.2; gene symbol: MUT) deficiency. In this study, mutations in the MUT gene were determined in unrelated Chinese mut type MMA patients by PCR-based sequencing analysis. Eleven mutations, designated c.316A>C (T106P), c.323G>A (R108H), c.682C>T (R228X), c.683G>A (R228Q), c.1050C>G (H350Q), c.1106G>A (R369H), c.1280G>A (G427D), [c.1630G>T+c.1631G>A] (G544X), c.1741C>T (R581X), c.1046-058del (A349delX368), and IVS9-1G>A, were identified in ten unrelated mut type patients. Among which, the c.316A>C, c.323G>A, c.1050C>G,

c.1280G>A, [c.1630G>T+c.1631G>A], c.1741C>T, c.1046-058del, and IVS9-1G>A alterations are novel mutations in the MUT gene. None of 100 alleles for 50 unrelated normal Chinese were found to have these novel alterations. These data indicated that these alterations identified in Chinese patients might be disease-causing mutations in mut type MMA.

The allele frequency of both c.1280G>A and [c.1630G>T+c.1631G>A] mutations were 15 % (3/20) in Chinese mut type MMA. A microsatellite marker, namely D6S269, near by the MUT gene was then applied to investigate whether the founder effect would contribute to c.1280G>A and [c.1630G>T+c.1631G>A] mutations in Chinese mut type MMA.

These two mutations were all linked to the 190 bp allele of D6S269 marker while the 190 bp allele of D6S269 was found to be a rare allele (<10 %) in normal Chinese population. The allele frequency of 190 bp allele in Chinese mut MMA with c.1280G>A

or [c.1630G>T+c.1631G>A] mutations was statistically different from that in the normal Chinese population. These data suggested that c.1280G>A and [c.1630G>T+c.1631G>A] mutations might have founder effects in Chinese mut type MMA patients.

9th ASIAN-EUROPEAN WORKSHOP OF INBORN ERRORS OF METABOLISM

Inborn errors of metabolism in Latvia

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The aim of the paper is to present developing approach to the identification of Inborn Errors of Metabolism (IEM) in Latvian population.

The Republic of Latvia is a country of 2,3 millions' inhabitants. The annual birth rate has decreased in recent ten years from +40 000 to +20 000 per year. Neonatal Screening Program in Latvia was started since 1987 as a mass screening for phenylketonuria (PKU). Neonatal screening for congenital hypothyroidism (CH) was introduced in 1996. For PKU newborn screening is used fluorometric determination of phenylalanine from blood specimens dried on filter paper. Neonatal hTSH (congenital hypothyroidism screening) is performed by enzyme immunoassay with fluorometric detection for determination of human thyrotropin from blood specimens dried on filter paper.

From 1987 to 2003 a total 425149 newborn infants were tested for PKU and 154194 (from 1996 to 2003) – for CH, among which 50 PKU cases and 26 CH cases were found. The screening coverage rate is about 98 %.

PKU incidence in 1987–2003 is about 1:8500, CH incidence in 1996–2003 is about 1:6000.

Since 1995 Biochemical Laboratory of the Latvian State Med-

ical Genetics Center are introduced: amino acid HPLC analysis (Waters PICO-TAG Amino Acid Analysis System); organic acid profile analysis by GC (Capillary column gas chromatograph Buck Scientific 910); dimethylmethylene blue (DMB) based spectrophotometry of glycosaminoglycans (GAG) in urine; electrophoresis of GAG; monosaccharides and disaccharides thin layer chromatography (TLC). The number of investigated patients for IEM by specific metabolic tests is about 250 per year. This number is limited by economical problems. During 2001–2003 there are 741 investigated patients for IEM by specific metabolic tests. We found and confirm diagnosis of IEM for 17 cases (about 2.3 %): 3 cases of nonketotic hyperglycinemia, 2 cases of LCHAD, homocystinuria, transient renal glycosuria, transitory hypertyrosinemia, 3 cases of mucopolysaccharidosis, alkaptonuria, 5 cases of phenylketonuria (PKU). Some of investigated patients have a specific laboratory findings, but unclear diagnosis.

All the cases of diagnosed genetic disorders and congenital malformations are registered in the Latvian State Register of Birth defects.

All genetic services, testing and treatment are free of charge for IEM patients.

9th ASIAN-EUROPEAN WORKSHOP OF INBORN ERRORS OF METABOLISM

Genotype and phenotype correlation of Pompe disease

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Glycogen storage disease type II (Pompe disease) is caused by deficiency of lysosomal-glucosidase (GAA), resulting in impaired degradation and lysosomal accumulation of glycogen. The GAA gene has been mapped to chromosome 17q25.2–25.3. Lesions in the gene lead to complete or partial inactivation of the en-

zyme. The early-onset form of the disease manifests shortly after birth and presents with progressive and generalized muscle weakness. Cardiac and respiratory insufficiency leads to death before 2 years of age. Patient with childhood and juvenile/adult form of the disease present with skeletal muscle weakness and respirato-

ry failure later in life, although some of them show cardiac enlargement. In this report we have comprised biochemical parameters in relation with mutation data in a total of 18 German and Turkish patients (10 females and 8 males), 6 infantile, 2 childhood, 5 juvenile and 5 adult patients. Patients with childhood and juvenile/adult form show a various degree of a residual GAA activity in muscle, fibroblasts and in leukocytes in contrast to the infantile form. Except in one patient with the childhood form we were able to identify mutations of the GAA gene in both alle-

les of all patients. 7 novel mutations, A237V in exon 4, G293R in exon 5, L355P in exon 6, IVS6-2A>G in intron 6, H568L in exon 12, A611V and R620Q in exon 13 were detected. Among German patients, IVS1-13T>G in intron 1, 525delT in exon 2 and delExon18 are most common, and among Turkish patients c.2741AG>CAGG was most prevalent. The spectrum of the mutations can be correlated with the GAA activity expressed, which again reflects the clinical severity of the disease.

9th ASIAN-EUROPEAN WORKSHOP OF INBORN ERRORS OF METABOLISM

Phenylketonuria and adulthood – what is the actual situation in the east slovakia region?

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The neurological and mental status, the occurrence of neuropathological and psychopathological symptoms, the dietary compliance and the life long metabolic compensation were analyzed in the group of 14 adult PKU patients followed up in the Center for long term follow up of PKU patients in Children's Hospital Košice.

The knowledge on the dietary treatment and basic physiology of PKU and the ability of its practical application was assessed by the questionnaire. The social aspect of the metabolic error and its influence on individuals was also included.

The correlation between the level of the theoretical knowledge, IQ and metabolic compensation was observed and the main lack in the knowledge and application was pointed out. The overall knowledge of PKU was quite good. The practical use of the theoretical information in life was associated with the higher mental capacity and the family background and was found to be a strong predictor of self – sufficiency and independency.

Personal problems connected with metabolic error and their influence on the quality of life of PKU patients were summarized in the study.

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Accumulation of cytosolic NADH as the prime cause of various symptoms in deficiency of citrin, a liver-type mitochondrial aspartate glutamate carrier

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Adult-onset type II citrullinemia (CTLN2) is characterized by a liver-specific ASS (argininosuccinate synthetase) deficiency with no abnormalities in hepatic ASS mRNA and the ASS gene. CTLN2 patients suffer from neurological symptoms, such as disorientation, abnormal behaviors, seizure, coma and potentially death from brain edema, associated with hyperammonemia and fatty liver. We have identified SLC25A13 on chromosome 7q21.3 as the causative gene for CTLN2. The gene encodes calcium-

binding mitochondrial solute carrier, named citrin. Furthermore, we have clarified that citrin and an analogue, aralar encoded by SLC25A12, are isoform of aspartate glutamate carrier (AGC). Since citrin is mainly expressed in liver, heart and kidney, while aralar is in skeletal muscle, brain, heart and kidney, we designate the former as a liver-type AGC and the latter as a muscle-/brain-type AGC. AGC transports mitochondrial aspartate to cytosol in exchange of glutamate and proton, and plays a role in transport

of NADH reducing equivalent from cytosol to mitochondria as a member of malate aspartate shuttle, which is essential for aerobic glycolysis. In the case of liver-type AGC, citrin also functions in urea synthesis and gluconeogenesis from lactate. Mutations of SLC25A13 gene cause not only severe CTLN2 at adult age (11–79 years), but also idiopathic neonatal hepatitis associated with intrahepatic cholestasis (NICCD) in neonatal period. Symptoms of NICCD are also variable: fatty liver, cholestatic jaundice, multiple aminoacidemia (citrulline, threonine, methion-

ine, and tyrosine), galactosemia, hypoproteinemia, and hypoglycemia. A variety of the symptoms in CTLN2 and NICCD may be due to accumulation of cytosolic NADH. NICCD is generally not severe, and symptoms disappear within one year of age. On the other hand, citrin-knockout mice showed no symptoms. These results suggest some adaptation and/or compensation, and other NADH shuttles may be able to decrease cytosolic NADH accumulated in the liver of citrin deficiency.

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Clinical outcome of PKU patients – correlation to the type of HPA, metabolic compensation and age

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Introduction: Phenylketonuria (PKU) belongs to the oldest known inborn errors of metabolism. Thanks to the newborn screening and effective treatment it is a model example of the medical approach to genetic metabolic diseases. The treatment also nowadays is based on the phenylalanine-restricted diet with pharmacological protein and other nutrient supplementation. The optimal metabolic compensation is joined with normal mental development without neurological damage. On the other hand an inadequate metabolic compensation is associated with neurological and mental deterioration. The most vulnerable is the early childhood.

Patients and methods: Neurological and psychological outcome of 66 patients in relationship to the type of hyperphenylalaninemia (HPA), metabolic compensation and age was studied in a group of children and adults with PKU and non PKU HPA from “The Centre for Long-Term Follow up of Patients with PKU in Children's Hospital Košice”.

Results: No clinical abnormalities were seen in the group of non PKU HPA patients. Higher number of both neurological and psychological abnormalities was seen in older group. Etiopathogenesis is probably multifactorial. Ideal and middle degree of the metabolic compensation was associated with significantly lower incidence of clinical abnormalities. On the contrary insufficient metabolic compensation was related to the high incidence of pathological clinical abnormalities (90 % incidence of psychopathological and 67 % incidence of neurological abnormalities).

Conclusion: The metabolic error and consequently the low phenylalanine diet and long-term follow up bring many restrictions and difficulties to the patients and his family. But the treatment is effective, the metabolic compensation is obtainable and result is optimistic – normal development without neuropsychological deterioration.

9th ASIAN-EUROPEAN WORKSHOP OF INBORN ERRORS OF METABOLISM

Free L-carnitine in diagnosis and monitoring of inherited metabolic diseases

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Introduction and aim: L-carnitine (L-3-hydroxy-4-N-trimethylaminobutyrate) is ubiquitous in mammalian tissues and is derived mainly from exogenous sources (75 %) and in part from endogenous synthesis. Carnitine allows the transfer of long-chain

fatty acids from the cytoplasm to the mitochondrial matrix where their β -oxidation occurs, with resultant energy production. Measurement of its total and free forms is essential in clinical evaluation of carnitine deficiency that can arise from disorders either

primary or secondarily. The aim of the study was to evaluate the effectiveness of free L-carnitine estimation for differential diagnosis of inherited metabolic diseases. Currently, measurement of carnitine is carried out by colorimetric, kinetic, fluorometric, radioimmunological and radioenzymatic methods. It can also be measured using high performance liquid chromatography, gas chromatography and electrospray tandem mass spectrometry.

Method: We used the enzymatic UV test (Roche Diagnostics GmbH, Mannheim, Germany) for the measurement of serum free L-carnitine concentration in patient's samples. The method is based on the transformation of carnitine to acetyl-carnitine catalysed by carnitine acetyltransferase. Amount of NADH consumed during the reaction is equivalent to amount of free L-carnitine and it is determined spectrophotometrically, on the basis of its absorption at 340 nm.

Patients: Over the period from January 2000 to July 2004, 1322 patients referred to our department with suspicion of inherited metabolic disease, were examined. Subsequent follow-up of patients with confirmed diagnosis was performed.

Results: Significantly reduced level of free L-carnitine was detected in 308 patients. There was 20 new diagnosed cases of inherited metabolic diseases. Further specialized laboratory

examinations revealed urea cycle disorders in 6 cases, fatty acid β -oxidation disorders in 6 cases, mitochondrial impairment in 4 cases, organic acidurias in 2 cases, aminoacidopathy in 1 case and phospholipid metabolic disorder in 1 case. Moderately or even markedly reduced free L-carnitine level was observed in 47 patients with valproate treatment. The serum free L-carnitine concentration was monitored in 22 patients on low-protein diet with or without carnitine supplementation. The particular group of 219 patients was represented by undiagnosed patients with suspicion of inherited metabolic diseases. This group comprised patients with other disorders such as failure to thrive or children on total parenteral nutrition. A significant increase in free L-carnitine level was observed in 2 patients with carnitine treatment.

Conclusion: Our experience with the described free L-carnitine determination indicates that this spectrophotometric method is relatively fast, simple and reliable. Precision and accuracy of the method was confirmed by ERNDIM quality control. The free L-carnitine examination can be important contribution to differential diagnosis of inborn metabolic error in laboratories without the expensive laboratory instruments such as tandem mass spectrometer.

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Universal screening of cholesterolaemia in Slovakia – current state

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Since 1st July 2003 health insurance in Slovakia has covered the assessment of total cholesterolaemia (TCH) especially for patients of general practitioners attending children and adolescents, within a preventive examination in the 11 or 17 year.

This decision is based on results of a pilot study evaluating a representative sample from almost 24,000 children.

There have been considered as risk values of cholesterolaemia the value <2.85 mmol/l and >4.85 mmol/l. In the first screening the risk values were found in 23 % of children, in the second sample (rescreening) such values were verified in 12 % of them. A part of children (about 5 % of the total population – those with values 4.85–5.4 mmol/l and negative family history data) remains observed with their GP's. The rest (7 %) are subject to closer checking by paediatric specialists (an expanded biochemical testing and examination of family members).

In the present phase a preparation of special out-patient ward begins: full-area pediatric, cardiology, endocrinology, and gas-

troenerology out-patient departments. Children with TCH over 5.5 mmol/l (i.e. about 0.5 % of all here examined children) and a part of children sent by specialists should be examined narrowly and specifically in specialized metabolic health centres. The number and tasks of such centres is now a topic of current workshops.

Such metabolic centres shall be considered as competent to indicate a particular pharmacotherapy for hyperlipidaemia.

The purpose of this general screening of hypercholesterolaemia is besides searching for threatened children also a broad engagement of the first-contact pediatricians and particular paediatric specialists in primary prevention of cardiovascular affections of children.

Such approach can result in effective influencing such serious medical and social problems as the incidence of cardiovascular diseases presents.

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Plasma levels of total homocysteine and methylenetetrahydrofolate reductase C677T polymorphismŠkodová J¹, Holešová I¹, Fabriciová K², Bzdúch V², Petrovič R³, Ponec J¹, Behúlová D¹¹Department of Clinical Biochemistry, ²1st Department of Pediatrics, University Children's Hospital, Bratislava and ³Centre of Medical Genetics, University Hospital, Bratislava, Slovakia

Background and aims: Plasma levels of total homocysteine (tHcy) can be significantly increased as a result of interaction of genetic and acquired factors. Related to genetic factors, the most common cause of hyperhomocysteinaemia is deficiency of cystathionine- β synthase and methylenetetrahydrofolate reductase (MTHFR) polymorphism C677T – termolabile form of the MTHFR enzyme. Elevated plasma tHcy is a sensitive marker of folate and cobalamine deficiency. Hyperhomocysteinaemia (HHC) is a risk factor for stroke, myocardial infarction and venous thrombosis. The aim of our report is to present means for early detection of patients with HHC and MTHFR polymorphism in order to start their treatment in a preclinical state.

Patients and methods: Patients with elevated level of total homocysteine in plasma detected through selective screening for inherited metabolic diseases were examined for MTHFR poly-

morphism C677T. Two four-member families are reported. The concentration of plasma tHcy was determined by high-performance liquid chromatography (method according to Araki A., Sako Y., J. Chromatogr., 1987, 422, 143–52). Genotyping was performed by PCR-RFLP analysis.

Results: Findings of plasma tHcy and genotype are demonstrated in Table 1.

Patients homozygous for MTHFR polymorphism treated with folic acid reduced their tHcy level to normal values (5–15 $\mu\text{mol/l}$).

Conclusions: Authors demonstrate in two families that patients with hyperhomocysteinaemia should be investigated in detail, including genotyping. Effective treatment in positive cases is available. It reduces the risk of vascular damage causing clinical manifestation of severe arterial and venous diseases and their complications.

Tab. 1. Plasma levels of total homocysteine and genotypes in two families.

Family 1					
Member	Age (y)	Sex	Genotype MTHFR	tHcy [$\mu\text{mol/l}$] initial	Hcy [$\mu\text{mol/l}$] under treatment
1	54	M	+/-	22	8
2	49	F	+/+	16	-
3	24	M	+/+	32	7
4	21	M	+/+	39	7
Family 2					
Member	Age (y)	Sex	Genotype MTHFR	tHcy [$\mu\text{mol/l}$] initial	Hcy [$\mu\text{mol/l}$] under treatment
5	51	F	+/-	8	-
6	49	M	+/-	12	-
7	20	M	+/+	28	8
8	18	F	-/-	8	-

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The effect of blood spot card variability on the detection limit analysis and the linearity of a galactose newborn screening kitTijen Tanyalcin¹, George Reclos²¹Tanyalcin Tip Laboratuvari, Izmir, Turkey, ²R&D Diagnostics LTD, Holargos, Greece

The effect of blood spot card variability on the detection limit analysis and the linearity of a galactose newborn screening kit is very important in the quantitative enzymatic assays since fluctuations in the pH of the reaction are crucial because of the limited „optimal“ pH range of the enzyme used by most kits in the market today. Different mean absorbances were obtained from disks of the same size obtained from the various filter paper blood spot cards and this observation showed that the empty cards (without blood) did not give the same detection limits, probably a result of different absorption/retention qualities of the filter paper.

The linearity of this total galactose screening method was also evaluated according to the NCCLS EP6-A Protocol. Linearity evaluation includes a lot more than drawing the line connecting the ODs of the standards. In our study, linearity evaluation included a series of regression analyses starting with a standard curve (simple linear regression graph) subsequently checked by two other regression statistical models (2nd order polynomial and 3rd order models). For the linearity assessment of this galactose kit, a series of calibrators ranging from 3,75–120 mg/dL were used. In this process, the linear (1st order) model was compared with the highest-order models in which the nonlinear term was significant (2nd or 3rd order). The difference between the models was less than the predetermined goal (5 %). The red graph falls within the predetermined limits as shown in the fig-

ure. Therefore for this galactose screening test kit, nonlinearity was declared as unimportant and the method was “sufficiently linear” up to 120 mg/dL leaving a large safety margin since most of the galactosemic babies’ galactose levels are found to be over the cut off of 14 mg/dL.

As a conclusion we would like to emphasize our finding concerning the large fluctuation in the performance of various paper filters coming from different manufacturers and/or sources. This finding adds one more factor in the list of things which should be evaluated when performing newborn screening. This is especially so when monitoring analyte levels in patients under treatment which may result in low values of the analyte of interest or when reporting your results. Thus, it is preferable to give results as “<detection limit” instead of reporting values which are definitely below it. Finally, we would like to propose that all manufacturers of filter paper used in newborn screening reach an agreement and produce one type of filter paper or at least similar types with the same characteristics. Since this is not immediately applicable, we would suggest that every lab should perform the detection limit analysis using empty (not spotted) filter paper identical (same manufacturer and Lot number) to the one used by the kit manufacturer to spot the standards and controls which are included in the kit. Most kit manufacturers will be glad to supply you with enough sheets of their filter paper.

9th ASIAN-EUROPEAN WORKSHOP OF INBORN ERRORS OF METABOLISM

Nonketotic hyperglycinaemia in SlovakiaVajnerová Z¹, Behúlová D¹, Holešová D¹, Škodová J¹, Holešová I¹, Ponec J¹, Bzdúch V², Fabriciová K², Gregová E³, Hálková K³¹Department of Clinical Biochemistry and ²First Department of Paediatrics, University Children’s Hospital, Bratislava, ³Department of Clinical Biochemistry, F.D. Roosevelt Hospital, Banská Bystrica, Slovakia

Introduction and aim: Nonketotic hyperglycinaemia (NKH, glycine encephalopathy) is an inborn error of glycine (Gly) degradation. The primary biochemical defect is in the glycine cleavage system, a multienzyme complex. This autosomal recessive disorder is characterized by rapidly progressing neurological symptoms mostly in neonatal period. A minority of NKH patients develop

symptoms later in life. A large quantities of glycine accumulate in all tissues, including the central nervous system. Our aim was to review six known cases of NKH in Slovakia and to present our clinical and biochemical approach to recognition of this disorder.

Patients and methods: Six NKH patients (3 boys and 3 girls) were detected in Slovakia over the period of the last 10 years. Bio-

chemical diagnosis was established on the basis of increased ratio of Gly in cerebrospinal fluid (Csf) to Gly in plasma (P) (normal values below 0.04). Determination of amino acids was performed by two methods: using automatic analyzer of amino acids and reversed-phase high-performance liquid chromatography with o-phthalaldehyde pre-column derivatization. Organic acids in urine (U) were assayed in all cases to exclude a ketotic hyperglycinaemia.

Results: Significant clinical and biochemical findings in six NKH patients are presented in Tables 1 and 2.

Conclusion: In the series of NKH cases detected in Slovakia five patients showed typical severe neonatal manifestation. Clinical course in one boy was atypical, his neurological symptoms developed at the age of 3 months and were more severe than it has been described in the late-onset type of NKH so far. No case of transient neonatal NKH was found. Biochemical diagnosis was set up in all cases quickly and reliably, however further enzyme and DNA testing were not performed to confirm NKH.

Tab. 1. Clinical manifestation, treatment and outcome in six NKH patients.

Patient No/Sex	First symptoms	Treatment	Outcome
1/M	3 d - hypotonia, apnoe, coma	ventilation, Na-benzoate, dextromethorphan	34 d - death
2/M	3 d - hypotonia, apnoe, coma	ventilation	13 d - death
3/M	3 m - hypotonia, coma	ventilation, Na-benzoate, dextromethorphan	7 y - moderate neurological damage
4/F	4 d - hypotonia, apnoe, coma	ventilation, low-protein diet	6 y - death
5/F	3 d - hypotonia, apnoe, coma	ventilation, Na-benzoate, dextromethorphan	6 y - severe neurological damage
6/F	2 d - hypotonia, apnoe, coma	ventilation	?

Tab. 2. Initial biochemical findings in six NKH patients.

Patient No/Sex	Age	P-Gly ($\mu\text{mol/l}$)	Csf-Gly ($\mu\text{mol/l}$)	Csf -Gly/P-Gly	U-Gly ($\mu\text{mol/mmol creat}$)
1/M	3 w	1640	blood cont.	blood cont.	19 307
2/M	10 d	2156	356	0,17	14 050
3/M	3 m	1084	200	0.19	7 645
4/F	8 d	1755	603	0.34	---
5/F	3 d	1859	225	0.12	10 886
6/F	5 d	1618	150	0.093	8 966

blood cont. - Csf contaminated with blood

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Genotype – phenotype correlation in Korean patients with ornithine transcarbamylase deficiency

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The urea cycle, consisting of a series of six enzymatic reactions, plays key roles to prevent the accumulation of toxic nitrogenous compound and synthesize arginine de novo. The ornithine transcarbamylase (OTC) deficiency is one of the most common inborn error of urea cycle, inherited in x-linked manner. In this study, we aimed at identifying mutations and correlating a genotype with a phenotype in patients with OTC deficiency. DNA isolated from peripheral leukocytes was amplified with intronic

primers flanking each exon, and subsequently sequenced. Each mutation was constructed by site-directed mutagenesis. For transient expression of each mutant construct in vitro, COS-7 cells were transfected. The OTC activity was measured in cell lysates using HPLC and Western blot was performed using the OTC monoclonal antibody. We identified 21 different mutations in 23 unrelated patients and most mutations are novel and private; L9X, R26X, R26P, T44I, R92X, G100R, R141Q, N161S, G195R,

M 205T, M206R, H214Y, K221N, D249G, R277W, F281S, R320X, V323M, 853delC, 796–805del. The L9X, R26P, R26X lead to the disruption of leader sequences, required for directing mitochondrial localization of the OTC precursor. They are asso-

ciated with severe neonatal-onset and CRIM-negative. The G100R, V323M, and R277W are found in late-onset OTC deficiency patients. In vitro transfection study, they are CRIM-positive mutations and have residual enzyme activities.

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Quantification of globotriaosylceramide in human plasma and urine by electrospray ionization tandem mass spectrometry for therapeutic monitoring of Fabry disease

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Measurement of globotriaosylceramide (Gb3, ceramide trihexoside) in plasma and urine has clinical importance for monitoring enzyme replacement therapy in Fabry disease. The disease is an X-linked lipid storage disorder that results from a deficiency of the enzyme α -galactosidase A (α -Gal A). The lack of α -Gal A causes an intracellular accumulation of glycosphingolipids, mainly Gb3. A rapid analytical method for Gb3 in plasma and urine was developed without labor-intensive pretreatment in high sensitivity and specificity. Only simple 50-fold dilution of plasma or deproteinated urine is necessary for the preparation of plasma and urine. Gb3 in diluted plasma or urine was dissolved in acetone: methanol (1:1)

containing C17:0 Gb3 as an internal standard. After centrifugation it was directly injected and analyzed through guard column by electrospray ionization MS/MS in combination with multiple reaction monitoring mode. All the species of Gb3 in the chromatogram were well resolved from plasma/urine matrix. C16-Gb3 occupied 50 % of total Gb3 as a major component in plasma while C24-Gb3 in urine. The linear range was between 0.028 ng and 2.8 ng and limit of detection (S/N=3) was 1.5 pg for C16-Gb3. Correlation coefficient of linearity was 0.9975. This method could provide method of choice as a rapid and sensitive diagnostic or monitoring tool for Fabry disease.

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Acid sphingomyelinase deficiency: molecular studies of a series of 25 Czech and Slovak patients with prevalence of intermediate phenotype

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A multi-approach study in a series of 25 Czech and Slovak patients with acid sphingomyelinase deficiency revealed a broad variability within Niemann-Pick disease type A and B. The clinical phenotype of only nine patients fulfilled the historical classification: 5 with the rapidly progressive neurovisceral infantile type A and 4 with a slowly progressive visceral type B. Sixteen patients (64 %) represented a hitherto scarcely documented “intermediary type” (IT). Twelve patients showed a protracted neurovisceral course with overt or mild neurological symptoms, three a rapidly progressing fatal visceral affection with rudimentary

neurological lesion. One patient died early from a severe visceral disease. The genotype in our patients was represented by 4 frameshift and 14 missense mutations. Seven were novel (G166R, P184L, R228H, A241V, D251E, D278A, A595fsX601). The Q292K mutation (homoallelic, heteroallelic) was strongly associated with a protracted neurovisceral phenotype (10 of 12 cases). The sphingomyelin loading test in living fibroblasts resulted in total degradation from less than 2 % in classical type A to 70–80 % in classical type B. In the intermediary group it ranged from 5 % to 49 % in a 24h chase. The liver storage showed three

patterns: diffuse, zonal (centrolobular), and discrete submicroscopical. Our series points out a notable variability in both the neurological and visceral lesions as well as in their proportionality and synchrony, and demonstrates a continuum between the historical “A” and “B” phenotypes of ASM deficiency. We suggest extension of the current classification which would recognize the important position of the intermediate type in ASM de-

ficiency. We propose type I for the classic type A, type II for the classic type B and type III for the intermediate type. Recognition of an intermediate phenotype referring to protracted neuropathic variants with overt, borderline or subclinical neuropathic features is important in view of future enzyme replacement therapy. This phenotype appears more common in Central Europe.