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RĪGAS STRADIŅA UNIVERSITĀTE

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Rare inborn errors of metabolism in children in Latvia

Summary of Doctoral Thesis
Speciality – Medical Genetics

Riga, 2011

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Doctoral theses elaborated in Medical Genetics Clinic of Childrens' University Hospital, Riga, Latvia

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CONTENTS

1. INTRODUCTION

Inborn errors of metabolism (IEM) or inherited metabolic diseases comprise a large class of genetic diseases. In most of the disorders, problems arise due to accumulation of substances which are toxic and can cause acute or chronic intoxication, hypoglycaemia or other metabolic disturbances.

First clinical manifestation can be seen already in antenatal period or later in newborn, children, juvenile or even in adult period. Clinical presentation in most cases is unspecific and in the neonatal period or infancy could be misdiagnosed as manifestation of sepsis, birth trauma, encephalitis, sudden infant death syndrome or other disease. In childhood or juvenile period IEM may manifest as schizophrenia, epilepsy, progressive mental retardation, unspecific hepatitis, eye, kidney, cardiac and other organ pathology. IEM are rare individually but collectively they are common, and numbers of them are rising as diagnostic techniques are improved. The number of described IEM currently is close to 2000. Data from the literature suggest that risk for a baby to be born with any of IEM is about 1:500. It means, that there have to be about 40 newborns with IEM in Latvia every year (presuming that each year there are about 20 000 newborns in Latvia).

Disorders that are not included in newborn screening are more difficult to diagnose due to clinical variety. These disorders are diagnosed using selective screening (specialised genetic analyses done only for individuals with clinical symptoms of IEM or positive family history). Many countries have enlarged newborn screening with more treatable IEM and also cystic fibrosis (CF), that still is one of lethal disorders (Sommerburg, 2010). CF is included in newborn screening because early diagnosis and adequate therapy before clinical signs of disease delays development of bronchectasis and other severe complications, that in longer period gives possibility to elongate patient's quality of life and lifetime (Farell, 2005, Grosse, 2006).

The precise frequency of two IEM – phenylketonuria and congenital hypothyroidism is known in Latvia due to newborn screening. The newborn screening for phenylketonuria started since year 1987 and for congenital hypothyroidism since 1996. Other IEM that are not included in newborn screening very often are not diagnosed or diagnosed very late. A delayed diagnosis can cause physical and mental retardation, invalidity and patient's early death. The diagnosis of untreatable IEM is also important, because it gives opportunity for a family to

recieve a qualitative genetic consultation, including a calculation of a recurrency risk for birth of an affected individual in the family and allows to take preventive actions.

The decision of European Parliament and Council No 1295/1999/EC (it was accepted on 29 April 1999) was adopting a programme of Community action on rare diseases within the framework for action in the field of public health (1999 to 2003). It was declared that rare IEM are disorders that affect less than 5 in 10 000 people. According to this document a work group was organised in Latvia in November of 2010 to develop the strategic plan in the field of rare IEM (The decision of Health Ministry No 229 (15.11.2010). The accepted plan contains 5 major directions: (1) to increase awareness of rare IEM, (2) prevention of rare IEM and its early diagnostics, (3) treatment, (4) integration of social and health care, (5) training for patients, their families and health care professionals.

In 2009 the government of the Republic of Latvia assigned one year budget of 700 000 LVL for medication for children with rare IEM.

Hypothesis

Most of patients with rare IEM are not diagnosed or diagnostics is delayed in Latvia.

The aim of the study

To evaluate situation in the field of rare inborn errors of metabolism in Latvian children.

Tasks of the study

1) The selection of patients with diagnosed rare inborn errors of metabolism, excluding phenylketonuria and congenital hypothyroidism;
2) The analysis of clinical symptoms and laboratory data of patients with rare inborn errors of metabolism in Latvia and data comparison with data from other countries;
3) The determination of prevalence for rare inborn errors of metabolism in Latvia;
4) The efficiency evaluation of the diagnostics of inborn errors of metabolism in Latvia;
5) The development of indications for further investigations in case of possible inborn error of metabolism.
6) The development of suggestions for the diagnostic improvement of rare inborn errors of metabolism in children in Latvia.

Scientific novelty

1) The data about children with rare inborn errors of metabolism in Latvia will be summarised for the first time. The data will include clinical and laboratory data of patients with diagnosed rare inborn errors of metabolism, which is important for further disease investigation and worldwide recognition.
2) The calculation of prevalence for rare inborn errors of metabolism in Latvia will be done for the first time.

Actuality of subject

1) Increased attention payed to rare disorders in Latvia from 2010 (The decision of Health Ministry No 229 /2010 of developing the strategic plan in the field of rare disorders in Latvia).
2) The information about rare IEM in children in Latvia currently has not been summarised.
3) Most of children with rare IEMs are not diagnosed and treated, which increases children's mortality and disability in Latvia.
4) There are no informative materials about rare IEM, no guidelines for investigations or management of acute metabolic crisis in Latvian.

2. REVIEW OF LITERATURE

2.1. Inborn errors of metabolism

Inborn errors of metabolism (IEM) or inherited metabolic diseases comprise a large class of genetic diseases involving disorders of metabolism caused by several enzyme defects. Most of IEM are genetically determined monogenic disorders that are usually inherited in autosomal recessive manner, but can show also other modes of inheritance. IEM are classified by main disorder groups (Table 2.1. (Pons Ruiz, 2007)).

Table2.1.

Classification of inborn errors of metabolism (Pons Ruiz, 2007)

Disease group	Specific diseases
IEM of amino acids	Hyperphenylalaninemia or phyenylketonuria, tyrosinaemia, non-ketotic hyperglycinemia, homocystinuria, maple syrup urine disease etc.
IEM of organic acids	3-methylglutaconic aciduria, glutaric aciduria, methylmalonic aciduria etc.
IEM of carbohydrates	Glycogenosis, fructose intolerance, galactosaemia, glucose transport defects, defects of glycerol metabolism
Fatty acid and ketone body metabolism	Fatty acid oxidation and ketogenesis disorders
Lysosomal storage disorders	Mucopolysaccharidoses, oligosaccharidoses, sfingolipidoses, mucolipidoses, lipid storage disorders, lysosomal transport defects, neural ceroid lypofuscinoses, glycogenosis type II (Pompe disease)
Mitochondrial disorders	Pyruvate dehydrogenase complex deficiency, Leigh syndrome etc.
Protein glycosylation disorders	Congenital disorders of glycosylation
Peroxisomal defects	Adrenoleukodystrophy, Refsum disease etc.
Sterol metabolism defects	Smith – Lemli – Opitz syndrome, Antley- Bixler syndrome etc.
Lipoprotein metabolism defects	Hypercholesterolaemias, hyperlipidaemias, hypertriglyceridaemias
Purine and pyrimidine metabolism defects	Lesch – Nyhan syndrome, podagra etc.
Urea cycle disorders	Ornitine transcarbamylase deficiency, citrullinaemia, argininaemia etc.
Neurotransmitter defects	Tetrahydrobiopterin deficiency, tyrosine hydroxylase deficiency etc.
Metal metabolism disorders	Wilson disease, Menkes disease
Vitamin metabolism disorders	IEM of folate, cobalamine, biotine, B 6, etc.
Membrane transport system defects	Renal tubular acidosis, cystic fibrosis

The growing number of IEM makes their classification difficult, that is why some authors (Saudubray, 2006; Pons Ruiz, 2007) suggest to classify IEM into three main groups from a pathophysiological point of view (2.2. table).

Table2.2.

Physiopathological groups (Saudubray, 2006; Pons Ruiz, 2007)

	Mechanism	Clinical aspect	Diseases
Group I	Acute and progressive intoxication	Neurological involvement. Hepatic failure. Growth failure. Cardiomyopathy	Aminoacid diseases, organic acidurias, urea cycle disorders, intolerance to sugars
Group II	Deficiency in energy production or usage	Hypotonia, myopathy, failure to thrive, hepatic failure, infant sudden death syndrome	Glucogenosis, defects in glucogenesis, congenital lactic acidemias, defects in beta oxidation, diseases of the mitochondrial respiratory chain
Group III	Alteration of complex molecule synthesis, deposit of complex cells	Permanent and progressive	Lysosomal, peroxisomal, transport diseases with intracellular processing

Further in this review some of rare IEM from each group will be described in more detail: Long-chain hydroxyacyl-CoA dehydrogenase deficiency (LCHAD) deficiency, urea cycle disorders (UCD) and lysosomal storage disorders (LSD). These disorders can be diagnosed in Latvia, and the treatment is also available (it can be applied to group III only partially).

2.2. Long-chain hydroxyacyl-CoA dehydrogenase deficiency

Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency (OMIM 609016) is a rare inherited metabolic disease, one of the fatty acid oxidation disorders, described for the first time in 1989 (Wanders et al., 1989). The combined prevalence of fatty acid oxidation disorders makes about 1: 9000, but for isolated long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency it is within the range from 1: 75 654 to 1: 750 000 (Lindner et al., 2010). The disease is inherited in the autosomal recessive manner; males and females equally affected. The disease is caused by mutations in the HADHA gene, located on the short arm of the second chromosome at position 2p23.3-2p24.1. The most prevalent mutation is 1528G>C,

traced in homozygous form in up to 87% of the total number of patients (Kahler et al., 2010). The HADHA gene's encoded protein is long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD), an enzyme required for long-chain fatty acid oxidation for energy production. The disease affects the Krebs cycle and adenosine triphosphate (ATP) synthesis, therefore the metabolic crisis is accompanied by nonketotic hypoglycemia.

Hypoglycemia, elevated liver transaminases and elevated creatine kinase level in blood are observed in the acute disease period. Moderately elevated levels of lactate and ammonia in blood are observed quite often. Nonketotic hypoglycemic acidosis and absence of ketons in the urine are observed during coma. Dicarboxylic and 3-hydro-dicarboxylic acid excretion in the urine, as well as changes in the acylcarnitines profile in blood, both being specific diagnostic criteria are found in metabolic crisis. The diagnosis is confirmed by DNA tests and finding two mutant alleles of the *HADHA* gene or by lowered enzyme activity in skin fibroblasts.

Early diagnostics and an adequate therapy might prevent a sudden death of patients; in most cases normalization of clinical symptoms is possible, except in the cases of progressive retinopathy and peripheral myopathy. Therefore, in many countries of the world a comprehensive newborn screening has been started, including fatty acid oxidation disorders, among them long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency.

2.3. Urea Cycle disorders

Urea cycle disorders (UCD) are among the most common IEM with cumulative incidence approx. 1:8000 (Zchocke, 2004). First symptoms can manifest at any age: starting from neonatal age to adult period, but most frequently in newborns, todlers and during puberty (Leonard, 2006). Although urea cycle disorders are considered easy to diagnose, the clinical symptoms quite often are not recognised, which results in delayed diagnosis. Urea cycle disorders have a characteristic symptom triad: encephalopathy, respiratory alkalosis, hyperammonaemia (Brusilow, 1996). The manifestations of clinical symptoms in different ages are shown in table 2.4. (Leonard, 2006). There is a wide variation of clinical symptoms even in one family with the same mutation. Early diagnostics is very important, because majority of patients with urea cycle disorders are treatable using diet with decreased protein amount and medication normalising ammonia level.

Table2.3.

Clinical manifestation of urea cycle disorders in different ages (Leonard, 2006)

Age	Symptoms
Neonatal period	For a full term newborn, if there were no complications during pregnancy, first symptoms can manifest in 24 – 72 hours after birth: - respiratory disturbances (hyperventilation, respiratory alkalosis) - lethargy (somnolence, loss of appetite) - instability of thermoregulation (hypo- and hyper thermia) - seizures - progressive encephalopathy and coma - brain oedema - intracranial haemorrhage
Late infantile period	- failure to thrive - vomiting - instability of thermoregulation (hypo- and hyperthermia) - chronic neurological symptoms - episodic encephalopathy with lethargy - ataxia - seizures - coma - brain oedema
Juvenile and adulthood	- irritability - vomiting - headaches - behavioural problems - learning disturbances - disorientation in time and space (mainly in older patients) - acute or chronic neurological disturbances - psychotic disturbances - recurrent encephalopathy - seizures - come - brain oedema

Hyperammonaemia is always a life threatening symptom that requires immediate and adequate therapy. The more pronounced hyperammonaemia and the longer the period without treatment, the worse are the results (Utchino, 1998; Gropman, 2004). Untreated hyperammonaemic coma in most cases is lethal. The highest mortality of patients with UCD is in the first hyperammonaemic crisis. Ammonia produced in metabolism of amino acids later step by step by 6 different enzymes is degraded in liver to neutral substance (urea) that is excreeted with urine. In the case of a defect in some of those enzymes, a hyperammonaemia is observed. One of those enzymes is ornithine transcarbamylase (OTC). This disorder OTC

deficiency will be described futher, because there are 6 patients diagnosed with this disorder in Latvia.

Ornitine transcarbamylase deficiency (OMIM 311250) is the most frequent urea cycle disorder in Europe and worldwide with frequency 1:14 000 newborns. Enzyme coding gene *OTC* is located on the short arm of X chromosome (Xp21.1), that's why clinical symptoms are most severe in boys and girls may be clinically unaffected. Clinical symptoms are similar for all urea cycle disorders, but there is an important fact that girls in the neonatal period usually are clinically healthy, while in boys the clinical symptoms manifest already 24 – 72 hours after birth. A characteristic symptom for OTC deficiency in the neonatal period is hyperammonaemia with respiratory alkalosis. During a metabolic crisis there are elevated glutamine, alanine and lysine concentrations in blood aminoacid spectrum, but citrulline and in some cases arginine level is decreased. A diagnostic symptom is an elevated orotic acid level in urine. If there are laboratory changes characteristic of OTC deficiency, there is a necessity of DNA diagnostics for confirmation of the diagnosis. If the mutation is not found, it's possible to measure enzyme activity in liver tissue and decreased enzyme activity also confirms the diagnosis (Zchocke, 2004).

2.4. Lysosomal storage disorders

There are 50 – 70 different rare genetic diseases (Staretz-Chacham, 2009; Aerts, 2011) in lysosomal storage disorders (LSD) group, caused by absence of lysosomal enzymes, which are involved in degradation of complex molecules. The prevalence of each LSD separately is in the range from 1:20 000 to 1:100 00, but combined prevalence is about 1: 5 000 to 1: 10 000 (Poorthuis, 1999; Aerts, 2011). Due to an enzyme deficiency, undegraded products of metabolism are stored in lysosomes, resulting in cells' and organs' enlargement and their impaired function (Moog, 2010). In most cases undegraded substrate is stored in central nervous system, which causes progressive mental retardation. Sphingolipidoses, mucopolysacchariodoses, olygosaccharidoses, mucolipidoses, lipid storage disorders, lysosmal transport defects, glycogen storage disorders type II or Pompe disease are the main LSD groups (Zschocke, 2004).

LSD is a group of slowly progressive disorders without acute metabolic crises (Moog, 2010). In most cases the neonatal period is without pathology; in some cases *hydrops fetalis*,

face dysmorphism, umbilical or inguinal hernia and cardiomegaly is observed. Quite often there are early hypotonia and motor development delay, later a progressive mental retardation, organomegaly, coarse facial features, skeletal changes (*dysostosis multiplex*) are evident. There are no characteristic changes in routine biochemical analyses, that's why for most of LSD diagnostics are difficult. The clinical variability is the main reason why some of those disorders are diagnosed late or misdiagnosed. As most of LSD are rare, there are no data about definite incidence, but in many countries the prevalence of total and individual LSD is calculated by using total diagnosed patient number in known period of time (Poupětová, 2010; Pinto, 2004; Poorthuis, 1999). For most of LSD the therapy is still not discovered. Enzyme replacement therapy is available for Gaucher disease, Fabry disease, Pompe disease and for MPS types I, II and VI (Kakis, 2001, Muenzer 2002, Harmatz 2004).

Mucopolysaccharidoses (MPS) are the most frequenly diagnosed LSD pathologies in Latvia.

MPS are caused due to the absence of glycoasminoglycans (mucopolysacharides) degrading enzymes. MPS are classified in several types (I, II, III, IV, VI, VII, IX) and subtypes. Usually MPS is inherited in an autosomal recessive manner, except MPS type II, which is X- linked. Clinical variability is significant between MPS types and even in one type depending from enzyme activity. The diagnostics is started primarily by the quantative analysis of glycosaminoglycans (GAG) in urine and GAG electrophoresis and confirmed by enzyme analysis.

3. MATERIAL AND METHODS

Retrospective analysis of clinical and laboratory findings of diagnosed 108 children with rare IEM. From 4600 children, that were sent to Medical Genetics Clinic of Children's University Hospital during 1997 to 2010 years with unclear possible genetic pathology, later after examination, excluding chromosomal, neuromuscular and other syndromic cases, 2500 patients were sent to selective screening for IEM and in 108 of them a diagnosis of rare inborn error of metabolism was confirmed.

The disease prevalence was calculated, using the methods, published by Poorthuis BJ (1999), Pinto R (2004), Poupetova H (2010). The prevalence reflects the number of patients with IEM per 100 000 live births. The total number of patients with particular disease is divided by the total number of live births in the given period. The birth period is the time span between the year of birth of the oldest diagnosed patient and the year of birth of the youngest patient. The total live births within the years 1997-2010 were calculated by using the data of the Central Statistical Bureau of the Republic of Latvia (http://www.csb.gov.lv/).

As IEM are rare diseases it was not possible to accumulate a sufficient information amount for applying either the Fisher test or ANOVA for data comparison within the period of 10-20 years. Consequently, the analysis of clinical and laboratory findings was done by using descriptive statistics.

For diagnostics of rare IEM selective screening was usually used, when patient with suspicion of IEM (clinical symptoms, laboratory data or family anamnesis) was sent to specialised genetic analyses. Genetic biochemical analyses that are available in Medical Genetics Clinic of Childrens' University hospital in Latvia are summarised in table 3.1.

Research was done in Medical Genetics Clinic of Childrens' University hospital. Study was approved by Riga Stradins university Ethical committee.

Research was supported by ESF grant No.2009/0147/1DP/1.1.2.1.2/09/IPIA/VIAA/009.

Table3.1.

Investigations in Genetic Biochemical laboratory of Medical Genetics Clinic, Childrens' University hospital

Detectable substrate	Material	Method	Refference
Free aminoacids	Blood, urine	Reverse phase liquid chromatography, Waters Pico-Tag method	Coen, 1989
Organic acid	Urine	Gas chromatography of organic acids using masselective detection	Sweetman, 1991
Glycosaminoglycans (mucopolysaccharides)	Urine	Dimethylmetilene blue based spectrophotometric detection	Blau, 2008
Glycosaminoglycans fractions	Urine	Single dimension electrophoresis	Blau, 2008
Mono- and disacharides	Urine	Thin layer chromatography	Blau, 2008
Oligosaccharides	Urine	Thin layer chromatography	Blau, 2008
Orotate	Urine	Spectrophotometry	Harris, 1980
Serum transferrine	Serum	Transferine isoelectrofucusing	Blau, 2008

Some IEM are confirmed by DNA analyses in Latvia (summarised in table 3.2.).

Table3.2.

DNA investigations in Latvia

Disease	Analysed mutations	Method	Participation in external quality control schemes
Childrens' University, Medical Genetics Clinic, DNA laboratory			
MCAD[a] deficiency	A985G	PCR, RFLP (Andersen, 1994)	-
LCHAD deficiency	1528G>C	PCR, RFLP (den Boer, 2000)	-[b]
Riga Stradins university Scientific laboratory of molecular genetics			
Cystic fibrosis	dF508	PCR, PAGE (Rommens, 1990)	CF Network[c]
	50 Caucasion mutations	Elucigene CF EU2 (Commercially available kit)	
Wilson disease	H1609Q	BI Pasa PCR (Polakova, 2006)	Rfb[d]
Gaucher disease	N370S	PCR, RFLP, PAGE (Beutler, 1990)	-[b]
OTC deficiency[e]	*OTC* gene mutations	Gene sequencing	-[b]

[a] MCAD – Medium chain hydroxyacyl-CoA dehydrogenase deficiency; [b] There is no available external quality control schemes, data from www.eurogenetest.com; [c] CF Network – www.cfnetwork.net; [d] Reference institut fur bioanalytik; [e] OTC – ornithine trancarbamylase

4. RESULTS

Summary of diagnosed 108 rare IEM shown in table 4.1.

Table 4.1.

Diagnosed rare IEM in children in Latvia

	Disorder group	Total number of patients	Girls	Boys
Aminoacid disorders	Homocystinuria	2	2	0
	Non-ketotic hyperglycinaemia	5	2	3
	Lysinuric protein intolerance	3	2	1
	Hyperprolinaemia type II	1	1	0
	Urea cycle disorders	7	4	3
	Cystinuria	2	1	1
Organic acid disorders	3 methylglutaconic aciduria	2	1	1
Carbohydrate disoders	Glycogenosis	4	1	3
	Galactosaemia	1	1	0
	Glycerolkinase deficiency	2	0	2
Fatty acid disorders	LCHAD deficiency	7	2	5
Lysosomal storage disoredrs	MPS	11	2	9
	Olygosaccharidoses (alpha mannosidosis, sialidosis)	3	3	0
	Sphingolipidoses (gangliosidosis type I, gangliosidosis type II, Gaucher disease)	3	1	2
Mitochondrial disorders	Pyruvate dehydrogenase complex deficiency	1	1	0
Protein glycosylation defects	Congenital disorders of glycosylation (CDG 1a)	2	1	1
Sterol metabolism disorder	Antley- Bixler syndrome	1	0	1
Lipoprotein metabolism defects	Familial hypercholesterolaemia	2	1	1
	Familial hypertriglyceridaemia	2	0	2
Purine metabolism disorders	Lesch- Nyhan syndrome	4	0	4
Metal metabolism disorders	Wilson disease	7	3	4
Membrane transport defects	Cystic fibrosis	36	15	21
	Total	108	43 (40%)	64 (60%)

4.1. Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency

Summarized results about 7 patients with LCHAD deficiency. The prevalence of LCHAD deficiency in Latvia is 1: 48 320 or 2.07: 100 000 live births.

Family history and pregnancy, the early neonatal period (summary shown in table 4.2).

Table 4.2.

Anamnesis of pregnancy and family history of LCHAD deficiency patients

Patient No	1*	2*	3	4	5	6	7
Gender	Male	Male	Male	Female	Male	Male	Female
Pregnancy No	2	4	2	1	1	3	2
Gestational week	40	37	40	42	33	32/33	39/40
Birth weight	3000 g	2950 g	3040 g	2978 g	1575 g	1180 g	3080 g
Birth length	52 cm	51 cm	50 cm	50 cm	38 cm	36 cm	52 cm
Family anamnesis	1st pregnancy HELLP**, child died after birth, 2nd pregnancy – patient 1 (died after birth), 3rd pregnancy missed abortion in 20 weeks				AFLP***, 33rd week pre-eclampsia	1st pregnancy 33 weeks AFLP, stillborn, 4th pregnancy 37 weeks, weight 2240 g, died on 2nd day autopsy - hypoxia	1st pregnancy missed abortion in 11 weeks

* patients 1 and 2 are siblings; **HELLP syndrome (haemolysis, elevated liver enzymes and low platelets); ***AFLP syndrome (acute fatty liver of pregnancy)

Clinical symptoms developed starting from the age of 3 to 21 months, average at the age of 6.5 months. Summary shown in table 4.3.

Table 4.3.

Clinical findings of patients with LCHAD deficiency

Patient No.	1	2*	3	4	5	6	7
The age of first clinical symptoms	6,5 months	5 years*	3 months	3 months	4 months	21 (?) months	4,5 months
Vomiting	+	-	+	+	+	-	+
Poor weight gain	-	-		+	-	-	+
Poor appetite	-	-	-	-	-	-	+
Diarrhea	-	-	-	-	-	-	+
Lethargy	+	-	+	+	+	+	+
Hypotonia	+	+	-	-	+	-	-
Hepatomegaly	+	-	+	+	+	-	+
Icterus	-	-	-	-	+	-	-
Seizures	+	-	+	-	-	+	-
Unconsciousness	-	-	+	-	-	+	-

End of table 4.3.

Patient No.	1	2*	3	4	5	6	7
Coma	+	-	+	-	-	+	+
Cardiomyopathy	n.d.	-	-	-	-	-	+
Psychomotor development	n.d.	N	N	N	N	delay	N
Rethinopathy	-	+	+	+	-	+	-
Rhabdomyolysis	-	+	-	+	-	-	-

* Diagnosis confirmed before appearance of clinical symptoms; patient is a sibling of the patient No 1.;
+ if symptom was observed; - if symptom was absent; n.d. no data; N normal range

LCHAD deficiency patients had changes in laboratory data (table 4.4.).

Table 4.4.

Laboratory findings for patients with LCHAD deficiency

Patient No	1	2	3	4	5	6	7	Normal range
Hb (g/dl)	n.d.	10.1	8	8.2	9.2	8.2	7.6	10.8 - 12.8
ALT (U/L)	n.d.	191	148	156	179	38[a]	123.9	0-56
AST (U/l)	n.d.	183	192	147	504	91[a]	123.1	0-84
CK (U/L)	n.d.	118[b]	6	1092	470	4127	165	32-171
LDH (U/L)	n.d.	n.d.	735	n.d.	492	592.6	562	110-248
Ammonia (µmol/l)	n.d.	n.d.	n.d.	57	87.9	66.66	38 - 153	0-48
Lactate (mmol/l)	n.d.	2.60	3.00	3.6	4.80	1.59	2 - 10.55	0.4-2.0
Glucose (mmol/l) min/max value	1.7/32	4.0	1.4/ 3.1	1.6	3.8	1.1	1.2/ 4.1	2.8-4.4
Mutation	1528G>C/ 1528G>C[c]							
Ketones in urine	n.d	-	-	-	-	-	-	
*Organic acid urine profile	n.d.	+	+	+	+	+	+	
Acylcarnitine profile:[d]								
↑C14-OH	n.d.	-	+	-	-	-	-	
↑C14:1	n.d.	-	-	-	-	-	+	
↑C14:2	n.d.	-	-	-	-	-	+	
↑C16-OH	n.d.	+	+	+	-	+	+	
↑C18-OH	n.d.	+	+	+	+	+	-	
↑C18:1- OH	n.d.	+	+	+	+	+	+	

n.d. no data; N normal; mo months, y years
*Dicarboxyl -aciduria and 3-hydroxydicarbo -xylic aciduria
[a] normal range adjusted by age ALAT/ASAT 0-33/0-33, [b] CK – creatinine kinase level after 6 month of age and later during metabolic decompensation was elevated; [c] for 3rd patient analysis was done in Heidelberg university Klinik (Prof. J. Zschocke); [d] acylcarnitine profile for 2nd patient was done in Erasmus university in Rotterdam (Dr. J. G.M. Huijmans), for other patients analyses were done in Freiburg university Clinocal Biochemical laboratory (Prof. J. O. Sass)

4.2. Urea cycle disorders

Here follow data summarised about seven patients with UCD; six of them were confirmed with OTC deficiency. Patients were from two unconsanguineous families. All OTC deficiency patients were from one family – the patients' mothers were unaffected sisters (see figure 4.1.).

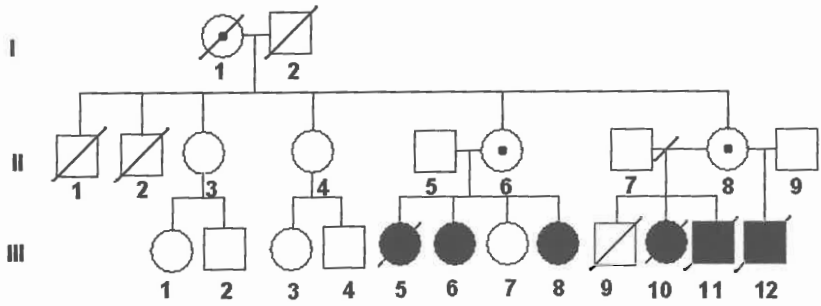

Fig.4.1. Pedigree tree of OTC deficiency patients
Used symbols: circle – female, square – male, white colour – healthy individual, black colour – affected individual, crossed out symbol – individual that is dead, circle with spot – healthy female mutation carrier

Calculated UCD prevalence in Latvia is 1,49 : 100 000. Calculation of prevalence of OTC deficiency wouldn't be correct, because patients are from one family.

The patients with OTC deficiency had positive family history – unexplained sudden deaths of newborns, infant and child. A characteristic sign is that all boys died in early neonatal period (2nd or 3rd day of life). The brothers of patients' mothers died also on the first and second day of life. All children were born as full term newborns in normal deliveries, one of newborns was large for gestational age.

The manifestation of clinical symptoms for patients with OTC deficiency was from second day of life till age of 3,5 years with distinct difference between boys and girls (Table 4.5.) The manifestation of clinical symptoms in girls in average was 1 year and 10 months (from 9,5 months till 3,5 years). The manifestation of clinical symptoms in boys (patients No. 2 and 3) started on the second day of life, when fast deterioration was observed with rapid superficial breathing, lethargy, coma and death.

Table 4.5.

Clinical findings of UCD patients

Patients (No.)	1[a]	2[a]	3[a]	4[b]	5[b]	6[b]	7[c]
Gender	female	male	male	female	Female	female	male
In figure 4.1.	III-10	III-11	III-12	III-5	III-6	III-8	
Beginning of first clinical symptoms	9,5 months	2nd day of life	2nd day of life	4 years	?	15 months	from birth
Clinical symptoms							
Vomiting	+	-	-	+	-	+	+
Lethargy	+	+	-	-	-	-	+
Respiratory disturbances	+	+	+	-	-	-	-
Termoregulation disturbances	+	-	-	-	-	-	-
Hepatho-megaly	+	-	-	+	-	+	-
Behavioral changes	-	-	-	+	-	+	
Coma	+	+	-	+	-	-	-
Seizures	-	-	+	-	-	-	-
Change in appetite	+	-	-	-	+	-	+
Other symptoms	-	-	Focal and generalised seizures	-	Sometimes headaches	-	Nystag-mus
Situation at the moment	Death at age of 10 months	Death at 3rd day of life	Death at 2nd day of life	Death at 5 years of age	20 years of age, normal psycho-motor development	4,5 years of age, normal psychomotor develop-ment	Death at age of 10,5 months

[a] siblings in one family; [b]siblings in other family; [c] UCD not precise

The ammonia level and aminoacid profile in blood were examined only in three patients (5, 6 and 7) and all three patients had elevated glutamine level, but patients 6 and 7 had hyperammonaemia 210 and 304µmol/l (normal range till 48 µmol/l) during acute manifestation of the disease. During metabolic crisis all girls had elevated liver enzymes, and respiratory alkalosis was observed in boys.

Doctors who sent the patients to hospital in an acute period of disease and those who admitted them in hospital had no suspicion of IEM. The diagnosis in most of cases was confirmed after the death (Table 4.6.).

Table 4.6.

The confirmation of diagnosis in UCD patients

Patients (No.)	1[a]	2[a]	3[a]	4[b]	5[b]	6[b]	7[c]
Gender	female	male	male	female	female	female	male
In figure 4.1.	III-10	III-11	III-12	III-5	III-6	III-8	
Beginning of clinical symptoms	9,5 months	2nd day of life	2nd day of life	4 years	?	15 months	from birth
Diagnosis confirmed	12 years after death	10 years after death	14 months after death	13 years after death	19 years of age	3,4 years of age	4.5 months unspeci-fied UCD

[a] siblings in one family; [b]siblings in other family; [c] patient has undiagnosed UCD

Patient No 6 with unclear hepatitis was consulted by geneticist on the 6th day of hospitalisation, when hyperammonaemia was discovered. Due to suspicion of OTC deficiency later *OTC* gene sequencing was done and discovered genotype R141Q/N, that confirmed diagnosis in our patient. Patient No 3 was consulted by geneticist on the first day of life, because of positive family history (at that moment patient No 6 already received ammonia level decreasing drugs). It was recommended to check ammonia level in any acute situation, but ammonia level was not measured when deterioration was observed and patient died on the second day of life. Later DNA analysis from autopsy material was done and discovered genotype R141Q, that confirmed the diagnosis of OTC deficiency. The pathogenic mutation was identified also for mother of patients No 1, 4, 5 and 6 and also for patient No 6.

4.3. Lysosomal storage disorders

The following results are summarised about 17 patients with lysosomal storage disorders (LSD). Patients were from 15 families, born in nonconsaguineous marriges. The diagnosed LSD and types of mucopolysaccharidoses (MPS) are shown in figure 4.2.

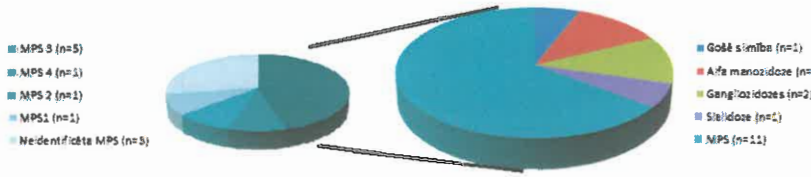

Fig4.2.In Latvia diagnosed LSD

Recurrent affected sibling births with MPS were in two families. Caesarian section was done in five patients, three of them due to pelvic presentation and two due to insufficiency of labour activity. Umbilical hernia after birth was diagnosed in 7 patients (41%). The manifestation of clinical symptoms was from birth (patient with sialidosis) till the age of seven years, on average 3 years. All symptoms except umbilical hernia and cardiomyopathy in patient with sialidosis developped progressively and that was a reason that many of parents were not able to tell a precise age of beginning of the clinical symptoms. Clinical symptoms of LSD patients are shown in Table 4.7.

Table 4.7.

Clinical symptoms of patients with LSD

Clinical symptoms	MPS	α – mannosi-dosis	Sialidosis	GM_1	GM_2	Gaucher disease
Number of patients	11	2	1	1	1	1
Beginning of clinical symptoms	5 mo till 7 y	7 mo	From birth	6 mo	3 y	2 y
Umbilical/ inguinal hernias	5 /2	2 /-	-/+	-/-	-/-	-/-
Hepatosplenomegaly	10	2	+	+	-	+
Coarse features	10	2	+	+	+	-
Deafness	10	2	n.d.	n.d.	-	-
Macrocephaly	9	2	-	-	-	-
Skeletal changes	11	2	-	+	+	+
Cataract	1	1	-	+	-	-
Cardiac pathology	5	-	+	+	-	-
Eye pathology	1	1	1	1	-	-
Mental retardation	7	2	+	+	+	-
Consultation of geneticist (age)	1,5 mo till 17 y of age	2,5 y 5 y	7 days	11 mo	7 y	3 y
Diagnosis confirmed enzymatically	See table 4.8.	4,11 y 5 y	n.d.	1 y	9 y	3 y
Patient's age at the moment	See table 4.8.	7 and 22 years	Death at 10 mo	Death at 21 mo	16 y	6 y
Received therapy	See table 4.8.	ST	ST	ST	ST	ERT

mo month; y year, GM_1 gangliosidosis type I; GM_2 gangliosidosis type II; n.d. no data; ST – symtptomatic therapy, ERT – ensyme replacement therapy

As most of LSD patients had mucopolysaccharidoses (total number 11), than more details are shown in table 4.8.

Table 4.8.

Clinical findings of MPS patients

Clinical symptoms	MPS I	MPS II	MPS III *	MPS IV	MPS ?	MPS ?**	MPS ?**
Number of patients	1	1	5	1	1	1	1
Beginning of symptoms	9 mo	1,5 y	3 - 4 y	5 mo	3 y	7 y	6 y
Umbilical/ inguinal hernia	+/-	+ /-	+ (2p.) /-	-/-	+/+	-/-	-/+
Hepatomegaly	++	++	+	-	++	+	+
Coarse features and hair structure	+++	++	+	-	+	+	+
Hirsutism	++	++	+	-	+	+	+
Breathing difficulties	++	++	-	-	-	-	-
Deafness	++	++	+	-	+	+	+
Macrocephaly	++	++	+	-	+	+	+
Skeletal changes	+++	++	+	++++	++	+	+
Cataract	++	-	-	-	-	-	-
Cardiac defects	+	+	-	-	++	+	+
Mental retardation	+++	++	+++	-	-	-	-
Genetisist consultation	1,5 mo	2,8 y	3,5;3,4; 4,2;7; 8,5	8 mo	17 y	14 y	16 y
MPS diagnosis/ confirmed type of MPS	1 y/ 6,5 y	2,8 y/ 2,10 y	6 y/6,2 y; 3,4 y3,6y; 4,2 y/4,3 y 7 y / 7,5 y 8,5 y/after death	2,5 y/ 2,5 y	17y/-	14 y/-	16 y/-
Age at the moment	Died at age of 11 y	8 y	2 patients died (13 y, 14 y) 1patient – 18 y, 2 patients (8y, 11y)	15 y – severe kypho-scoliosis	Died at 19 y	23 y	21 y
Received therapy	-	ERT: Elaprase from 6 y	Sympto-matic therapy	Sympto-matic therapy	-	Sympto-matic therapy	Sympto -matic therapy

mo month; y year; ERT – enzyme replacement therapy; p patient; + slight symptoms; ++ medium symptoms; +++ severe symptomsi; * 2 of patients are brothers; ** patients are brothers

Patients didn't have characteristic changes on rutine biochemical analyses, except anaemia and thrombocytopenia observed for Gaucher patient. Gaucher patient had also eleveted chitotriosidase activity in serum. GAG quantitative detection in urine was done in suspicion of MPS and GAG electrophoresis if GAG level was elevated. Patients with alpha mannosidosis, gangliosidosis type I and II and sialidosis had typical changes in olygosaccharides in urine. The detection of enzyme activity was done in foreign laboratories (mainly in Poland), because these investigations are not available in Latvia. X chromosome inactivation and deletion of *IDS* gene in other X chromosome was found for girl with MPS II. Prevalence of LSD in Latvia is 1,931: 100 000 live births (table 4.9.) and it's lower compared to other countries (table 4.10).

Table 4.9.

Prevalence of LSD in Latvia

Disorders	No of patients[a,b] 1997-2010	Years of birth	No of live births[c]	Prevalence per 100,000	CI 95%
LSD total	17	1980 - 2010	880 527	1,931	1,162-3,028
MPS total	11 (9)	1980 - 2003	728 315	1,510	0,794-2,625
MPS III	5 (4)	1989 - 2003	373 032	1,340	0,490 – 2,971
MPS I	1*	1997 - 2010	289 920	0,350	0,017 – 1,700
MPS IV	1*	1997 - 2010	289 920	0,350	0,017 – 1,700
α-manno-sidosis	2	1989 - 2004	393 464	0,508	0,080 – 0,679
GM_1	1*	1997 - 2010	289 920	0,350	0,017 – 1,700
GM_2	1*	1997 - 2010	289 920	0,350	0,017 – 1,700
Sialidosis	1*	1997 - 2010	289 920	0,350	0,017 – 1,700

[a] number of patients who were diagnosed in time from 1997 – 2010; [b] in brackets there is shown number of families; [c] the data from Central Statistical Bureau of Latvia (http://www.csb.gov.lv/); CI confidence interval; * If there was only one diagnosed patient than in calculation there were used number of live births in inevstigated period.

Table 4.10.

Prevalence of LSD in Latvia comparing with other countries [a,b]

Diseases	Prevalence Latvia	Prevalence Netherlands	Prevalence Portugal	Prevalence Australia	Prevalence Czech Republic	Prevalence Germany
LSD total	1,93	14,00	25,00	12,90	12,25	n.d.
MPS total	1,51	4,50	4,80	4,44	3,72	3,53
MPS III	1,34	1,89	0,84	1,42	0,91	1,57
MPS I	0,35	1,19	1,33	1,14	0,72	0,69
MPS IV	0,35	0,36	0,60	0,59	0,73	0,38
α-manno-sidosis	0,51	0,09	0,12	0,10	0,38	n.d.
GM_1	0,35	0,41	0,62	0,26	0,26	n.d
GM_2	0,35	0,34	1,49	0,26	0,19	n.d
Sialidosis	0,35	0,05	0	0,02	0,07	n.d

n.d. no data; [a] prevalence calculated on 100 000 live births; [b] the data of other countries (Poupětová 2010);

The recognition of LSD among doctors is insufficient, because only two patients (with suspicion of MPS and Gaucher disease) were sent to geneticist. One patient with alpha mannosidosis came to us with already confirmed diagnosis abroad. Time period from first clinical symptoms till first consultation of mediacl geneticist was in average 3 years (from 7 days to 17 years), and median time of consultation till confirmation the diagnosis was two years.

4.4 Results of diagnosed rare IEM in children in Latvia.

Prevalence of IEM, except that of LSD are shown in table 4.11.

Table 4.11.

Prevalence of IEM in Latvia and Orphanet data

Diseases	Number of patients [a,b]	Years of birth	No of live births[c]	Prevalen-ce per 100,000	CI 95%	Orphanet data[d]
LSD total	17 (15)	1980 - 2010	880527	1.931	1.162-3.028	-
LCHAD deficiency	7 (6)	1997 - 2010	289920	2.070	0.838-4.304	1
UCD	7 (2)	1990 - 2009	467103	1.499	0.655-2.964	1
Non-ketotic hyperglycinaemia	5 (4)	1997 - 2010	289810	1.725	0.632-3.824	0.2
Homocystinuria	2	1991- 2003	296192	0.675	1.13-22.31[e]	0.4
Lysinuric protein intolerance	3	1984 - 2005	620831	0.483	1.23-13.15[e]	-
Lesch – Nyhan syndrome	4 (2)	1996 - 2007	244748	1.634	0.519-3.942	0.38

End of table 4.11.

Diseases	Number of patients [a,b]	Years of birth	No of live births[c]	Prevalence per 100,000	CI 95%	Orphanet data[d]
Glycerolkinase deficiency	2	2001- 2009	193707	1.032	0.173-3.411	-
Cystinuria	2	1989 - 2010	525244	0.380	0.63-12.58[e]	14
Wilson disease	7	1990 - 2004	354444	1.975	0.863-3.907	5.84
3-methylglutaconic aciduria	2	2001 - 2009	193707	1.032	0.173-3.411	-
Pyruvate dehydrogenase complex deficiency	1*	1997 - 2010	289810	0.344	0.17-17.01[e]	-
Congenital disorders of glycosylation (CDG 1a)	2 (1)	1997 - 2010	289810	0.689	1.15-22.79[e]	-
Familial hypertriglyceridaemia	2	2000 - 2007	148082	1.351	0.226-4.462	-
Familial hypercholesterolaemia	2	1999 - 2007	187726	1.065	0.179-3.520	-
Glycogenosis	4 (3)	1997 - 2007	224966	1.778	0.565-4.289	-
Hyperprolinaemia type II	1*	1997 - 2010	289810	0.345	0.17-17.01[e]	-
Antley- Bixler syndrome	1*	1997 - 2010	289810	0.345	0.17-17.01[e]	-

[a] number of patients diagnosed during 1997 – 2010; [b] number of families shown in brackets; [c] the data from Central Statistical Bureau of Latvia (http://www.csb.gov.lv/; [d] Orhanet data summarise prevalence data in Europe, if there are no available data, that is because of small diagnosed number of patients * If there was only one diagnosed patient, number of live births in inevstigated period was used in calculation; [e] CI 95% calculated for prevalence 1: 1 000 000.

Clinical symptoms of diagnosed IEM patients is shown in table 4.12.

Table 4.12.

Clinical symptoms of diagnosed IEM patients in Latvia

Disorders	LCHAD deficiency	UCD	Lysinuric protein intolerance	LSD	Nonketotic hyper-glycinemia	Homocystinuria
Number of patients	7	7	3	17	5	2
Beginning of clinical symptoms (average age)	3 mo - 21mo (6,5 mo)	2 d. – 4y (11mo)	7mo- 24mo (14 mo)	From birth till 7y (2y)	2 d. – 3d. (2d.)	10 mo – 16 mo (13 mo)
Vomiting	5 (7)	4 (7)	3 (3)	2 (17)	0 (5)	0 (2)
Poor weight gain	4 (7)	2 (7)	3 (3)	2 (17)	4 (5)	2 (2)
Poor appetite	4 (7)	7 (7)	3 (3)	2 (17)	5 (5)	2 (2)
Diarrhea	1 (7)	0 (7)	3 (3)	0 (17)	0 (5)	0 (2)
Lethargy	6 (7)	5 (7)	2 (3)	0 (17)	5 (5)	0 (2)
Coma	4 (6)	4 (7)	1 (3)	0 (17)	4 (5)	0 (2)
Hypotonia	2 (7)	4 (7)	0 (3)	2 (17)	5 (5)	1 (2)
Hepatomegaly	5 (6)	3 (7)	3 (3)	15 (17)	0 (5)	1 (2)
Icterus	1 (7)	0 (7)	1 (3)	2 (17)	0 (5)	0 (2)
Seizures	3 (6)	2 (7)	0 (3)	0 (17)	5 (5)	1 (2)
Skeletal pathology	0 (7)	0 (7)	1 (3)	16 (17)	0 (5)	1 (2)
Eye pathology	4 (6)	0 (7)	0 (3)	5 (17)	0 (5)	2 (2)
Cardiac pathology	2 (6)	0 (7)	0 (3)	7 (17)	0 (5)	0 (2)
Deafness	0 (7)	0 (7)	0 (3)	12 (17)	n.d.	0 (2)
Kidney pathology	0 (7)	0 (7)	1 (3)	0 (17)	0 (5)	0 (2)
Dysmorphism	0 (7)	0 (7)	0 (3)	15 (17)	0 (5)	0 (2)
Mental retardation	1 (7)	0 (7)	0 (3)	12 (17)	4 (5)[a]	2(2)
Sudden death	2 (7)	4 (7)	0 (3)	0 (17)	0 (5)	0 (2)
Age at confirmation the diagnosis	3 mo-21mo (1 after death)	3,4y, 19y (4- after death)	16 mo – 11y	10 mo – 17 y	12 d – 1 mo (15 d)	16 mo, 10 y
Positive family history	4 (7)	6 (7)	0 (7)	4 (17)	2 (5)	2 (2)

d day; mo month,;y year

Continuation of table 4.12.

Disorders	Lesch Nyhan syndro -me	Wilson disease	3 methyl-glutaconic aciduria	Pyruvate dehydroge-nase complex deficiency	Gala-ctosaemia	Glycoge nosis	Congenital disorder of glycosilation type Ia
Number of patients	4	7	2	1	1	4	2
Beginning of clinical symptoms (average age)	6 mo – 9 mo (7,5 mo)	3 y – 5 y (4 y)	2 d. – 3 y (15 mo)	From birth	6 d	6 mo – 3 y (1,5 y)	1 y – 3 y (2 y)
Vomiting	0 (4)	1 (7)	1 (2)	+	+	1(4)	0 (2)
Poor weight gain	2 (4)	2 (7)	1 (2)	+	+	1 (4)	2 (2)
Poor appetite	1 (4)	2 (7)	1 (2)	+	+	1 (4)	0 (2)
Diarrhea	1 (4)	0 (7)	0 (2)	-	+	0 (4)	0 (2)
Lethargy	0 (4)	0 (7)	1 (2)	+	-	1 (4)	1 (2)
Coma	0 (4)	0 (7)	0 (2)	-	-	1 (4)	0 (2)
Hypotonia	4 (4)	0 (7)	2 (2)	+	+	0 (4)	2 (2)
Hepatomegaly	0 (4)	7(7)	1 (2)	+	+	4 (4)	0 (2)
Icterus	0 (4)	0 (7)	1 (2)	+	+	0 (4)	0 (2)
Seizures	0 (4)	0 (7)	1 (2)	+	-	1 (4)	0 (2)
Skeletal pathology	3 (4)	0 (7)	1 (2)	+	-	1 (4)	2 (2)
Eye pathology	0 (4)	0 (7)	1 (2)	+	+	0 (4)	2 (2)
Cardiac pathology	0 (4)	0 (7)	0 (2)	-	-	0 (4)	0 (2)
Deafness	0 (4)	0 (7)	1 (2)	+	-	0 (4)	0 (2)
Kidney pathology	4 (4)	0 (7)	0 (2)	-	-	0 (4)	0 (2)
Dismorphism	0 (4)	0 (7)	1 (2)	+	-	1 (4)	2 (2)
Mental retardation	4 (4)[b]	0 (7)	1 (2)	+	-	1 (4)	2 (2)
Sudden death	0 (4)	0 (7)	0 (2)	-	-	0 (4)	0 (2)
Age at confirmation the diagnosis	9 mo-4 y	5 y – 16 y	8 mo – 7 y	10 mo	1,5 mo	10 mo - 10 y	10 y – 12 y
Positive family history	4 (4)	1 (7)	0 (2)	-	-	2 (4)	1 (2)

d day; mo month,;y year

End of table 4.12.

Disorders	Lipid metabolism disorder	Hyper-prolinaemia type I	Antley Bixler s.	Cystinuria	Glycerol kinase complex deficiency	CF	**Number of all patients (%)**
Number of patients	4	1	1	2	2	36	**108**
Beginning of clinical symptoms (average age)	no	1 y	From birth	3 y – 4 y (3,5 y)	1 week-3 week (2 week)	From birth – 4 y (10 mo)	
Vomiting	0 (4)	-	-	0 (2)	2(2)	10 (36)	**29 (27%)**
Poor weight gain	0 (4)	-	-	0 (2)	2 (2)	30 (36)	**59 (55%)**
Poor appetite	0 (4)	-	-	0 (2)	2 (2)	0 (36)	**30 (28%)**
Diarrhea	0 (4)	-	-	0 (2)	0 (2)	24 (36)	**29 (27%)**
Lethargy	0 (4)	-	-	0 (2)	1 (2)	0 (36)	**22 (20%)**
Coma	0 (4)	-	-	0 (2)	0 (2)	0 (36)	**14 (13%)**
Hypotonia	0 (4)	+	-	0 (2)	2 (2)	0 (36)	**27 (25%)**
Hepatomegaly	0 (4)	+	-	0 (2)	0 (2)	12 (36)	**56 (52%)**
Icterus	0 (4)	-	-	0 (2)	1 (2)	0 (36)	**8 (8%)**
Seizures	0 (4)	+	-	0 (2)	0 (2)	0 (36)	**15 (14%)**
Skeletal pathology	0 (4)	-	+	0 (2)	1 (2)	5 (36)	**33 (31%)**
Eye pathology	0 (4)	-	-	0 (2)	0 (2)	0 (36)	**14 (13%)**
Cardiac pathology	0 (4)	-	-	0 (2)	0 (2)	1 (36)	**10 (9%)**
Deafness	0 (4)	-	-	0 (2)	0 (2)	0 (36)	**14 of 103 (14%)**
Kidney pathology	0 (4)	-	-	2 (2)	0 (2)	2 (36)	**9 (8%)**
Dysmorphism	0 (4)	+	+	0 (2)	1 (2)	0 (36)	**23 (21%)**
Mental retardation	0 (4)	+	-	2 (2)	2 (2)	0 (36)	**33 (31%)**
Sudden death	0 (4)	-	-	0 (2)	0 (2)	0 (36)	**6 (5%)**
Age at confirmation the diagnosis	1 y – 8 y	8 y	1 mo	9 y and 17 y	2,5 mo and 2 y	1 mo - 15 y	
Positive family history	4 (4)	-	-	0 (2)	1 (2)	2 (36)	**33 (31%)**

d day; mo month,;y year

Clinical symptoms in neonatal period started in 28 patients (24%) and in 31 patients (28%) – before the age of one year. The most frequent symptoms in early infancy were vomiting, diarrhea, poor weight gain, lethargy with coma and seizures. Progressive mental

retardation (mostly for LSD), skeletal changes and hepatomegaly were observed quite often in patients aged 2 and 3 years.

The most frequent changes in biochemical analyses of our patients with IEM are shown in table 4.13.

Table 4.13.

Biochemical analyses of patients with IEM

Disorders	Patient number	ALT/ AST ↑ U/L	Glucose ↓ mmol/l	NH_3 ↑ µmol/l	CK[a] ↑, U/L	LDH[b] ↑, U/L	Hb[c] ↓, g/dl	Lactate ↑, mmol/l	Cholesterine ↑	TG[d] ↑
LCHAD deficiency	7	6 (6)	6 (7)	4 (5)	6 (6)	6 (6)	6 (7)	5 (6)	2 (6)	2 (6)
UCD	7	3 (6)	0 (7)	2 (3)	1 (3)	2 (3)	2 (6)	2 (6)	0 (3)	0 (3)
Lysinuric protein intolerance	3	2 (3)	0 (3)	2 (3)	2 (3)	3 (3)	2 (3)	1 (3)	2 (3)	2 (3)
LSD	17	5 (17)	0 (6)	1 (5)	1(5)	2 (11)	3 (17)	2 (10)	3 (7)	2 (5)
Non-ketotic hyperglycinemia	5	0 (5)	0 (5)	1(2)	n.d.	n.d.	0 (5)	n.d.	n.d.	n.d.
Homocystinuria	2	1 (2)	0 (2)	n.d.	0 (2)	1 (2)	2 (2)	0 (2)	0 (2)	0 (2)
Glycerlol kinase deficiency	2	0 (2)	1(2)	1(2)	2 (2)	1 (2)	1 (2)	1 (2)	0 (2)	0 (2)
Cystinuria	2	0 (2)	0 (2)	n.d.	0 (2)	0 (2)	0 (2)	n.d.	0 (7)	0 (7)
Lesch – Nyhan s.	4	0 (4)	0 (4)	n.d.	2 (2)	1 (2)	0 (4)	2 (2)	n.d.	n.d.
Wilson disease	7	7 (7)	0 (7)	1 (3)	2 (7)	0 (7)	2 (7)	2 (7)	n.d.	n.d.
3-methylglutaconic aciduria	3	2 (2)	1 (2)	1 (2)	1 (2)	0 (2)	1 (2)	0 (2)	0 (2)	0 (2)
Pyruvate dehydrogenase complex deficiency	1	+	+	-	+	-	+	-	-	-
Galactosaemia	1	+	+	-	+	+	-	-	-	-
Glycogenosis	4	4 (4)	2(4)	1 (4)	1 (4)	0 (4)	1 (4)	1 (4)	4 (4)	4 (4)

End of table 4.13

Disorders	Patient number	ALT/ AST ↑ U/L	Glucose ↓ mmol/l	NH_3 ↑ µmol/l	CK[a] ↑, U/L	LDH[b] ↑, U/L	Hb[c]↓, g/dl	Lactate ↑, mmol/l	Cholesterine ↑	TG[d] ↑
Congenital disorders of glycosilation type Ia	2	0 (2)	0(2)	0 (2)	2 (2)	0 (2)	1 (2)	0 (2)	n.d.	n.d.
Lipid metabolism defects	4	2 (4)	0 (4)	0 (4)	0 (4)	0 (4)	0 (4)	0 (4)	4 (4)	4 (4)
Hyperprolinaemia type II	1	+	-	-	-	-	-	-	+	-
Antley Bixler syndrome	1	-	-	n.d.	-	-	-	-	-	-
Cystic fibrosis	36	16 (36)	0 (36)	n.d.	n.d.	n.d.	7 (36)	2 (36)	n.d.	n.d.
Total	108	51 out of 106 (48%)	12 out of 97 (12%)	16 out of 38 (42%)	17 out of 48 (35%)	17 out of 54 (31%)	29 out of 107 (27%)	18 out of 90 (20%)	16 out of 44 (36%)	14 out of 42 (33%)

[a]CK creatinine kinase,; [h]LDH lactate dehydrogenase,; [i]Hb hemoglobine,; [j]TG triglycerides

The most frequent biochemical change in IEM patients were elevated liver tranaminases (48% of patients), that were detected in almost all patients.

5. DISCUSSION

5.1. Long–chain 3–hydroxyacyl–CoA dehydrogenase (LCHAD) deficiency

LCHAD deficiency prevalence in Latvia constitutes 2.07: 100 000, this being relatively higher than average in Europe where it is 1: 100 000 (Orphanet Report Series, 2010). Differing from Western Europe where the most frequent fatty acids oxidation disorders are medium chain hydroxyacyl-CoA dehydrogenase deficiency, in Latvia, presumably, like in Poland, the Czech Republic an Russia, patients with long-chain hydroxyacyl-CoA dehydrogenase deficiency predominate.

The data analysis of our patients reveals that in the cases of identical genotype most of the clinical and laboratory data are similar. Differences do appear, but they are less pronounced; the fact being stressed also by a part of researchers (den Boer, 2002, Spiekerkoetter, 2010). However, it should be noted that due to the small number of patients categorical statements can't be done. Hypoglycemia developed in five out of six patients or in 83% cases during the first metabolic crisis. Hypoglycemia may not develop at the initial stage of metabolic crisis, and the fact makes diagnosing difficult and might cause lethal consequences as it was in the case with patient No 1, when stating moderate hyperglycemia, insulin therapy was prescribed as a result of which within less than an hour developed hypoglycemic coma and seizures and later death. It is likely that in the stress situations due to the hormones secreted by the adrenal glands for a short period a normal or even elevated glucose level in the blood might be observed, but the organism already experiences intracellular hypoglycemia. All available sources refer to hypoglycaemia as a characteristic symptom of LCHAD deficiency, but the possibility of a short-term hyperglycemia is overlooked. Though the literature data mention creatine kinase as a sensitive marker in patients with LCHAD deficiency our data reveal that up to the age of 6 months lactate dehydrogenase is a more sensitive indicator for the acute period of the disease. A sudden worsening of general health, accompanied by vomiting, lethargy, hypoglycemia with the absence of ketone substances in the urine are important symptoms that might indicate of possible disorders of fatty acids metabolism. The Latvian doctors have poor information and knowledge about the clinical and laboratory symptoms and emergency therapy in the cases of acute decompensation period of LCHAD deficiency. As acylcarnitines profile in the blood is not tested in Latvia and doing the test is possible only

abroad on the basis of individual agreement the given fact has led to extension of the diagnosis confirmation. Thus, lately molecular diagnostics of the most frequent mutation in *HADHA* gene 1528G>C is done to patients with clinical symptoms and changes in the organic acid spectrum, as a result of which the diagnosis may be confirmed within a period of five to seven days.

Early diagnostics of the disease before the manifestation of clinical symptoms is very important for decreasing the patients' mortality rate and the number of complications. It might be possible if a comprehensive newborn screening included testing for disorders of fatty acids oxidation, including LCHAD deficiency. It might also provide a possibility for measuring the real disease frequency or incidence in Latvia. However, under the present conditions it is important to improve the doctors' knowledge about LCHAD deficiency, so as on the basis of clinical and laboratory data they might identify the patients and timely do the required tests for confirmation the diagnosis and adequate therapy.

5.2. Urea cycle disorders

The clinical data of our patients with UCD are similiar to those of other countries. The evident difference of clinical symptoms between girls and boys was noticed in children with OTC deficiency. The first characteristic clinical symptom in boys was respiratory failure, and later, if hyperammonaemia persisted, lethargy and coma appeared. The repeated episodes of vomiting before acute decompensation was characteristic sign in girls. There are no precisely data what is the proportion between symptomatic and asymptomatic females with OTC deficiency in literature. The time of clinical presentation in our girls was earlier compared to data of M. Summar (Summar, 2008). The letality among our girls with OTC deficiency was higher (50%), compared to median letality in Europe (11%). The data were collected about 110 female patients from 19 metabolic centers in 11 Eurpean countries (Häberle, 2010). The peculiarity of clinical signs and severity among patients in one family, more likely is due to difference in X chromosome inactivation. Our four patients with OTC deficiency died during first undiagnosed hyperammonaemic coma. The reason of diagnostic failure was impossibility to detect ammonia level in Latvia in patients 1, 2 and 4. Respiratory alkalosis was diagnosed in three patients, elevated liver enzymes were observed in all girls during metabolic decompensation. It's difficult to summarise laboratory results because ammonia level,

aminoacids and orotic acid haven't been done in most of patients. The family pedigree with sudden death in boys during neonatal period showed the connection with X chromosome, that's why DNA analysis of *OTC* gene was done. The mortality of our patients with UCT is 71.4%, including OTC deficiency - 66.6%, but in UK – 14,4% and 15% (Chakrapani, 2010). The highest mortality is reported during neonatal period: from 32% to 36% (Summar, 2008; Chakrapani, 2010), but in Latvia it runs to 66,6 %. Latvian data show a serious problem which is necessary to start solving. We could affirm that most of patients with UCD are still not diagnosed in Latvia. It's confirmed by increasing number of patients in countries where diagnostics of UCD is included in newborn screening. The majority of doctors have no knowledge about clinical symptoms of hyperammonaemia and it's risks. For instance in year 2007, when patient Nr.7 was in Intensive Care Unit of Children's University Hospital for three weeks, the ammonia level was checked only after geneticist consultation. The geneticist was informed about the result of ammonia level - 214 μmol/l (normal range below 48 μmol/l) only on the fifth day and to that day no therapy to decrease ammonia level had been prescribed. The tactic was wrong also in case with patient Nr.3, when his health condition became worse, the ammonia control and appropriate therapy wasn't done and boy died on the second day of life. Sudden health aggravation as respiratory failure, lethargy, vomiting episodes, acute neurological disturbances, coma could indicate the possibility of UCD independently of age and ammonia level must be checked immediately or child transported to other hospital where it's available. The parameters of mortality with UCD in Latvia are 50% – 70% higher compared to data of other countries.

The lack of knowledge about UCD among doctors in Latvia leads to late diagnosis and high mortality in many cases. Presently it's important to improve the awareness about UCD among doctors in order to recognise patients and take analyses and start adequate therapy on time. The protocol about activities in case of hyperammonaemia is necessary.

5.3. Lysosomal storage disorders

Clinical symptoms of patients with LSD in Latvia are similiar to those in other countries. In spite of fact that clinical symptoms are persistent and slowly progressive, the diagnostic process is often extended and difficult, because some symptoms appear in later period of life. Comparatively high amount of revealed MPS is due to possibility to do GAG quantitation and

electrophoretic separation of different GAGs and relatively pronounced progressive clinical symptoms. The pathological findings of heart in all cases developed secondary as a complication of LSD. However comparatively small number of all patients with LSD (20% compared to Chech Republic, Portugal, Netherlands and others) definetely shows, that many patients with LSD are still not diagnosed. Partially it's because the only method of diagnostics for many LSD is enzyme studies in leukocytes or fibroblasts, which is not done in Latvia. The clinical variability and lack of knowledge about LSD among doctors causes diagnostic difficulties. The proper diagnosis is important for family planning and prenatal diagnosis, but at the moment the most important thing is to find patients with treatable LSD. Patients with hepatosplenomegaly, coarse facial features, and skeletal changes must be examined to exclude LSD. The symptoms of LSD could be also behavioural problems, regress of psychomotor development and hearing impairment. Gaucher disease must be excluded in patients with unknown etiology of spleno- or hepatosplenomegaly and trombocytopenia.

It will be advisable to start checking chitotriosidase activity in Latvia, because it's a good marker for several LSD.

5.4. Diagnosed rare IEM in Latvia

The results of studies showed that incidence of CF in Latvia is similiar to incidence in Europe 1:3300 (Krumina, 2001). It means that in Latvia about six patients are born every year, but only two patients or one third are diagnosed in a year. It was confirmed also by pilotproject done in Latvia (Lace, 2009) when from 7000 screened newborns, two new patients with CF were found. During last fifteen years only one patient was found with classical galactosaemia in Latvia and this also indicates diagnostic problems in our country. In Estonia during time from 1986 through 2008 nine patients with galactosaemia were diagnosed (Ounap, 2010). Diagnostic difficulties are also with mitochondrial disorders, because immunohistochemistry or enzyme histochemistry of muscle biopsy is not done in Latvia. Diagnostic problems are connected also with quickly increasing number of new IEM during last years. For example, the first defect of congenital disorders of glycosylation (CDG) was discovered in 1980, but now the number is over 50 (Lefeber, 2011).

There are two main preconditions in diagnostics of IEM: examination facilities in Latvia to confirm the disease and awareness of doctors about IEM. Many investigations are not

available, also the knowledge of doctors about IEM is poor in Latvia. Only with improving these both things, we could hope for better results. First of all it's important to improve diagnostics of treatable IEM, accordingly decrease mortality and disability. For improving the knowledge of practitioners of medicine about IEM it's necessary to provide more information in Latvian and Medical Genetics Association of Doctors must organise courses and workshops about IEM. As half of our patients had symptoms already during first year of life, training of neonatologists and pediatricians is very important.

6. CONCLUSIONS

1. Clinical symptoms in children with rare IEM are similiar with data from other countries.
2. Patients with LCHAD deficiency at the beginning of metabolic crises for short period may have normoglycemia or hyperglycemia.
3. LDH is a good marker for metabolic decompensation in LCHAD deficiency for infants before six months of age.
4. The prevalence of IEM in most cases is lower compared to average data of other European countries.
5. Rare IEM in most cases are not recognised or diagnosed too late, which leads to early death and severe complications.
6. The knowledge about clinical symptoms, diagnostics and therapy of rare IEM among Latvian doctors are insufficient.

7. RECOMMENDATIONS HOW TO IMPROVE DIAGNOSTICS OF RARE INBORN ERROR OF METABOLISM IN LATVIA

1. To extend a comprehensive newborn screening embraced testing for relatively more frequent and treatable disorders, including disorders of fatty acids oxidation, UCD, galactosaemia, cystic fibrosis.
2. The ammonia level in blood must be checked immediately to all patients with sudden health aggravation, lethargy, coma, seizures (an informative letter about hyperammonaemia has been created).
3. A possibility to check ammonia level in Riga's maternity home and in all prenatal centers of our country must be organised.
4. Common guidelines about measures in case of hyperammonaemia must be developped.
5. Newborns with sepsis and hepatopathy must be examined to sugars reducing substances and in case of positive result selective screening for galactosaemia must be done rapidly.
6. For infants with LCHAD deficiency before six months of age it is recommended to examine lactatdehydrogenase, which presents evidence of metabolic decompensation.
7. Sweat test must be done for children with unknown etiology of malnutrition, recurrent obstructive bronchitis for excluding cystic fibrosis.
8. Medical Genetics Association must facilitate the knowledge about IEM organising lectures and workshops among neonatologists, paediatricians and others practitioners of medicine.

APPROBATION

Publications:

1. **Krūmiņa Z.,** Daneberga Z., Piekuse L, Kreile M., Valeiņe S., Lace B., Lugovska R. Long-chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency in Latvia. Proceedings of the Latvian Academy of Sciences, 2011, (submitted).
2. **Krumina Z.** Lizosomālās uzkrāšanās slimības. Latvijas Ārsts, 2011, 7-8: 54-56.
3. Wortmann S.B., Vaz F.M., Vissers L.E.M., Gardeitchik T., Schuurs-Hoeijmakers J.H.M., Kulik W., Lammens M, Christin C., Kluijtmans L.A.J., Rodenburg R., van Hasselt P.M., Kloosterman W., Baric I., Pronicka E., Kalkan S., Naess K., Singhal K., **Krumina Z.**, van Bokhoven H., Veltman J.A., Smeitink J.A.M., Lefeber D.J., Wevers R.A., Morava E., de Brouwer A.P.. *SERAC1* mutations cause MEGDEL syndrome, a phospholipid remodeling disorder with mitochondrial dysfunction and impaired intracellular cholesterol trafficking. *SERAC1* mutations cause MEGDEL syndrome, a phospholipid remodeling disorder with mitochondrial dysfunction and impaired intracellular cholesterol trafficking. Journal „ Nature Genetics", 2011, (submitted).
4. Jurecka A., **Krumina Z.**, Zuber Z., Rodzynski-Swiakowska A., Kloska A., Tylki-Szymanska A. MPS Type II in Females and Response to Enzyme Replacement Therapy. American Journal of Medical Genetics, 2011 (accepted).
5. Pētersons A., Ābola Z., Villeruša., Pilmane M., Lugovska R., Proņina N., Daneberga Z., **Krūmiņa Z.**, Šterna O., Kreicberga I., Rezebega D., Lubaua I. Uz modernām tehnoloģijām balstītu iedzimtu patoloģiju diagnostikas un ārstēšanas algoritmu izstrāde bērniem.- Latvijas iedzīvotāju dzīvildzi un dzīves kvalitāti apdraudošās slimības, Zinātniskā analīze un galvenās rekomendācijas. V. Pīrāga redakcijā. Rīga, 2009, pp.77-94.
6. Jurecka A., Popowska E., Tylki-Szymanska A., Kubalska J.,Ciara E., **Krumina Z.**, Sykut-Cegielska J., Pronicka E. Hypoxanthine-guanine phosphoribosylotransferase deficiency – clinical, biochemical and molecular characteristics of patients. Przeglad Pediatryczny 2008, vol.38, Nr.3, 227-236
7. Jurecka A., Popowska E., Tylki-Szymanska A., Kubalska J.,Ciara E., **Krumina Z.**, Sykut-Cegielska J., Pronicka E. Hypoxanthine-guanine phosphoribosylotransferase deficiency-The spectrum of Polish mutations. JIMD Short Report # 136 (2008) Online.

8. Krūmiņa A., Keiss J., Sondore V., Chernusenko A., Zarina A., Micule I., Piekuse L., Kreile M., Lace B., **Krumina Z.**, Rozentāle B. From clinical and biochemical to molecular genetic diagnosis of Wilson disease in Latvia. – Genetika, 2008, 44(10),pp.1379-1384.
9. Krumina A, Kroshkina V, Krumina L, Svabe V, **Krumina Z**, Tamane I, Baumanis V (2001) Cystic fibrosis mutation dF508 in the Latvian population. RSU/AML Scientific Proceedings, 161-166.
10. **Krumina Z.**, Lugovska R., Vevere P. Long chain 3 hydroxyacyl- Co A dehydrogenase deficiency – case report. Balcan Journal of Medical Genetics, International J.of Medical Genetics, 1999,Vol.2(1),pp.37-3

The oral presentations in International conferences:

09.2011.	LCHAD deficiency in Latvia. Baltic Paediatric Ophthalmology conference, Riga, Latvia
06.2011.	Patient with Alexander disease – case report. 11th International conference of the Baltic child neurology association, Riga, Latvia
05.2009.	The infantile form of GM1 gangliosidosis: case report. 10th International conference of the Baltic child neurology association, Tartu, Estonia
10.2008.	Situation with rare diseases in Latvia. MPS and rare diseases conference. Warsaw, Poland
09.2008.	Rare inborn errors of metabolism in Latvia. 9th Baltic Congress of Laboratory Medicine, Jurmala, Latvia
03.2007.	Experience with vitamin B6 non-responsive homocystinuria patients in Latvia. Meeting of Baltic Metabolic Specialists, Talinn, Estonia
03.2005.	Lysinuric protein intolerance.10. AEWIEM European-Asian conference of metabolic diseases, Cairo, (Egypt)
09.2003.	Difficulties in diagnosis of CF in Latvia. 7th International Symposium for Cystic Fibrosis, Bratislava, Slovakia

Theses:

1. **Krumina Z.**, Kreile M., Daneberga Z., Piekuse L., Vevere P, Krumina A, Lugovska R. Large pedigree of ornithine transcarbamylase (OTC) deficiency. Case report. – J Inherit Metab Dis (2011) 34 (suppl3): S93
2. **Krumina Z**, Daneberga Z, Kreile M, Lugovska R. Long-chain-3-hydroxyacil-CoA dehydrogenase deficiency - the most frequent fatty acid oxidation disorder in Latvia: 7 cases. - European Society of Human Genetics Conference, 2011, Abstract book, pp. 124-125.
3. **Krūmiņa Z**, Daneberga Z, Lugovska R. Garo ķēžu taukskābju hidroksiacilkoenzīma A dehidrogenāzes nepietiekamība – biežākais diagnosticētais taukskābju defekts bērniem Latvijā. - Rīgas Stradiņa universitātes zinātniskā konference, 2011., Rīga, Latvija, tēžu grāmata 231 lpp.
4. **Krūmiņa Z**, Miklasevics E, Berzina D, Lugovska R. Patient with Alexander disease – case report. - 11th International conference of the Baltic child neurology association, Riga, Latvia, 2011, Abstract book, p 15.
5. **Krumina Z**. Enzyme replacement therapy in a girl with Hunter syndrome.- Starptautiskā konferencē par lizosomālajām slimībām, 2010, Prāga, Čehija. Abstract book.
6. Kreile M, Dzivite- Krisane I, **Krumina Z**, Daneberga Z, Piekuse L. Long chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency with unusual presentation of extremely low vitam D level. J Inherit Metab Dis, 2010, 33 (Suppl 1): S161.
7. Osipova O, Pronina N, Piekuse L, **Krumina Z**, Strautmanis J, Krumina A, Baumanis V. Quantitative analysis of mitochondrial DNA in patients with suspected mitochondrial depletion syndrome. 5th Baltic Sea reagion conference in medical sciences, 2010, Abstracts, pp125-126.
8. **Krumina Z**, Daneberga Z, Czartoryska B, Kornejeva A, Lugovska R. The infantile form of GM1 gangliosidosis: case report. 11th International conference of the Baltic child neurology association, 2009, Abstract book, p
9. Krūmiņa A, Laganovska G, Baumane K, Pliss L, Brakmanis A, Lāce B, **Krūmiņa Z**, Baumanis V. Mitohondriālās DNS analīze cilvēka patoloģijas diagnostikā. RSU 2008.gada zinātniskā konference. Tēzes, Rīga, 102 lpp

10. **Krumina Z**, Lace B, Lugovska R, Vevere P, Daneberga Z, Grauduma I, Kornejeva A, Pronina N, Micule I, Kornejeva L, Locmele Dz, Zarina A, Jasko J. Rare inborn errors of metabolism in Latvia. Laboratorine Medicina (ISSN 1392-6470), 2008, Vol. 10, Special Suppl, p. 14.
11. **Krūmiņa Z**, Vēvere P, Korņejeva L, Lugovska R. Lizinūriskā proteīnu nepanesība. Klīniskais gadījums. RSU 2008.gada zinātniskā konference. Tēzes, 97 lpp.
12. Lugovska R, **Krumina Z**, Lace B, Vevere P, Pronina N, Daneberga Z, Kornejeva A. Newborn screening and diagnosis of inborn errors of metabolism in Latvian children. Laboratorine Medicina, 2008, Vol. 10, Special Suppl, p. 15.
13. **Krūmiņa Z**, Czartoryska B, Grauduma I, Lugovska R. Hunter syndrome in a girl: case report. – Journal of Inherited Metabolic Disease, Volume 30, Suppl. 1, August 2007, p.98
14. **Krumina Z,** Muceniece Z, Lugovska R. Two patients with LCHAD deficiency in Latvia.- Abstract book 6th International Congress on Fatty Acid Oxidation Clinical, Biochemical and Molecular Aspects, 2005, Abstract book, p.39
15. Muceniece Z, **Krūmiņa Z**, Vēvere P, Pronina N, Lugovska R. Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency: Biochemical and mutation analysis in Latvia. European Journal of Human Genetics Vol. 13 Suppl. 1, 2005, pp.269-270
16. Pronina N, Lugovska R, Muceniece Z, Olhovaya O, **Krumina Z**, Lace B, Balode. Experience of molecular diagnostics for genetic disorders in Latvia. European Journal of Human Genetics Vol. 13 Suppl. 1, 2005, p.286
17. Lugovska R, **Krumina Z**, Vevere P, Lace B, Pronina N, Muceniece Z, Grauduma I, Detection of inborn errors of metabolism in Latvia. Abstract "International workshop on Advances in Pediatrics Metabolomics", 2005, Tartu, p.27
18. **Krumina Z**, Vevere P, Lugovska R. Two patients with B6 non-responsive homocystinuria – case report. "International workshop on Advances in Pediatrics Metabolomics", 2005, Tartu, Abstract book, p.57
19. Lugovska R., **Krumina Z.**, Vevere P., Pronina N., Muceniece Z., Purina G., Lace B., Grauduma I. Inborn errors of metabolism in Latvia. – Abstr. Bratislava Medical Journal 105(9) , 2004. p. 332

20. Muceniece Z., **Krumina Z.**, Sass J. O., Vevere P., Grauduma I., Lugovska R. Laboratory findings of Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency – case report. Abstr. The 7th Baltic Congress in Laboratory Medicine. Parnu, Estonia, 2004. p.127

21. Lugovska R., **Krumina Z.**, Vevere P., Muceniece Z., Purina G., Lace B., Grauduma I.Screening for Inherited Metabolic Diseases in Latvia. – Dietary Management of Inborn Errors of Metabolic Disease. Inborn Errors Review Series No. 14. London, 2004, p. 36

22. **Krumina Z.**, Grauduma I., Muceniece Z., Lugovska R.. Difficulties in diagnosis of MPS in Latvia. Conference of MPS, Mainz, Germany, 2004. (stenda referāts)

23. Muceniece Z., **Krumina Z.**, Sass J.O., Vevere P., Grauduma I., Lugovska R.. Laboratory findings of Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency- case report. Abstr. The 7th Baltic Congress in Laboratory Medicine, Parnu, Estonia, 2004. (stenda referāts)

24. **Krumina Z.**, Sass JO, Muceniece Z., Lugovska R. Long-Chain Hydroxyacyl-CoA Dehydrogenase (LCHAD) Deficiency- case report. - Dietary Management of Inborn Errors of Metabolic Disease. Inborn Errors Review Series No. 14. London, 2004, p.33

25. **Krumina Z.**, Krumina A., Krumina L., Svabe V. Difficulties in diagnosis of CF in Latvia. Abstr. 7th International Symposium for Cystic Fibrosis, Bratislava, Slovakia, 2003, p.7

26. Maliseva-Lace B., **Krūmiņa Z.**, Tamane I., Krūmiņa A, Baumanis V. Alfa-1-antitripsīna nepietiekamības klīniskā gadījuma apraksts bērnam. – RSU Zinātniskā conference, 2002, Riga, tēzes, p 75

27. Muceniece Z., Lugovska R., Vevere P., **Krumina Z.**, Purina G., Lace B., Grauduma I. Biochemical screening methods for inborn errors of metabolism in Latvia. - Laboratorine Medicina (ISSN 1392-0), 2002, Special supplement , pp. 52-53; 63

28. **Krumina Z.**, Vevere P., Lace B., Lugovska R., Late diagnosis of homocystinuria (case report) Laboratorine Medicina (ISSN 1392-0), 2002, Special supplement , p. 49

29. Krumina A., Krumina L., Lazdins M., Svabe V., **Krumina Z.**, Teibe U., Baumanis V.. CA repeats in the first intron of the CFTR gene in cystic fibrosis patients and healthy Latvians. European Journal of Human Genetics Vol. 10 Suppl. 1, May 2002, p.213.

30. Krumina A., Kroshkina V., Svabe V., **Krumina Z.**, Krumina L., Tamane I.,The major cystic fibrosis mutation in Latvia; frequency, origin and age of the dF508 mutation, Journal of the European Society of Human Genetics, 2001, Vol.9.,Suppl.,1.,p. 276

REFERENCES

1. Aerts J, Kallemeijn W, Wegdam W, Ferraz M, Breemen M, Dekker N, Kramer G, Poorthuis B, Groener J, Brinkman J, Rombach S, Hollak C, Linthorst G, Witte M, Gold H, Marel G, Overkleeft S, Boot R (2011) Biomarkers in the diagnosis of lysosomal storage disorders: proteins, lipids, and inhibodies. J Inherit Metab Dis 34: 605-619
2. Andersen BS, Jensen TG, Bross P, Knudsen I, Winter V, Kolvraa S, Bolund L, Ding JH, Chen YT, Van Hove J, Curtis D, Yokota I, Tanaka K, Kim JP, Gregersen N (1994) Disease-causing Mutations in Exon 11 of the Medium-Chain Acyl-CoA Dehydrogenase Gene. Am J Hum Genet 54: 975-988
3. Bachmann C (2003) Outcome and survival of 88 patients with urea cycle disorders: a retrospective evaluation. Eur. J Pediatr. 162:410-416
4. Beutler E, Gelbart T, West C (1990) The facile detection of the NT 1226 mutation of glucocerebrosidase by "mismatched" PCR. Clin Chim Acta 194:161–166
5. Blau N, Duran M, Gibson KM (2008 a) Laboratory guide to the methods in biochemical genetics. Springer-Verlag, pp 287-324, pp 325 – 333
6. Brusilow S, Maestry N (1996) Urea cycle disorders: diagnosis, pathophysiology and therapy. Advances in Pediatrics, 43: 127-139
7. Chakrapani A, Champion B, Grunevald S, Lachmann R, Shortland G, Williams M, Morris A (2010), Experience of urea cycle disorders in the UK. Poster V6: Layout 1
8. Coen SA, Meys M, Tarvin TD (1989) The Pico-Tag Method. A Manual of Advanced Techniques for Amino Acid Analysis. Waters, Millipore Corporation.
9. den Boer ME, Ijlst L, Wijburg FA, Oostheim W, van Werkhoven MA, van Pampus MG, Heymans HS, Wanders RA Heterozygosity for the common LCHAD mutation (1528G>C) is not a major cause of HELLP syndrome and the prevalence of the mutation in the Dutch population is low. Pediatric Research, 2000, Vol 48, No 2: 151-154
10. den Boer ME, Wanders RJA, Morris AA, IJlst L, Heymans HS, Wijburg FA (2002) Long-chain 3–hydroxyacyl–CoA dehydrogenase deficiency: clinical presentation and follow up of 50 patients. Pediatrics 109: 99-104

11. Farrell PM, Lai HJ, Li Z, Kosorok MR, Laxova A, Green CG, Collins J, Hoffman G, Laessig R, Rock MJ, Splaingard ML (2005) Evidence on improved outcomes with early diagnosis of cystic fibrosis through neonatal screening: enough is enough! J Pediatr 147: S30 – S36
12. Gropman A, Batshaw M (2004) Cognitive outcome in urea cycle disorders. Molecular Genetics and Metabolism 81(1): 58-62
13. Grosse SD, Rosenfeld M, Devine OJ, Lai HJ, Farell PM (2006) Potential impact of newborn screening for cystic fibrosis on child survival: a systematic review and analysis. J Pediatr 149: 362-366
14. Häberle J, Lachmann R (2010) Diagnosis and treatment of OTC in females. European Metabolic Group Workshop proceedings 2010., Milupa, Metabolics, pp 49-59
15. Harmatz P, Whitley CB, Waber L et al (2004) Enzyme replacement therapy in mucopolysacharidosis VI (Maroteaux-Lamy syndrome). J Pediatr 144:574-580
16. Kahler S (2010) LCHAD (Including Trifunctional Protein) Deficiency. In: Hoffmann G, Zschocke J, Nyhan L (eds) Inherited Metabolic Diseases. A clinical approach. Springer-Verlag Berlin Heidelberg pp 171-172
17. Kakkis ED, Muenzer J, Tiller GE, Waber L, Belmont J, Passage M, Izykoxski B, Phillips J, Doroshow R, Walot I, Hoft R, Neufeld EF (2001) Enzyme-replacement therapy in mucopolysaccharidosis. N Engl J Med 344:182–188
18. Krumina A, Kroshkina V, Krumina L, Svabe V, Krumina Z, Tamane I, Baumanis V (2001) Cystic fibrosis mutation dF508 in the Latvian population. RSU/AML Scientific Proceedings, 161-166.
19. Lace B, Grīnblate S, Korņejeva L, Svabe V, Grauduma I,Vevere P, Lugovska R, Martinsons A (2009) Neonatal Cystic Fibrosis screening in Latvia: a pilot project. Proceedings of the Latvian Academy of Sciences, Section B. Natural, Exact, and Applied Sciences, 63 (4/5): p 24
20. Lazovskis I (2001) Klīniskie simptomi un sindromi. Clinical signs, symptoms and syndromes. Nacionālais medicīnas apgāds, Rīga, Latvija
21. Leonard J (2006) Disorders of the Urea Cycle and and Related Enzymes. In: Fernandes J, Saudubray JM, van den Berghe G, Walter JH (eds) Inborn Metabolic Diseases 4th edition, Springer Medizin Verlag Heidelberg pp 264-272

22. Lindner M, Hoffmann G, Matern D (2010) Newborn screening for disorders of fatty-acid oxidation: experience and recommendations from an expert meeting. J Inherit Metab Dis 33: 521-526.
23. Moog U, Zchocke I, Grünewald S (2010) Lysosomal Storage Disorders. Disturbed degradation of macromolecules in lysosomes. In: Hoffmann GF, Zschocke J, Nyhan WL (edit) Inherited metabolic diseases. A clinical approach, Springer-Verlag Berlin Heidelberg, pp 220-225
24. Muenzer J, Lamsa JC, Garcia A, Dacosta J, Garcia J, Treco DA (2002) Enzyme replacement therapy in mucopolysaccharidosis type II (Hunter syndrome): a preliminary report. Acta Paediatr Suppl 91:98–99
25. Munnich A (2006) Defects of the Respiratory Chain. In: Fernandes J, Saudubray JM, van den Berghe G, Walter JH (eds) Inborn Metabolic Diseases 4th edition, Springer Medizin Verlag Heidelberg pp 198-208
26. Orphanet Report Series – Prevalence of rare diseases: Bibliographic data – May 2010 – Number1;http://www.orpha.net/orphacom/cahiers/docs/GB/ Prevalence_of_rare_ diseases_by_alphabetical_list.pdf
27. Ounap K, Joost K, Temberg T, Krabbi L, Tonisson N (2010) Classical galactosemia in Estonia: selective neonatal screening, incidence, and genotype/ phenotype data of diagnosed patients. J Inherit Metab Dis 33: 175-176
28. Pinto R, Caseiro C, Lemos M et al (2004) Prevalence of lysosomal storage diseases in Portugal. Eur J Hum Genet 12(2): 87-92
29. Polakova H, Minarik G, Ferekova E, Ficek A, Baldovic M, Kadasi L (2007) Detection of His1069Gln mutation in Wilson disease by bidirectional PCR amplification of specific alleles (BI-PASA) test. Gen Physiol Biophys 26(2): 91-6
30. Pons Ruiz M, Sanchez-Valverde V, Dalmau Serra J (2007) Classification. Nutritional treatment of inborn errors of metabolism. Nutricia, Madrid pp 7-11
31. Poorthuis BJ, Wevers RA, Kleijer Wj et al (1999) The frequency of lysosomal storage diseases in The Netherlands. Hum Genet 105(1-2): 151-156
32. Poupětová H, Ledvinová J, Berná L, Dvoráková L, Kozich V, Elleder M (2010) The birth prevalence of lysosomal storage disorders in the Czech Republic: comparison with data in different populations. J Inherit Metab Dis 33: 387-396

33. Rommens JM, Kerem BS, Greer W, Chang P, Tsui LC, Ray P (1990) Rapid nonradioactive detection of the major CF mutation. Am J Hum Genet 46:395-396
34. Saudubray J, Desguerre I, Sedel F, Charpentier C (2006) Classification of inborn errors of metabolism. In: Fernandes J, Saudubray J, van den Berghe G, Walter J (eds) Inborn Metabolic Diseases 4th edition, Springer Medizin Verlag Heidelberg pp 5-6
35. Sommerburg O, Lindner M, Muckenthaler M, Kohlmueller D, Leible S, Feneberg R, Kulozik A, Mall M, Hoffmann G (2010) Initial evaluation of a biochemical cystic fibrosis newborn screening by sequential analysis of immunoreactive trypsinogen and pancreatitis-associated protein (IRT/PAP) as a strategy that does not involve DNA testing in a Northen European population. J Inherit Metab Dis 33 (Suppl 2):S263-S271
36. Staretz-Chacham O, Lang TC, LaMarca ME, Krasnewitch D, Sidransky E (2009) Lysosomal storage disorders in the newborn. Pediatrics 123(4):1191-1207
37. Summar M, Dobbelaere D, Brusilow S, Lee B (2008) Diagnosis, symptoms, frequency and mortality of 260 patients with urea cycle disorders from a 21-year multicentre study of acute hyperammonaemia episodes. Acta Paediatrica, 97: 1420-1425
38. Sweetman L (1991) Techniques in Diagnostic Human Biochemical Genetics: A laboratory Manual. Wiley-Lics, Inc.pp 143-176
39. Tuchman M (2001) Proceedings of a Consensus Conference for the Managment of patients with Urea Cycle disorders. J Pediatr 138 (Suppl 1):S1-S80
40. Utchino T, Endo F, Matsuda I (1998) Neurodevelopmental outcome of long-term therapy of urea cycle disorders in Japan. J Inherit Metab Dis 21:151-159
41. Wanders RJ, Duran M, Ijlst L, de Jager JP, van Gennip AH, Jakobs C, Dorland L, van Sprang FJ (1989) Sudden infant death and long chain 3-hydroxyacyl-CoA dehydrogenase. Lancet 2:52-53
42. Zschocke J, Hoffmann G (2004) Disorders of ammonia detoxification – urea cycle defects. Allopurinol test. Vademecum Metabolicum. Manual of Metabolic Paediatrics, Milupa, Heidelberg, p 51, pp 61-63, p.96, pp. 111-123
43. Lefeber DJ, Morava E, Jaeken J (2011) How to find and diagnose a CDG due to defective N-glycosylation. J Inherit Metab Dis 34:849-852

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THE STRUCTURE OF WORK AND VOLUME

The doctoral thesis are written in Latvian. There are introduction, rewiev of literature, materials and methods, results, discussion, conclusions, practical recommendations, and refferences (101). The volume of thesis are 104 pages, apart from appendix, 32 tables and 15 figures.