Special genotype-phenotype correlations in rare diseases

PhD Thesis

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1. Introduction

A rare disease, also referred to as an orphan disease, is any disease that affects a small percentage of the population. There is no single, widely accepted definition for rare diseases. The European Commission on Public Health defined rare diseases as life-threatening or chronically debilitating diseases which are of such low prevalence that special combined efforts are needed to address them. The term low prevalence has been later defined as generally meaning fewer than 1 in 2,000 people. The European Organization for Rare Diseases (EURORDIS) estimates that there exist between 5,000 and 7,000 distinct rare diseases. Although each individual disease is rare, the sheer number of individual rare diseases results in between 6% and 8% of the population of the European Union that means, that 30 million EU citizens being affected by a rare disease. Rare diseases usually are genetic, hence chronic. It is estimated that at least 80% of them have an identified genetic origin. Other rare diseases are supposed to be a result of infections and allergies, or due to degenerative and proliferative causes. 75% of rare diseases affect children, whom 30% do not live until 5 years of age. Examination of rare diseases' molecular mechanism exercises a strong influence on our knowledge of diagnosis, pathological behavior and treatment of common diseases.

2. Studied diseases

2.1.CDG syndrome

The group of congenital disorders of glycosylation (CDG) is a family of inherited metabolic diseases caused by defects in the synthesis, assembly and processing of glycans. About half of the body proteins contain carbohydrate chains essential for protein structure and function, and about 200-300 genes have been found to be involved in glycosylation processes. Most type of CDG described so far arise from defects in the N-glycosylation pathway. The clinical presentation of CDG patients includes psychomotor and mental retardation, muscle hypotonia, seizures, ophthalmological anomalies, neurological signs, failure to thrive, endocrine and coagulation abnormalities and variable dysmorphic features.

Based on the localization of the metabolic defect within the cell, two main groups of CDG can be distinguished by their specific transferrin isoelectric focusing (TIEF) pattern:

CDG I and CDG II. The clinical spectrum of the unsolved patients studied, classified as CDG Ix.

2.2. Rett syndrome

Rett syndrome (OMIM 312750) is a severe neurodevelopmental disorder affecting females almost exclusively. Besides the classic form of RTT, many variants are known.

Classic Rett syndrome shows an X-linked dominant pattern of inheritance and has a relatively high incidence of approximately 1 in 10,000 among females. Mutations in the methyl-CpG binding protein, *MECP2* gene, were identified as disease-causing and according to data published, *MECP2* mutations account for approximately 80-96% of the classical Rett cases and 40-50% of atypical Rett manifestations. The lack of *MECP2* mutation in a small percentage of clinically well defined Rett syndrome patients with infantile spasms suggested the involvement of another gene locus. Indeed, mutations of the cyclin-dependent kinase-like 5 (*CDKL5* also known as serine threonine kinase 9, *STK9*) and in the *FOXG1* gene were identified in patients with atypical RS.

2.3. Mitochondrial disorders

Mitochondrial diseases are rare multisystem disorders affecting tissues with high energy demand and are clinically, genetically and biochemically highly variable. On the basis of clinical symptoms they can be divided into syndromic and non-syndromic forms.

2.3.1. Hearing impairment

Hearing impairment is one of the most common disease with a frequency of at least 1/1000 births. The onset of the disease may be before or after beginning of speech (pre- or post-lingual hearing impairment). The disease can present as a non-syndromic isolated hearing impairment or as associated with other abnormalities (syndromic). Half of the cases may attribute to genetic defects. The mutations of the mitochondrial DNA play significant role in both syndromic and non-syndromic hearing impairment. The most common mitochondrial mutation in the A1555G transition has been identified in association with a non-syndromic hearing loss.

Mutations of the gene encoding the Ser^{UCN}-tRNA have also been implicated in the development of a sensorineural hearing loss.

2.3.2. Leigh syndrome

One of the most well-known syndromic mitochondrial disease is Leigh syndrome, first described by Denis Leigh in 1951 in a patient with focal necrosis and capillary proliferation in the brainstem. The onset of Leigh syndrome is usually in childhood and is characterized by developmental regression, hypotonia, lactic acidosis, failure to thrive, seizures and typical lesions in the brainstem and basal ganglia. The estimated prevalence of Leigh Syndrome is 2.05 cases per 1,00,000, and the preschool incidence of Leigh syndrome is 1 out of 32,000.

Clinical symptoms depend on which areas of the central nervous system are involved. Several mitochondrial mutations have been shown to involve in pathogenesis of Leigh syndrome.

3. Aims of the study

As a result of laboratory techniques development, acceleration in the detection of molecular mechanisms and alterations has been achieved. This stated the new tasks to assign the most precise phenotypic characteristics to these molecular variations. Our aims were to collect clinical data in four clinical entities to define their symptoms and clinical course and the workflow for the achievement of these aims may be divided in four task groups as follows:

1. Compare the clinical data of the examined CDG Ix patients, and based on the dysmorphic signs, neurologic and specific clinical symptoms to determine the follow-up chart and prognosis assessment.
2. Provide an analysis of *MECP2*, *CDKL5* and *FOXG1* genes to determine the genetic alteration in the Hungarian Rett patients. Following the molecular investigations to explore the genotype-phenotype correlations and determine a diagnostic algorithm.
3. Describe a new phenotypic variant caused by mt7445A>G mutation.
4. Describe a new phenotype caused by mt11777C>A mutation.

4. Patients

According to the proposal of the European Union for the homogeneous and appropriate supply of rare diseases Centers should be established in every country. In

Hungary this task and all the surrounding facilities are provided by our Institute, together with the coordination and global diagnostic work. Therefore the prevalence of patients with rare disease is relative frequent, giving us also the opportunity to detect recurrently phenotypic variations.

We reviewed the clinical presentations of 10 patients diagnosed with CDG type Ix. In all patients, an abnormal TIEF profile was observed with a decreased amount of tetrasialo- and increased amounts of asialo- and disialotransferrin.

Blood DNA samples were collected from 158 patients with suspected Rett syndrome individuals after informed consent. All of them were originally recruited from patients who had been referred to our institute for *MECP2* testing. In the second stage of screening all those patients negative for a detectable *MECP2* mutation were selected and screened for mutations of the *CDKL5* gene. Similarly, patients with no detected *MECP2* or *CDKL5* mutations were tested for *FOXG1* mutations.

We examined a Hungarian family with 16 members affected by hearing loss. While in Hungarian hearing-loss patient our detection was the first, we compared our patients' data to those found in the literature

In the case of our LS patient after the molecular genetic investigations we explored the phenotype with the analysis of previous clinical descriptions and of finding of examinations (brain MRI, cardiolgic investigations, BERA, VEP) We determine the genotype in different tissues from the proband and form six other family member.

5. Methods

5.1. Polymerase chain reaction (PCR)

The genomic DNA was obtained from peripheral blood leucocytes using a standard salting out method. The examining parts of the available DNAs were amplified with polymerase chain reactions. The GenBank reference sequence signed by AY422949 accession number was applied for the study design. The amplifications in all molecular analyses were carried out in a final volume of 50 µl containing 200 µM of each dNTP, 0.2 mM of each primer, 5 µl of reaction buffer (containing 500 mM KCl, 10 mM Tris-HCl, 14 mmol/L $MgCl_2$, pH 9.0), 1U of Taq polymerase (10 U/µl) and 1 µg extracted DNA as template. The sequences

of oligonucleotide primers applied and circumstances of PCR reactions were reviewed in *Table 1.*

5.2. Restriction fragment length polymorphism (RFLP)

For RFLP assays 10-15 microlitres of PCR products were digested with 1U of appropriate restriction endonuclease with 10x enzyme buffer incubating on the appropriate temperature. The primers were designed to create obligatory cleavage sites of the proper restriction enzymes in the amplicons independently of the genotype to control the accuracy of the digestion. The bands received were electrophoresed through an ethidium-bromide-stained 3% agarose gel and were analyzed with UVIdoc gel documentation system.

5.3. Bidirectional DNA sequencing and analyses

To validate our genotyping results bidirectional sequencing was performed for some samples. The examinations were carried out using ABI Prism 3100 Avant Genetic Analyzer. The sequence alignments were made using Winstar genetic program.

5.4. MLPA (Multiplex Ligation-dependent Probe Amplification) analysis

Genomic DNA was purified from peripheral blood leucocytes as previously described. Screening for MECP2 and CDLK5 single and multi-exon deletions was perfomed using the SALSA P015-E1 MLPA assay (MRC-Holland, Amsterdam, The Netherlands), asinstructed by the manufacturer. This assay is containing probes for MECP2, CDKL5, ARX and NTNG1. An aliquot of ,100 ng of denatured genomic DNA was used in the overnight annealing of the exon-specific probes and subsequent ligation reaction. PCR was carried out with FAM-labelled primers using 10 ml of ligation reaction. Separation and relative quantification of the amplification products were carried out using an ABI Prism 3100 Genetic Analyzer (Applied Biosystem, Foster City, California, USA). The peak area for each fragment was measured with GeneScan Analysis software V.3.7 (Applied Biosystems), and normalised by dividing it by the combined area of all peaks in that lane. This normalised peak area was then divided by the average normalised peak area from five normal controls. With this method, the results given are allele copy numbers compared with normal controls, and a

ratio of ,1 should be obtained if both alleles are present. A reduction or increase in the peak area values to ,0.7 or .1.3 was considered an indication of a deletion or a duplication, respectively. DNA samples showing such a reduction or increase in the MLPA peak area values were reanalysed by MLPA, and only the samples showing consistent results between the two experiments were considered positive for a deletion or duplication.

6. Results

6.1. CDG Ix clinical spectrum

In those patients, who were diagnosed as being CDG Ix ones, two determined signs were present: developmental delay and axial hypotonia. Beside these, other non-specific symptoms as, dysmorphic features, abnormal clotting factors, hypoalbuminemia, liver disease, and various ocular abnormalities were also detected.

For their clinical importance several patients were highlighted, who presented unusual signs, while the typical presence of CDG Ix was obvious:

- In four patients unique association of optic nerve atrophy and blindness were observed with movement disorder.
- In other two patients cataract and epileptic seizures were combined with liver disorder.
- One patient presented microcephaly seizures, ascites, hepatomegaly, nephritic syndrome and severe developmental delay, while in another unique findings included arthrogryposis, macrocephaly, polyneuropathy and cystic kidneys.

An overview of all clinical symptoms showed that two main subgroups could be distinguished based on the severity of the disease course: one with a pure neurological presentation and the other with the neurological-multivisceral form. The clinical features of all CDG Ix patients are summarized in Table 1.

6.2. Genotype-phenotype examinations in Rett syndrome

In the first stage of the screening we performed mutation analysis of *MECP2* by direct sequencing. Exons 2-4 of *MECP2* were amplified by polymerase chain reaction. In the second stage of screening all those patients negative for a detectable *MECP2* mutation were selected

and screened for mutations of the *CDKL5* gene. Similarly, patients with no detected *MECP2* or *CDKL5* mutations were tested for *FOXG1* mutations.

A total of 22 different known *MECP2* alterations were identified in 42 subjects: 7 frameshift-deletions, 4 nonsense mutations, 10 missense mutations, and in one patient one nucleotide insertion. We also detected a missense c.925C>T (Arg309Trp) change whose role in Rett pathogenesis is unknown. Interestingly we identified a 18bp deletion 1162_1179del18 in a father and his daughter (R/34). The daughter also carried the frameshift mutation 276insG. The detailed correlations among the genotype-phenotype obtained throughout the *MECP2* examinations are presented in Table 2.

Following the *MECP2* mutation screening we tested all patients without any *MECP2* alteration for *CDKL5* mutations. During the mutation analysis we have identified previously described polymorphism c.2327A>C (Gln791Pro) in 8 subjects as well as both the c.3003C>T (His1001His) and c.3084G>C (Thr1028Thr) known polymorphisms in one of the patients. In addition, we discovered two novel nonsense mutations:, c.607G>T that results in a premature termination codon at amino acid position 203 disrupting the catalytic domain of CDKL5 protein, and c.536C>T (p.Ser179Phe) leading to a premature stop at amino acid position 570 of the C-terminal region involved in either the catalytic activity or the subcellular localization.

Finally, we screened our patients for *FOXG1* mutations using direct sequencing, but we did not find any alterations affecting this gene.

6.3. Phenotypic variants of the deafness-associated mt7445A>G mutation

The mutation above was revelaed in only six families. In Table 3. different families, found in the literature, with the same mutation, were compared with the Hungarian family detected in our Institute.

6.4. Phenotype of mitochonmdrial DNA 11777C>A mutation associated Leigh syndrome

In male proband restlessness and vomiting were seen on first days of life, and on the 5th day of life, myoclonus was also observed. In the checkup records taken at 7 months age,

infantile spasticity, limb convulsions, and cerebral paresis with hypotonia were documented. The electroencephalogram showed hypsarrhythmia, West syndrome was diagnosed and treatment with vigabatrin was initiated The following period was without any attacks, and the EEG examination performed at the age of 16 months did not show any epileptic disorder. During 1.5-T MRI, gray matter spectroscopy exhibited increased signal intensity within almost the entire mesencephalon and medulla oblongata. Increased lactate signal was detected by proton spectrometry. The clinical features suggested mitochondrial encephalomyopathy. Rapid progression developed, the child showed fast general deterioration; with somnolence and several attacks of vomiting. Hypertrophic obstructive cardiomyopathy was revealed by cardiac examination. Due to cardiorespiratory arrest, the child died at the age of 17 months. The most common mitochondrial mutations associated with Leigh syndrome and mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome tests in the proband, by direct sequencing were negative. DNA samples isolated from liver, blood, and muscle were also examined for the 11777C>A mitochondrial mutation, which was detected in heteroplasmic form in all of these tissues. The quantity of the mutant load was estimated by densitometry analysis of RFLP agarosegels. The percentage of the 11777A allele was 50% in blood and 60% both in the liver and the muscle DNA. Although the 11777C>A mtDNA mutation is cited in reviews and is recognized as a widely detected mutation, only four individuals are to be found reported in the PubMed, the major clinical data are summarized in Table 4.

7. Discussion

7.1. CDG Ix

The clinical records of 23 CDG Ix patients were reviewed.

- Inverted nipples and abnormal fat distribution, characteristic of CDG Ia, may be found in patients with other subtypes, although they are much less common.

- In the patient detected dysmorphic features such as retrognathia, low-set ears and club foot are common, non-specific findings that could be observed in patients with congenital CNS anomalies.

- Abnormal clotting is an important indicator of a glycosylation disorder as indicated by the high number of CDG Ix patients presenting with at least abnormal laboratory results. Therefore, coagulation parameters should be evaluated in each patient suspected for CDG even if no clinical symptoms are visible.

- The review of CDG Ix patients shows that additionally bilateral cataract, glaucoma and optic nerve atrophy can be specific features in CDG. Our data indicated that the diagnosis of CDG should be considered in each patient with hypotonia, dysmorphic features and developmental delay accompanied by cataract. The combination of optic nerve atrophy and dystonic movements is extremely rare. It is common in mitochondrial disorders and has been observed in hereditary Leber optic neuropathy, but had not been described so far in any of the solved types of CDG I. The rare association of these symptoms, accompanied by relatively mild multivisceral presentation, may suggest a new CDG I subtype. On the other hand, it might indicate the need for CDG screening among patients with suspected Leber hereditary optic neuropathy. CDG Ix patients could be divided in two subgroups corresponding to clinical symptoms: the solely neurological form and a neurological-multivisceral presentation. The course of the disease in the patients with neurological presentation was usually milder, whereas in the patients with multivisceral form the symptoms occurred early and progressed rapidly.

7.2. Rett syndrome

According to data in the literature, common point mutations of *MECP2* are present in 60-70% of the cases. We have only detected MECP2 mutations in 27.6% of Rett patients studied, a ratio that is likely because of the limitations of direct sequencing method used for mutation analysis, but possibly attributable to a high percentage of gross rearrangements of the MECP2 gene that are not detectable by sequencing. It is important to point out that among the MECP2 –negative patients only 17% showed classical Rett phenotype. In all, 8% of these patients showed the features of congenital form of Rett syndrome, 12% were male patients and 4% have no neurological symptoms. Among the subjects screened, we identified 2 patients with ovel CDKL5 mutation, but we did not find any alterations affecting the FOXG1 gene, that may be because of overall low frequency of FOXG1 mutations in patients with Rett phenotype.

7.3. Deafness- associated mt7445A>G mutation

The disease appears either with deafness only, or combined with palmoplantar keratoderma. Penetrance and expressivity of the disease are different within the pedigrees. The mt7445A>G mutation was for the first time found in Hungarian patients in our Institute. The findings in our Hungarian family also suggest that other mitochondrial or nuclear factors and environmental agents may contribute to the development of hearing loss. The mutation was present in homoplastic or in heteroplastic form in the families, however, previously no pedigrees were found in which homo-and heteroplastic forms of the mutation appeared simuiltaneously. The analysis of the A7445G alteration was performed from only blood leukocytes, other tissues were examined neither in this work, nor in previous studies. However, the proportion of the normal and mutant alleles may vary for example in the auditory cells. The normal allele which was found in the grandmother in low proportion did not segregate to the mother and the proband at all; while in the other branch of the pedigree the heteroplasmy was transmitted at a very low level. In spite of detecting huge differences of level of heteroplasmy, there were clinically affected individuals on both sides of the family tree which shows that the degree of the mutated DNA alone does not necessarily determine the development of clinical symptoms.

7.4. Mitochondrial DNA 11777C>A mutation associated Leigh syndrome

Several differen types of gene-determined metabolic defects can lead to Leigh disease or Leigh-like symptoms and several mitochondrial mutations have been shown to involve in pathogenesis of Leigh disease. Albeit the 11777C>A mtDNA mutation is cited in reviews and is recognized as a widely seen mutation, only four individuals are reported in the PubMed. Our patient was 7 months old, which is the earliest among the patients presented to date. The main clinical features were West syndrome, toxic vomiting, and hypertrophic obstructive cardiomyopathy. The progress was aggressive, leading relatively early fatal outcome. These together clearly differentiates our case from the others presented, supporting the wide clinical phenotype spectrum associated with this mutation.

8. Conclusions

The following exceptional genotype-phenotype associations were described in the thesis of this work:

1. In CDG Ix syndrome:

- developmental delay and axial hypotonia are dominant features, non-specific craniofacial dysmorphic signs (micro/retrognathia, hypertelorism, prominent and low-set ears) are observed.
- the prevalence of abnormal clotting is high, presented sometimes only by abnormal laboratory results. Therefore, coagulation parameters should be evaluated in each patient suspected for CDG even if no clinical symptoms are visible.
- ophthalmological findings are common additional symptoms, optic nerve atrophy is the most frequent one.
- in some cases the diagnosis of CDG Ix arises upon the rare association of symptoms.

2. In Rett syndrome we specified:

- in details the distribution and frequency of mutations detected by MECP2 screening in Hungarian patients with Rett syndrome phenotype.
- the phenotypic signs and disease course relation to the genotype.
- at the screening of CDKL5 gene mutations two novel nonsense mutations: in the first case a c.607G>T change that results in a premature termination codon at amino-acid position 203, and c.1708G>T in a second patient that leads to a premature stop at amin-acid position 570 of the C-terminal region.

Based on our observation we suggest to perform the *CDKL5* gene screening in case of *MECP2* negativity even as a first investigation in patient with therapy-resistant seizures.

3. In deafness associated with mtDNS 7445A>G mutation:

- we first described in Hungarian patient this rare mutation
- In spite of the huge differences in the level of heteroplasmy, there were clinically affected individuals on both sides of the family tree, indicating that the proportion

of mutated mtDNA in the blood DNA alone does not necessarily determine the development of the clinical symptoms.

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4. mt11777C>A mutation associated Leigh syndrome:

- new phenotype was described, as in our patients symptoms appeared at exceptionally early age and the main clinical features; West syndrome, toxic vomiting and hypertrophic obstructive cardiomyopathy also differed from the already known ones and showed a rapid progression.
- examining the phenptype of our patients and that of the ones found in the literature we found that there is a wide clinical phenotype spectrum associated with mt11777C>A mutation.

Table 1. Overview of the clinical symptoms observed in CDG Ix patients

Symptoms	Patient																	
	1	2	3[a]	4	5	6[b]	7[a]	8	9[a]	10	11-13	14	15	16-17	18	19	20-21	22-23
Dysmorphic features	+	+	-	+	-	+	+	-	-	+	-	+	-	+	-	-	+	+
Hypotonia	+	-	-	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+
Psychomotor retardation	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+/-
Retinitis pigmentosa	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Glaucoma	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
CKatarakt	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Optic atrophy	-	-	+	+	+	-	-	-	+	-	-	-	+	-	-	-	-	-
Extrapyramidal symptoms	-	-	+/-	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-
Stroke-like episodes	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-
Seizures	-	+	-	-	-	+	-	-	-	-	+/-	+	-	+/-	-	+	+	-
Cardiomyopathy	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Muscle weakness, increased CK	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-
Polyneuropathy	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Cerebellar atrophy	-	+	-	+	-	-	-	-	+	+	-	-	-	+	-	+	-	+
Arthrogryposis	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Failure to thrive	+	+	-	-	-	+	+/-	-	-	-	-	+	-	-	+	+	-	+
Cystic kidney disease	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Respiratory failure	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-
Diarrhoea/vomiting	+	-	-	-	-	+	-	-	-	-	+/-	+	-	+	-	-	-	+
Liver involvement	-	+	+/-	-	+	+	-	-	-	-	+/-	+	-	+	+	-	+	+
Hypoalbuminaemia	+	+	-	-	-	+	-	-	-	-	+	+	-	+	+	-	-	+
Coagulation anomalies	-	+	+/-	+/-	+/-	+	-	-	-	-	-	+	-	+	+	-	-	-
Thrombocytopenia	-	-	-	-	-	-	-	-	-	-	+/-	+	-	+	-	-	-	+
Recurrent infections	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Neurological presentation	-	-	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	-
Neurovisceral presentation	+	+	-	-	+	+	+	+	-	-	+	+	-	+	-	-	+	+

[a] transient liver function abnormalities

Patient diagnosed with CDG Ik.

11-13: De Lonlay et al (2001). 14: Mention et al (2001). 15: Prietsch et al (2002). 16-17: Acarregui et al (1998). 18: Zentilin et al (2003). 19: Assmann et al (2001). 20-21: Cohn et al (2006). 22-23: McKenzie et al (2007)

Table 2. Genotype-phenotype correlations in connection with *MECP2*

	Mutant (43)	Polymorphic (8)	Negative (74) – 9 boy
I. stadium			
Uneventful	44,2%	50%	27,39%
Slow development	51,2%	37,5%	71,23%
Hypotonic muscles	4,65%	12,5%	1,36%
Inflowing of II st.	19,9 month (6-48)	14,6 month (8-24)	21,39 month (3-66)
Not determinable (in)		25%	19,17%
Dysmorphic signs			
Microcephaly	74,4%	37,5%	41%
Little feet	46,5%		12,3%
Facial asymmetry	34,8%		
Scoliosis	32,5%		23.2%
Dyscrania			15%
Negative	4,65%	12,5%	17,8%
Neurological signs			
Stereotypic/compulsive	76,7%	62,5%	49%
Seizure	44,1%		42,4%
Abnormal reflex	37,2%		
Hypotonic muscles	34,8 %	50%	50,6%
Autistic features		37,5%	
Neuroradiol. sign			21,9%
Negative			4%
Mild phenotype	4,65%		
Classic phenotype			16,2%
Age at examination	6,865 years (2-21)	5,5 years (2-9)	5,32 years (0,75-18)

Nationality of the family	Number of patients/affected	Status of mautation	Onset	Additional symptom	Additional findings	Gene
Scottish	14/13	homo/heteropl	3-18 years	-	Polimorph..	MT-CYB
New-Zealand	10/18	Heteroplasmic	Second decade	-	Two sec. Leber's mut.	MT-ND
Japanese	3/17	homoplasmic	childhood	PPK	-	-
French	2/4	homoplasmic	>6 years	PPK	Not tested	-
Ukrainian	5/7	homoplasmic	6-15 years	-	-	-
Portuguese	8/10	homoplasmic	No data	PPK	Not tested	-
Hungarian	5/2	homo/heteropl.	childhood	-	Polimorph.	16S rRNS, MT-ND, Mt-CYB, MT-COI-II

Table 3. Comparison of families with the A7445G mitochondrial mutation

Table 4. Review of the clinical data of patients with the 111777C>A mitochondrial mutation

Nationality/sex of the patient	Examined tissues	11777C>A mutation load (%)	Onset of first symtoms	Additional symptoms	Additional findings		
					Mitokondriális mutáció	haplotype	
Japanese female 1	Muscle blood myoblast fibroblast	83 40 78 57	3 years and f5 months (short stature)	Exotropia (right eye) Mildly reduced muscle tone Elevated lactate in blood and CSF MRI: abnormal signals int he bilateral midbrain and thalamus	73A>, 150C>T, 151C>T, 263A>G, 303delC, 310insC, 489T>C, 527C>G, 1107T>C, 1438A>G, 2706A>G, 3106delC, 4200A>T, 4216T>C, 4317A>G, 4769A>G, 4833A>G, 4985G>A, 5178C>A, 5301A>G, 5442T>C, 5554C>T, 7028C>T, 7129A>G, 7669C>T, 8580C>T, 8860A>G, 10397A>G, 10398A>G, 10400C>T, 10873T>C, 11394T>C, 11719G>A, 12705C>T, 12810A>G, 13984C>T, 14783T>C, 14927A>G, 15043G>A, 15301G>A, 15326A>G, 15622T>C, 15737G>A, 16184insC, 16190delC, 16233C>T, 16291C>T, 16311T>C, 16316A>G, 16362T>C	D 5	
Japanese female 2	muscle blood myoblast	76 52 76	3 years (dystonia)	Exotorpia (left eye) short stature, hyperactive reflexes, dystonia, Elevated lactate in blood/CSF Mri: abnormal signals int he bilateral basal ganglia, substantia nigra and cervical cord	73A>G, 143G>A, 152T>C, 204T>C, 263A>G, 489T>C, 709G>A, 1438A>G, 3106delC, 3426A>G, 4769A>G, 4833A>G, 4985G>A, 5108T>C, 7028C>T, 7621T>C, 8701A>G, 8854G>A, 8860A>G, 9540T>C, 10398A>G, 10400C>T, 10873T>C, 11335T>C, 11719G>A, 12705C>T, 14569G>A, 14766T>C, 15043G>A, 15301G>A, 15326A>G, 15746A>G, 16233C>T, 16274G>A, 16362T>C	HV-N-L3	
Italian female	muscle	60	6 years (bilateral optic atrophy)	Leigh – like symptoms, normal lactate, MRI: transient involvement int he rostral midbrain, abnormal signals int he basal nuclei		ND	
British male	Muscle brain	93 67-81	67 years (left-sided resting temor)	Hemiparesis, epilepsy, pancreatitis, elevated lactate in blood/CSF MRI: hyperintense lesions in frontal, parietal lobe and cerebellar hemisphere		ND	
Hungarian male	muscle blood liver	60[a] 50[a] 60[a]	7 moths (spasticity, hypotonia, cerebral paresis)	Hypsarrhytmias, West syndrome MRI: frontal atrophy, ventricular dilatations, increased signal intensity within the whole area of the medulla oblongata Presence of lactate int he brain Toxic vomiting, hypertrophic obstuctive cardiomyopathy	4216T>C, 11719G>A, 12453T>C, 13708G>A, 15452C>A	J	

LIST OF PUBLICATIONS

Publications related to the thesis:

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