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ZNANSTVENI PREGLED
SCIENTIFIC REVIEW

THE CLINICAL SPECTRUM OF MITOCHONDRIAL DISEASES IN NEONATES AND YOUNG CHILDREN AND APPROACH TO DIAGNOSIS

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Mitochondrial diseases are becoming more frequently diagnosed in neonates and small children. Different clinical presentations are possible and specific investigations need to be performed in order to get to the right molecular diagnosis necessary for further genetic counseling. MtDNA or nuclear genes can be investigated after biochemical work-up in the affected tissue. In the neonate, severe presentation may lead to early death, so taking the possible mitochondrial disease in consideration is essential, especially in case of severe lactic acidosis, encephalopathy, myopathy, cardiomyopathy or hepatopathy. In infancy, there are three syndromes that can occur more frequently: Pearson's syndrome due to deletion of mitochondrial DNA; Leigh's encephalopathy, a progressive neurodegenerative disorder with many different mitochondrial gene mutations; and Alpers' disease, progressive gray matter degeneration due to mutations in the POLG gene. Different organ involvement is seen in mtDNA depletion syndromes and finally one should also consider CoQ10 deficiency in certain clinical presentations.

Descriptors: DNA, MITOCHONDRIAL; LEIGH DISEASE; MITOCHONDRIAL ENCEPHALOMYOPATHIES; LACTIC ACIDOSIS

INTRODUCTION

Mitochondrial encephalomyopathies include a large group of diseases in which different genetic inheritance can take place. The term mitochondrial disease refers to any disorder affecting the respiratory chain and oxidative phosphorylation (OXPHOS) system, a series of five multi-subunit enzyme complexes in the mitochondria. This system involves the transfer of electrons along the mitochondrial respiratory chain, through a series of oxidation and reduction reactions resulting in the consumption of oxygen. This leads to a proton gradient, which is then spread through complex V resulting in the formation of ATP. Complexes I, III, IV and V are under the influence of both mitochondrial and nuclear genomes, whereas complex II is entirely coded by nuclear DNA (1).

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There is epidemiological evidence suggesting that at birth, the prevalence is 1/7434 life births and the lifetime risk of developing a mitochondrial disease is approximately 1/5000 (2, 3).

Patients present with various combinations of dysfunction of muscle and other organs, especially tissues that require more energy such as brain, retina, kidney and heart.

Initial investigation of the patient can lead to suspicion, but there is no single diagnostic test. We will discuss different phenotypes in the neonate and infant, and look at the biochemistry strategies and molecular analysis. The choice of which tissue to investigate is also an important point. We will not discuss clinical syndromes occurring at older ages such as MELAS, MERRF and MNGIE.

Mitochondrial DNA

Mitochondria are the only subcellular organelles with their own DNA (mtDNA), which are capable of synthesizing a set of proteins. Human mtDNA is a small (16.5 kb), circular, double-stranded molecule and has been completely sequenced (4). It encodes 13 structural proteins, all of which are subunits of respiratory chain complexes (complex I ND1,2,3,4,4L,5,6; complex III cyt b; complex IV COX 1, 2, 3 and complex V (ATP6, ATP8), as well as two rRNAs and 22 tRNAs needed for translation. It is present in hundreds or thousands of copies *per* cell and is transmitted by maternal inheritance. If there is a mutation in some mtDNA in the ovum or zygote, this may be passed on randomly to subsequent generations of cells, some of which will receive few or no mutant genomes, others will receive primarily or exclusively mutant genomes, and others will receive a mixed population of mutant and wild-type mtDNAs (heteroplasmy). In the last case, the phenotypic expression of a mtDNA mutation will depend on the relative proportion of mutant to wild-type genomes with a minimum critical number of mutant genomes being necessary for expression (threshold effect) in each tissue. So, wild and mutant mtDNA is called heteroplasmy; threshold is the minimum of mutant mtDNA before dysfunction and the threshold for disease is lower in tissues highly dependent on oxidative metabolism such as brain, heart,

skeletal muscle, retina, kidney and endocrine glands. The threshold for clinical signs is different for each mutation. This means that analysis should be done in the affected tissue such as muscle/liver, whereas in the others analysis can be done in blood or fibroblasts.

Patients with pediatric mitochondrial diseases have mutations in the mtDNA in 20%-25% of cases.

Nuclear DNA

The majority of mitochondrial proteins are encoded by nDNA, synthesized in the cytosol and then imported into mitochondria. There can be mutations in structural nuclear genes of complex I, II, III, IV, or in assembly genes for complexes I, II, III, IV and V. Further, there are mutations described in genes of enzymes necessary for replication and integrity of mDNA and translation and genes involved in abnormal mitochondrial motility, fission and fusion (5). Clinically, we will look at the presentation at different age of onset and make a differential diagnosis (Figure 1).

Some patients present prenatally and there are several reports on associated congenital anomalies, e.g., VATER syndrome, microcephaly. Others have prenatal brain anomalies such as pontocerebellar hypoplasia, intracerebral calcifications, porencephaly, dysgenesis of corpus callosum, cortical atrophy or polymicrogyria (6).

NEONATAL PRESENTATION

In the neonate, several clinical presentations are possible. Firstly, there can be an acute lactic acidosis with or without hypoglycemia, sometimes associated with hyperammonemia. Others present with hypotonia, with signs of myopathy or cardiomyopathy, or liver failure. Epileptic encephalopathy with seizures and apnea, dysmorphism, deafness or cataracts, or any combination of involvement of different tissues should also lead to suspicion of a mitochondrial disease. Neonatal cardiomyopathy due to OXPHOS defects has recently been summarized (7). A common splice site mutation and an isolated frameshift mutation have been described in the *TMEM70* gene (complex V), particularly in a homogeneous ethnic group (Romanies), with a clinical phenotype characterized by neonatal mitochondrial encephalocardiomyopathy, lactic acidosis and dysmorphic features (8).

In the neonate, there is often a severe and isolated deficiency and a more severe clinical presentation, mostly due to mutations in nuclear genes, such as in complex I, in assembly genes of III, IV, and V, or as a result of a mtDNA depletion syndrome with a decrease of several complexes.

Complex 1 deficiency is the most commonly identified biochemical deficit accounting for 25% of all mitochondrial disease cases presenting in childhood (9). A muscle or liver biopsy to measure different complexes, including polyacrylamide blue native gel electrophoresis (BN-PAGE) and determination of the amount of mtDNA will then lead to investigation of the gene involved.

A clinical picture with fetal failure to thrive, postnatal lactic acidosis, hypoglycemia, coagulopathy, and cholestasis, especially in combination with neurological symptoms or renal tubulopathy, should alert the physician to direct investigations to mitochondrial disorders. A combination of lactic acidosis, liver involvement, and Fanconi type renal tubulopathy is common for mutations in the complex III assembly factor BCS1L (10).

Mutations in several nuclear DNA genes have been identified that lead to mitochondrial hepatopathy, e.g., mitochondrial depletion syndrome caused by *DGUOK, MPV17, SUCLG1, POLG1* mutations and are associated biochemically with combined OXPHOS deficiencies (11). Recessive *PEO1* (C10ORF2) mutations affecting the Twinkle helicase involved in mtDNA replication can cause severe epileptic encephalopathy (12).

Mitochondrial protein translation is a complex process performed within mitochondria by an apparatus composed of mitochondrial DNA (mtDNA) encoded RNAs and nuclear DNA-encoded proteins. Mutations in nuclear translation factor genes (*TRMU, EFG1 and EFTu*) have been reported (13), and have a se-

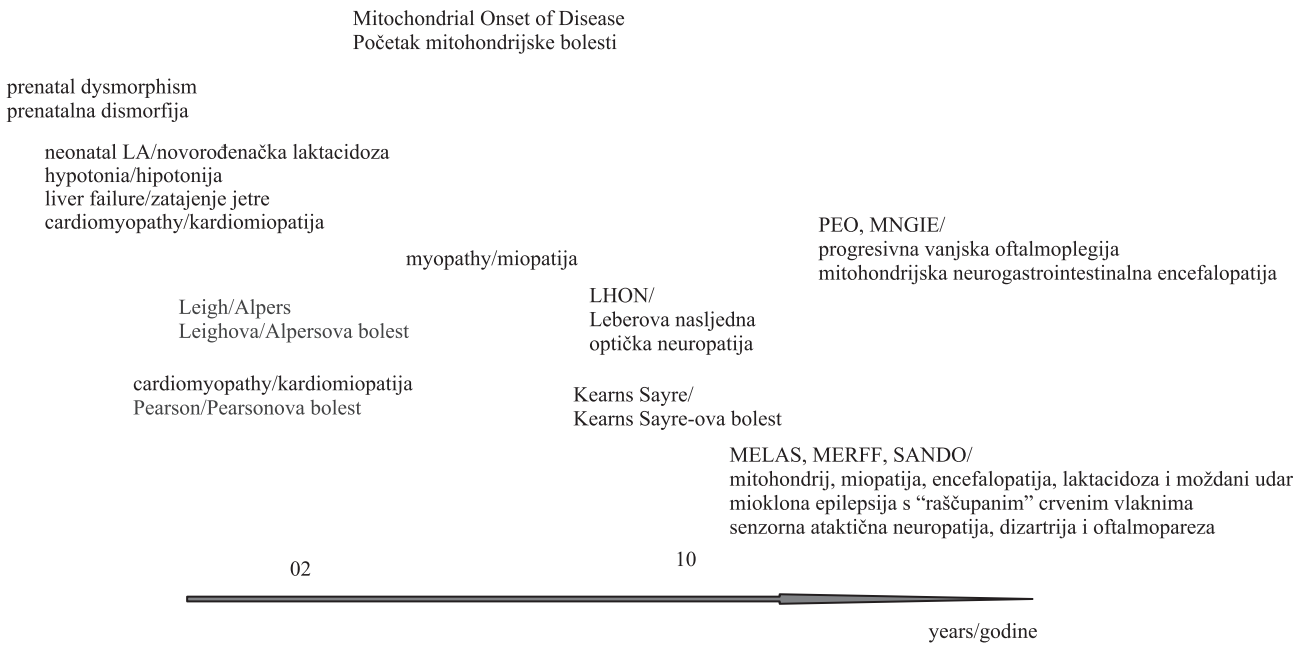

Figure 1. *Clinical presentation of mitochondrial disease*
Slika 1. *Kliničke manifestacije mitohondrijske bolesti*

vere and early onset presentation with lactic acidosis and rapid fatal encephalopathy, early-onset Leigh's or macrocystic leukodystrophy with polymicrogyria.

CLINICAL SYNDROMES IN INFANCY

In infancy, clinical presentation can be failure to thrive, food refusal and diarrhea, or neurological deterioration as seen in Leigh's encephalopathy or Alpers' disease, progressive myopathy or hypertrophic or dilated cardiomyopathy.

Pearson's syndrome due to a deletion in mtDNA presents with refractory sideroblastic anemia with vacuolization in bone marrow precursors and failure to thrive, and will lead to other organ failure (pancreas insufficiency, kidney failure), growth hormone insufficiency and photosensitivity. The mtDNA deletions can be found in leukocytes by Southern blot. After infancy, these children may develop neurological deterioration with a phenotype of Leigh's encephalopathy or Kearns-Sayre syndrome (14).

Leigh's encephalopathy (or subacute necrotizing encephalopathy) is the most common presentation in mitochondrial diseases. The onset of symptoms is usually between 3 and 12 months of age, but can also start earlier or later in life. Signs and symptoms include psychomotor retardation, hypotonia, brainstem dysfunction and signs of extrapyramidal dysfunction. Seizures, dystonia, abnormal eye movements, apnea and hyperventilation can be part of the clinical picture.

On magnetic resonance imaging (MRI), Leigh's encephalopathy is characterized by progressive basal ganglia and brainstem involvement with symmetrical hyperintensities on T2 weighted images in basal ganglia, brainstem, diencephalon, cerebellum and spinal cord.

There are a number of different etiologies causing Leigh's disease with mutations in nuclear genes and in mtDNA (Figure 2). The number of different genes increases every year.

A clue for the diagnosis can be lactate increase in cerebrospinal fluid (CSF), family history (consanguineous parents, maternal inheritance), increased hair growth (Surf 1, assembly gene for complex IV)) or retinitis pigmentosa (ATPase 6). Mutations in the ATPase gene have maternal transmission and the mothers can have clinically a NARP picture with neurogenic weakness, ataxia and retinitis pigmentosa, while the infants develop

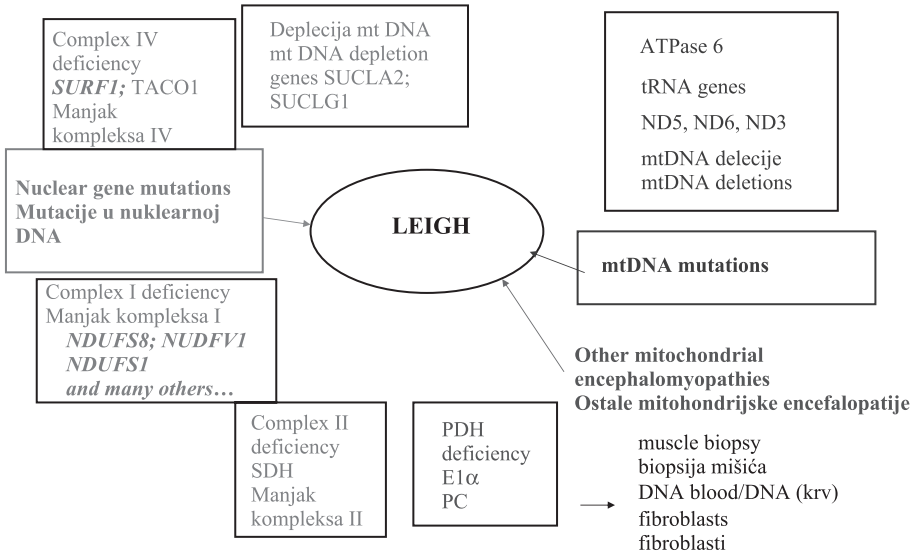

Figure 2. *Genetic causes of Leigh's encephalopathy*
Slika 2. *Genetski uzroci Leighove encefalopatije*

maternal inherited Leigh's encephalopathy or MILS. Mutations are usually in T8993C/G or T9176G in the *ATPase 6* gene (15).

There are a number of point mutations in subunits of complex 1, in the mitochondrial DNA, mostly of complex 1, and most frequently involving *ND1, ND3, ND5 or ND6* genes. In one study, the G13513A mutation (*ND5*) was found in 21% of patients with Leigh's syndrome and complex I deficiency (16).

Another frequent presentation of a mitochondrial disease in infancy is Alpers' disease.

Clinical features are initial normal development, early onset intractable seizures, cortical blindness and development of liver failure with micronodular cirrhosis aggravated by valproate ingestion. Symptoms start between 2 and 4 years of age (but can be earlier or later in life). The syndrome is characterized by developmental delay and periodic regression, intractable epileptic encephalopathy and liver failure. Convulsive status epilepticus can be the first sign of Alpers' disease (17). Seizures can start as focal clonic and complex-focal and develop into epilepsia partialis continua. Initial EEG can show unilateral occipital rhythmic high-amplitude delta with superimposed (poly) spikes (RHADS) (18). Regression of the cognitive functions increases during intercurrent infections.

On MRI, gliosis of the cerebral cortex, most commonly in the occipital lobes, will develop into global cortical atrophy.

POLG1 analysis should belong to the first-line DNA diagnostic tests for children with an encephalitis-like presentation evolving into epileptic encephalopathy with liver involvement (Alpers' syndrome), even if brain MRI and morphology, respiratory chain activities, and the amount of mitochondrial DNA in the skeletal muscle are normal. Also importantly, *POLG1* analysis should precede valproate therapy in pediatric patients with a typical phenotype.

Biomarkers for Alpers are high CSF protein and increased alpha fetoprotein, which can also be seen in other mtDNA depletion syndromes.

The diagnosis is made by muscle biopsy (biochemistry; RT-PCR for mt-DNA depletion) and confirmed by mutation search in DNA polymerase gamma. Common combined mutations are homozygous A467T, homozygous W748S or compound heterozygotes A467T/W748S. Mutations in *POLG* have been identified in a wide range of mitochondrial diseases including isolated clinical syndromes with fatigue, muscle weakness and muscle pain (19). Recessive mutations tend to cause mtDNA depletion and are present in childhood, whereas dominant mutations tend to cause adult-onset disease with multiple secondary deletions of mtDNA. Defined clinical syndromes described in patients with *POLG* mutations may also include chronic progressive external ophthalmoplegia (CPEO), infantile spinal muscular atrophy (SMA), myo-

Table 1. *Mitochondrial depletion syndromes*
Tablica 1. *Sindromi mitohondrijske deplecije*

MPV17	Early liver failure, leukoencephalopathy, polyneuropathy, increased lactate Rano zatajenje jetre, leukoencefalopatija, polineuropatija, povišena koncentracija laktata
DGUOK	Early onset leukoencephalopathy, hepatopathy, increased lactate Rani razvoj leukoencefalopatije, oštećenja jetre, povišena koncentracija laktata
TK2	Infantile myopathy with respiratory failure, isolated myopathy, SMA-like, increased lactate, CK, red ragged fibers Infantilna miopatija s respiratornim zatajenjem, izolirana miopatija, nalik na SMA, povišena koncentracija laktata, CK, crvena čupava vlakna
SUCLA2, SUCLG1	Leigh-like, deafness, dystonia, fatal infantile lactic acidosis, mild MMA Nalik na Leighovu bolest, gluhoća, distonija, letalna infantilna laktacidoza, blaga MMA
RRN2B	Fatal infantile lactic acidosis, renal and CNS disease Letalna infantilna laktacidoza, bolest bubrega i središnjeg živčanog sustava
POLG	Infants, children: Alpers, myoclonic epilepsy/encephalopathy, isolated therapy refractory epilepsy Djeca, dojenčad: Alpersova bolest, mioklona epilepsija/encefalopatija, izolirana, na terapiju rezistentna epilepsija Adults: Progressive external ophthalmoplegia, ataxia Parkinson, neuropathy, epilepsy Odrasli: Progresivna vanjska oftalmoplegija, ataksija Parkinsonova bolest, neuropatija, epilepsija
TP	MNGIE Mitohondrijska neurogastrointestinalna encefalopatija
Twinkle	Severe epilepsy, hepatopathy Teška epilepsija, oštećenje jetre

clonic epilepsy with ragged red fibers (MERRF), mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS), mitochondrial neurogastrointestinal encephalopathy disease (MNGIE), mitochondrial recessive ataxia syndrome (MIRAS) or sensory ataxic neuropathy with dysarthria and ophthalmoparesis (SANDO) presentations, and finally an epileptic syndrome with initial features of occipital lobe epilepsy in adolescence (20).

In patients with well-defined clinical syndromes that are consistent with *POLG* mutations, it is recommended to check for two common pathogenic alleles A467T and W748S in blood DNA.

Finally, clinical picture can change over time from Leigh's encephalopathy towards Alpers' syndrome in POLG mutations (21).

The different mtDNA depletion syndromes can have some overlap clinically and are increasingly published (Table 1). In case of mutations in the subunits of the succinyl-CoA synthase (*SUCLA2, SUCLG1*) will also have moderate methylmalonic aciduria (22).

Finally, CoQ10 deficiency is an important group as there might be a response to treatment. Defective biosynthesis of the lipophilic electron carrier CoQ10 presents with decreased activities of I and III and II and III in muscle biopsy or other affected tissue. Seizures are frequent manifestations and can start in infancy, but early-onset multiorgan failure and nephrotic syndrome have also been described. Different phenotypes also include childhood onset cerebellar ataxia and atrophy, lactic acidosis, mental retardation and seizures, or an isolated myopathic form with exercise intolerance, proximal weakness and elevated CKs has also been described in an overview by Raman et al. (23).

Investigations in Mitochondrial Diseases

First line investigations in case of suspicion of a mitochondrial disease, after careful physical examination and detailed pedigree history, include lactate, pyruvate in blood and CSF, urinary organic acids, aerobic exercise test in older children, brain MRI and MRS spectroscopy, and search for other tissue involvement, with ophthalmology, audiology and echocardiography.

Further investigations will then include muscle biopsy or biopsy of other involved tissue. Ragged red fibers (RRF) or ultrastructural alterations of mitochondria in muscle biopsy provide an important diagnostic clue (24).

In BN-PAGE gels, using histochemical staining methods, enzymatic activity of the complexes I, II, IV and V can be evaluated in heart and skeletal muscle, liver and cultured skin fibroblasts. In samples from patients with a severe deficiency, almost complete absence of the corresponding enzyme band is observed after catalytic staining in the gel. In patients with known partial deficiency, a milder decrease of the corresponding enzyme band is demonstrated (25). Complex V, the site of the final step in oxidative phosphorylation, uses proton gradient across the inner mitochondrial membrane for the production of ATP. It is a multi-subunit complex composed of a catalytic domain (F (1)) and a membrane domain (F (0)) linked by two stalks. The presence of sub complexes of complex V on BN-PAGE is a valuable indicator in the detection of mtDNA defects (26). The same is possible in other tissues, liver, fibroblasts or kidney. In case of suspicion, CoQ10 can be measured in blood, muscle or fibroblasts.

In conclusion, the number of mitochondrial diseases in neonates and infants remains a challenge to get to the definitive diagnosis. The important point is to consider the diagnosis and to investigate the right tissue.

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Authors declare no confilct of interest.

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S a ž e t a k

KLINIČKI SPEKTAR MITOHONDRIJSKIH BOLESTI KOD NOVOROĐENČADI I MALE DJECE I DIJAGNOSTIČKI PRISTUP

L. De Meirleir

Mitohondrijske bolesti sve se češće dijagnosticiraju u novorođenčadi i male djece. Zbog varijabilnosti kliničke slike potrebne su specifične pretrage kako bi se postavila ispravna molekularna dijagnoza koja je pak neophodna za daljnje genetičko savjetovanje. MtDNA ili nuklearni geni mogu se ispitivati nakon biokemijskih pretraga zahvaćenog tkiva. Kod novorođenčeta teško stanje pri dolasku može dovesti do rane smrti pa je zato presudno u obzir uzeti moguću mitohondrijsku bolest, osobito u slučaju teške laktične acidoze, encefalopatije, miopatije, kardiomiopatije ili hepatopatije. U dojenačkoj dobi moguća je češća pojava slijedećih triju sindroma: Pearsonova sindroma zbog delecije mitohondrijske DNA; Leighove encefalopatije, progresivne neurodegenerativne bolesti s mnogo različitih mitohondrijskih genskih mutacija; i Alpersove bolesti, progresivne degeneracije sive tvari zbog mutacija u genu POLG. Kod sindroma deplecije mtDNA zahvaćeni bivaju različiti organi, pa na kraju treba u obzir uzeti i deficijenciju CoQ10 kod određenih kliničkih manifestacija.

Deskriptori: MITOHONDRIJSKA DNA; LEIGHOVA BOLEST; MITOHONDRIJSKE ENCEFALOMIOPATIJE; ACIDOZA, LAKTIČNA

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