Spinal Dysraphism Associated with Congenital Heart Disorder in a Girl with MELAS Syndrome and Point Mutation at Mitochondrial DNA Nucleotide 3271

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We describe a case of mitochondrial encephalopathy, lactacidosis, and stroke-like episode (MELAS syndrome) associated with ventricular septal defect and meningocele at the L3 level in a 5-year-old girl. Mitochondrial DNA analysis showed point mutation at nucleotide 3271 T>C. The occurrence of heart and neural tube defects in association with usual features of the MELAS syndrome might be explained by either defective high-energy metabolism during early embryogenesis or a common genetic cause.

Key words: DNA, mitochondrial; heart septal defects, ventricular; MELAS syndrome; meningocele; spinal dysraphism; point mutation

Mitochondria are energy-producing cellular organelles. Oxidative metabolism of carbon substrates yields energy through sequentially ordered redox reactions catalyzed by enzymes constituting the respiratory chain complexes I, II, III, and IV. The energy is ultimately used to phosphorylate ADP to ATP. The mitochondrial respiratory chain is under dual genetic control – nuclear and mitochondrial. The human mitochondrial genome is a 16,569 base-pair long circular chromosome composed of double-stranded DNA. It contains 37 genes, which encode transfer RNA, ribosomal RNA, and polypeptide chains (1,2). Several human diseases have recently been attributed to pathogenic mutations of mitochondrial DNA (mtDNA) (3,4). Due to stochastic distribution of defective mitochondria during embryonic cell division and development, any tissue may be affected and any association of the defects may occur, but due to high energy requirements, the central nervous system and skeletal and cardiac muscles are particularly vulnerable and thus most commonly involved. Approximately one third of the patients manifest clinical symptoms during the first months of life and one half during the first two years, although the onset of the symptoms may occur at any age (5-7).

The MELAS (mitochondrial myopathy, encephalopathy, lacticacidosis, and stroke) syndrome is a multisystem mitochondrial disorder characterized by variable degrees of encephalopathy, lacticacidosis, stroke-like episodes, and mental deterioration. Additional clinical features include epilepsy, short stature, various endocrinologic disorders, and nephropathy. Recurrent headache is one of the first clinical symptoms, which is usually registered in late infancy and adolescence. The majority (80%) of patients with MELAS syndrome have a characteristic point mutation at the nucleotide (nt) pair A>G 3243 in mtDNA, coding for transfer RNA gene of leucine (UUR). The mutation is biochemically associated with the deficiency of complex I respiratory chain enzymes in different tissues. Other mtDNA mutations associated with MELAS occur at nt 3256, 3260, 3271, and 3291 in the tRNA gene for leucine (UUR) (7,8). Somatic cells may contain varying proportions of normal and wild type mtDNA – a phenomenon called heteroplasmy. Although most diseases causing mutations are heteroplasmic, diseases with homoplasmic mitochondrial mutations are clear examples of maternal transmission (8,9). In addition to mtDNA mutations, mutations of nuclear genes may also cause structural and functional abnormalities of respiratory chain complexes (enzymes). When there is a family history, inheritance appears to be autosomal recessive or dominant, suggesting the defect is in a nuclear rather than mitochondrial gene.

The course of MELAS is variable, depending on the amount of mutant mitochondrial genome and on the age at the onset. The higher the proportion of mutated mtDNA, the earlier the onset of symptoms. Also, the course of the disease is more severe. Mental abilities and neurological functions deteriorate with repeated attacks of stroke-like episodes (7). During febrile illnesses, patients with MELAS are prone to stroke-like attacks due to cellular energy deficiency. Treatment includes vitamins K1, C, nicotinamide, and riboflavin, as well as L-carnitine and coenzyme Q10 or idebenone. The aim is to improve mitochondrial electron transfer and generation of high-energy
phosphate and to prevent further mitochondrial genomic damage.

We present a case of a 5-year-old girl with MELAS syndrome associated with ventricular septal defect and spinal dysraphism.

**Case Report**

The girl was born in 1992 on term, but was a hypotrophic, small-for-date newborn (body mass 1.73 kg, length 41 cm, head circumference 31 cm). The mother had vaginal bleeding in early pregnancy and a urinary infection. Paternal grandmother had insulin-dependent diabetes mellitus. At birth, the patient was found to have bilateral clubfoot and systolic heart murmur. At the age of 4 months, bilateral hip dislocation was diagnosed. At the age of 6 months, orthopedic foot surgery and corrective hip extension were performed.

The heart ultrasound examination revealed a small perimembranous ventricular septal defect (VSD) 4 mm in diameter with a gradient of 60 mm Hg, located in the outlet part of the right ventricle. There were no hemodynamic consequences and no indication for invasive diagnostic procedures (catheterization, angiocardiography) or operation during a 7-year follow-up.

Somatic and psychomotor developmental delay had been noticed since early infancy. At the age of 14 months, her body mass was 5.8 kg, length 65 cm, and head circumference 41 cm, all below the 3rd centile. Due to febrile and afebrile epileptic seizures occurring at the age of 18 months, phenobarbitone therapy was introduced. Electroencephalography (EEG) showed continuous slow wave activity and focal discharges. At the age of 4 years, a generalized convulsive attack terminating in *status epilepticus* occurred during a febrile illness. The patient was admitted to the intensive care unit in postictal cerebral coma, with right hemiplegia and respiratory insufficiency. Computerized tomography (CT) of the brain showed ischemic cerebrovascular thromboembolic insult in the irrigation area of left medial cerebral artery, followed by massive hemorrhagic transition (Fig. 1). After 2 weeks of artificial ventilation, the patient regained consciousness. EEG showed asymmetrical continuous slow wave activity over the left hemisphere. Right hemiplegia gradually improved. Control EEG showed mild focal discharges, predominantly over the right frontotemporal region. The follow-up CT scans of the brain, 2 and 16 weeks later, showed chronic vascular lesion with left hemisphere atrophy (Figs. 2 and 3). On the basis of clinical course, a tentative diagnosis of MELAS syndrome was considered.

Laboratory investigations were performed 5 months later. Lactate in the whole blood was measured by enzymatic method (Sigma-Aldrich, Steinheim, Germany) after deproteinization with ice-cold 8% perchloric acid. Muscle biopsy from quadriceps femoris was performed under local anesthesia, with parents’ consent. The analysis showed increased serum lactate (from 1.45 to 2.45 mmol/L; reference range 0.33-1.33 mmol/L) and slightly decreased total carnitine level (31.0 μmol/L; reference range 40.7-47.5 μmol/L). Alpha-fetoprotein level was 2.2 IU/L (reference range, 0-10). Homocysteine and methionine levels were normal.

Karyotype was normal. Ocular fundoscopy showed optic nerve atrophy. Visual evoked potentials revealed signs of neuronal lesion. Brainstem evoked potentials were normal at the age of 3 years. Electromyography showed chronic neurogenic lesion in short extensor and anterior tibial muscle of the leg.

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**Figure 1.** Axial computed tomography scan 24 hours after the beginning of the epileptic state. Segmental hypodense areas in frontal and occipital regions of the left hemisphere, indicating an acute ischemic lesion (arrows).

**Figure 2.** Follow-up CT scan 2 weeks after the stroke-like episode. Demarcation of the ischemic lesion in the left hemisphere (arrow).
with normal motor nerve conduction velocities on both lower extremities. The finding was compatible with the diagnosis of L5-S1 radiculopathy. Nuclear magnetic resonance (NMR) showed a meningocele at L3 (Fig. 4). Brain NMR at the age of 7 showed a huge cavity attributable to malacia after stroke in the irrigation area of the left medial cerebral artery, and enlargement of the left ventricle. Muscle biopsy showed normal morphology under a light microscope, and scarce nonspecific subsarcolemmal accumulations of mitochondria on electron microscopy.

Polymerase chain reaction (PCR) was performed on the DNA from peripheral blood leukocytes, by use of internally synthesized primers 5'-3150 and 3'-3305, covering the mutation spot (3271 T → C). The specific PCR product was digested by restriction enzyme Dra I and analyzed in acrylamide/bisacrylamide gel containing ethidium bromide. Mitochondrial DNA analysis detected point mutation 3271 T → C, a specific finding associated with the MELAS syndrome (Fig. 5). No mutation was detected in patient’s mother, father, sister or brother. The more common mutation of mtDNA in MELAS, 3243 A → G, was negative.

The patient is currently on antiepileptic treatment with diphenylhydantoin and primidone, together with creatine monophosphate, L-carnitine, and vitamins C and K1. Muscle weakness has improved. Stroke episodes have not recurred during the follow-up period of 5 years. Brief partial epileptic seizures of the left side occur occasionally. Now, at the age of 8 years, the patient is short, below the 5th centile (109 cm), cannot walk without assistance, and has a pronounced delay of speech development. The psychomotor developmental quotient is 25-35.

**Discussion**

The diagnosis of the MELAS syndrome in our patient was established on the basis of clinical presentation: epileptic seizures, myopathy, and eventually a stroke-like episode, as well as by laboratory evidence of specific mtDNA mutation at nt 3271 T → C. In addition to the common clinical features of the MELAS syndrome, spinal dysraphism and congenital heart disorder were present. Multigorgan involvement is a clinical hallmark characterizing mitochondrial disorders (7) but, to our knowledge, the association of spinal dysraphism and congenital heart disorder with the MELAS syndrome has not yet been reported.

Neural crest cell abnormality attributable to chronic hypoxia may play an important role in the
pathogenesis of central nervous system anomalies and congenital heart disorders (9). Spinal dysraphism encompasses a wide variety of spinal anomalies that result from imperfect midline fusion of the embryonic neural tube. Spinal dysraphism, congenital heart disorder, and skeletal anomalies are the most common features of diabetic embryopathy (7,11,12), suggesting impairment during early embryogenesis, before the 7th week of gestation. Pathogenesis of this group of disorders is not known and has been investigated in vitro and in experimental animals. Feiglbaum (12) has reported the association of diabetic embryopathy with mitochondrial disorder. In our example, normoglycemia of the mother during pregnancy argued against gestational diabetes as a possible cause of the patient’s phenotype.

Bilateral clubfoot, present at birth in our patient, is a common finding in patients with spinal dysraphism or MELAS syndrome (7). Increased laxity of ligaments with joint hypermobility, which in our patient resulted in bilateral hip dislocation, is usually associated with inherited neuromuscular disorders.

The genetics of neural tube defects has not been elucidated. Gene polymorphism of the folate and homocysteine pathways, e.g., 5,10-methylenetetrahydrofolate reductase 677 C > T and 1298 A > C and cystathionine beta-synthase 844 ins 68, has been implicated in the etiology of spinal dysraphism, together with environmental factors, such as folate, vitamine B12, and methionine deficiencies (13). Normal levels of methionine, folate, and homocystein in our patient excluded the association of her clinical features with methylenetetrahydrofolate reductase mutation.

Genetics of cerebral strokes includes metabolic disorders, vasculopathies, connective tissue disorders, and inherited coagulation defects. Stroke in the MELAS syndrome has been attributed to abnormal mitochondrial metabolism in platelets and vessel walls. Mitochondria have been found to accumulate in cerebral capillaries and choroid plexus, implicating that narrowing of the vessels might be sufficient to cause infarction (14). Creatine monophosphate was effective in prevention of stroke episodes in the MELAS syndrome (our unpublished observations).

According to published reports, congenital heart defects are occasionally associated with inherited neuromuscular, central nervous system, and gastrointestinal disorders. Association with aneuploidic chromosomopathies (Down, Patau, or Turner) as well as with microdeletion and translocation disorders (CATCH 22) is considerable.

The possibility that abnormalities of different organ systems were caused by common genetic defect(s), such as mitochondrial nucleotide mutations, has to be considered in our patient. According to the etiopathogenetic classification of congenital heart disorders by Clark, ventricular septal defect (VSD) may arise due to four causes: 1. migration defects of ectomesenchymal tissue (subaortic VSD), 2. extracellular matrix defect (endocardial cussion VSD), 3. cell death (muscular VSD), and 4. abnormal intracardial flow (perimembranous VSD) (15-17). In our patient, the cardiac defect was situated at the right ventricular outlet, and may be attributed to incomplete myocardialization due abnormal migration of the neural crest cells.

Diagnosis of the MELAS syndrome in our patient was confirmed by DNA analysis. We found mutation at nucleotide position 3271 in mtDNA, but not at nt 3243, which is more common (7). However, there is no difference between 3243 and 3271 mutations with regard to clinical symptoms, muscle pathology or the impairment of oxidative phosphorylation. Both mutations are associated with complex I and IV deficiencies in the mitochondrial respiratory chain (18). The fact that mtDNA mutation was not detected in (clinically unaffected) mother and siblings of the patient might be explained as follows: (a) a new mutation occurred in the patient; (b) the mother was a silent carrier and heteroplasmy accounted for undetectability of mutated mtDNA in her leukocytes and in the siblings, and (c) the mother was silent carrier of another point mtDNA mutation, not recognizable by the used primers (12).

Mitochondrial enzymopathies are relatively common neurometabolic disorders in children (19). Mitochondrial mutation mostly affect postmitotic cells with high metabolic rate, such as muscle cells and neurons. Abnormal oxidative phosphorylation and lack of high energy phosphates impair cellular proliferation, migration, differentiation and final function, especially in tissues and organs containing higher amounts of mitochondria and having high metabolic rates, such as the cardiac and skeletal muscles and the central nervous system. From the clinical point of view, a defect in the mitochondrial respiratory chain should be considered in patients presenting an unexplained association of neuromuscular symptoms involving seemingly unrelated organs or tissues, and a variable, often rapidly progressive course. Disease having an infantile onset usually evolves with a more severe course.

Association of the heart and neural tube migration defects with the inherited metabolic disorder, as in our patient, might be attributed to mitochondrial compartmentalization and insufficient mitochondrial respiratory chain function in early embryogenesis, resulting in shortage of high energy phosphates in vulnerable tissues. Another possibility would be that the malformations were due to a genomic DNA mutation coexisting with the mtDNA mutation, and that both of them occurred de novo. More refined analysis of the genetic material would be needed to assess that possibility.
References


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