“Drop attacks” as first clinical symptoms in a child carrying MTTK m.8344A>G mutation

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Abstract
We describe a child with dyslexia and difficulty in school who, at the age of 13 years, began to suffer from several head injuries resulting from falls of uncertain cause. Two years later, the patient developed symptoms of a severe mitochondrial disorder (involving bulbar-pyramidal paralysis, ophthalmoplegia, and hyperlactatemia) that coincided with VPA administration. Brain MR imaging revealed rapidly developing Leigh syndrome (LS), and muscle biopsy showed ragged blue fibres (RBF). A diminished expression of the E1α subunit of pyruvate dehydrogenase was found in muscle homogenate (signal 28.7% of normal).
The accurate diagnosis of mitochondrially inherited LS (MILS) and the identification of an almost homoplasmic m.8344G>A mutation in the MTTK gene was delayed due to an initial incorrect diagnosis of epilepsy, misdiagnosis of neuroinfection, and failure to note LS on the first brain MRI.
Periods of exacerbation or improvement were observed in association with the administration of certain drugs or procedures (VPA administration or intensive rehabilitation associated with worsening; ketogenic diet associated with remission). However, the random association of these factors with natural disease fluctuations cannot be excluded.
Conclusions: 1) To improve the early detection of mitochondrial disorder, we recommend screening for mtDNA (and nDNA) mutations in all patients with LS present on brain MRI. 2) Brain MRI protocols should include diffusion-weighted and T2-weighted imaging, and LS-like changes should be analysed by a neuroradiologist experienced in the field. 3) Additional controlled studies are urgently needed to assess the causal relationship between management strategies and the natural history of the disease. Until the association between VPA and disease exacerbation can be ruled out, VPA should be avoided in patients with these symptoms unless the mitochondrial disorder has been excluded.

Key words: Leigh syndrome, MERRF mutation, pyruvate dehydrogenase dysfunction, ketogenic diet.

Introduction
Mitochondrial diseases (MID), which develop due to either mitochondrial (mtDNA) or nuclear DNA mutations [13], are rare. Therefore, the knowledge of these diseases among physicians may be poor, and the diagnosis of these diseases can be delayed. The extremely
The patient, are discussed.

cations used to modify the energetic metabolism of may have influenced the natural course of the dis ease. The mitochondrial m.8344A>G mutation. Several factors that patient with late-onset Leigh syndrome caused by mutations in nuclear DNA (the SURF1 gene) [6] and is inherited as an autosomal recessive trait. The mitochondrially inherited form (MILS) represents only 10-20% of all LS cases [6,7,10].

Although the m.8344A>G mutation in the mtDNA lysine gene (MTTK) is a “common” mtDNA mutation, its prevalence was estimated as relatively low (< 1 : 300 000) [12]. This mutation was originally described in patients with MERRF (myoclonic epilepsy with rag ged red fibres) syndrome [14], but multiple heterogeneous non-MERRF presentations have been reported [2,3,5]. There have also been a number of descriptions of MILS related to the m.8344A>G mutation [1,15,16].

The aim of this paper is to describe the first Polish patient with late-onset Leigh syndrome caused by an MTTK m.8344A>G mutation. Several factors that may have influenced the natural course of the disease, including delayed diagnosis, stress and medications used to modify the energetic metabolism of the patient, are discussed.

Material and methods

Case report

A 15-year-old boy on a respirator, who had been diagnosed with encephalomyelitis, was sent to CMHI for the inclusion in the programme of artificial ventilation at home.

The boy had developed normally during the neo natal period and infancy. His family history was negative; the parents and two older siblings are all healthy. From early childhood, the patient was under the care of a neurologist, speech therapist and psychologist due to dyslexia and school difficulties.

Beginning at 13 years of age, the patient suffered from repeated falls of uncertain cause (“drop attacks”), which were associated with several head injuries. Cardiac involvement was not observed. Valproic acid (VPA) was administered at 14 years due to suspicion of epilepsy, despite the lack of epileptiform discharges on EEG. Approximately five months after VPA administration, limb weakness, double vision, difficulties in swallowing, dyspnoea and stool incontinence appeared and rapidly developed over several weeks. Respiratory failure occurred and required artificial ventilation. Episodes of seizures, impaired consciousness, and difficulty in controlling blood pressure were observed at a local paediatric ward. Plasma lactate levels were slightly elevated (24 mg/dl, ref. < 20 mg/dl). Liver enlargement (6 cm), mild transaminase elevation (< 100 U/l) and hyperammonaemia (300 µg/dl) were found. Initially, neuroinfection was suspected based on the results of borreliosis testing (IgG + antibodies), and relevant therapy was applied. Repeated attempts at extubation failed.

At the age of 15 years, the patient was admitted to the CMHI intensive care ward; his physical development was normal (height 180 cm, weight 63 kg). Due to the initial suspicion of undetermined neuroinfection, the patient was treated with intravenous immunoglobulins, antibiotics, and steroids. VPA administration was discontinued, and carnitine, vitamin B1 and coenzyme Q were administered. His clinical condition improved quickly, and after ten days, the boy was weaned from mechanical ventilation and transferred to the neurological ward. Neurological examination revealed the severe bulbo-pyramidal syndrome. Visual evoked potentials were abnormal.

Laboratory examination did not reveal any signs of infection. The lactate concentration in the plasma (58.3 mg/dl) was high, and that in the cerebrospinal fluid was in the upper range of normal (22.6 mg/dl). The organic acid profile revealed an increased excretion of lactate and Krebs cycle metabolites as well as the presence of VPA derivatives in the urine.

This time, a mitochondrial disorder was suspected, and an open muscle biopsy was performed, confirming mitochondrial pathology. The clinical condition of the patient showed further gradual improvement (he became able to walk with support), and he was discharged home.

A few months later, the boy was re-admitted to CMHI due to further deterioration in speech and movement as well as dyspnoea. Bulbar and pseudobulbar palsies, tetraparesis, generalised hypotonia, absent deep tendon reflexes, and pathologic reflexes were exacerbated during the implementation of an intensive rehabilitation program. The rehabilitation was then stopped, and his neurologi-
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In vivo multi-voxel proton magnetic resonance spectroscopy (1H-MRS) was also performed. We used a PRESS 2D-chemical shift imaging (CSI) sequence with the following parameters: TR = 1500; TE = 30 with planar resolution; FOV, 230 mm; matrix size, 16 x 16 cm²; section thickness, 2 cm. Full analyses of the spectra were performed in LC Model software.

Muscle biopsy

A mitochondrial study of the muscle biopsy was performed according to histochemical and spectrophotometric methods commonly used in our laboratory. The assessment included light microscopic examination of frozen sections in the following stains and histochemical reactions: haematoxylin and eosin (HE), modified Gomori trichrome, oil red O, succinate dehydrogenase, NADH dehydrogenase, cytochrome c oxidase (COX), acid phosphatase, myosin ATP-ase preincubated at pH 4.3, 4.6 and 9.4. The activity of respiratory chain complexes I-IV was measured in the muscle homogenate as described [6].

DNA analysis

Total DNA was extracted from the whole blood of the patient, his two siblings and his mother using a standard phenol/chloroform extraction procedure. The SURF1, MTATP6 and MTTK genes were amplified and sequenced in 3130 Genetic Analyzer with BigDye Terminators (Applied Biosystems). Multiplex Ligation Probe Amplification (MLPA) analysis was performed with the SALSA MLPA P125-B1 Mitochondria kit (MRC-Holland) according to the manufacturer’s protocol.

Samples preparation for Western blot

Tissue samples kept at –80°C, were defrost, cut into small pieces and resuspended in cold lysis buffer (50 mM Tris pH 7.5, 150 mM NaCl, 1% Triton, 0.1% SDS, 1% sodium deoxycholate) containing inhibitors of proteases (1 mM PMSF and the protein protease inhibitor cocktail). Skeletal muscles were homogenized on ice. Samples were incubated on ice for 20 min and centrifuged at 600 x g for 5 min at 4°C to remove insoluble material. The supernatants were collected and centrifuged at 16 000 x g for 20 min at 4°C. Protein concentration in lysates was determined using Bradford method. Samples for SDS-PAGE were denatured in reducing Laemmli loading buffer at 95°C for 5 min.
Western blotting

Tissue lysates (20 μg protein) from muscles (patient and healthy control) were separated electrophoretically in 8% SDS polyacrylamide gels and transferred into PVDF membrane (BioRad). Membranes were blocked and proteins were detected using MitoProfile® Pyruvate dehydrogenase (PDH) WB Antibody Cocktail (abcam) followed by appropriate secondary AP-conjugated antibodies (1:1000, Bio-Rad).

Results

Electrophysiological findings

The available EEG recordings from the patient demonstrate progressive pathology: periodic epileptiform discharges (PEDs) at the age of 15 years, triphasic periodical waves and no spikes after few weeks, and the presence of frontal intermittent rhythmic delta activity (FIRDA) two months later.

Evoked potentials examined at the first rapid deterioration, at the age of 15 years, revealed abnormal visual evoked potentials. Auditory potentials were not recorded.

Magnetic resonance imaging and spectroscopy

MR examination was performed three times over a year to document the clinical progression of the disease. Re-assessment of these MR images showed that lesions suggesting LS were already present at the first examination, at the age of 14.5 years (not shown). They were milder than those found 6 months later.

The MR examinations at the age of 15 years are shown in the upper portion of Fig. 1 (panels A-D). The co-occurrence of bilateral, symmetrically located lesions in the thalamic region were not mentioned in the first description of the results, although they are clearly visible in diffusion-weighted images (Fig. 1B). Spinal cord imaging performed 10 days later (Fig. 1C-D) showed a diffuse abnormal signal in the medulla oblongata, cervical spinal cord and upper portion of the thoracic spinal cord (Th1-Th3).

Follow-up brain and spinal canal MR exams taken at the age of 15.5 years revealed a remarkable progression of both localisations. The bilateral thalamus, putamen, midbrain, pons, medulla oblongata, and entire length of the spinal cord were involved. The lesions were observed predominantly on T2- and diffusion-weighted images in the brain (Fig. 1E-F) and on T2-weighted images in the spinal cord (Fig. 1G-H).

MR spectroscopy with the voxel localised in the right putamen demonstrated significantly elevated lactate levels and relatively low levels of Cho (Cho/Cr = 0.26) and NAA (NAA/Cr = 0.94). The levels of Glx (Glx/Cr = 2.7) and ml (ml/Cr = 0.81) were relatively elevated (Fig. 2).

Histochemical and biochemical findings

Histchemistry of muscle samples revealed few distinct ragged blue fibres (RBF) (Fig. 3) accompanied by a pattern suggestive of mosaic COX deficiency and mild to moderate lipid accumulation. No classical ragged red fibres (RRF) were observed.

Spectrophotometry of a muscle homogenate showed an increased citric synthase activity (267.5 nmol/min/mg protein, ref. < 180 nmol/min/mg protein) and normal activity for respiratory chain complexes I-IV (not shown).

Immunoblotting with pyruvate dehydrogenase (PDH) complex antibodies showed a diminished signal representing the steady state level of the E1α subunit in the muscle homogenate (28.7% of the reference value), consistent with PDH dysfunction (Fig. 4).

DNA analysis

A search for the common mutations responsible for LS in the SURF1 and MTATP6 genes was negative. MLPA analysis demonstrated a lack of ligation of the m.8344A>G-specific probe. Further, MTTK gene sequencing confirmed the presence of the m.8344A>G mutation in 96% of his mtDNA. The heteroplasmy levels of this mutation in the patient’s sister and mother were 80% and 70%, respectively (all family members were asymptomatic).

Discussion

The patient bearing an m.8344G>A mutation (proband) rapidly developed a full complement of symptoms of the mitochondrial disorder, including muscle weakness, loss of acquired skills, bulbar-pyramidal syndrome, ophthalmoplegia, Leigh syndrome, stroke-like episodes, elevated lactate concentration, and ragged blue muscle fibres. The first exacerbation (with transient liver enlargement and dysfunction) coincided with VPA administration. The course of the
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Disease had overlapping symptoms of MILS, MERRF and MELAS (mitochondrial encephalopathy, lactic acidosis, stroke-like episodes).

Leigh syndrome (LS) is a frequent presentation of the mitochondrial disorder in children, and it occurs regardless of the molecular aetiology of the mitochondrial pathology. The disease has a peculiar topography and specific pathological morphology [7]. It is not yet understood why LS develops rarely in mitochondrial tRNA-mutated patients [16], more frequently in PDH-deficient patients, and most frequently in SURF1-mutated patients [6].

According to our hypothesis, episodes of hyperventilation and hypocapnia increase the risk of LS development [9]. A transient elevation in the intracellular pH of neurons (respiratory alkalosis) could trigger apoptosis in specially structured regions of the brain affected in LS.

We documented the occurrence of respiratory alkalosis in SURF1-related LS and also described this phenomenon in an LS patient with another MTTK m.8363 mutation [10]. Alkalosis and hypocapnia were not documented in the reported patient, but the occurrence of hyperventilation episodes due to anxiety over his unexpectedly rapid deterioration cannot be excluded.

The proper diagnosis of our patient was delayed by an incorrect recognition of epilepsy and the administration of VPA (a known mitochondrial suppressor), misdiagnosis of infectious encephalomyelitis, and the failure to note the LS in the first brain MRI performed at the local paediatric ward. It should be
emphasised that the m.8344A>G mutation could be detected much earlier because the variant is included in DNA screening panel recommended for LS [6]. Early diagnosis could protect the patient from unnecessary procedures and harmful medications. Further, early diagnosis might have prevented (slowed down or even stopped) the rapid progression of LS changes demonstrated in subsequent brain MR examinations.

Interestingly, some features of PDH deficit, including glucose intolerance and low E1alpha subunit expression, which is also known to cause LS [7,11,17], were observed in our patient. Similarly impaired PDH activity was previously described in a patient with an MTTL m.3243A>G mutation [18]. Both of these mtDNA mutations disrupt mitochondrial tRNAs (for lysine and leucine, respectively) and impair mitochondrial protein synthesis [1,18,19]. A disruption of the import and/or the misassembly of cytosolic synthesised polypeptides was suggested to explain the PDH defect in the m.3243A>G mutated cells [18].

The undulating course of the disease, which is a major hallmark of mitochondriopathies, was evident in the reported patient. This severely complicated the evaluation of the therapeutic approaches used. The periods of exacerbation or improvement in our patient were apparently associated with the drugs or medical procedures applied. It is tempting to suggest a causal relationship, e.g. between the administration of VPA [8] (or intensive rehabilitation) and the clinical deterioration or between the diet manipulation [4,17] and the remission; however, neither could be conclusively proven in this patient. It should be remembered that random independent fluctuations in the clinical course may imitate a good or bad response to parallel treatments.

Our expanding knowledge of the natural history of m.8344A>G-related disease reveals a very broad range of symptoms and severity [2,3,5], regardless of the heteroplasmy level. Multiple environmental factors (favourable or damaging) may influence the course of disease in individual patients [4]. This allows speculation that avoiding harmful factors can slow down disease progression, and vice versa. Until

Fig. 2. Multi-voxel proton spectroscopy, PRESS acquisition (TR/TE 1500/30) at the level of right putamen at the age of 15.5 years demonstrates elevated lactate levels.

Fig. 3. Skeletal muscle biopsy. Succinate dehydrogenase reaction showing few ragged blue fibers. Original magnification 200×.
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the nature of these relationships is finally established, the potential impacts of treatment must be considered in the management of patients with suspected mitochondrial disease [4].

In summary: We recommend molecular screening for mtDNA (and nDNA) mutations in all patients with symmetric lesions in the subcortical nuclei and other locations characteristic of LS present on brain MR examination.

The brain MRI protocol should obligatorily include diffusion-weighted (DWI), and T2-weighted (T2-WI) imaging; LS-like changes should be assessed (or at least consulted on) by a neuroradiologist experienced in the field.

Valproic acid (VPA) should be avoided as an anticonvulsant drug in patients with a suspected mitochondrial disorder. In cases of severe epilepsy, especially associated with other symptoms of encephalopathy, a mitochondrial pathology of any kind should be carefully considered (i.e. according to Nijmegen criteria) before VPA introduction.

The benefit of a ketogenic diet for mitochondrial tRNA-mutated patients cannot be assessed until prospective blinded studies in larger groups of patients are performed.

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References


