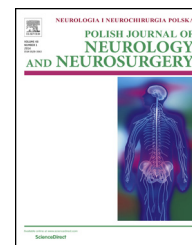


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Case report

Mitochondrial encephalomyopathy: Towards diagnosis. A case report

Małgorzata Gawel^{a,*}, Biruta Kierdaszuk^a, Katarzyna Tońska^b, Magdalena Kaliszewska^b,
Justyna Kubiszewska^a, Zygmunt Jamrozik^a, Ewa Bartnik^{b,c}, **Hubert Kwieciński^a**,
Anna M. Kamińska^a

^a Department of Neurology, Medical University of Warsaw, Warsaw, Poland^b Institute of Genetics and Biotechnology, Faculty of Biology, University of Warsaw, Warsaw, Poland^c Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

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ABSTRACT

Mitochondrial diseases may cause a wide range of central and peripheral nervous system disorders, as well as muscle disorders. The diagnostic workup routinely includes electrophysiological, morphological, neuroimaging and genetic studies. In some cases, the diagnosis may be ascertained only when mitochondrial DNA (mtDNA) examination in the muscle is performed. We report on a case of a 24-year-old woman, with a 7-year history of slowly progressive cerebellar syndrome and bilateral ptosis. Mitochondrial encephalomyopathy was suspected, based on the clinical picture and results of examinations, but the typical red ragged fibers were not found in the muscle biopsy. The results of molecular analysis of mtDNA showed a mtDNA deletion in the muscle and, on a level detectable only with polymerase chain reaction method, in blood leukocytes. This case emphasizes the important role of mtDNA studies in muscle in nonspecific multisystem mitochondrial disorders, even without clinical muscle involvement.

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1. Introduction

Mitochondria are organelles involved in cellular energy production, which contain their own DNA (mtDNA) encoding a small number of essential polypeptides of the oxidative phosphorylation system (OXPHOS). The coding sequences for 2 rRNAs, 22 tRNAs and 13 polypeptides are contiguous and without introns [1]. However, most of the 88 protein subunits of the mitochondrial respiratory chain (RC) complexes as well

as the mtDNA replication and most of the expression systems are encoded by the nuclear genome. Thus, the proper operation of the respiratory chain depends on interactions between numerous genes. Mutations in nuclear DNA are transmitted according to Mendelian rules. They occur in genes encoding RC subunits and in genes encoding assembly and auxiliary factors of the RC as well as proteins that affect maintenance and expression of mtDNA or that have functions indirectly linked to OXPHOS [2]. Until now more than 200 mtDNA point mutations, numerous mtDNA deletions as well

* Corresponding author at: Klinika Neurologii, Warszawski Uniwersytet Medyczny, ul. Banacha 1a, 02-097 Warszawa, Poland.
Tel.: +48 22 599 28 58; fax: +48 22 599 18 57.

E-mail address: mgawel@wum.edu.pl (M. Gawel).

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as hundreds of mutations in nuclear genes have been described [3]. However, it is still impossible to establish the genetic defect in 80–95% of patients suspected of having a mitochondrial disorder [4].

Mitochondrial disorders are a heterogeneous group of multi-system disorders resulting from impaired OXPHOS [5]. They may affect multiple organs, but the clinical manifestations vary in respect to age of onset, course and disease severity. Symptoms are diverse and often non-specific [6,7], making it difficult to establish a precise genotype–phenotype correlation and obtain a definite diagnosis [8,9]. The true prevalence of mitochondrial disorders is difficult to assess. It is estimated that up to 9.2 in 100,000 adults aged less than 65 years may be affected [9]. At the genetic level, mitochondrial disorders may result from mutations either in nuclear genes or in mitochondrial DNA (mtDNA). Therefore, the recognition of mitochondrial disorders remains challenging and diagnostic procedures require a wide range of investigations.

In the diagnostic work-up of mitochondrial disorders the most important first step is the precise recognition of the patient's symptoms and signs. Then, the various laboratory tests, electrophysiological studies (electromyography, nerve conduction studies and electroencephalography) and neuroimaging studies including magnetic resonance spectroscopy may be applied. Usually, during further investigation, skeletal muscle or skin biopsies are performed [10–12]. This makes it possible to obtain tissues for morphological, biochemical and supplementary genetic studies.

2. Case report

We present a case report of a 24-year-old woman, a student of psychology, with a 7-year history of ataxia with slurring and scanned speech, and with unremarkable family history. She was referred to the Department of Neurology, Medical University of Warsaw for the first time when she was 17 years old. The first symptoms – arm tremor and difficulties in pronouncing words – occurred when she was 14 years old and slowly progressed. The patient's mother reported compulsory laughter and crying occurring also for 3 years. When she was 16, she started complaining of progressive difficulties with stability and nausea with vomiting.

The neurological examination showed bilateral ptosis, more pronounced in the left eyelid, pseudobulbar syndrome and cerebellar syndrome (scanned speech, tremor of the head, intention tremor of hands, with slight positional tremor, ataxia of upper and lower extremities, walking with slightly widened base). There was no significant cognitive impairment.

During the first hospitalization, the diagnostic methods including laboratory tests in blood and cerebrospinal fluid, neuroimaging and electrophysiological examinations were used. Slight hypocalcemia (2.16 mmol/L; normal range 2.2–2.75 mmol/L) and a decreased level of magnesium (0.67 mmol/L; normal range 0.7–1) were found in routine laboratory tests. The parathormone level was decreased (11.97 pg/mL; normal range 15–95 pg/mL), so hypoparathyroidism was diagnosed. The level of lactic acid in blood was normal. However, an elevated level of protein (155 mg/dL) with a normal level of glucose and normal cytosis in cerebrospinal fluid (CSF) was found. Densitometry of the lumbar spine revealed the first symptoms of decalcification and prophylaxis of osteoporosis was initiated. Cardiomyopathy and cardiac arrhythmias were excluded by echocardiography and 24-h ECG tests. Audiological tests demonstrated significant sensorineural hearing loss in the right ear. Ophthalmologic examination excluded retinitis pigmentosa. Electrophysiological studies revealed normal conduction parameters in peripheral nerves and normal motor unit potentials in muscles. EEG showed generalized discharges of delta waves 3 Hz and theta waves 4–7 Hz with sporadic sharp waves. Magnetic resonance imaging (MRI) of the brain disclosed diffuse involvement of the white matter (Fig. 1A and B). Magnetic resonance spectroscopy (MRS) revealed a high concentration of lactates in white matter of both hemispheres, in basal ganglia and in cerebellar hemispheres. The levels of N-acetylaspartate and choline were low.

Viral infections (Epstein-Barr virus, cytomegalovirus, herpes simplex virus), mycosis and neuroborreliosis were excluded using appropriate tests as well as some forms of leukoencephalopathy as metachromatic leukodystrophy, adrenoleukodystrophy and Krabbe disease.

The history of slowly progressed ataxia with scanned speech and arms tremor, bilateral ptosis, pseudobulbar syndrome with increased level of protein in cerebrospinal fluid, diffuse involvement of the white matter showed in MRI

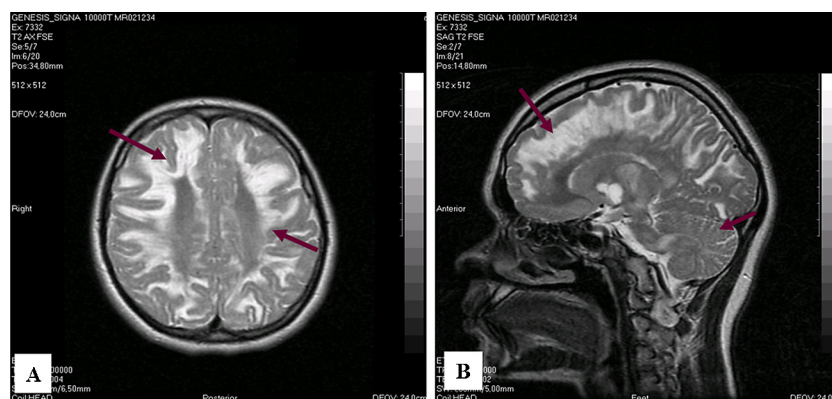


Fig. 1 – (A) MRI. Diffuse hyperintense changes in white matter of both hemispheres (arrows). (B) MRI. Diffuse hyperintense changes in white matter of hemispheres, in cerebellar hemispheres (arrows) and in basal ganglia.

and increased peak of lactates in MRI spectroscopy suggested the diagnosis of mitochondrial encephalomyopathy. The next step in diagnosis was muscle biopsy to find ragged-red fibers.

The patient was given coenzyme Q10 and supplements of calcium and vitamin D3.

2.1. Muscle biopsy

Skeletal muscle biopsy of the quadriceps was performed under local anaesthesia. Cryostat sections of fresh-frozen muscle were stained with routine histological and histochemical methods. Another part of the muscle was routinely prepared for electron microscopy [13]. Moreover, respiratory chain complex activity in the muscle homogenate was checked with reference to the activity of citrate synthase.

Minor nonspecific myopathic changes were detected in light microscopy, with no typical ragged-red fibers (Fig. 2A and B). In electron microscopy, small collections of abnormal mitochondria with irregular cristae were detected in some fibers (Fig. 2C and D). Additionally, biochemical studies of the respiratory chain complexes showed only a slight and insignificant reduction in complex IV activity.

2.2. Molecular analysis

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) tests for common mitochondrial DNA point mutations: 3243A>G, 8344A>G, 8993T>G and 8993T>C were performed as described in our previous paper [14].

A PCR test for the so-called common deletion of 4977 bp was performed as described previously by Soong and Arnheim [15]. Long PCR with primers located in the mtDNA major arc (forward primer 6730–6750, reverse primer 16,545–16,526) was performed using the Expand Long template PCR System (ROCHE). PCR was carried out in 25 µL volume consisting of 2.5 µL 10× PCR number 2 buffer, 12.5 mM of each dNTP, 1.875U polymerase, 6 nM of each primer and 100 ng of DNA template. PCR was performed at 94 °C for 2 min, 10 cycles at 94 °C for 10 s, 58 °C for 30 s, 68 °C for 8 min and 25 cycles at 94 °C for 10 s, 58 °C for 30 s, 68 °C for 8 min + 20 s per cycle and final elongation at 68 °C for 20 min. For Southern analysis, total DNA (1000–1200 ng) were separately digested with BamHI and PvuII overnight at 37 °C. After electrophoresis in 0.8% agarose gel, digested DNA samples were transferred onto Hybond membrane and hybridized with previously labeled with ³²P probe, complementary to the D-loop region of mitochondrial DNA. Restriction mapping of the long PCR product was performed using BclI, HpaI, SnaBI, AuaI and EcoRV restriction enzymes. The PCR product obtained with primers amplifying the region 7349–14928 [16] encompassing the deletion borders was sequenced.

Molecular analysis performed on DNA isolated from peripheral blood for common mitochondrial DNA point mutations: 3243A>G, 8344A>G, 8993T>G and 8993T>C gave negative results as did a PCR test for the so-called common deletion of 4977 bp. Long PCR with primers located in the mtDNA major arc performed on DNA isolated from peripheral blood was positive suggesting a deletion greater than 6 kb

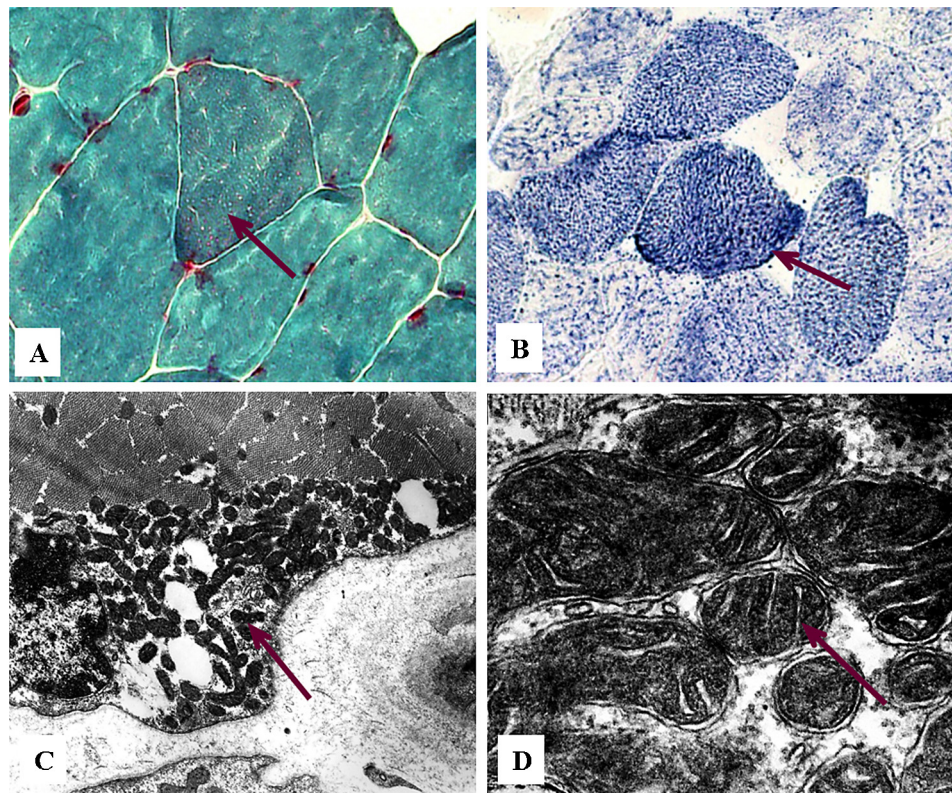


Fig. 2 – (A) Gomori trichrome 200×. Fiber with abnormal reddish rim (arrow). (B) Succinic dehydrogenase (SDH) 200×. Slightly increased activity of SDH in some fibers (arrow). (C) ME 20,000×. Accumulation of abnormal mitochondria (arrow). (D) ME 50,000×. Mitochondria with irregular cristae (arrow).

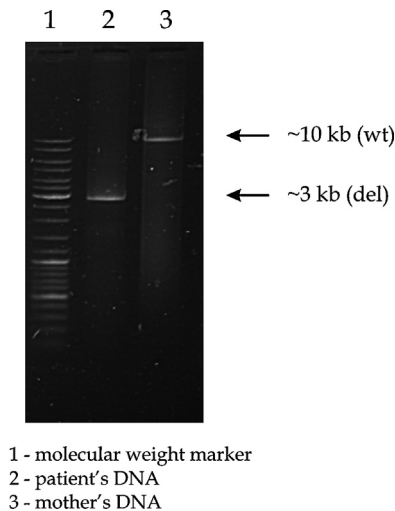


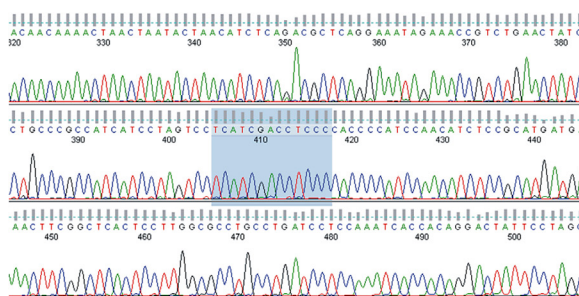
Fig. 3 – Long polymerase chain reaction for blood leukocyte DNA.

(Fig. 3). Southern analysis for mitochondrial rearrangements was negative for the blood sample.

Deletion screening was repeated for DNA isolated from muscle biopsy. Southern analysis revealed the existence of a single large mtDNA deletion in muscle with 24% heteroplasmy. Long PCR product mapping, followed by sequencing of the PCR product comprising the deletion borders, enabled us to determine the precise deletion size. It has a length of 6976 nucleotides located between nucleotides 7823 and 14,799 and contains direct imperfect border repeats of 12 nucleotides (Fig. 4). This result confirmed the diagnosis of a mitochondrial encephalomyopathy.

Long PCR analysis was performed for the patient's mother peripheral blood DNA with a negative result. The PCR test for the common deletion and Southern hybridization were performed for patient's brother. We also carried out the PCR with primers comprising the patient's deletion borders. All methods gave negative results.

Part of the determined sequence



3. Discussion

The ubiquitous nature of mtDNA and the peculiar rules of mitochondrial genetics contribute to explaining the extraordinary clinical heterogeneity of mitochondrial disorders. However, due to the fact that the muscle and nerve tissues are highly dependent on oxidative phosphorylation, they are usually severely affected. According to the recently published guidelines by Wong et al. [4] in the first diagnostic step the most common point mutations and large scale deletions should be checked. The most commonly used mtDNA samples are from blood leukocytes, however, according to the literature, the muscle tissue is the best one for screening for mtDNA rearrangements [10].

Deletions of the mtDNA, first described by Holt et al. in 1988 [11], are mostly heterogeneous and vary in length and location. The so-called common deletion encompasses a 4977 bp fragment and includes several tRNA and protein coding genes [3]. Usually the deletions are associated with the chronic progressive external ophthalmoplegia (CPEO), Kearns-Sayre syndrome (KSS) and Pearson marrow-pancreas syndrome (PS). CPEO is characterized by progressive external ocular muscle impairment accompanied very often by ptosis. Kearns-Sayre syndrome usually begins in the second decade of life, and apart from progressive external ophthalmoplegia, pigmentary retinopathy as well as cerebellar ataxia, cardiac conduction defects and elevated cerebrospinal fluid protein concentration occur. In contrast, Pearson syndrome is associated with multisystem manifestations such as sideroblastic anaemia, pancytopenia, exocrine pancreatic insufficiency, malabsorption, nephropathy and hepatopathy [10]. Moreover, mtDNA deletions have also been described in many other conditions such as deafness and diabetes as well as in neurodegenerative disorders [3].

Because of their heterogeneity, it is difficult to describe characteristic symptoms for mtDNA deletions. Our patient presented some features typical for Kearns-Sayre syndrome: cerebellar syndrome, mild ptosis and elevated level of protein in cerebrospinal fluid. However, retinitis pigmentosa and cardiac conduction defects were excluded and RRF were not

Alignment with the reference sequence

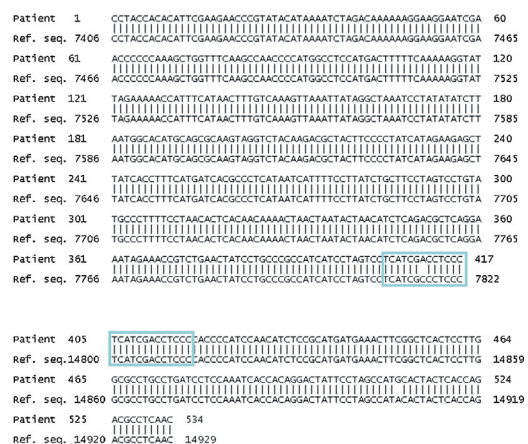


Fig. 4 – Determination of deletion borders by sequencing.

found in muscle tissue. The type of deletion found in our patient has not been described so far.

Yamashita et al. [12] reported a group of 136 patients with mtDNA deletions and with the predominance of CPEO and KSS in clinical manifestations. The length and location of the deletions were very heterogeneous. Moreover, the length of the deletion and number of deleted tRNA genes were directly correlated with the earlier onset of the disease and with the more severe involvement of the central nervous system [12]. The exact pathological mechanism is not known, but it is thought that mtDNA rearrangements are caused by inappropriate mechanisms of mtDNA repair processes [3].

The presented case emphasizes the role of mtDNA studies in muscle in the diagnosis of atypical multisystem mitochondrial disorders, even without evident clinical muscle involvement. The results of molecular analysis of mitochondrial DNA show the presence of single, large deletion in the mtDNA in the patient's muscle and, on a level detectable only by PCR methods, in blood leukocytes. This is a frequent situation for large mtDNA deletions, which are often at a low level or even undetectable in blood. The percentage of mutated mtDNA molecules in the patient's muscle biopsy is rather moderate. This is in agreement with the histopathology, which has shown only slight accumulation of mitochondria with no typical ragged-red fibers, and with slightly increased succinate dehydrogenase activity. Negative deletion screening of the patient's mother and brother is concordant with the sporadic rather than inherited character of single mitochondrial deletions. Hypoparathyroidism was the additional feature, as it is sometimes reported in encephalomyopathies.

The case of our patient confirms the necessity of molecular analysis of mtDNA in the patient's muscle in uncertain diagnosis. It points out that the diagnosis of mitochondrial disorder requires various investigations and neurological, cardiological, endocrine and ophthalmological follow-up is advised.

Conflict of interest

None declared.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical

Association (Declaration of Helsinki) for experiments involving humans; Uniform Requirements for manuscripts submitted to Biomedical journals.

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