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Case report

A new mutation in the mitochondrial tRNA^{Pro} gene associated with early-onset neuromuscular phenotype and ragged-red fibers

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Abstract

An 11-year-old boy with psychomotor delay, exercise intolerance, ptosis and growth delay had a muscle biopsy showing typical mitochondrial alterations (60% of ragged-red fibers and 90% of cytochrome-c oxidase-deficient fibers). Next-generation sequencing revealed a novel heteroplasmic mutation (m.15958A>T) in the *MTTP* gene that encodes tRNA^{Pro}. The mutation was not present in the accessible non-muscle tissues of the patient's asymptomatic mother. Mutations in the rarely affected *MTTP* gene are responsible for different clinical presentations. We report the third early-onset case associated with a mutation in this gene. The severity of myopathy is likely related to the high mutation rate (96%) found in the patient's muscle. The clinical heterogeneity associated with *MTTP* mutations illustrates the value of the next-generation sequencing in routine diagnosis of mitochondrial diseases.

Keywords: Mitochondrial myopathy; mtDNA; *MTTP* gene; Neuromuscular disease; New mutation; NGS

1. Introduction

Mitochondrial tRNA gene mutations are an important cause of human disorders and are associated with a large clinical spectrum ranging from isolated organ-specific diseases such as myopathy or hearing loss to multisystemic disorders with encephalopathy, ptosis, ophthalmoplegia, diabetes mellitus, cardiomyopathy, developmental delay. . . Around 200 pathogenic point mutations in mitochondrial tRNA (*MTT*) genes have been identified to date [1,2]. Several *MTT* genes are hotspots for mutations associated with mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) or diabetes mellitus (*MTTL* (*UUR*)), myoclonic epilepsy associated with ragged-red fibers (MERRF) (*MTTK*) or hearing loss (*MTTS1* (*UCN*)). Some *MTT* genes have been rarely associated with human disease including the *MTTP* gene encoding tRNA^{Pro}.

We report a young boy who harbored a novel pathogenic m.15958A>T point mutation in the *MTTP* gene. The severe mitochondrial alterations found in the patient's muscle were associated with a high level of mutation load. The significant mutation rate found in non-muscle tissues of the patient likely explains the association with severe growth delay and the cognitive impairment.

2. Case report

The patient was the second child of healthy unrelated parents and was born at full term. There was no family history. The patient had normal development until the age of 4 years. Then, he presented with myalgia, and weakness during effort mostly in the lower limbs. He also developed failure to thrive. At the age of 9, the symptoms worsened. Episodes of myalgia and weakness were more frequent. He presented with a right ptosis and a dysarthria mainly at the end of the day. The failure to thrive increased (weight −2 SD, height −3 SD and OFC −1.5 SD).

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At 11 years of age, weight and height were 23 kg (−3 SD) and 129 cm (−3 SD), respectively. The patient could not walk more than a few dozen meters because of weakness, muscle cramps and tachycardia. He had learning difficulties and had repeated a grade in school. He had the support of a special needs teaching assistant and his educational program included speech and psychomotor therapy. Neurological examination revealed moderate amyotrophy in the lower limbs with mild proximal and distal muscle weakness associated with a muscle strength score of 4/5. There was no muscle atrophy and no loss of muscle strength of the upper limbs. Gowers' sign was negative. The deep tendon reflexes were present. There was a right ptosis without ophthalmoparesis. There was no sensory loss, no ataxia, no movement disorder, no loss of balance and the rest of the clinical examination was normal. Laboratory investigations revealed increased levels of plasma lactate (5.3 mM/l, 0.5 < normal < 2.0), an elevated lactate to pyruvate ratio (36, normal < 20), and normal creatine kinase levels. The electromyography (EMG) revealed myopathic features, with short-duration, polyphasic, and high-pitch Motor Unit Action Potential (MUAPs) displaying a characteristic early recruitment pattern. Nerve conduction studies excluded denervation disorders. The echocardiogram was normal and brain MRI showed no abnormality.

Blood and tissue samples were obtained after the parents had given informed consent. Enzymatic measurements of the individual respiratory chain complexes and citrate synthase were performed at 37 °C on muscle crude homogenates as previously described [3] and proteins were measured according to Bradford microassay [4]. Polarographic studies on fibroblasts of intact cell respiration and digitonin (0.004%) permeabilized cells mitochondrial substrate oxidation were carried out as previously described [3].

For molecular analyses, total DNA was extracted using standard phenol chloroform procedure. Long-range PCR and determination of mtDNA copy number were performed as previously described [5,6]. For targeted next-generation sequencing (NGS) with Ion PGM System (Ion Torrent), whole mtDNA was amplified from 250 ng of genomic DNA, using the Roche Expand Long Template Kit (Roche Applied Science), in two overlapping long range PCR amplicons (primers spanning positions 446-16220 and 9411-9027). Library preparation was performed following the manufacturer's instructions (Library Builder – Ion Xpress plus Fragment Library kit, Life Technologies) for 200 bp single-end reads. Emulsion PCR was performed on pooled libraries (Ion PGM Hi-Q Chef kit; Life Technologies). Samples were prepared according to the instructions provided with Ion PGM Hi-Q Sequencing Kit (Life Technologies) protocol and were loaded on an Ion 316 chip. Sanger sequencing was performed by standard procedure on an ABI 3130XL automated sequencer (Applied Biosystems).

To assess quantitatively the level of the m.15958A>T mutation, Quantitative Multiplex PCR of Short Fluorescent fragments (QMPSF) was performed with a reverse primer (nucleotides (nts) 16172-16151) and a forward labeled primer (nts 15836-15857) on DNA extracted from muscle, urinary epithelial cells, blood, buccal mucosa or skin fibroblasts. A 337 base-pair (bp) mtDNA fragment was amplified, then cut by *DdeI*. After digestion, the wild-type labeled fragment was 161 bp long and the mutant mtDNA labeled fragment was 122 bp long (Fig. 1B). The quantification of heteroplasmy level was performed with an ABI 3130 system (Applied Biosystems).

Muscle biopsy revealed a typical mitochondrial myopathy with 60% of ragged-red fibers and 90% of cytochrome oxidase (COX)-deficient fibers. The activities of the respiratory chain complexes were measured in the patient's muscle homogenates. The activities of complexes I, III and IV were decreased when referred to muscle proteins (Table 1A). However, these activities became significantly lower when corrected for abnormal mitochondrial proliferation, i.e., after normalizing respiratory chain complex activities to the activity of citrate synthase, a mitochondrial enzyme which is a good index of mitochondrial volume. This observation also highlights the importance of respiratory chain enzyme assessment on crude homogenates in the affected tissue. Determination of mtDNA amount by qPCR revealed an increase in copy number (202%) that is consistent with the presence of a mitochondrial proliferation. The presence of large-scale mtDNA rearrangements in the patient's muscle was excluded by long-range PCR. The analysis of the entire mitochondrial genome by targeted next-generation sequencing (NGS) revealed a m.15958A>T heteroplasmic transition in the *MTTP* gene that encodes the mitochondrial $tRNA^{Pro}$ gene. The presence of this variant was confirmed by Sanger sequencing (Fig. 1A). This change was not reported in the URLs of mtDB-Human Mitochondrial Genome Database (http://www.mtdb.igp.uu.se/), MITOMAP (http://www.mitomap.org/MITOMAP), and in house databases.

QMPSF analysis revealed that the highest mutation load level was found in muscle (94%) with lower levels present in urinary epithelial cells (78%), skin fibroblasts (48%) and blood (9%). The mutation was not detected in buccal mucosa (Fig. 1B,C). Spectrophotometric assay in the patient's fibroblasts revealed a complex III deficiency (Table 1B) and polarographic analysis showed normal oxygen consumption and mitochondrial substrate oxidation (Table 1C). Both spectrophotometric and polarographic assays were performed on the same culture passage number. Analysis of urinary epithelial cells, blood and buccal mucosa from the proband's clinically unaffected mother failed to identify the m.15958A>T variant suggesting a *de novo* mutation event. Unfortunately, there was insufficient material to perform single muscle fiber analysis.

3. Discussion

The clinical presentation of our patient associated with elevated lactate levels and high lactate/pyruvate ratios in blood suggested a mitochondrial disease. The mitochondrial abnormality was confirmed by muscle biopsy, which revealed 60% of ragged red fibers (RRF) and 90% of COX-deficient fibers. Such percentage of RRFs, rarely found in young children, was correlated with the severity of clinical signs and the presence of a multiple respiratory chain complex deficiency.