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Case report

A novel sporadic mutation G14739A of the mitochondrial $tRNA^{Glu}$ in a girl with exercise intolerance

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Abstract

We describe a 7-year-old girl who presented with loss of appetite, weakness and excercise intolerance. Enzyme investigation of the respiratory chain in muscle tissue revealed a combined complex I, III and IV deficiency. A novel heteroplasmic G → A exchange at nucleotide position 14739 was found in the *MTTE* gene of the tRNA glutamic acid. The mutation load in muscle was 72%, urine sediment 38%, blood 31% and fibroblasts 29% and it correlated with COX-negative fibres. Our patient presented with a predominantly myopathic phenotype. The G14739A mutation is the third reported in the mitochondrial tRNA glutamic acid gene, and it occurred in a sporadic case.

Keywords: Mitochondria; mtDNA; tRNA; Mitochondrial myopathy

1. Introduction

Disorders of the mitochondrial energy metabolism are clinically heterogeneous and can affect various organs. Highly energy dependent tissues such as muscle, heart and the central nervous system are most frequently involved [1,2]. The respiratory chain and the ATP synthase consist of five enzymes which all contain multiple subunits. Thirteen of these subunits are encoded by the mitochondrial genome (mtDNA). Furthermore mtDNA encodes 22 tRNAs and two rRNAs necessary for the translational process within mitochondria.

Since the first identifications of pathogenic mutations of the mtDNA in the late 1980s [3,4] more than 150 point mutations and rearrangements have been reported to date. Mutations within tRNAs usually result in combined respiratory chain deficiency of complexes I, III as well as cytochrome *c* oxidase (COX) and affect the ATP synthase to a various degree, but spare complex II which is devoid of mtDNA encoded subunits.

Here we describe a novel mutation within the aminoacyl acceptor stem of the mitochondrial $tRNA^{Glu}$, which was identified in a 7-year-old girl with non specific symptoms of poor appetite and weakness.

2. Case report

After an uneventful pregnancy the girl was born normally at term. Free sitting was possible at the age of 7–8

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months, free walking at the age of 17 months. Her mother reported that she was slower at running than children of the same age. At the age of 7 years she was referred to our gastroenterologist due to loss of appetite, weakness and slight growth retardation. Her body weight shifted from the 50–75th percentile from the age of 5 years to the 10th percentile at the age of 7 years, similarly the body length from the 75–90th percentile to the 25–50th percentile. Loss of appetite and weight loss were only transient. Weight and length gain are at the moment adequate with values both on the 25th percentile. Neurological investigation revealed a discrete muscular atrophy. Slightly elevated glutamate-oxaloacetate transaminase, lactate dehydrogenase and creatine kinase were found. Bicycle ergometry showed an endurance performance of 25 W (70% of age-matched controls), at a heart frequency of 195 per minute, and a pathological increase in lactate (from 4.0 to 8.1 mmol/l). Electromyographic investigation revealed a clear myopathic pattern. Electrocardiography and echocardiography as well as ophtalmological investigations were normal. Muscle biopsy was performed at the age of 7.5 years. Brain MRI at the age of 8 years was normal. She is successfully attending a normal school. There is no family history of neurologic or muscle diseases.

2.1. Morphological and biochemical analysis of the muscle

A muscle biopsy of the *M. vastus lateralis* was performed. Morphological and enzyme-histochemical analyses [5] showed numerous ragged-red (Fig. 1a) and COX-negative fibres (Fig. 1b). Electron microscopy revealed irregular mega-mitochondria with tubular structures and focally subsarcolemmal glycogen accumulations (Fig. 1c). Respirometric investigation of intact mitochondria in digitonine-permeabilised muscle-fibres [6] was normal in relation to muscle wet weight but reduced in relation to citrate synthase (Table 1). Biochemical investigation [6] of respiratory chain enzymes, citrate synthase and pyruvate dehydrogenase complex in muscle showed elevated activities of citrate synthase, complex II and the oligomycin-sensitive ATPase in relation to the protein content. Complexes I, III and IV were moderately reduced in relation to citrate synthase activity. This shifted enzymatic pattern suggested a defect within the mitochondrial DNA.

Table 1
Biochemical investigation of native digitonine-permeabilised muscle fibres by respirometry and measurement of oxidative phosphorylation enzymes and pyruvate dehydrogenase in muscle 600 g supernatant

	Patient[a]	Controls[a]	Patient[b]	Controls[b]
Pyruvate + Malate + ADP	1.38	1.19–2.2	64	107–204
Succinate + ADP	1.50	1.17–2.25	70	106–212
TMPD/ascorbate	2.60	2.41–5.49	121	229–517
	Patient[c]	Controls[c]	Patient[d]	Controls[d]
Citrate synthase	391	160–310		
Complex I	47	30–65	0.12	0.17–0.26
Complex I + III	58	27–58	0.15	0.13–0.19
Complex II	136	53–100	0.35	0.30–0.36
Complex II + III	83	41–75	0.21	0.20–0.26
Complex III	421	230–490	1.08	1.30–1.96
Cytochrome *c* oxidase	324	205–739	0.83	1.24–2.38
Oligomycin-sens. ATPase	294	78–180	0.75	0.24–0.70
Pyruvate dehydrogenase	12.9	5.3–19.8	0.033	0.026–0.079

TMPD, *N*,*N*,*N*′,*N*′-tetramethyl-p-phenylenediamine.
[a] nmol O_2/min/mg fibres.
[b] nmol O_2/min/Unit citrate synthase.
[c] Units/g protein.
[d] Units/Unit citrate synthase.

2.2. Mutation analysis

The 22 mitochondrial tRNAs were amplified by PCR from genomic DNA from muscle and analysed by DHPLC [7]. In the DNA fragment including the $tRNA^{Glu}$ an additional peak was found (Fig. 2a). The other 10 PCR fragments showed no abnormalities. Sequence analysis (Fig. 2b) on a CEQ-8000 system (Beckman Coulter, Fullerton, CA, USA) revealed a heteroplasmic exchange $G \rightarrow A$ at nucleotide position 14739 (G14739A) which affects the aminoacyl acceptor stem of the $tRNA^{Glu}$ (Fig. 2c). In order to study the distribu-

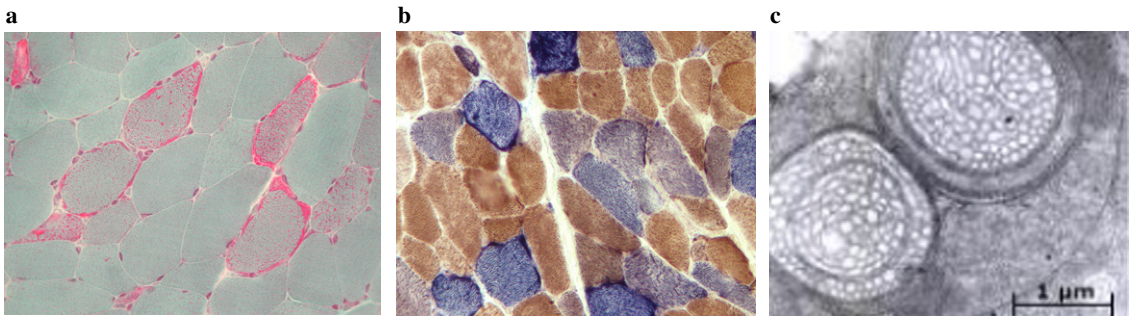

Fig. 1. Morphological investigations of the muscle: Gomori trichrome staining showing numerous ragged-red fibres (a), succinate dehydrogenase and cytochrome c oxidase (COX) double-staining demonstrating COX-negative (blue) muscle fibers (b), and electron microscopy demonstrating large mitochondria with tubular cristae (c).