Biochemical and Biophysical Research Communications 412 (2011) 518–521

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Mitochondrial myopathy in a child with a muscle-restricted mutation in the mitochondrial transfer RNA^{Asn} gene

Claudio Bruno [a,*], Denise Cassandrini [b], Fabiana Fattori [c], Marina Pedemonte [a], Chiara Fiorillo [b], Giorgia Brigati [a], Giacomo Brisca [a], Carlo Minetti [a], Filippo M. Santorelli [b]

[a] Unit of Muscular and Neurodegenerative Disease, IRCCS G. Gaslini Institute, Genova, Italy
[b] Unit of Molecular Medicine, IRCCS Stella Maris, Pisa, Italy
[c] Unit of Molecular Medicine for Neuromuscular and Neurodegenerative Disorders, IRCCS Bambino Gesù Hospital, Rome, Italy

ARTICLE INFO

Article history:
Received 20 June 2011
Available online 29 June 2011

Keywords:
Mitochondrial myopathy
mt-tRNA gene
Exercise intolerance

ABSTRACT

We report an 11-year-old boy with exercise-related myopathy, and a novel mutation m.5669G>A in the mitochondrial tRNA Asparagine gene (mt-tRNA^{Asn}, *MTTN*). Muscle biopsy studies showed COX-negative, SDH-positive fibers at histochemistry and biochemical defects of oxidative metabolism. The m.5669G>A mutation was present only in patient's muscle resulting in the first muscle-specific *MTTN* mutation. Mt-tRNA^{Asn} steady-state levels and *in silico* predictions supported the pathogenicity of this mutation. A mitochondrial myopathy should be considered in the differential diagnosis of exercise intolerance in children.

1. Introduction

Exercise intolerance, a common presentation of disorders of glycogen and lipid metabolism, has been associated in the last decade with specific mutations in protein encoding genes of mitochondrial DNA (mtDNA), and in different mitochondrial tRNAs [1].

As in other functional impairment of substrate utilization in muscle, defects in the mitochondrial respiratory chain damage energy production and cause recurrent, reversible muscle dysfunction, manifesting as exercise intolerance, myalgia, and cramps. Muscle weakness is an alternative manifestation, often affecting extraocular muscles resulting in ptosis and in progressive external ophthalmoplegia (PEO), or limb girdle muscles with proximal myopathy [2].

Here, we report a child with a mitochondrial myopathy, characterized by mild exercise intolerance and mild weakness since age 10 years, who harbored a novel muscle-restricted mtDNA mutation (m.5669G>A) in the tRNA^{Asn} (*MTTN*) gene.

2. Materials and methods

2.1. Case report

The patient, a full-term healthy infant, is the third child of a family with neither consanguinity nor family history of neuromuscular disease. His psychomotor developmental milestones were within the normal range. Since early childhood he suffered from unexplained easy fatigability. Serum CK levels were slightly increased (2–3×). At 10-years of age he started to complain of muscle pain triggered by intense exercise. In one occasion, there was a rise of CK levels (up to 2000 U/l; normal <150) after intense exercise but he never presented myoglobinuria.

Neurological examination at age 11 showed a slight decrease in muscle bulk, particularly at the scapular girdle and distally in his legs. His strength was decrease (4/5 MRC) distally in the legs. He did not have ptosis and his ocular motility was normal. Deep tendon reflexes, sensory examination, cerebellar function and cognition were normal. Serum CK and blood lactate levels were mildly elevated (350 U/l, normal <150; 2.9 mmol/l, normal <2.1, respectively). Metabolic laboratory investigations were normal. Electromyography (EMG) showed a myogenic pattern. Electrocardiogram and echocardiogram were normal. Two older sibs, the mother, and known maternal relatives are clinically normal.

A muscle biopsy from *m. vastus lateralis* was performed at the age of 11 years and was analyzed histochemically and spectrophotometrically for alterations of oxidative metabolism as described [3].

2.2. mtDNA analysis

Total DNA was obtained from tissues using a standard method. Sequencing of mtDNA was performed on an ABI3500 automatic sequencer using the Big Dye terminator Labeling Kit (Applied BioSystems, Foster City, CA).

* Corresponding author. Address: Laboratory of Muscle Pathology, Unit of Muscular and Neurodegenerative Disease, IRCCS Giannina Gaslini Institute, Largo G. Gaslini 5, 16147 Genova, Italy. Fax: +39 010 3538265.
E-mail address: claudiobruno@ospedale-gaslini.ge.it (C. Bruno).

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doi:10.1016/j.bbrc.2011.06.155

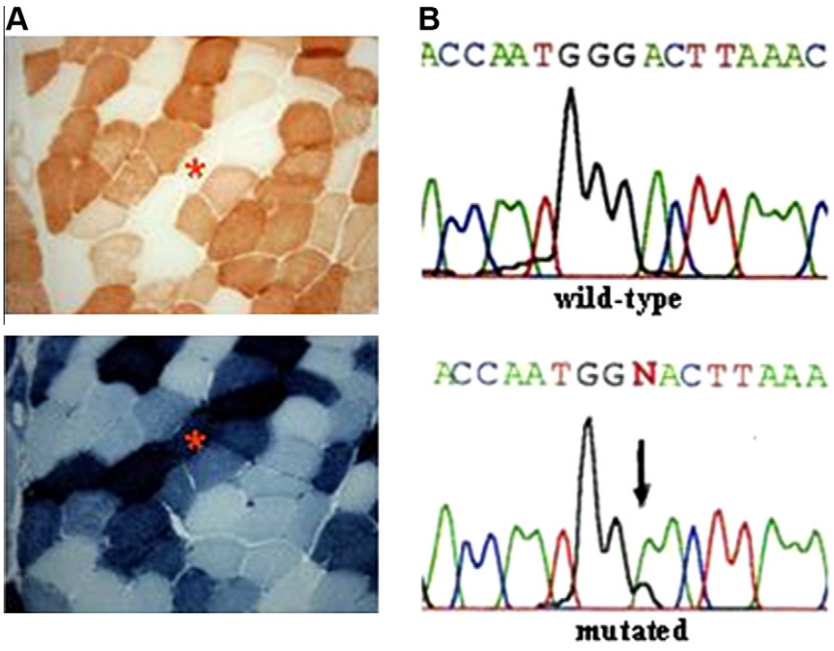

Fig. 1. (A) Serial muscle sections stained for cytochrome *c* oxidase (COX) (top panel) and succinate dehydrogenase (SDH) (bottom panel) reactions. Several fibers showed absence of COX activity (star) and marked mitochondrial proliferation, as shown by their strong SDH reaction (star). (B) Mutation affecting the mt-tRNA^{Asn} gene. Sequence electropherogram encompassing the mt-tRNA^{Asn} in patient's skeletal muscle DNA and in a control. The m.5669G>A mutation is arrow-headed.

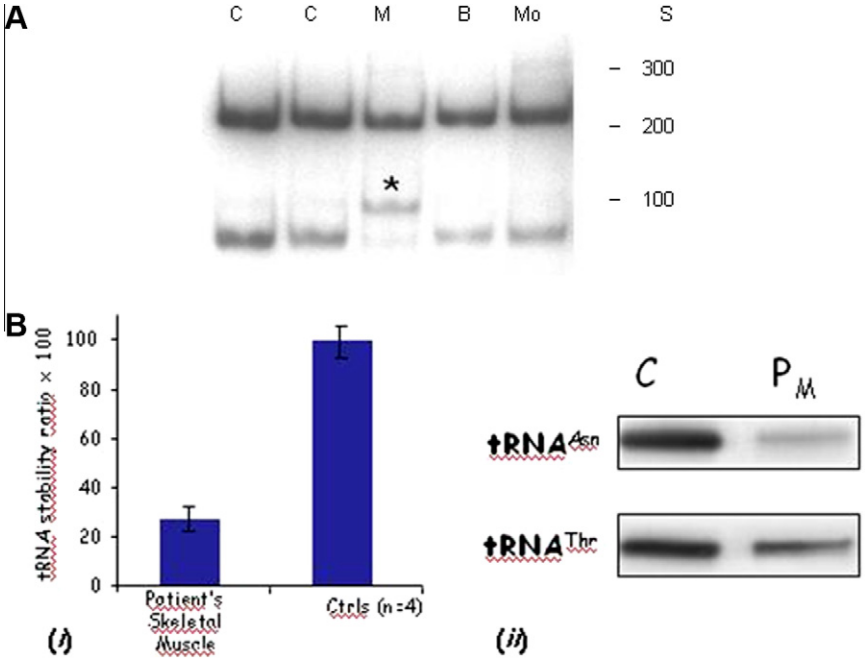

Fig. 2. (A) Quantification of the m.5669G>A mutation in the patient (M, muscle; B, blood), his mother (Mo), and two healthy controls (C) by PCR-RFLP (restriction-fragment length polymorphism) analysis employing the endonuclease *Bsl*I (New England Biolabs, Hitchin, UK). U, uncleaved fragment; S, 100-bp DNA marker size. Using PCR and primers (5′–3′) MTF5630 (nt 5630–5666; AGCCACTTTAATTAAGCTAAGCCCTTACTCCACCAAT) (mismatched nucleotides are underlined) and MTR-5990 (nt 5966–5990; TAGGACTCCAGCTCATGCGCCGAAT), we amplified a 361-bp fragment encompassing the mutation. PCR was performed with a "last hot-cycle" in the presence of alpha-^{32}P deoxycytidinetriphosphate. Equal amounts of products were cleaved with 10U *Bsl*I, separated through a 12% non-denaturing polyacrylamide gel, and radiolabeled products were quantified upon PhosphorImager analysis (Amersham Biosciences, Little Chalfont, UK). Wild-type amplicons are normally cleaved into fragments sized 249-, 76- and 36-bp (not shown). The m.5669G>A mutation removes a site of cleavage, resulting I fragments sized 249- and 112-bp (star). B. Determination of mt-tRNA^{Asn} steady-state levels using high-resolution Northern blot. Total RNA (1 μg) was separated through a 13%, 8 M urea denaturing polyacrylamide gel, electroblotted onto membranes, and hybridised with alpha-^{32}P-radiolabeled probes specific for mt-tRNA^{Thr} (endogenous control) and mt-tRNA^{Asn} transcripts as in [4]. (i) Illustration of the relative mt-tRNA^{Asn}/mt-tRNA^{Thr} ratio in patient and control muscles (Ctrls). (ii) Representative determination of mt-tRNA transcripts in patient's skeletal muscle (P_M) and in an appropriate control sample (C).

Levels of mutant mtDNAs in different tissues from the child, and in blood from his mother were determined using a "last hot" labeled PCR method and the endonuclease *Bsl*I (New England Biolabs, Hitchin, UK). To investigate the functional effect of the mutation on tRNA stability, mt-tRNA^{Asn} steady state levels were determined by high resolution Northern blot analysis in muscle tissue and cultured skin fibroblasts, as described previously using the mt-tRNA^{Thr} levels as endogenous control for loading [4]. *In sil-*