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Novel mitochondrial tRNA$^{Leu(CUN)}$ transition and D4Z4 partial deletion in a patient with a facioscapulohumeral phenotype

Massimiliano Filosto [a,b], Paola Tonin [a], Mauro Scarpelli [a], Chiara Savio [a], Francesca Greco [c], Michelangelo Mancuso [d], Gaetano Vattemi [a], Vittorio Govoni [e], Nicolò Rizzuto [a], Rossella Tupler [c], Giuliano Tomelleri [a,*]

[a] *Department of Neurological Sciences and Vision, Section of Clinical Neurology, University of Verona, Italy*
[b] *Clinical Neurology, University Hospital "Spedali Civili", Brescia, Italy*
[c] *Department of Biomedical Sciences, University of Modena and Reggio Emilia, Italy*
[d] *Department of Neurosciences, University of Pisa, Italy*
[e] *Institute of Clinical Neurology, University of Ferrara, Italy*

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Abstract

Point mutations in mtDNA-encoded tRNA genes frequently cause isolated myopathies but rarely cause the facioscapulohumeral phenotype.

We report on a patient affected with chronic progressive weakness of facioscapulohumeral/peroneal muscles whose muscle biopsy showed a mitochondrial myopathy. mtDNA direct sequencing and RFLP analysis revealed a heteroplasmic transition T12313C which disrupts a conserved site in the TΨC stem of the tRNA$^{Leu(CUN)}$ gene and fulfills the accepted criteria of pathogenicity.

A partial deletion of the nuclear DNA D4Z4 region with residual repeat sizes of 25 kb was also found in the patient and in her mother.

This is the first reported case of mitochondrial myopathy/facioscapulohumeral muscular dystrophy (FSHD) "double trouble".

Keywords: mtDNA; Mitochondrial myopathy; FSHD

1. Introduction

Mitochondrial DNA (mtDNA) mutations are associated with a wide spectrum of disorders involving different tissues, particularly brain and muscle [1]. While tRNA$^{Leu(UUR)}$ is a "hot spot" for mutations that cause different clinical pictures, a relatively small number of pathogenic changes have been reported in the tRNA$^{Leu(CUN)}$ gene from patients with more homogeneous neurological presentation, such as chronic progressive external ophthalmoplegia and myopathy [1–12].

Facioscapulohumeral muscular dystrophy (FSHD), an autosomal dominant neuromuscular disorder, is characterized by progressive weakness of the facial, shoulder girdle and upper limb muscles, often involving peroneal and pelvic girdle muscles. The molecular bases of the disease are unknown, but the disorder is associated with deletions of specific 3.3 kb tandem repeats, termed D4Z4 repeats, on chromosome 4q35 [13]. These deletions lead to inappropriate transcriptional derepression of proximal genes located upstream of D4Z4, including FRG1, FRG2, and adenine nucleotide translocator (ANT1) [13].

We report on a patient with facioscapulohumeral and peroneal muscle weakness who had a new pathogenic mutation in the TΨC stem of the mtDNA tRNA$^{Leu(CUN)}$

* Corresponding author. Tel.: +39 045 8074285; fax: +39 045 585933.
E-mail address: giuliano.tomelleri@univr.it (G. Tomelleri).

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and a partial deletion of the repetitive elements D4Z4 on chromosome 4q35. The clinical and the molecular data are consistent with a diagnosis of mitochondrial myopathy/FSHD "double trouble".

2. Patient and methods

2.1. Patient

A 45-year-old teacher came to our observation for fatigue in lifting her arms above the head and difficulty in walking. She reported incapacity to whistle and scapular winging from adolescence. She did not describe fatigue, myalgia or cramps, even for long trekking, up to 30 years of age. When she was 34 she began complaining of weakness and easy fatigability of the right arm while writing on the blackboard. Two years later she noticed left foot drop and difficulty in climbing stairs.

At 45 years the first neurological examination showed scapular winging more evident on the right side; because of weakness, abduction of the right arm was limited to 30 degrees with poor scapular fixation, while the left arm was abducted beyond 40 degrees; orbicularis oculi and orbicularis oris muscles were weak; the patient had a stepping gait and hyperlordosis with mild prominence of the abdominal wall and used the Gower's manoeuvre to stand up from the floor; shoulder-girdle and left tibialis anterior muscles were atrophic; deep tendon reflexes and sensation were preserved; external ocular movements were full and fundus exam was normal; hearing was spared.

Family history was not contributory; the patient's 76-year-old mother and a 16-year-old daughter were symptomless and their neurological examination was normal.

Blood and urine assays, including resting blood lactate, were normal except for sCK levels that were 2–3 times the normal values in repeated determinations. Pure tone audiometry showed mild bilateral sensorineural deafness at 8000 Hz. EMG recording showed low-amplitude and short duration of motor unit potentials in the four limbs. Electrocardiography and echocardiography were normal. A biopsy of vastus lateralis muscle was performed.

Six years later both neurological examination and EMG recording were unchanged. Electrocardiography was normal while echocardiography showed mild prolapse and insufficiency of the mitral valve.

2.2. Methods

Morphological study of the muscle biopsy and assay of mitochondrial respiratory chain enzyme activities were done as described [14,15]. No specimen for electron microscopy was available.

DNA was extracted from muscle by Puregene™ DNA purification Kit (Gentra Systems, MN, USA).

All 22 mitochondrial tRNA genes were amplified using suitable nucleotide primers [16]. The resulting PCR fragments were directly sequenced using an Autoload Sequencing Kit (Amersham Pharmacia Biotech).

To confirm the mutation, Restriction Fragment Length Polymorphism analysis (RFLP) was performed on mtDNA from the proband's muscle, blood and urine and from her mother's and her daughter's blood and urine [16]. We amplified a 210 bp mtDNA fragment using the following primers: F 12124–12144 CGGGTTTTCCTCTTGTAA ATA and R 12334–12314 CTTTTATTTGGAGTTG CACGA; a "mismatch" base at the position 12315 (C for G) of the reverse primer was introduced to create a restriction site for the endonuclease BSSs I.

PCR conditions for both tRNA sequencing and RFLP analysis were 94 °C for 5 min, followed by 30 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min with a final extension step at 72 °C for 7 min. For RFLP analysis one extra cycle was performed after the addition of 250 nM fluorescein dCTP [17]. PCR products were digested with BSSs I overnight; RFLP analysis was performed on a 12% non-denaturing acrylamide gel and, for quantification by AlleleLinks software (Amersham Pharmacia Biotech), on a 6% non-denaturing gel on an ALFexpress sequencer. Single-fiber PCR was performed using 30 μ thick muscle sections and the same primers as described above and was followed by RFLP analysis and quantification [18].

Genetic analysis for facioscapulohumeral dystrophy (FSHD) was performed as follows: seven μg of genomic DNA, extracted from peripheral blood lymphocytes, were digested with restriction enzyme EcoRI and double digested with EcoRI/BlnI, according to manufacturer's directions (Fermentas). DNA was separated on a 0.4% agarose gel, for 45–48 h at 35 V. After electrophoresis, the gel was stained with ethidium bromide and the DNA was fragmented with a 0,25 N HCl solution. After denaturation with a 0,5 N NaOH solution, the DNA was transferred to a Zeta-Probe GT Genomic Tested Blotting Membrane (Bio-Rad). The probe used for hybridization, p13E-11, a 0.8 kb subclone of cosmid 13E, was labeled by random priming with α^{32}P-dATP, using the Prime/IT II random primer lab kit (Stratagene). Hybridization was performed overnight at 65 °C and the filter was washed first to a stringency of 2× SSC/0,1% SDS at room temperature and then to a stringency of 0.5× SSC/0,1% SDS at 63 °C, followed by autoradiography for 3 days at −80 °C using Kodak X-OMAT LS film with an intensifying screen. After exposure, the alleles were assigned to their respective chromosomes based on their BlnI sensitivity. Sizes were estimated according to a 8–48 kb marker (Bio-Rad).

3. Results

Muscle biopsy showed few atrophic fibers, mild focal increase of connective tissue and numerous ragged red fibers (RRFs) (Fig. 1A). Thirty nine percent of the fibers were cytochrome c oxidase (COX)-negative (Fig. 1B); on longitudinal sections the histochemical defect was segmental.